

Severe Acute Respiratory Syndrome Coronavirus 2 Antibody Prevalence in Blood in a Large School Community Subject to a Coronavirus Disease 2019 Outbreak: A Cross-sectional Study

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Background. A severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) outbreak affecting 52 people from a large school community in Santiago, Chile, was identified (12 March) 9 days after the first case in the country. We assessed the magnitude of the outbreak and the role students and staff played using self-administered antibody detection tests and a self-administered survey.

Methods. The school was closed on 13 March, and the entire community was placed under quarantine. We implemented a home-delivery, self-administered, immunoglobulin (Ig) G/IgM antibody test and survey to a classroom-stratified sample of students and all staff from 4–19 May. We aimed to determine the overall seroprevalence rates by age group, reported symptoms, and contact exposure, and to explore the dynamics of transmission.

Results. The antibody positivity rates were 9.9% (95% confidence interval [CI], 8.2–11.8) for 1009 students and 16.6% (95% CI, 12.1–21.9) for 235 staff. Among students, positivity was associated with a younger age ($P = .01$), a lower grade level ($P = .05$), prior real-time polymerase chain reaction (RT-PCR) positivity ($P = .03$), and a history of contact with a confirmed case ($P < .001$). Among staff, positivity was higher in teachers ($P = .01$) and in those previously RT-PCR positive ($P < .001$). Excluding RT-PCR-positive individuals, antibody positivity was associated with fever in adults and children ($P = .02$ and $P = .002$, respectively), abdominal pain in children ($P = .001$), and chest pain in adults ($P = .02$). Within antibody-positive individuals, 40% of students and 18% of staff reported no symptoms ($P = .01$).

Conclusions. Teachers were more affected during the outbreak and younger children were at a higher risk for infection, likely because index case(s) were teachers and/or parents from the preschool. Self-administered antibody testing, supervised remotely, proved to be a suitable and rapid tool. Our study provides useful information for school reopenings.

Keywords. SARS-CoV-2; school; outbreak; antibodies; seroprevalence.

As of 30 May 2020, reported coronavirus disease 2019 (COVID-19) cases had reached 5 817 385 globally, including 362 705 deaths [1], with the majority of infections occurring in adults 18–64 years of age (74%) [2]. Children under 18 years of age have made up a relatively small proportion of all cases (3% in the United States) [2], and children under 5 years of age account for less than 1% of reported cases [3]. However, early studies from China suggested that as the epidemic progressed in a large community, children became infected at higher rates

[4]. Further information from Europe and the Americas also seems to indicate that children could play a minor role in the infection process, at least in terms of detected cases and transmission [5, 6].

Since the true percentage of the childhood population infected with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is not known, the role that children play in transmission of the virus is unclear. However, they may interact with a greater number of contacts, as compared to adults, under normal non-social distancing conditions [7]. Additionally, they may have higher viral loads as compared to adults [8], suggesting that they may still be transmitters of disease, similar to other respiratory viruses [9]. The possibility of children transmitting infection, especially to adult populations, led many countries worldwide to implement school closures as an important component of SARS-CoV-2 transmission mitigation policies [10]; the impact of such measures is still being explored [11–14].

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The reference method for screening and diagnosing COVID-19 infections is real-time polymerase chain reaction (RT-PCR); nevertheless, the detection of antibodies against SARS-CoV-2 (immunoglobulin [Ig] G, IgM, and IgA) plays a complementary role, with particular importance in providing epidemiological information [15]. Seroprevalence has been extensively explored in COVID-19 patients confirmed by RT-PCR, as recently reviewed [16]. A few studies have assessed seroprevalence, primarily in asymptomatic individuals, using different methods, with rates among health-care workers who had direct contact with COVID-19 patients ranging from 1.6–44% [17, 18] and rates in the general population ranging from 2.8–5% [19–21].

COVID-19 was first detected in Chile on 3 March 2020; over the following 2 weeks, cases increased slowly, largely via travelers arriving from Asia and Europe [22]. The first communities to report an increase in detected cases were in eastern Santiago, and the first COVID-19 outbreak to be reported in Chile was in a private school in this area. The school outbreak began on 12 March, 9 days after the index COVID-19 case was detected in the country. The school year began on 4 March, and parent-teacher meetings were held nearly every evening during the first week of school (Figure 1). On 13 March, when there were 58 confirmed cases nationwide, 2 cases were reported within the school community: 1 teacher and 1 other staff member. That same day, the Chilean Ministry of Health declared quarantine for the entire school community. In the

next few days, confirmed symptomatic cases (RT-PCR) continued to appear, primarily amongst staff and parents, and to a lesser extent within the student population. At the time of the study, PCR testing was available with a medical referral when patients were symptomatic or had contact with a known case. As of 6 April, 52 members of the school community had been confirmed positive for SARS-CoV-2, and there had been 1 associated death. The cases were distributed as follows: 7 (13%) students, 18 (35%) staff, and 27 (52%) parents. The index case was a staff member who worked with the entire preschool and elementary school staff, and was present at all of the parent-teacher meetings for prekindergarten through fourth grade.

The school outbreak provided a unique opportunity to determine the role of children as potential asymptomatic transmitters of SARS-CoV-2. While children are believed to play a minor role in detected symptomatic cases, if a large number of students were asymptotically infected, this could be determined after the fact by testing for anti-SARS-CoV-2 antibodies. Thus, we designed a contact-free field study using finger-prick chromatographic-based IgM/IgG antibodies tests, in order to evaluate a large number of students and school staff at 8–10 weeks after the start of the outbreak.

Our primary aim was to determine the overall SARS-CoV-2 antibody prevalence in blood during the outbreak, and to characterize the antibody prevalences among students by grade level and among school staff. Secondary aims were to retrospectively

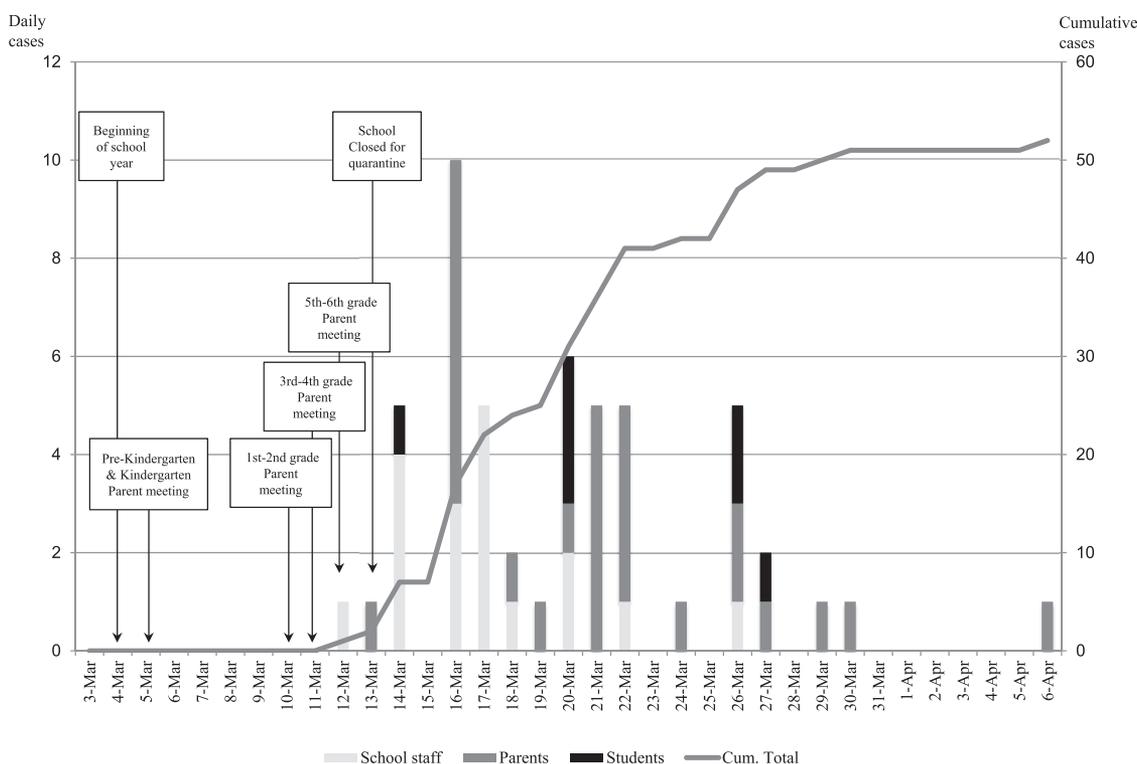


Figure 1. Description of the school outbreak, including selected parent-teacher meetings and the daily and cumulative number of confirmed cases (by RT-PCR) among parents, staff, and students. Abbreviation: RT-PCR, real-time polymerase chain reaction.

identify symptoms, confirmed COVID-19 cases, and contact history within the school community, in order to correlate the symptoms with antibody positivity and explore possible transmission dynamics within the school community.

METHODS

School Community

The school is coeducational, located in the community of Vitacura (population 85 000 from the 2017 census), in the Metropolitan Region (population 7.1 million). The school has 2616 students in 14 grade levels. There are 318 staff members, of which 195 are teachers. Classrooms in the preschool have 2 head teachers and 25–27 students; in the rest of the school, the classes are 36–38 students, and there is 1 teacher each in grades 3–12. The academic researchers presented the study proposal to the school authorities in mid-April, followed by submission to the Ethical Committee for Research in Human Subjects of the Faculty of Medicine, Universidad de Chile, which was approved on 30 April.

Self-application of Questionnaire and Antibody Test

All school staff and a randomized selection of students, evenly distributed by classroom, were invited to participate in the study. Participants were sent an email describing the study and asking them to provide their address if they were interested in participating. In the case of students, parents and/or legal guardians were initially contacted; if a student's parent/guardian declined to participate, a random replacement was selected. After indicating an intention to participate, packages containing a flyer with simple step-by-step instructions ([Supplementary Data](#)) and the antibody testing supplies (test card, lancet, a cryovial with diluent, 2 capillary tubes, and an alcohol pad) were distributed to each household using a georeferenced home delivery service (Uber Flash). The flyer included a link/quick-response code that directed participants to a web page where study data were collected using Research Electronic Data capture (REDCap) tools hosted at the Medical School of the Universidad de Chile [23]. Using the REDCap platform, participants could read and digitally sign the informed consent and, in the case of children 8 years and older, assent. Once these forms were signed, a copy was emailed to participants for their records and they were directed to a secure survey that (1) asked basic demographic questions; (2) requested information on any previous RT-PCR test for SARS-CoV-2 and potential contact with any COVID-19-positive cases; and (3) asked about symptoms experienced since the outbreak (date and duration, in days, of each symptom). Next, a detailed video ([Supplementary Data](#)) that was prepared by the research team demonstrated how to perform the antibody test. Participants were asked to attach a photo of the test after 15 minutes had elapsed and self-report the appearance of the 3 lines: G (IgG), M (IgM), and C (test control). After completion, participants were told they would

receive results, as interpreted by the research team, within the next few days. All tests were to be performed within 1 hour of opening the sealed aluminum foil bag.

SARS-CoV-2 Antibody Test

The Novel Coronavirus (2019-nCoV) IgG/IgM Test Kit (Colloidal gold) from Genrui Biotech Inc, China, was used according to the manufacturer's specifications.

Determination of Antibody Positivity

For details on training staff in interpreting photos of the test cards, see the [Supplementary Data](#). In a first screening phase, the study nurse and/or technician viewed the photo provided by the participant, along with the participant's self-report as to the visibility of the 3 bands, and determined whether the tests were IgG+, IgM+, IgG & IgM+, Negative, Invalid, or Indeterminate. In this screening phase, all self-reported positive responses, in addition to any visual presence according to the study nurse and/or technician, no matter how faint, were to be considered. All samples considered positive, including those where parents/participants and study staff did not agree, and indeterminate samples (doubt of line presence), along with a random selection of negative samples, were then individually reviewed by a 3-physician panel to determine their final positivity/negativity (details in [Supplementary Data](#)).

Statistical Analysis

Data were analyzed in STATA version 15 [24]. Statistical differences for categorical variables were calculated using Pearson's Chi-squared test or Fisher's exact test; continuous variables were tested using the Mann-Whitney U test or Kruskal-Wallis test. Sample size calculations and details on model adjustment are in the [Supplementary Data](#).

RESULTS

Overall Results of Antibody Test Performance

Parents of 1224 children were contacted by email; 1105 manifested interest in participating and were sent a study package. A total of 1029 children/parents signed consent and participated in the study, of whom 20 (1.9%) were excluded from analysis due to invalid or indeterminate results. This resulted in 1009 students for inclusion in the analysis, representing 38% of the entire student body ([Supplementary Figure 1A](#)). All staff ($n = 318$) were contacted by email, and a total of 272 responded and were sent packages. A total of 240 staff members completed the online consent process, and 235 were included in the final analysis, representing 74% of the entire school staff ([Supplementary Figure 1B](#)).

The overall antibody positivity rates were 9.9% (95% confidence interval [CI], 8.6–11.5) for students and 16.6% (95% CI, 12.1–21.9) for school staff ([Table 1](#)). High school students had a lower positivity rate as compared to students in younger

levels. There were no differences in positivity between women and men in either students or staff, or between teachers and support staff.

Population Characteristics and Self-reported Symptoms by Serology Status

Among students, antibody-positive children were younger, had a higher PCR positivity rate (in those who underwent PCR testing during the outbreak), and were more likely to self-report contact with 1 or more confirmed cases, as compared to seronegative children (Tables 2 & 3). In staff, the only variable that differed by antibody positivity was a previous history of PCR-positive results; most individuals reported contact with 1 or more COVID-19 cases, and there was no difference by antibody positivity (74 vs. 54%).

Overall, 490/1009 (49%) students and 171/235 (73%) staff declared the presence of 1 or more symptoms since the start of the outbreak (Figure 2). In children, abdominal pain and fever were significantly more common among antibody-positive children (21% and 17%, respectively) than antibody-negative children (9% and 6%, respectively). After removing children that were PCR positive, these differences remain significant (22% and 14%, respectively, compared to 9% and 5%, respectively).

Among school staff (Figure 2), antibody-positive individuals reported significantly higher frequencies of weakness (54% vs. 30%, respectively), abdominal pain (21% vs. 7%, respectively), fever (36% vs. 7%, respectively), myalgia (46% vs. 24%, respectively), dyspnea (26% vs. 6%, respectively), chest pain (33% vs. 8%, respectively), and hyposmia (31% vs. 5%, respectively) than antibody-negative individuals. After removing PCR-positive individuals, these differences remain significant only for fever and chest pain (14% vs. 6%, respectively, and 25% vs. 7%, respectively). When comparing symptoms between staff and students, significantly higher frequencies of headache, weakness, fever, myalgia, dyspnea, chest pain, sore throat, and hyposmia were observed in staff. Within antibody-positive individuals, 40%

(95% CI, 30–50%) of students and 18% (95% CI, 8–34%) of staff reported no symptoms ($P = .01$).

Regarding the timing of onset of reported symptoms, in the staff the median date was significantly earlier in antibody-positive versus -negative participants ($P = .02$). There was greater dispersion in the timing of symptom onset in seronegative participants (March–May), while in antibody-positive subjects the symptoms predominantly occurred during March (closer to the school outbreak). In students, the median date of symptom onset was also significantly earlier in antibody-positive children ($P = .04$). The median duration from self-reported symptom onset to sampling tended toward fewer days for an IgM-positive result (44 days for students and 51 for staff), compared to either an IgM/IgG-positive result (51 and 60 days, respectively) or an IgG-positive result (58 and 56 days, respectively; student $P = .06$; staff $P = .17$). For seropositive individuals, symptoms occurred earlier in adults, compared to students (median: 15 March vs. 21 March, respectively; $P = .03$).

Student Antibody Prevalence by Classroom and Teacher Infection Status

Antibody-positive students were distributed across all grade levels at the school (Figure 3), but were significantly less common in high school students. There was a significantly greater number of teachers that were antibody positive and/or had a history of positive RT-PCR results in the preschool, as compared to the other levels.

The median percent of antibody-positive students per classroom was 8.3% (interquartile range, 1.6–14.3%). In 7 classrooms, over 25% of students were positive for antibodies, of which 4 had a primary teacher who was antibody positive and/or RT-PCR positive.

Antibody-positive children had an average of 1.8 contacts with a COVID-19 case that was confirmed by RT-PCR, while antibody-negative children had 1.4 contacts ($P = .01$). We found that the greater the number of contacts, the greater the probability that the child was antibody positive (odds ratio [OR], 1.4; $P = .05$; bivariate logistic model; response variable, positive/negative test result; explanatory variable, number of COVID-19 positive contacts). The most common COVID-19 contact in antibody-positive versus antibody-negative children was their teacher (21% vs. 12%, respectively; $P = .022$), followed by a household relative (11% vs. 2%, respectively; $P < .001$), a classmate (9% vs. 4%, respectively; $P = .03$), and a home caregiver (4% vs. 0.1%, respectively; $P < .001$). Sources with the greatest likelihood of possible contagion in students were a home caregiver (OR, 27.9), a household relative (OR, 5.4), a classmate (OR, 3.2), and a teacher (OR, 2.2).

DISCUSSION

The SARS-CoV-2 antibody prevalences were 10% in students and 17% in staff from a large school in Santiago, Chile, at 8 to 10 weeks after a COVID-19 outbreak that affected mostly staff and parents, and affected students to

Table 1. Overall Antibody Test Results in Students and Staff

	n	Age		Positivity rate
		Years (SD)	Female	% (95% CI)
Students, total	1009	10.8 (4.1)	46%	9.9 (8.6–11.5)
Preschool	147	4.7 (.7)	49%	12.3 (7.8–18.6)
Elementary	286	7.9 (1.5)	43%	10.8 (7.8–14.7)
Middle school	295	11.9 (1.2)	47%	11.9 (8.8–15.9)
High school	281	15.8 (1.4)	46%	5.7 (3.6–8.9)
Staff, total	235	42.8 (10.4)	73%	16.6 (12.1–21.9)
Teachers	165	40.6 (10.0)	76%	20.6 (14.7–27.6)
Support staff	70	48.0 (9.3)	66%	7.1 (2.4–15.9)

The positivity rate was defined as an IgM and/or IgG line being visible according to the medical review panel. There was no difference in positivity between women and men in either students (9.5% vs. 10.2%, respectively; $P = .7$) or staff (18.1% vs. 12.5%, respectively; $P = .3$). Students vs. staff: $P = .003$, Fisher's exact test; high school students vs. all other students: $P = .01$, Pearson Chi-squared test; teachers vs. support staff: $P = .01$, Fisher's exact test.

Abbreviations: CI: confidence interval; Ig, immunoglobulin; SD: standard deviation.

Table 2. Summary of School Population Included in Analysis by Antibody Positive/Negative Status

Variable	Antibody Positive	Antibody Negative	P
Students			
n	100	909	
Age, years, mean (range)	9.8 (4–18)	10.9 (4–18)	.01 ^a
Gender, female	44 (44)	417 (45.9)	NS
Number of siblings attending the school, median (IQR)	1 (1–2)	1 (1–2)	.05 ^a
Grade05 ^b
Preschool	18 (18)	129 (14.2)	
Elementary	31 (31)	255 (28.1)	
Middle school	35 (35)	260 (28.6)	
High school	16 (16)	265 (29.2)	
History of PCR-confirmed SARS-CoV-2	3/9	2/40	.03 ^c
Reported contact with ≥1 PCR-confirmed COVID-19 case	36 (36)	186 (20.5)	<.001 ^b
Staff			
n	39	196	
Age, years, mean (range)	43.2 (26–57)	42.8 (23–64)	NS
Gender, female	31 (79.5)	140 (71.4)	NS
History of PCR-confirmed SARS-CoV-2	11/17	3/23	<.001 ^c
Reported contact with ≥1 PCR-confirmed COVID-19 case	29 (74.4)	107 (54.6)	NS

Data are shown as n (%) unless otherwise indicated. The data are from n = 1005 students and n = 235 staff.

Abbreviations: COVID-19, coronavirus disease 2019; IQR, interquartile range; NS, not significant; PCR, polymerase chain reaction; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

^aMann Whitney Test.

^bPearson's Chi-squared test.

^cFisher's exact test.

a lesser extent. These prevalence rates are higher than reports from community settings, where the rates reported to date are about 5% [19–21]. However, due the fact that not

everyone who develops COVID-19 develops an immune response [25], the true infection rates may have been even higher. Consistent with the detection of PCR-confirmed

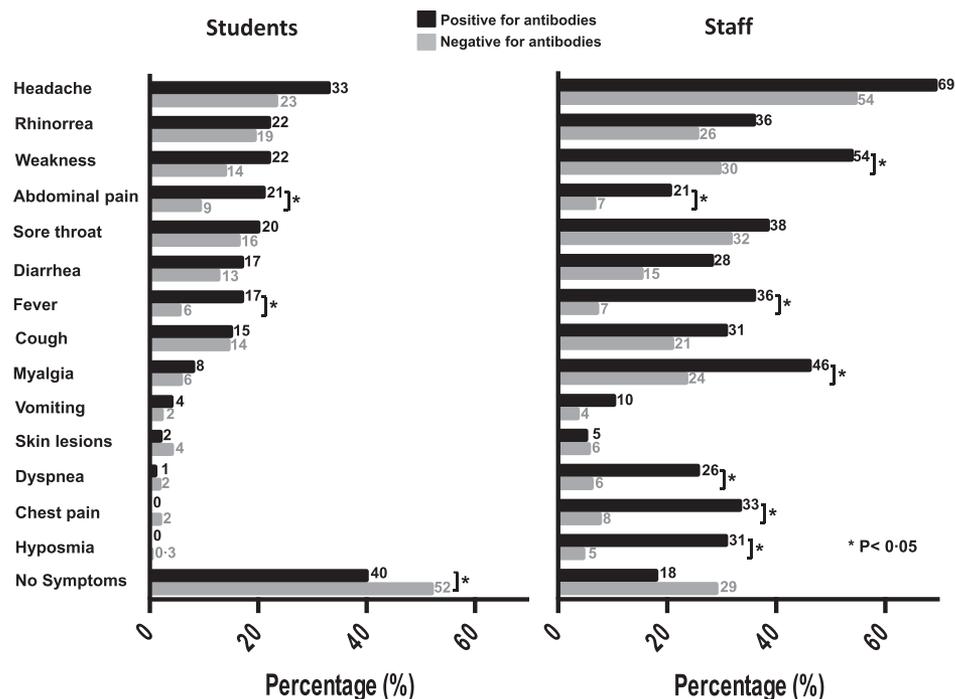


Figure 2. Differences in reported symptoms by antibody presence in students and staff.

Table 3. Multivariate Association Between Antibody Test Results and Variables That Differed by Antibody Status

	OR	95% CI	P
Students			
Myalgia	1.14	1.01 1.28	.04
Greater number of siblings who attend the school	1.30	1.01 1.68	.05
History of performing a RT-PCR test	2.37	1.08 5.18	.03
Symptomatic	1.83	1.17 2.85	.01
Age, years	.93	.88 .98	.01
Staff			
Headache	1.32	.97 1.79	.08
Fever	1.35	1.07 1.7	.01
History of performing a RT-PCR test	5.97	2.73 13.07	<.001

In the logistic model, the response variable was a positive/negative test result. The models had the discrimination power of an antibody-positive versus antibody-negative result of 67% (ROC, 0.67) for students and 73% (ROC: 0.73) for staff. ROC curve: students = 67% and staff = 73%; Wald test: students and staff, *P* values < .001; Hosmer and Lemeshow's test: student *P* = .51 and staff *P* = .04.

Abbreviations: CI, confidence interval; OR, odds ratio; ROC, receiver operating characteristic; RT-PCR, real-time polymerase chain reaction.

cases during the outbreak, more adults than students were antibody positive. Among students, antibody positivity was higher in younger children (pre-high school) and among teaching staff, as compared to nonteaching staff. Increased positivity in younger children was likely due to the fact that the index case was a member of the preschool community.

Factors associated with antibody positivity in children were SARS-CoV-2 PCR positivity during the outbreak and a history of contact with an infected case; in staff, only PCR positivity was significantly associated with antibody positivity. Overall, both PCR positivity and contact history were significantly higher in staff, as compared to student; in addition to the higher antibody positivity observed in this study, this supports the more significant role of adults within the outbreak, in proportion to the overall population. There were 19 participants with prior positive PCR results, of which 14 (73.7%) were also antibody positive.

This outbreak has particular epidemiological features that may differ from other current or future school outbreaks. The index case(s) was (were) incoming travelers, teachers, parents, and possibly students. Initial cases in nontraveling members of the community were mostly related to parent-teacher meetings, especially among adults related to the preschool. It is possible that a future outbreak, with a different index case(s), may result in a different age distribution of infection within a school. The duration of the virus' circulation period within the school lasted a maximum of 10 days from the beginning of the school year to the school closure (if an infected individual circulated on opening day). The virus most likely continued to circulate in the households of infected individuals, and a thorough investigation of intra-familial outbreaks was not performed. Additional external exposures both during and following the outbreak period, while possible, seem less significant, as a majority of

families in the area were under relatively strict stay-at-home orders imposed on 20 March.

Our findings, if replicated in other schools, have several potential implications for the prevention of future COVID-19 outbreaks in schools. In this outbreak, school closure occurred within 2 days of detection of the first 2 cases and within a few days of the start of the school year. Nevertheless, newly detected cases occurred mostly after the school closure, with a declining trend in school staff and an increase in parents and students. We hypothesize that adults, mainly through adult-to-adult contact, seem to have been most affected during this outbreak. Students, in comparison, were most likely to be infected by adults, either their teacher or parent, and parents were most likely infected during parent-teacher meetings.

Thus, when reopening schools under scenarios in which community transmission levels may be as low as during the pre-epidemic phase, the focus should be on avoiding new cases among teachers. Assuring the household detection of cases in adults will also be important. General recommendations for reopening schools are focused on maximizing person-to-person distancing as much as possible given the reality of a school setting (reducing both the number of students and hours within classrooms, limiting group activities), rapidly identifying and isolating symptomatic cases, encouraging robust parent-school reporting of cases, and creating action plans in case of outbreaks, among others. Our study does not contradict these recommendations, but may lead to the prioritization of certain actions, especially focusing on reducing adult-to-adult transmission. Certainly, school-related outbreak studies from other settings and situations, especially after the first wave, are required to further increase our knowledge of transmission dynamics within schools in order to pinpoint those recommendations which may end up being most effective. It is likely that current infection awareness will allow the early identification of future school outbreaks and the implementation of school measures within a short period, which should rapidly curtail a potential outbreak.

Antibody detection tests have limitations. Although manufacturers claim sensitivities and specificities of 72–100% and 98.7–100%, respectively [26], how these values relate to the true infection statuses of individuals in a community setting is not fully clear. According to the manufacturer, any visible band at the IgG and/or IgM level indicates a positive test. Nevertheless, the visibility of the band is subject to individual interpretation, as evaluated in our pilot testing. Bands can range from clearly visible to faintly visible. To deal with this issue, we defined criteria for positivity, with 4 levels of visibility based on a medical panel's review of photos of the test card. Overall, there was agreement between parents and the medical panel in 931/1009 student samples (92%) and 213/235 (91%) staff samples. Discrepancies largely occurred in samples deemed negative by parents but positive by the panel. Antibody positivity

due to a city-wide quarantine that is still in place to date. Importantly, the self-applied tests were successful in terms of self-application, with only 3.7% determined to be invalid and 0.5% indeterminate (1.9% were invalid/indeterminate after the option to perform a second test). While it would have been ideal to test parents as well, due to funding limitations this was not possible.

Symptoms were retrospectively reported, with the known risk of recall bias. Importantly, we asked participants to report symptoms prior to viewing the instructional sampling video; thus, it is unlikely that the test results influenced the self-report of symptoms. The fact that both antibody-negative students and staff reported symptoms at later dates leads us to conclude that their symptoms were due to other causes.

Self-application of the antibody test, delivered using a home delivery system, in combination with the use of electronic data capture via the participant's cell phone in a secure web platform, proved to be an effective, contact-free epidemiological tool in the current pandemic setting, where limiting person-to-person contact is a relevant public health measure. Such methodology could be considered in other settings worldwide, including in remote areas.

CONCLUSIONS

In this school-based COVID-19 outbreak in Chile, affecting nearly 50 people among school and household members, antibody positivity rates based on a self-administered test were 10% and 17% among students and staff, respectively. Adults, mainly through adult-to-adult contact, seem to have been most affected during this outbreak. Self-administered, remotely supervised antibody testing allowed us to determine the magnitude of infection and characterize outbreak features.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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