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Serological surveys to estimate cumulative incidence of SARS-CoV-2 infection in adults (Sero-MAss study), Massachusetts, July–August 2020: a mail-based cross-sectional study

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ABSTRACT

Objectives To estimate the seroprevalence of anti-SARS-CoV-2 IgG and IgM among Massachusetts residents and to better understand asymptomatic SARS-CoV-2 transmission during the summer of 2020.

Design Mail-based cross-sectional survey.

Setting Massachusetts, USA.

Participants Primary sampling group: sample of undergraduate students at the University of Massachusetts, Amherst (n=548) and a member of their household (n=231).
Secondary sampling group: sample of graduate students, faculty, librarians and staff (n=214) and one member of their household (n=78). All participants were residents of Massachusetts without prior COVID-19 diagnosis.

Primary and secondary outcome measures Prevalence of SARS-CoV-2 seropositivity. Association of seroprevalence with variables including age, gender, race, geographic region, occupation and symptoms.

Results Approximately 27 000 persons were invited via email to assess eligibility. 1001 households were mailed dried blood spot sample kits, 762 returned blood samples for analysis. In the primary sample group, 36 individuals (4.6%) had IgG antibodies detected for an estimated weighted prevalence in this population of 5.3% (95% CI: 3.5 to 8.0). In the secondary sampling group, 10 participants (3.4%) had IgG antibodies detected for an estimated adjusted prevalence of 4.0% (95% CI: 2.2 to 7.4). No samples were IgM positive. No association was found in either group between seropositivity and self-reported work duties or customer-facing hours. In the primary sampling group, self-reported febrile illness since February 2020, male sex and minority race (Black or American Indian/Alaskan Native) were associated with seropositivity. No factors except geographic regions within the state were associated with evidence of prior SARS-CoV-2 infection in the secondary sampling group.

Conclusions This study fills a critical gap in estimating the levels of subclinical and asymptomatic infection. Estimates can be used to calibrate models estimating levels of population immunity over time, and these data are critical for informing public health interventions and policy.

Strengths and limitations of this study

- ► Our study collected serological samples in a well-defined rigorous sample frame in a contactless (mail-based) survey in an early stage of the pandemic in an area of high SARS-CoV-2 burden.
- ► A range of potentially associated demographic, occupational and behavioural factors were surveyed to contextualise seropositivity across geographic regions within the state.
- ► Our study sampled from populations affiliated with a large public university in Massachusetts, and may not be generalisable to the general population.

INTRODUCTION

Since emergence in late 2019, the SARS-CoV-2 virus has severely impacted the entire globe. The state of Massachusetts was heavily impacted in the earliest stages of the pandemic, and a 'super-spreader' event in the state in April 2020 may have seeded large case clusters throughout the country.[1] However, the trajectory of the early stages of transmission in the state, as well as across the USA remain poorly understood due to changes in case definitions and limited testing of both symptomatic and asymptomatic persons during the summer of 2020.[2] To assess seroprevalence across the state, a mail-based serosurvey was implemented July–August 2020. At the time of this survey, the Massachusetts Department of Public Health had reported over 109 143 confirmed COVID-19 cases and over 8081 deaths.[3] Seroepidemiological studies are a critical tool to explore infection dynamics, especially where asymptomatic or subclinical infections are common, as for SARS-CoV-2.[4] This study helps to fill

a critical gap in estimating the levels of subclinical and asymptomatic infection to inform consequent levels of population-immunity.[5]

Concurrent with this study, a number of seroprevalence studies were conducted on the East Coast of the USA; these studies focused on specific populations at highest risk and found varying results. A survey in April 2020 in a convenience sample of 200 asymptomatic residents of Chelsea, MA found an estimated seroprevalence of 31.5% (17.5% IgM+/IgG+, 9.0% IgM+/IgG− and 5.0% IgM−/IgG+).[6] This study used a small convenience sample and did not include any randomisation.[6] A study with a larger sample of over 28,000 clinical patient samples in New York City, USA found an IgG seropositivity prevalence of 44% with over 50% of participants reporting no symptoms.[7]

Other seroprevalence surveys across the USA found generally low to moderate prevalence in a diverse set of study populations. A study of 790 university students in Los Angeles, California conducted in April and May of 2020 estimated a prevalence of SARS-CoV-2 IgG antibody of 4.0% (95% CI: 3.0% to 5.1%).[8] During May–April of 2020, a cross-sectional study in St. Louis found IgG seropositivity to be estimated at 1.71% (95% CI: 0.04% to 3.38%) in paediatric patients and 3.11% (95% CI: 0.92% to 5.32%) in adult patients. In the most comprehensive serosurvey from the spring and summer months of 2020, 16,025 clinical samples were analysed with IgG spike protein seroreactivity ranging from 1.0% in the San Francisco Bay Area to 6.9% in New York City.[9] These disparate results highlight major geographic variability in the trajectory of infections, and reinforce the need for additional seroprevalence studies to more fully contextualise trends in immunity to SARS-CoV-2 targeting specific geographic regions.

Though community seroprevalence studies generally rely on serum samples collected in health facilities, the use of dried blood spot (DBS) samples is a practical and effective alternative.[10] DBS samples involve a small fingerprick sample self-collected by participants in their own homes. The use of dried blood samples for antibody assays has been validated in other work prior to the current pandemic,[10 11] and previous studies have evaluated the feasibility, validity and acceptability of using DBS samples for SARS-CoV-2 antibody testing.[12–15] This method of sample collection facilitates efficient population-level sampling while minimising social mixing and concurrent potential exposures.

This study estimated the prevalence of previous infection with SARS-CoV-2 in individuals who had not been diagnosed with COVID-19 and were asymptomatic with representative coverage across the entire state of Massachusetts, USA. Information from this study can provide knowledge regarding the seropositivity of this population and can be used to inform decision-making regarding community reopenings during the pandemic.

METHODS

Study design and participants

The study population included undergraduate students, graduate students, staff and faculty members currently affiliated with the University of Massachusetts, Amherst (UMass) and their household members. On-campus classes were suspended in mid-March 2020; consequently, undergraduates had exposure to the local epidemiology within their home communities from March until sampling in July–August throughout the state (primary sampling group). Conversely, graduate students, faculty, staff, librarians and their family members (secondary sampling group) generally reside in close proximity to Amherst, and broadly reflect transmission in the Western part of the state.

UMass affiliates were eligible to participate in this study if they were above the age of 18, had been living in Massachusetts for the past 8 weeks, had never received a COVID-19 diagnosis from a medical professional, and did not have a fever greater than 100.4 °F at the time of survey completion. Household members were eligible for inclusion if they met all of these same criteria and were between the ages of 23 and 78 (chosen to expand sampling beyond college-age population groups). Both UMass affiliates and their household members had to complete online consent forms in order to participate in the study. Upon meeting eligibility criteria, participants were directed to a consent form which they reviewed prior to providing their first and last name, the date and an electronic signature.

An institutional email list was provided by university administration for recruitment. Initial emails were sent out to UMass affiliates between 23 June 2020 and 26 June 2020 for participant recruitment. The email provided information about the study and links to a screening eligibility survey, informed consent document, initial survey regarding COVID-19 risk factors and information regarding shipping addresses. If the UMass affiliate had a household member interested in participating, a single household member was invited to complete the eligibility, consent and survey forms. The household member was invited to participate prior to analysing samples from the main participant. To increase participation rates, two reminder emails were sent to all non-respondents (day 3 and 6 after initial solicitation). All survey responses were collected and stored in REDCap.[16]

The survey was closed after a 3-week enrolment period, and a subset of participants were selected to receive a test kit containing supplies to collect an at-home DBS sample. To select a population representative of the broader UMass community across the entire state of Massachusetts, two sampling schemes were applied. The first consisted of all undergraduates and their associated household members (primary sampling group); the second sampling frame consisted of graduate students, staff, faculty members, librarians and their household members (secondary sampling group). Within the primary sampling group, selection for biosample

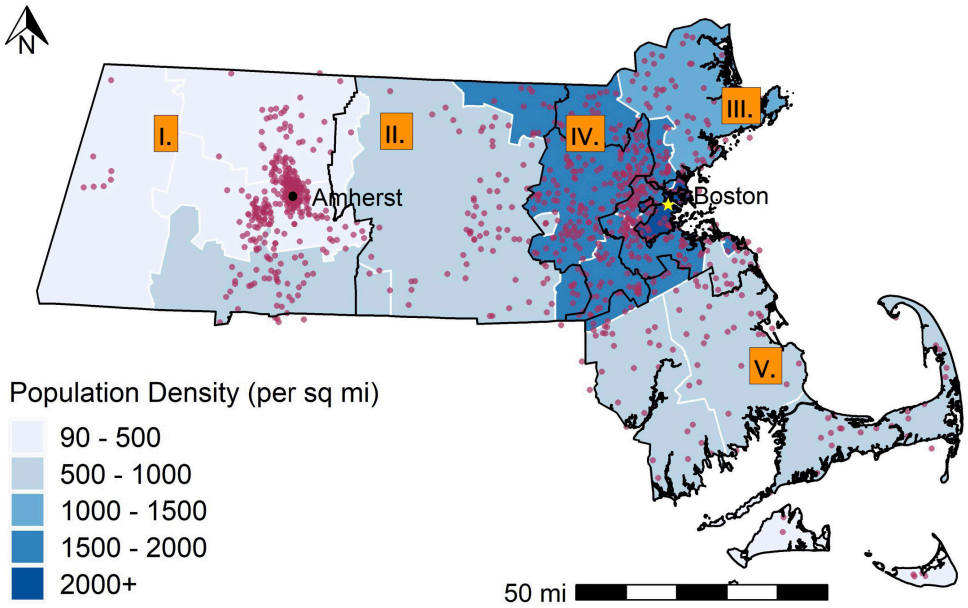

Figure 1 Study sampling frames for SARS-CoV-2 seropositivity surveys, Massachusetts, USA, July–August 2020. State-level emergency response regions are shown in orange; anonymised participant locations are shown as maroon markers (markers may be outside state borders due to jittering).

collection used probability proportional to population size, using the most recent census data aggregated to state-level emergency response regions due to sparse county-level populations (figure 1).[17] For the secondary sampling group, selection for biosampling was via simple random sampling.

The full sample frame selection is shown in figure 2. Briefly, an email invitation was sent to a total of 27 339 individuals, of which 4124 completed the screening, informed consent and initial survey forms. A total of 1001 individuals were then randomly selected to receive a sampling kit. Participants were mailed all materials to safely collect and return samples, including lancets, alcohol wipes, gauze, gloves, bandages, a bloodspot collection card, a prepaid shipping box and detailed printed instruction cards (which included a nurse call line).

When mailing out the test kits, participants were also emailed a link containing an instructional video on how to collect the DBS, along with a detailed survey form with demographics, risk factors and any current symptoms or COVID-19 diagnoses. No participants reported a COVID-19 diagnosis between the initial survey and sample collection several weeks later. All shipments utilised a Biological Substance Category B (UN3373) shipping box.

Sample preparation and ELISA analysis

On receipt of boxes, the DBS sample cards (Whatman Protein Saver 903) were heat-treated (30 min at 56 °C); a single blood spot per card was punched (0.25-inch diameter); and transferred to an ELISA plate. Plates were coated with 1 µg/mL of purified SARS-CoV-2 receptor-binding domain (RBD) diluted in phosphate buffered saline (PBS) overnight at 4 °C and blocked with tris-buffered saline with 0.1% Tween 20 (TBST) containing 5% non-fat dry milk. DBS samples were eluted in 500 µL of TBST overnight at 4 °C and 50 µL of each sample was added to the ELISA plate preloaded with 50 µL of TBST containing 2% non-fat dry milk. Samples were then assayed for SARS-CoV-2 antibodies according to published protocols.[18 19] The RBD protein was produced in-house via transfection of HEK293T cells using polyethylenimine (plasmid was a generous gift from Florian Krammer, Mt. Sinai School of Medicine). Batches were controlled for purity by SDS-PAGE followed by Coomassie staining and ELISA using an anti His-tag monoclonal antibody. Optical densities were read at 405 nm, and each 96-well plate contained seven negative controls and one positive control (serum from PCR-confirmed case at 1/100 dilution). Samples were tested against IgG, and positive samples were confirmed and then tested with anti-IgM antibodies. Optical density values were normalised to the mean optical density of negative controls daily.

Data analysis

Sample size and power

The study was designed to assess seropositivity within the primary sampling group with sufficient precision to inform policy. With 750 persons, and an assumed 5% positivity, the 95% CI for this estimate is 3.6% to 6.9%. Within the five emergency response subregions, at 5% seropositivity, the survey is powered for a precision of 2.3% to 10.2%. The secondary sampling group (n=250) sample size was based on logistic limitations, but was powered to a precision of 2.8% to 8.8%. All CIs are binomial exact, without adjustments for study design effects or non-response.

Analysis of serology data

Finite mixture models were used to determine seropositivity cut-offs. These latent-class models estimate

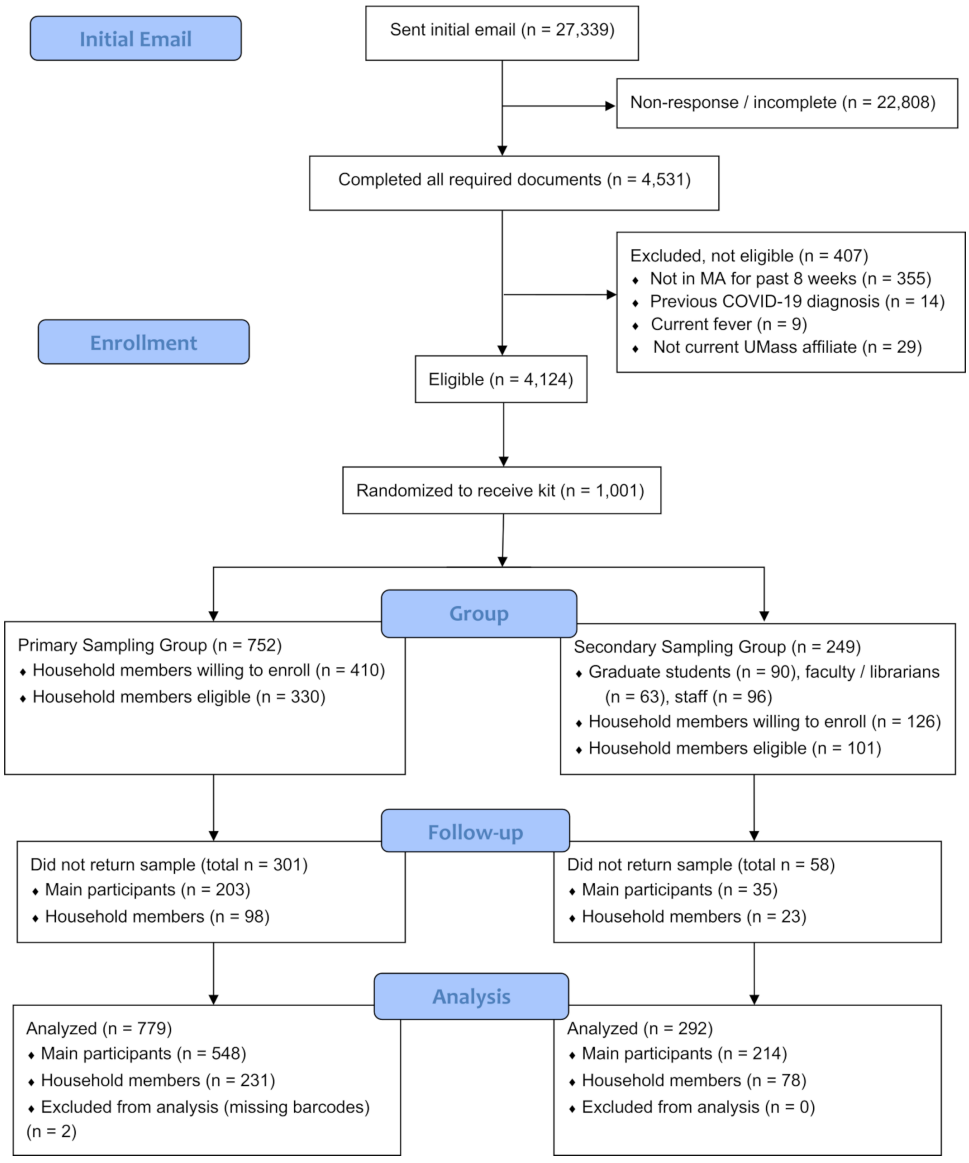

Figure 2 Participant enrolment diagram, SARS-CoV-2 serosurvey, Massachusetts, USA, July–August 2020. Reported using CONSORT, (Consolidated Standards of Reporting Trials) requirements.

breakpoints for seropositive and seronegative subpopulations, and have been applied to a range of pathogen serosurvey data, including rubella, pertussis and parvovirus.[20–22] From this analysis, all samples with an IgG optical density ratio ≥ 2.49-fold above daily background were considered positive for SARS-CoV-2 (distributions shown in online supplemental figure 1; and sensitivity analysis with alternative cut-points can be found in online supplemental table 2).

Adjusted estimates

All reported prevalences and prevalence ratio (PR) estimates are adjusted with non-response weights, which were estimated using inverse weighting. Briefly, logistic regression models were used to calculate propensity scores for each individual in the sample using reported gender and race categories. These were transformed to probabilities; a small number of individuals had extremely large weights due to sparse strata; these weights were truncated at 1/0.02.[23] Weights were then applied to all prevalence and PR estimations using the *survey* package in R.[24] The primary sampling group sample was self-weighting due to probability proportional to population size sampling. Sampling weights were not used in the secondary sampling group as selection utilized simple random sampling.

Multivariable analyses for prevalence ratios

PRs were estimated to assess factors associated with seropositivity, with individual Poisson models[25] for both of the two sampling groups, with robust (sandwich) errors to address clustering within households.

Bivariate analyses were performed for each factor separately. All variables with a p-value < 0.20 based on bivariate association with outcome were further evaluated for inclusion in final models. All final models were adjusted for age, race and gender (see table 1). Due to several

Table 1 Demographics of study populations, SARS-CoV-2 serology surveys in university-affiliated populations, Massachusetts July–August 2020

Characteristic	Primary sampling group (n=779)	Secondary sampling group (n=292)
Gender		
Female	499 (64.1%)	154 (52.7%)
Male	274 (35.2%)	136 (46.6%)
Gender diverse	5 (0.6%)	2 (0.7%)
Missing	1 (0.1%)	0 (0.0%)
Race		
AIAN	17 (2.2%)	1 (0.3%)
Asian	78 (10.0%)	34 (11.6%)
Black	12 (1.5%)	3 (1.0%)
Hispanic	36 (4.6%)	9 (3.1%)
Multiple	37 (4.8%)	11 (3.8%)
White	545 (70.0%)	217 (74.3%)
Missing	54 (6.9%)	17 (5.8%)
Age		
Mean	29.9	41.6
Median	21	39
Range	18–75	21–75
Education		
HS/GED	102 (13.1%)	5 (1.7%)
Some college	483 (62.0%)	24 (8.2%)
BA/BS	117 (15.0%)	78 (26.7%)
More than BA/BS	74 (9.5%)	183 (62.7%)
Missing	3 (0.4%)	2 (0.7%)
Essential worker status		
No	533 (68.4%)	224 (76.7%)
Yes	195 (25.0%)	51 (17.5%)
Missing	51 (6.6%)	17 (5.8%)
Self-reported attitude about COVID-19		
Strongest fear	135 (17.3%)	72 (24.7%)
Somewhat fearful	389 (49.9%)	122 (41.8%)
Neutral/missing	139 (17.8%)	63 (21.6%)
Somewhat not fearful	86 (11.0%)	23 (7.9%)
Not fearful	30 (3.9%)	12 (4.1%)
Self-reported febrile illness since February 2020		
No	534 (68.6%)	224 (76.7%)
Yes	188 (24.1%)	53 (18.2%)
Missing	57 (7.3%)	15 (5.1%)
Self-reported care seeking (if reporting illness since February 2020)		
No	112 (59.6%)	32 (60.4%)
Yes	75 (39.9%)	21 (39.6%)
Missing	1 (0.5%)	0 (0.0%)

The primary sampling group includes UMass undergraduates and household members, and the secondary sampling group includes UMass affiliated faculty, staff and graduate students and household members.
AIAN, American Indian/Alaska Native; BA/BS, Bachelor's degree; HS/GED, High school diploma or General Educational Diploma.

very sparse categories, some were combined in the final models. Specifically, all race/ethnicity categories and all geographic regions were not included in analysis of the secondary sampling group due to unstable estimates.

Model parsimony was evaluated using Akaike/Bayesian information criterion (AIC/BIC) and all tests were two-tailed, with $\alpha = 0.05$. R version 4.0.3 and SAS version 9.4 (SAS Institute, Inc, Cary, NC, USA) were used for analysis.

Patient and public involvement

All members of the university community were invited to participate, and serological testing was proved at no cost to either the sampled individuals or to their selected household contact.

RESULTS

A total of 1001 individuals were enrolled into the study; this included 752 undergraduate students, 90 graduate students, 63 faculty/librarians and 96 staff members (figure 2). Seventy-six percent of these (n=762) returned blood samples for analysis: 548 in the primary sampling group and 214 in the secondary sampling group. Of the 548 participants in the primary sampling group, 230 enrolled a household member. One household member submitted a sample without the sample of the main participant, bringing the total number of undergraduate household members to 231. Of the 214 participants in the secondary sampling group, 78 enrolled a household member. Two returned samples were excluded from analysis due to missing sample identifiers. A total of 1071 samples were included in the final analyses: 762 main participants and 309 household members (figure 2).

Demographic characteristics of both sampling groups are presented in table 1. Race categories do not total to 100% due to non-response and multiple possible answers. Age, gender and essential worker status were broadly similar between those invited to participate and those who completed the study (online supplemental table 1).

Of the total 1071 samples tested, 46 were positive for SARS-CoV-2 antibodies. Demographic results are stratified by IgG serostatus (table 2); no samples showed evidence for IgM positivity. Seropositivity was low-to-moderate across the survey groups, with several important exceptions. Variation

Table 2 Weighted seropositivity by main demographic variables, SARS-CoV-2 serology surveys in university-affiliated populations, Massachusetts July–August 2020

Characteristic	Primary sampling group	Secondary sampling group
Age in years, median (95% CI)	21 (20 to 21)	41 (38 to 44)
Prevalence of SARS-CoV-2 antibodies, by subgroup		
Overall population prevalence (95% CI)	5.3% (3.5 to 8.0)	4.0% (2.2 to 7.4)
Sex % (95% CI)		
Female	4.0% (2.4 to 6.6)	4.9% (2.2 to 10.7)
Male	8.7% (5.1 to 15.0)	3.0% (1.1 to 8.6)
Gender diverse/no response	0.0	0.0
Race % (95% CI)		
White	3.9 (2.6 to 5.9)	–
Multiple	6.3 (1.7 to 23.7)	–
Asian	6.2 (2.7 to 14.5)	–
Missing	1.9 (0.3 to 13.5)	–
Hispanic	5.4 (1.4 to 21.0)	–
Black/AIAN	21.0 (5.8 to 76.4)	–
White	–	4.2 (2.2 to 7.9)
Non-White	–	1.6 (0.2 to 11.7)
Essential worker status		
Yes	4.2 (2.0 to 8.8)	7.1 (2.3 to 21.3)
No	5.8 (3.5 to 9.7)	3.6 (1.7 to 7.6)
Missing response	3.5 (0.9 to 14.2)	0
Participant type		
University-affiliate	5.3 (3.5 to 8.1)	4.3 (2.1 to 8.6)
Household member	5.1 (2.4 to 11.2)	3.3 (0.8 to 13.0)
State emergency response region (see figure 1)		
Region 1	7.8 (3.9 to 15.6)	–
Region 2	1.6 (0.2 to 11.3)	–
Region 3	3.2 (1.0 to 10.7)	–
Region 4	5.7 (3.0 to 10.8)	–
Region 5	6.2 (2.3 to 16.5)	–
Region 1	–	3.1 (1.4 to 6.5)
Regions 2/3/4/5	–	11.3 (4.1 to 31.3)

All prevalences are adjusted for non-response.
AIAN, American Indian/Alaska Native.

is apparent by sex, race and across geographic regions; however, several strata have wide confidence intervals due to small sample sizes.

Of the 779 primary sampling group participants and their household members, 36 were positive for SARS-CoV-2 antibodies. This corresponds to an overall seroprevalence of 5.3% (95% CI: 3.1 to 7.5) of the population after adjustment for non-response and geographic location. In the secondary sampling group, of the 292 graduate students, staff, librarians, faculty members and their household members, 10 (adjusted 4.0 %, 95% CI: 1.6 to 6.5) had evidence for prior SARS-CoV-2 infection (table 2). Results were also further stratified by UMass affiliate versus household member. Of the 548 undergraduate students in the primary sampling group, 27 were positive for SARS-CoV-2 IgG antibodies (population positivity rate of 5.3% (95% CI: 3.1% to 7.6%)). Of the 231 household members of undergraduate participants, nine (adjusted 5.2%, 95% CI: 1.2 to 9.2) were positive for SARS-CoV-2 IgG antibodies. In the secondary sampling group, eight university affiliates (adjusted 4.3 %; 95% CI: 1.3 to 7.3) were positive for SARS-CoV-2 IgG antibodies. Of the household members in the secondary sampling group, two were seropositive, with a weighted seroprevalence of 3.3% (95% CI: 0.0% to 7.8%) (table 2). The overall distributions of measured IgG lognormal optical density ratios by subgroups are broadly similar (online supplemental figure 1).

Table 3 Multivariable associations for SARS-CoV-2 seropositivity, primary sampling group, Massachusetts, USA, July–August 2020

Characteristic	Prevalence ratio	95% CI	P value
Emergency response region			
Region 1	1.48	0.62 to 3.52	0.38
Region 2	0.34	0.05 to 2.45	0.28
Region 3	0.53	0.14 to 1.96	0.34
Region 4	Reference	–	–
Region 5	1.02	0.35 to 2.98	0.97
Age (years)	1.04	0.96 to 1.12	0.33
Gender			
Male	Reference	–	–
Female, gender diverse or no response	0.50	0.27 to 0.92	**0.027**
Race			
White	Reference	–	–
Multiple	1.91	0.46 to 7.98	0.38
Asian	1.66	0.66 to 4.16	0.28
Missing race	0.51	0.07 to 3.71	0.51
Hispanic	1.76	0.44 to 7.04	0.42
Black or AIAN	4.49	1.57 to 12.9	**0.005**
Febrile illness since February			
No	Reference	–	–
Yes	2.42	1.24 to 4.75	**0.010**
Missing response	0.33	0.04 to 2.45	0.28
Other household member			
No	Reference	–	–
Yes	0.29	0.01 to 7.69	0.46

Values in boldface are significant at p < 0.05.
AIAN, American Indian/Alaskan Native.

Table 4 Multivariable associations for SARS-CoV-2 seropositivity, secondary sampling group, Massachusetts, USA, July–August 2020

Characteristic	Prevalence ratio	95% CI	P value
Age (years)	1.03	0.99 to 1.07	0.17
Gender			
Male	Reference	–	–
Female or gender diverse	1.35	0.43 to 4.31	0.61
Race			
White	Reference	–	–
All other/multiple/missing	0.58	0.07 to 4.86	0.62
Febrile illness since February 2020			
No	Reference	–	–
Yes	2.56	0.68 to 9.67	0.17
Missing response	2.35	0.21 to 26.73	0.49
Emergency response region			
Region 1	Reference	–	–
Regions 2/3/4/5	4.08	1.09 to 15.33	**0.039**
Other household member			
No	Reference	–	–
Yes	0.70	0.18 to 2.72	0.61

Values in boldface are significant at p < 0.05.

After adjustments for age, gender, region and self-reported febrile illness since February 2020, the strongest association with seropositivity in the primary sampling group was Black or American Indian/Alaskan Native (AIAN) race (PR=4.49, 95% CI=1.57 to 12.9) (table 3). This indicates that individuals who reported being Black or AIAN had a prevalence 3.49 times higher than White individuals after adjustment. Additionally, after adjustments, females and those who are gender diverse were at a significantly lower risk of prior SARS-CoV-2 infection (PR=0.5; 95% CI=0.27 to 0.92) compared with males. Those who reported a febrile illness in February were more likely to be seropositive than those who did not report any febrile illness in this time period (PR=2.42, 95% CI=1.24 to 4.75). No significant associations were found across each of the five geographic regions in the primary sampling group; however, the prevalence of seropositivity was 48% higher in region 1 compared with region 4 (PR=1.48, 95% CI=0.62 to 3.52).

Within the secondary sampling population, after adjustments for age, race, gender, region, household member and self-reported febrile illness since February (table 4), participants who reported residing in either region 2, 3, 4 or 5 had greater than four times higher prevalence of SARS-CoV-2 antibodies as compared with those who resided in region 1 (PR=4.08, 95% CI=1.09 to 15.33). No other factors included in the model were significantly associated with seropositivity.

DISCUSSION

This mail-based serosurvey of two university-affiliated populations across Massachusetts in July and August 2020 found an estimated seroprevalence of ~5% of antibodies to SARS-CoV-2.

These results indicate that even with extensive morbidity and mortality across the state at the time of sampling, there had been limited exposure to SARS-CoV-2 at a population level. This estimated seroprevalence is lower than that detected with concurrent community-based studies in other states. An estimated 14.3% of the US population had been previously infected with SARS-CoV-2 by November 2020, as estimated in a pooled analysis of multiple seroprevalence surveys.[26]

Our estimates are substantially lower than some models of COVID-19 seroprevalence in Massachusetts. One model estimates a seroprevalence of 16.2% (no CIs provided) on 27 July 2020 (the closest modelled date to these surveys). These estimates are nearly double our measured seroprevalence with inclusion of 110 000 confirmed cases at

that date (ca 1.5%).[27] These differences may be due to several factors, including a non-representative population by age or geographic range, or waning of antibody titers. Without CIs, we are unable to evaluate coverage outside the reported point estimate. However, alternate nowcasting estimates suggest a total statewide attack rate on 31 July 2020 of 6.9% (95% CI: 5.5% to 8.4%) in Massachusetts,[28] which are closely aligned with our estimates.

The primary sampling group also showed increased risk of seropositivity with self-reported illness since February 2020; this association was not observed in analyses of the secondary sampling group in multivariable analyses. Within the surveyed groups, approximately 24% of the primary sampling group and 18% of the secondary sampling group reported illness since February. This finding may indicate that some participants in our study may not have been strictly asymptomatic, and were simply unable to obtain a COVID-19 test due to limited availability during the beginning of the pandemic. This finding reinforces results suggesting paucisymptomatic and subclinical illness are important contributors to the observed pandemic trajectories.

Contrasting antibody dynamics have been reported in other studies. A number of studies have found sustained antibody levels for over 3 months,[29 30] while others suggest IgG levels can remain 6 months or more.[31–33] An additional study has reported rapid waning of routine serological markers in individuals who had lower initial antibody responses.[34] Only 7.1% of those with high titers at baseline seroreverted to a level below the threshold for positivity within 60 days, compared with 64.9% of those with lower titers at baseline.[34] Evidence for IgM seropositivity was not detected in any of IgG positive samples, which is consistent with results from other surveys studies that included asymptomatic or subclinical populations due to rapidly waning titers.[32 35] Studies have shown that IgM levels decline more rapidly after infection than IgA and IgG levels,[30 36 37] and this is especially apparent with asymptomatic and sub-clinical infections.[32 35]

Trends in SARS-CoV-2 antibody levels are complex, and vary greatly depending on the measured antibody, timing of sampling and severity of disease.[31 32 35] Seroconversion times vary depending on the study, but one study found a median time-to-seroconversion for IgM of 8 days and median seroconversion for IgG of 10 days. Additionally, the SARS-CoV-2 IgG response generally begin around 10–15 days after symptom onset.[2] For this reason, repeated serial sampling of convalescent populations should be prioritised to more fully understand the dynamics of immune response.

SARS-CoV-2 seropositivity was associated with minority race status in this survey. While the total number of non-white participants was limited, the large effect size reinforces other studies suggesting that marginalised communities have been and continue to be disproportionately impacted by the pandemic. Results from the primary sampling group analysis suggest that self-reported Black race is a risk factor for previous SARS-CoV-2 infection, which is consistent with findings from other studies.[38–40] A number of factors may contribute to this significant association, including data suggesting that Black individuals are more likely to work in frontline industries or live in areas with a higher population density in many settings.[41] No parallel associations were found in the analysis of the secondary sampling group due to limited sample size in some strata. In the secondary sampling group, the aggregation of race categories into White race and Non-white race likely obscured meaningful associations between Race and seropositivity.

Results from the primary sampling group showed increased prevalence of IgG seropositivity among males. After adjusting for age, race and region, male gender remained a statistically significant risk factor for evidence of prior SARS-CoV-2 infection. Other studies have similarly found that males have higher rates of infection than females for asymptomatic infections.[42 43] These findings may reflect differences in care-seeking behaviour (recruitment biases); true biological differences; or differences in health behaviours such as smoking, alcohol use or COVID-19 prevention measures.[44] This association was not observed in the secondary sampling group.

This study was population-based and had broad eligibility criteria but is subject to several limitations. The exclusion of persons with prior confirmed diagnoses or any current symptoms (due to biosafety concerns) also inherently limited capture of subclinical infections. As such, the estimates are likely a lower bound. However, participants who suspected they may have been previously infected with SARS-CoV-2 might be more likely to participate compared with those that were less concerned about prior infections. This is a pervasive issue in community-based studies, where characteristics of those who volunteer to participate in community-based research differ from the general population.[45] Randomisation after a 3-week enrolment period helped to address this limitation, as using only the first participants to volunteer could have biased the sample to include those who were most motivated to receive their antibody test results. If participants were more motivated to receive their results because they suspected a prior exposure to SARS-CoV-2, this would have inflated the observed prevalence of seropositivity within the surveyed population.

Another limitation of the study is the self-reported response of the lack of a prior COVID-19 diagnosis and current fever. It is possible that some participants shielded their answer and submitted samples for analysis without meeting the eligibility criteria; this would have inflated our estimation of seroprevalence in asymptomatic groups. The limited number of non-white, and gender-diverse participants also limited some analyses and restricted our ability to assess any differences in prevalence across all racial groups. Next, while multiple studies have validated DBS sampling for SARS-CoV-2,[46] waning antibodies in asymptomatic individuals could be below the limited of detection of the ELISA assay. Additionally, the cut-off was determined in this study to be 2.49 for seropositivity.

When using a single cut-point for a continuous variable, there is the possibility of outcome misclassification; however, we attempted to reduce misclassification through the use of a finite mixture model. Finally, generalisability is limited due to the recruitment of a university-affiliated population in a relatively restricted geographic area.[8] The population in our study primarily included young adults, those of working age and their household members. Older age groups or those who reside in institutional settings would not have been recruited for our study. This population may not be representative of the broader US population and may be healthier, include few non-Whites, have higher education levels and may have differing sociopolitical attitudes with consequent health impacts. Additionally, samples were collected during the summer months of 2020 in Massachusetts during which the state was in phase 3 of the reopening plan. The state government implemented a strict 'lockdown' in March 2020 and progression to each reopening phase required a reduction in COVID-19 cases and hospitalisations. Massachusetts also had a mandatory mask order in all public spaces beginning 1 May 2020. Other states in New England followed similar timelines, however the implementation timelines and effectiveness differed widely across the USA. It is probable that the government policy measures on a state-wide level influenced seroprevalence, with stricter guidelines resulting in lower viral exposure.

In conclusion, this serosurvey estimates prevalence of prior SARS-CoV-2 infections in a university-affiliated population in Massachusetts, with adjusted prevalences of 5.3% in the primary sampling group and 4.0% within the secondary sampling group. Risk factors for IgG seropositivity included self-reported recent febrile illness, minority race status and male gender. This study reinforces the critical need for targeted serosurveys in highest-risk and marginalised communities, both in Massachusetts, and nationwide.

This study provides important estimates of seroprevalence in Massachusetts after the 'first wave' of SARS-CoV-2 infections in the spring of 2020. These are critical to benchmark modelling studies, and to more comprehensively understand the dynamics of population-level serostatus throughout the continuing pandemic, especially as vaccines become widely available.

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Contributors AL and DA led study conceptualisation and design. LB and NGR informed overall aims, sampling design and statistical analysis. JR, TS and EYC implemented all field implementation and associated data collection. DA organised, performed and reported all laboratory-based testing. JR, TS, EYC and AL performed data cleaning and analyses, and wrote the first draft. All authors contributed to revisions of the final manuscript. All authors read and approved the final manuscript.

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