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A Cross-Sectional Study Examining the Seroprevalence of Severe Acute Respiratory Syndrome Coronavirus 2 Antibodies in a University Student Population

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\textbf{ABSTRACT}

\textbf{Purpose:} The aim of the study was to determine the prevalence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) antibodies in a university student population.

\textbf{Methods:} This was a cross-sectional survey study based on the World Health Organization population-based seroepidemiological investigational protocol for SARS-CoV-2 conducted between April 29, 2020, and May 8, 2020, examining SARS-CoV-2 antibody prevalence among 790 university students in Los Angeles, CA. Participants completed a questionnaire on potential risk factors before blood sampling. Samples were analyzed using the EUROIMMUN Anti-SARS-CoV-2 ELISA (IgG) for the qualitative detection of IgG class antibodies to SARS-CoV-2 in human serum or plasma.

\textbf{Results:} The estimated prevalence of SARS-CoV-2 antibody was 4.0% (3.0%, 5.1%). Factors associated with having a positive test included history of anosmia and/or loss of taste (95% CI: 1.4—9.6). A history of respiratory symptoms, with or without fever, was not associated with a positive antibody test.

\textbf{Conclusions:} Prevalence of SARS-CoV-2 antibodies in the undergraduate and graduate student university population was similar to community prevalence.

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Because of widespread testing shortages and the varied range of clinical presentations for COVID-19, including asymptomatic infection, the true disease incidence in the U.S. since January 2020 is unknown. Given public health concerns with reopening IHE, serologic evaluation to determine the student population prevalence of antibodies to the virus is of utmost importance. In the fall of 2019, approximately 19.9 million students were attending IHE in the U.S., representing more than 5% of the U.S. population [4,5]. There is limited data on the number of infections with SARS-CoV-2 associated with IHE. Surveys done in the spring of 2020 indicate that <1% of college students report having a confirmed case of COVID-19 [6,7].

Early cases of COVID-19 infection were associated with international travel, yet by mid-March 2020, community spread was evident in many locations across the U.S. For many IHE, with diverse, interconnected, often residential populations, it was unclear what proportion of the population had been infected with SARS-CoV-2 before the closure of many campuses in March 2020. A national survey of college students conducted between March and May 2020 reported that 14% of college students indicated that they may or probably had COVID-19 based on health care provider assessment or symptoms but not confirmed by a test [6]. Given that the severity of the symptoms increases with increasing age and existence of medical comorbidities, many infections among undergraduate and graduate university students are expected to be asymptomatic or minimally symptomatic [6,8]. Thus, the presence of antibodies to SARS-CoV-2 is important in assessing the current prevalence of infection on college campuses.

The assessment of risk factors in students both with and without the presence of antibodies to SARS-CoV-2 will help guide IHE as they plan for reopening their physical campuses. Given the close proximity in which many university students live in dormitories or other off-campus high-density housing [9], the potential for rapid spread of SARS-CoV-2 is a relevant concern. University students are also expected to have different social connectedness compared with the general population, such as participation in athletics or social clubs [9,10]. In addition, the academic course structure of both undergraduate and graduate student education has been shown to have a high degree of connected networks, thus fostering the social conditions for the spread of an infectious disease such as SARS-CoV-2 [11]. A recent survey on the behaviors of college students who experienced symptoms consistent with COVID-19 found that 30.1% continued to attend classes [7].

Previously published seroprevalence studies have focused on community-level spread in cities or countries [12–14]. This study aimed to provide an estimate of infection in students attending a Los Angeles University with a diverse population, including a large number of international students. It also aimed to explore the risk factors for infection in this population. This information will offer evidence-based strategies for control measures, as IHE plan to reopen campuses.

Methods

Design

The study was a cross-sectional study examining the prevalence of COVID-19 antibodies from blood samples obtained from college students. The study design was based on the World Health Organization population-based seroepidemiological investigational protocol for COVID-19 virus infection [15]. Data were collected from April 29, 2020, to May 8, 2020, and prevalence estimates reflect this specific snapshot in time. It is not known how long antibodies persist after infection. There is also potential for some lag in ability to detect antibodies after infection, so prevalence estimates could reflect cumulative infection up to approximately late April 2020.

Setting

The study was conducted at a large urban University in Los Angeles, CA. Approval was obtained from the Institutional Review Board at this institution, and all participants provided electronic informed consent and Health Insurance Portability and Accountability Act authorization. On April 29, 2020, the County of Los Angeles, CA, reported 27,641 cumulative cases of SARS-CoV-2 infection and had 1,156 recorded deaths related to COVID-19 [16].

Participants

Participants were invited to participate in this study via an email invitation, which was sent to 9,135 students (25% of eligible students) enrolled in the Spring 2020 semester. Inclusion criteria were (1) participants were eligible to access services at the student health center (i.e., students must be enrolled in six or more credit hours for on-campus programs, not in online degree programs) and (2) students’ primary campus was the main campus for this institution (this was a practical consideration). Exclusion criteria included (1) students aged <18 years and (2) students who were not in the randomly selected pool of potential participants (i.e., students who heard of the study via word of mouth or other similar means were excluded from participating). Although not a criterion for receiving a study invitation, only students still living in the region were truly eligible to participate in this study, given that they had to come to the student health center for a blood draw.

A stratified random sampling approach was used with the following subgroups: female undergraduates, male undergraduates, female graduate students, and male graduate students. With the goal of having the sample distributions match distributions in the population, these strata were selected based on internal university data, indicating that females are more likely to participate in health-related research projects compared with males, and fewer undergraduates (36.4%) spent the end of the Spring 2020 semester in Los Angeles relative to graduate students (76.5%). Within each stratum, a random selection of students was invited to participate. Sign-ups were monitored, and demographic distributions of invitations were adjusted as needed. During recruitment, it was also determined that white domestic students were overrepresented in sign-ups, and students in this group were downweighted for random selection in later waves of invitations so that sufficient data could be collected from nonwhite domestic and international students. Five waves of invitations were sent.

The invitation email was sent to the student’s university email address through the student health electronic health record. Each email had a unique study code to ensure that only invited students participated in the study. Students accepted the invitation by scheduling an appointment at the student health center. The number of appointments available for students to participate in this study was capped at 800.
Materials

Assay

The EUROIMMUN Anti-SARS-CoV-2 ELISA (IgG) is an enzyme-linked immunosorbent assay intended for the qualitative detection of IgG class antibodies to SARS-CoV-2 in human serum or plasma. EUROIMMUN is licensed for use under the Food and Drug Administration’s Emergency Use Authorization. Testing requires 5 mL of serum or 2 mL of whole blood. Specimens were collected at the student health center by the laboratory staff and sent via courier within 24 hours of collection for testing. The validation program at the Frederick National Laboratory for Cancer Research determined that sensitivity of this test is 90% (27/30; 95% confidence interval [CI]: 74.4%–96.5%) and specificity is 100% (80/80; 95% CI: 95.4%–100%) [17]. This test classifies individuals into negative (ratio < .8 is considered negative—this is a normal result for patients who have no or early SARS-CoV-2 exposure), borderline (ratio ≥ .8 to <1.1), and positive (ratio ≥ 1.1) for Anti-SARS-CoV-2. In the independent clinical agreement validation study from the manufacturer borderline, results were counted as negative [17]. Individuals who were classified as borderline were asked to return for a follow-up test 14 days later.

Survey instrument

All participants completed a study questionnaire with questions on the following items: prior exposure to a person with suspected or confirmed COVID-19 infection; prior COVID-19 diagnosis; prior illness history and symptoms since January 1, 2020; prior loss of smell and/or taste since January 1, 2020; receipt of the influenza vaccine during this academic year; travel history since December 1, 2019, including international and domestic; living situation in early March 2020; and current living situation. Those who reported coughing, wheezing, shortness of breath or “other” respiratory symptoms were classified as having experienced respiratory symptoms.

Demographics

Demographic data on students were obtained from electronic health records, and demographic data in these records are provided by the university. Available data included sex (two categories available only: male and female), race/ethnicity/international status (the university combines these into one variable with levels including Asian/Pacific Islander, Black/African American, Hispanic/Latino, International from any non-U.S. country, multiracial/multiethnic, white/Caucasian, and other/unknown; at this school, the majority of international students are from China), and degree program category (collapsed into two categories: undergraduate and graduate).

Analyses

To reduce potential bias, weights were created for each participant using iterative proportional fitting to match demographics available on the overall university population. The following variables were included: sex by degree program category and race/ethnicity/international status by degree program category.

We used a Bayesian approach to estimate the antibody prevalence of COVID-19 in our student population by incorporating the sensitivity and specificity of the diagnostic test into the prevalence estimate [18]. To be consistent with the EUROIMMUN Anti-SARS-CoV-2 ELISA (IgG) manual [17], we classified a borderline test result as negative. Prior values for the sensitivity and specificity were taken from the validation program at the Frederick National Laboratory for Cancer Research [17]. The prior on the true prevalence was taken from a recent study on antibody prevalence in LA County [13] and reflects the community from which our sample was derived from. Of the 863 adults in LA county that consented to the study and were tested, 35 individuals tested positive. More information on the prior and model specifications are found in the Appendix.

Odds ratios (ORs; unadjusted for covariates in the model) were used to explore potential factors associated with a positive antibody test. For these analyses, positive cases were compared with borderline and negative cases combined. Given the exploratory nature of this work, adjustments were not made for multiple comparisons, so results should be interpreted within this context. Reference groups for demographic factors were female for sex, white/Caucasian for race/ethnicity/international status, and undergraduate for degree program category. For all other variables, the reference was not having the given characteristic.

Results

There were 790 students who participated in this study. The sample was 48.2% female, 30% international, and 52.2% undergraduate (Table 1). At the time of data collection, most students lived in off-campus housing, alone or with roommates/friends (61.3%); in their family home (26.5%); and in university-owned housing (9.1%). Initially, 23 individuals tested as borderline. All but four of these people returned for follow-up testing, and five people had a borderline test the second time, with one person having a positive test.

Estimates for the prevalence and its 95% credible intervals are reported in Table 2. The estimated prevalence of COVID-19 antibody positivity at the time of our study was 4.0% (3.0%, 5.1%). Posterior estimates for the sensitivity and specificity can be found in the Appendix. Furthermore, when stratified by academic status, the estimated prevalence was 4.0% (3.0%, 5.2%) and 4.0% (2.9%, 5.2%) for undergraduate and graduate students, respectively, suggesting that the two student groups may not have differing lifestyles that will make them more (or less) susceptible to infection.

Table 1: Demographic characteristics of participants from a Los Angeles University

| Sample (n = 790), n (%) |  |
|------------------------|--|
| Female                 | 381 (48.2) |
| Male                   | 409 (51.8) |
| Asian/Pacific Islander | 95 (12.0)  |
| Black/African American | 21 (2.7)   |
| Hispanic/Latino        | 47 (6.0)   |
| International          | 237 (30.0) |
| Multiracial/multiethnic| 126 (15.9) |
| White/Caucasian        | 219 (27.7) |
| Other/unknown          | 45 (5.7)   |
| Undergraduate          | 412 (52.2) |
| Graduate               | 378 (47.8) |
| On-campus residence    | 72 (9.1)   |
| Off-campus residence   | 718 (90.9) |
Our analysis treated borderline subjects (N = 10) as test negative. To investigate the influence of potentially misclassifying borderline subjects, we provide a supplementary analysis where we treat borderline subjects as test positive. This increased the prevalence slightly to 4.3% (3.2%, 5.7%). These results can be found in the Appendix. To assess the influence of prior specifications to the prevalence of COVID-19 antibody positivity, we ran the model, classifying borderline results as test negative, by placing a noninformative prior to the prevalence. With a noninformative prior, the estimated prevalence of antibody positivity was 3.6% (.7%, 6.0%).

There were three participants (reflecting 2.8 weighting observations) who reported that they had a previous COVID-19 diagnosis via a nasal or throat swab; all these participants also had positive antibody tests (reflects 8.8% of positive antibody cases). More than half (61.4%, weighted n = 19.4) of the positive cases did not report any prior illness with respiratory symptoms, and more than one third (37.0%) did not report any prior illness at all (others reported symptoms such as fatigue, headache, sore throat, and runny nose).

We report the unadjusted ORs and their 95% CIs in Table 3. In this sample of university students, the following factors were associated with having a positive test for SARS-CoV-2 antibodies: students with a history of loss of taste and/or smell were 4.0 (95% CI: 1.4–9.6) times more likely to have a positive test, compared with those without this history (95% CI: 1.4–9.6); students with confirmed or suspected exposure to a positive COVID-19 case were 3.3 times as likely to have a positive test, relative to those without confirmed or suspected exposure (95% CI: 1.4–7.0). We observed increased test positivity for those reporting international travel (OR = 1.6, 95% CI: .8–3.3) and domestic travel (OR = 2.1, 95% CI: 0.7–5.2) since December 1, 2019, although neither was statistically significantly. Of the 26 positive cases who reported domestic travel, only 10 reported regional travel within Southern California. Sex, race/ethnicity, academic level, current and prior living in university-owned housing, and history of flu shot during the 2019–2020 academic year were not significantly associated with having a positive antibody test (data not shown for all variables). A history of respiratory symptoms, with or without fever, was also not associated with a positive antibody test.

We perform a supplementary analysis by creating weights for each participant using iterative proportional fitting to match demographics available on the overall university population to reduce potential bias. The following variables were included in calculating these: sex by degree program category and race/ethnicity/international status by degree program category. We present the weighted unadjusted OR in the Appendix and note that the overall conclusions are consistent with what we previously observed.

Discussion

Seroprevalence of SARS-CoV-2 antibodies in a Los Angeles university student population as of May 8, 2020, was estimated to be 4.0%. This does not substantially differ from what has been reported in prior population studies from Santa Clara County and Los Angeles County. In the Los Angeles County study, done in April 2020, prevalence was estimated at 4.65% (95% CI: 2.8%–5.6%) [13]. Likewise, an analysis of the blood samples from approximately 3,300 people living in Santa Clara County in early April estimated prevalence at 2.8% (95% CI: 1.3%–4.7%) [14]. This study demonstrates that the prevalence of infection at this institute did not differ from the larger community. The low prevalence indicates that SARS-CoV-2 was not widely circulating

Table 2
Posterior estimates of COVID-19 antibody prevalence and their 95% credible intervals stratified by academic status

| Group          | Estimate | 95% CI             |
|----------------|----------|--------------------|
| Undergraduates | .040     | (.030, .052)       |
| Graduate       | .040     | (.029, .052)       |
| All            | .040     | (.030, .051)       |

Table 3
Unadjusted odds ratios with 95% confidence interval for associations with a positive antibody test

| Variable                          | Group          | Proportion testing positive, estimatec (95% CI) | Odds ratioa (95% CI) |
|-----------------------------------|----------------|-----------------------------------------------|----------------------|
| Exposures to suspected or confirmed COVID-19 case | Yes | 10.3% (3.9%, 16.7%) | 3.3 (1.4–7.0) |
| | No | 3.4% (2.1%, 4.8%) | Ref. |
| History of loss of taste and/or smell since January 1, 2020 | Yes | 13.0% (3.3%, 22.8%) | 4.0 (1.4–9.6) |
| | No | 3.6% (2.3%, 5.0%) | Ref. |
| Influenza vaccine this academic year | Yes | 4.5% (2.5%, 6.5%) | 1.2 (0.6–2.4) |
| | No | 3.9% (1.9%, 5.8%) | Ref. |
| Any travel (international and/or domestic) since December 1, 2019 | Yes | 7.3% (2.9%, 11.6%) | 2.1 (0.95–4.5) |
| | No | 3.5% (2.1%, 4.9%) | Ref. |
| International travel since December 1, 2019 | Yes | 5.6% (2.7%, 8.5%) | 1.6 (0.8–3.3) |
| | No | 3.5% (2.0%, 5.1%) | Ref. |
| Domestic travel since December 1, 2019 | Yes | 5.1% (3.2%, 7.0%) | 2.1 (0.9–5.2) |
| | No | 2.5% (1.7%, 4.4%) | Ref. |
| Currently living in university-owned housing | Yes | 4.2% (0.8%, 8.8%) | 1.0 (0.2–2.9) |
| | No | 4.2% (2.7%, 5.6%) | Ref. |
| Prior living in university-owned housing | Yes | 3.2% (1.1%, 6.2%) | 0.7 (0.2–1.9) |
| | No | 4.4% (2.8%, 5.9%) | Ref. |
| Respiratory symptoms with fever | Yes | 1.3% (0.3%, 3.8%) | 0.3 (0.1–1.3) |
| | No | 4.5% (4.0%, 6.0%) | Ref. |
| Respiratory symptoms without fever | Yes | 4.8% (2.2%, 7.5%) | 1.3 (0.6–2.6) |
| | No | 3.9% (2.3%, 5.5%) | Ref. |

CI = confidence interval; NA = not applicable; Ref = reference.

a Statistical significance in test positivity compared with the reference group (p < .05).

b Odds ratio comparing test positivity for the indicated group compared with the reference group.

c A comparison of Black/African American students to white/Caucasian students is not included because there was no variance in black student antibody data (i.e., all students in this group had a negative test).
in the student population before the closure of the physical campus in mid-March 2020.

This institute started the transition to virtual learning on March 11, 2020, with Spring Break the following week. For the remainder of the spring semester, the students completed all coursework virtually, and the physical campus was closed except for a small percentage of students who remained in university-owned housing. On March 19, 2020, Governor Gavin Newsom issued a stay-at-home order for the State of California. It is likely that these events played a part in the low seroprevalence of SARS-CoV-2 in this study.

With the low seroprevalence to SARS-CoV-2 in our population at the time of this study, there is not a strong argument for widespread antibody testing to inform decisions on reopening the college campuses. The reopening of IHE will rest on the ability to mitigate spread through continued physical distancing measures, environmental measures, promotion of behaviors that reduce spread, contact tracing, and access to testing. The Centers for Disease Control is collaborating with public health departments and private laboratories to use seroprevalence surveys in different locations and populations to help estimate the number of persons who may have been infected with SARS-CoV-2 and not included in official case counts [19]. Although widespread antibody testing at this time has limited usefulness, additional seroprevalence surveys from IHE will be important to estimate the number of infections associated with IHE and to further our understanding of risk factors for infection as physical college campuses reopen. Approximately 1% of the samples in our study fell in the borderline or indeterminate range. This may represent early infection with a rising antibody titer, prior infection with waning antibody production, or cross-reactivity with another virus.

Loss of smell and/or taste could be a relevant indicator in this population, which has also been reported by others [20]. This may be a screening tool relevant to IHEs, although more information on duration and intensity of lost smell/taste would be beneficial. Notably, 39.6% of negative cases reported respiratory symptoms, suggesting that their answers to this question likely reflected respiratory effects from seasonal conditions unrelated to SARS-CoV-2.

The presence of SARS-CoV-2 antibodies indicates prior infection, but it is still unclear whether this indicates immunity. We did not perform additional neutralization antibody assays to determine further characteristics of the antibodies. It is also unknown what percentage of students with asymptomatic or mild infections develop detectable antibody response. Prior studies with the Middle East respiratory syndrome (MERS-CoV) demonstrated that the severity of the disease correlated with the antibody response [21]. This consideration is important for IHE; if large numbers of the population have asymptomatic or mildly symptomatic infections, seroprevalence studies may under-represent the prior disease incidence. Further longitudinal serological studies on the college population are needed to determine ongoing disease incidence as well as the extent and duration of immunity to SARS-CoV-2.

There are several other potential limitations of this study. There is limited data available on the validity of the assay used in this study, although this uncertainty was accounted for in statistical analyses. Limitations in our understanding of borderline findings also make it challenging to draw conclusions. In analyses of potential risk factors, adjustments were not made for multiple comparisons, and estimated associations of each factor with prevalence were not adjusted for other risk factors, and so these findings should be interpreted with caution. Only students currently residing in the region were eligible to participate in this study, so true prevalence in this university population is unknown. Demographic data are limited in their availability, so there may be other biases present because of unmeasured or insufficiently measured variables. It is also possible that there are factors that drove interest in participating in this study, such as prior symptoms, which may affect prevalence estimates. Furthermore, it is not known how generalizable these findings may be to other university populations.

Although it is still unknown the frequency in which positive SARS-CoV-2 antibodies develop or are sustained in asymptomatic or subclinical infection or if antibodies confer protective immunity and how long that immunity will last, antibody testing remains a powerful tool to examine prevalence of a disease in a population after the initial infection. Over time, this information can be used to assess risk factors, monitor spread, and help administrators and public health officials plan for easing current mitigation and future health care needs. Our results reflect antibody positivity in this university population because of infection with SARS-CoV-2 in the early days of the pandemic and during the statewide shutdown. In addition to showing comparability with general population prevalence, our estimates serve as a useful comparison for future studies that may be conducted in this population as this university campus returns to normal operations.

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Supplementary Data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.jadohealth.2020.09.001.

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