FAST TRACK

Clustering and longitudinal change in SARS-CoV-2 seroprevalence in school children in the canton of Zurich, Switzerland: prospective cohort study of 55 schools

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ABSTRACT

OBJECTIVES

To examine longitudinal changes in severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) seroprevalence and to determine the clustering of children who were seropositive within school classes in the canton of Zurich, Switzerland from June to November 2020.

DESIGN

Prospective cohort study.

SETTING

Switzerland had one of the highest second waves of the SARS-CoV-2 pandemic in Europe in autumn 2020. Keeping schools open provided a moderate to high exposure environment to study SARS-CoV-2 infections. Children from randomly selected schools and classes, stratified by district, were invited for serological testing of SARS-CoV-2. Parents completed questionnaires on sociodemographic and health related questions.

PARTICIPANTS

275 classes in 55 schools; 2603 children participated in June-July 2020 and 2552 in October-November 2020 (age range 6-16 years).

MAIN OUTCOME MEASURES

Serology of SARS-CoV-2 in June-July and October-November 2020, clustering of children who were seropositive within classes, and symptoms in children.

RESULTS

In June-July, 74 of 2496 children with serological results were seropositive; in October-November, the number had increased to 173 of 2503. Overall SARS-CoV-2 seroprevalence was 2.4% (95% credible interval 1.4% to 3.6%) in the summer and 4.5% (3.2% to 6.0%) in late autumn in children who were not previously seropositive, leading to an estimated 7.8% (6.2% to 9.5%) of children who were ever seropositive. Seroprevalence varied across districts (in the autumn, 1.7-15.0%). No significant differences were found among lower, middle, and upper school levels (children aged 6-9 years, 9-13 years, and 12-16 years, respectively). Among the 2223 children who had serology tests at both testing rounds, 28/70 (40%) who were previously seropositive became seronegative, and 109/2153 (5%) who were previously seronegative became seropositive. Symptoms were reported for 22% of children who were seronegative and 29% of children who were newly seropositive since the summer. Between July and November 2020, the ratio of children diagnosed with SARS-CoV-2 infection to those who were seropositive was 1 to 8. At least one child who was newly seropositive was detected in 47 of 55 schools and in 90 of 275 classes. Among 130 classes with a high participation rate, no children who were seropositive were found in 73 (56%) classes, one or two children were seropositive in 50 (38%) classes, and at least three children were seropositive in 7 (5%) classes. Class level explained 24% and school level 8% of variance in seropositivity in the multilevel logistic regression models.

CONCLUSIONS

With schools open since August 2020 and some preventive measures in place, clustering of children who were seropositive occurred in only a few classes despite an increase in overall seroprevalence during a period of moderate to high transmission of SARS-CoV-2 in the community. Uncertainty remains as to whether these findings will change with the new variants of SARS-CoV-2 and dynamic levels of community transmission.

TRIAL REGISTRATION

NCT04448717

WHAT IS ALREADY KNOWN ON THIS TOPIC

The role of school children in transmitting severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection is a controversial issue; outbreaks in schools do occur and children can have similar seroprevalence as adults

Representative, prospective, and school based studies are needed to assess the overall spread of the virus in schools and clustering in classes

Understanding the transmission and impact of SARS-CoV-2 infection in school children is critical for implementing appropriate mitigation measures

WHAT THIS STUDY ADDS

Among 55 randomly selected schools and more than 2500 children, 2.4% of children had SARS-CoV-2 antibodies in July and 7.8% by November 2020 after a period of moderate to high community incidence

Seroprevalence did not differ among different age groups

Less than half of classes had at least one child who was seropositive; clustering of three or more children who were seropositive in a class was rare and was partly caused by unrelated infections at different time points

Introduction

The role school children have in transmitting severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection remains a controversial issue.

Infection rates in children, particularly adolescents, can be as high as in adults,[1 2] but children rarely develop clinically manifest coronavirus disease or severe health outcomes.[3-6] However, debate continues about the prevalence of infections in children with no symptoms or few symptoms, and the potential spread in schools. Many countries closed their schools during the first half of 2020 to curb the pandemic, which led to disrupted education for 1.5 billion learners in up to 172 countries as of April 2020[7]; on 2 February 2021, more than 200 million learners were still affected by school closures.[7]

The negative effects of school closures include an increase in social and economic inequality, and adverse long term educational, social, and health outcomes for children.[8-10] However, the epidemiological benefits of school closures for transmission of SARS-CoV-2 are uncertain. Even during periods of moderate to high community transmission, in many countries few outbreaks have been observed among children in schools where infection control measures have been implemented.[11-13] Although some outbreaks have been reported in educational settings during early 2020 (eg, in Israel[14] and the United States[15]), full or partial opening of schools in many countries in autumn 2020 has not resulted in increased outbreaks.[11 16-18]

Most studies in schools have focused on people with identified SARS-CoV-2 infection and subsequent contact tracing within schools. Therefore, children with SARS-CoV-2 infection who have no symptoms or few symptoms are probably still being missed, and the extent to which they contribute to spread of infection within schools is not clear. Additionally, the total eligible population is often not defined in studies focusing on outbreaks and contact tracing of index children in schools; the relative frequencies of outbreaks and children with SARS-CoV-2 infection in these studies remain unclear. Longitudinal, population and school based studies are needed with random sampling at class and school level to determine the frequency of outbreaks in classes and schools.

In this article, we present the results of a longitudinal cohort study (Ciao Corona) in the canton of Zurich, Switzerland. We measured SARS-CoV-2 antibodies and assessed symptoms in a cohort of more than 2500 children from 55 schools in June-July and October-November 2020 (referred to as T1 and T2). Schools were open in this most populous canton of Switzerland since 17 August 2020, with preventive measures in place. While the number of new infections stayed low until early October, Switzerland subsequently experienced one of the highest second waves of the pandemic in Europe in autumn 2020. By the time testing took place in October-November, children had been exposed to high levels of community transmission for 3-6 weeks. Ciao Corona is one of the few prospective, population, and school based studies of SARS-CoV-2 seroprevalence. The study offers unique insights into changes in clustering of children who were seropositive within classes and schools, and the association with self-reported symptoms.

The objectives of the study were to estimate longitudinal changes in seroprevalence at individual, school, district, and cantonal levels; to calculate the ratio of children diagnosed with SARS-CoV-2 infection to those who were seropositive; to assess the association of seropositivity with reported symptoms; to determine the frequency of clustering of children who were seropositive within classes and schools; and to investigate potential causes of observed clusters.

Methods

The protocol of this longitudinal cohort study (ClinicalTrials.gov identifier: NCT04448717) is reported elsewhere.[19] The study is part of the nationally coordinated research network in Switzerland, Corona Immunitas.[20] The study was based in the canton of Zurich, which has 1.5 million linguistically and ethnically diverse residents who live in urban and rural settings and comprise 18% of the Swiss population. In 2020, physical attendance of schools was interrupted between 16 March and 10 May, and then continued until the start of the summer holiday on 13 July. As in most of Europe, schools in Switzerland have been in continuous operation from the start of the school year on 17 August to the end of 2020. When schools reopened, preventive measures were introduced and gradually adjusted (eg, masks for school staff, masks for children in secondary schools, distancing rules in classrooms and teachers' rooms, no mixing of classes, reduction of large group activities, requirement for children to stay at home when ill). The measures varied, but all schools required children to stay at home if they were ill unless their symptoms were very mild (such as a runny nose or mild cough); they required adults to wear masks in school from 19 October, and secondary school children (older than 12 years) to wear masks from 2 November.

Specific contact tracing was implemented for schools, triggered by children or school staff testing positive for SARS-CoV-2. Subsequent action for the affected class and school was based on the assessment performed by the contact tracing team. Full classes were in general quarantined when two or more children were infected simultaneously within a class.

Population

We randomly selected primary schools from the list of all schools in the canton of Zurich, stratified by region, and matched the geographically closest secondary school (often in the same school building). Of 156 schools invited to participate, 55 schools agreed. We randomly selected classes within participating schools, stratified by school level: lower school level (grades 1 and 2; children aged 6-9 years); middle school level (grades 4 and 5; children aged 9-13 years); and upper school level (grades 7 and 8; children aged 12-16 years). We selected grades to ensure that the same cohorts would be in the classes until April 2021, when further testing is planned (children in grades 3, 6, and 9 often change class and school during the next

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academic year). The median number of children in invited classes was 20 (range 6-27, interquartile range 18-22). We aimed to enrol at least three classes and at least 40 children from each school level at the invited schools. A major exclusion criterion was suspected or confirmed SARS-CoV-2 infection on the testing day, which precluded the child attending school and therefore being tested at school.

Timeline of testing

Venous blood samples were collected from participants at schools during two testing rounds. The first round of testing (T1) from 16 June to 9 July 2020 included 2585 participants (serology results available for 2484), and the results are reported elsewhere.[1] An additional 18 eligible children who could not participate in June-July were tested from late August to early September (serology results available for 12), and the results were merged with the T1 testing round, resulting in a total of 2603 children. Figure 1 shows a flow chart of the study participants.

We invited the same cohort of classes to the second testing round (T2) from 26 October to 19 November 2020. For previously invited classes that had been restructured after the summer break, all children in the newly formed classes were eligible to participate. The third testing round is planned for March-April 2021; this round includes children and their parents, and school staff.[19]

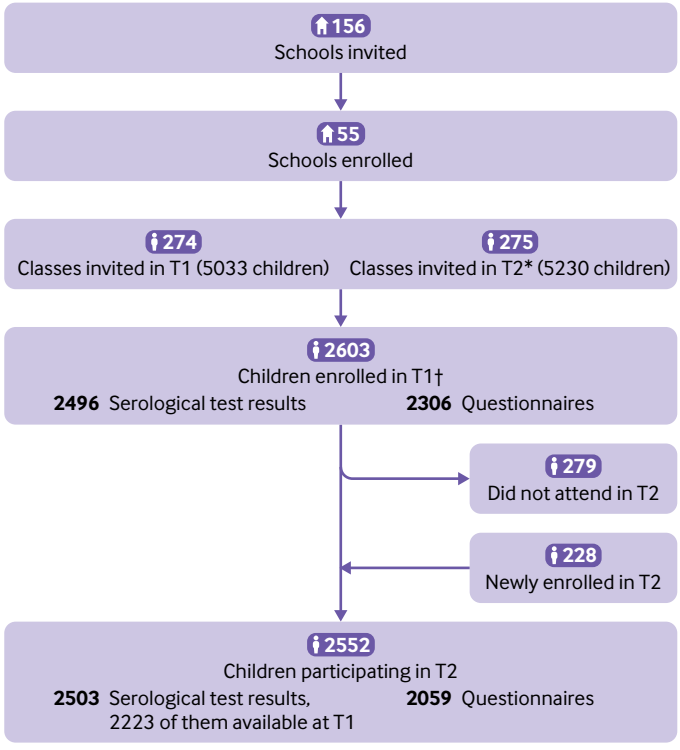

Fig 1 | Flowchart of study participants. T1—testing in June-July 2020; T2—testing in October-November 2020. *Some classes were split or rearranged into multiple classes after summer break. †Eighteen of these children were enrolled from late August to early September 2020 (12 serological results, 18 questionnaires)

Serological testing and outcomes

Venous blood samples were analysed with the ABCORA 2.0 binding assay (Institute of Medical Virology, University of Zurich), which is based on Luminex technology. This test has been described previously.[1] The test analysis immunoglobulins (IgG, IgM, and IgA) against four SARS-CoV-2 targets (receptor binding domain, spike proteins S1 and S2, and the nucleocapsid protein N) yielded 12 different measurements. Cut-off values were established against prepandemic plasma that allowed a sensitivity of 94.3% and specificity of 99.0% (see test description[21]; test parameters have been updated based on an expanded validation cohort). Samples were defined as seropositive for SARS-CoV-2 if at least two of the 12 parameters were above the cut-off value. Serological results were unavailable if venous blood could not be obtained during venipuncture or previously enrolled participants declined venipuncture during testing.

The results at T1 and T2 were combined and three serological outcomes were analysed. Table 1 summarises the results. The first outcome was the seroprevalence in June-July (T1). The second outcome was the seroprevalence in October-November among children who previously tested negative and those who were not tested in June-July (T2). Even though some of the children who were seropositive at T1 tested negative at T2, we excluded them from T2 results because of potentially persisting cellular immunity[22] and rare reports of reinfection in children, and thus a low likelihood of a repeated infection since the summer. The third outcome was the proportion of children who ever had a SARS-CoV-2 infection based on the serological test results by October-November. This outcome was defined by analysing the children tested at T2 (or seropositive at T1 and not retested at T2) and counting as positive those who tested positive at T1 (regardless of subsequent T2 results) or at T2. The seroprevalence at T1 and T2 does not add up to the proportion of T1+T2 seroprevalence because the populations included in the numerator and denominator of these three outcomes are not the same (see appendix 1 for explanation).

Statistical analysis

Descriptive statistics included summaries of participation rates at the school, class, and individual level. Key characteristics of participants (age, school level, sex) and their reported symptoms were summarised as median (range) or count (percentage). Children without serology results were not included in the analysis of the main outcomes. Children without a completed questionnaire were not included in the analysis of symptoms. No missing data were reported for age, sex, class, and school identity of participating children. The participation rate in a class was calculated as the ratio of enrolled children to all children in the class.

We used Bayesian logistic regression[2] to estimate seroprevalence, which was adjusted for participants' grade at school, sex, and geographical district of the school, and contained random effects for school

Table 1 | Definitions of seroprevalence outcomes

Abbreviation	Definition	Numerator	Denominator
T1 seroprevalence (seropositive in summer)	Seroprevalence in children in June-July 2020	Children who were seropositive in June-July	All tested children in June-July*
T2 seroprevalence (newly seropositive in autumn)	Seroprevalence in children in October-November 2020, excluding children seropositive in June-July	Children who were seropositive in October-November, excluding those who also tested seropositive in June-July†	All tested children in October-November, excluding those who also tested seropositive in June-July
T1+T2 seroprevalence (ever seropositive in summer or autumn)	Proportion of children who had been infected with SARS-CoV-2 by October-November 2020, as reflected by ever being seropositive	Children who tested seropositive at least once in June-July or October-November (ever tested seropositive)	All tested children in October-November and those who were seropositive in June-July but did not participate in testing in October-November

SARS-CoV-2=severe acute respiratory syndrome coronavirus 2.
*Included 18 children tested (serological results available for 12) from late August to early September.
†Included 22 children who tested positive at T2, who were not tested at T1.

levels (lower, middle, and upper). The Bayesian approach permitted adjustments for the sensitivity and specificity of the SARS-CoV-2 antibody test and the hierarchical structure of the cohort (individual and school levels). To compute seroprevalence estimates representative for the canton of Zurich, we stratified our results according to the total population size at the school level and geographical district.

We calculated the ratio of confirmed infections to total infections to assess how many SARS-CoV-2 infections are potentially missed in non-serological studies (eg, using official statistics of confirmed infections). The ratio was calculated by using the real time polymerase chain reaction confirmed cumulative incidence of SARS-CoV-2 infections from 30 June to 8 November 2020 (during autumn testing period) and the total cumulative incidence until 8 November, based on official statistics,[23] and the estimated autumn (T2) and overall (T1+T2) seroprevalence.

We determined whether schools or specific classes explained more of the variance in seropositivity by modelling individual level serology results in a multilevel logistic regression, with sex and school level as fixed effects, and class and school as random effects. Intraclass correlation was then used to compare the part of variance explained by class or school, allowing us to evaluate the potential association of seropositivity within classes or schools.

Cluster analysis focused on the class level. Clusters were defined as three or more children who were newly seropositive within a class during autumn testing (T2), regardless of class size. We chose this threshold because it is commonly associated with the spread of acute infection within classes and is used to initiate quarantine measures of the whole class. Although the temporal sequence of infections cannot be determined with serological testing, we aimed to increase the sensitivity of detection of potential clusters by using the smallest reasonable threshold.

Clustering was assessed in a subset of classes in which at least five children were successfully tested at the relevant time point and the participation rate was 50% or higher in the class. Therefore, classes with low participation rates were excluded because they were potentially underpowered to detect clusters. However, if a cluster was identified in a class with a participation rate lower than 50%, the class was included in the numerator and the denominator for the proportion of classes with clusters. We calculated the proportion of classes with a cluster of children who were seropositive by dividing their number by the total number of enrolled classes.

We also evaluated how many clusters would be expected if children within classes were infected independently at the same overall rate as in the entire sample. In a simulation study, we created 10 000 such hypothetical populations with the same number of classes and children that were tested at T2 in this study. In the simulation, an independent chance of seropositive results with a normal distribution (that is, no association of children who were seropositive within a class) was assumed, equal to the observed proportion of children who were seropositive at T2 among all children tested at T2 who were not positive at T1 (see table 1). We then compared the actual observed distribution of clusters with those in the simulated populations, and calculated the probability of observing clusters of at least three, at least four, and at least five children who are seropositive within a class. If the observed actual number of clusters is higher than the number of clusters in the simulation study, this would provide evidence against clustering of children who are seropositive within classes by chance.

To further investigate potential causes and associations of infections within the detected clusters, after T2 testing we performed semi-structured interviews with the principals of schools with classes that had clusters of children who were seropositive

Table 2 | Comparison of individual serological results in study participants in summer (T1) and autumn (T2) 2020

	Serological result at T2		
Serological result at T1	Negative	Positive	Not available
Negative	2044	109	269
Positive	28	42	4
Not available	258	22	55

T1: June-July 2020; T2: October-November 2020.

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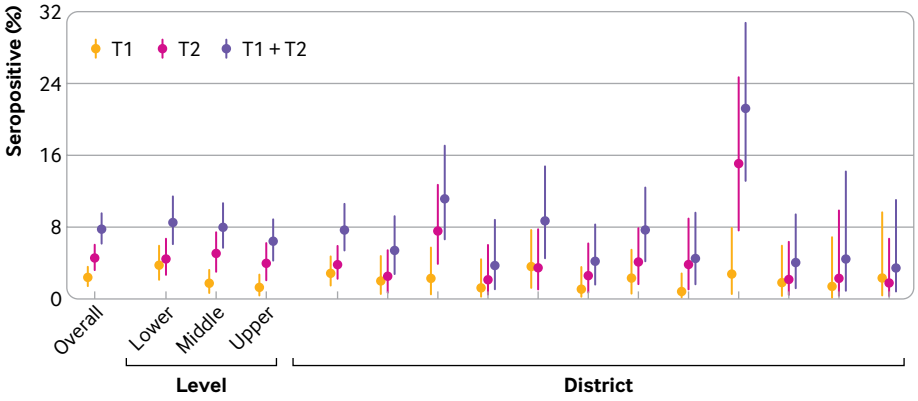

Fig 2 | Seroprevalence estimates in children in June-July 2020 (T1), among children who were newly seropositive in October-November (T2), and those who were ever seropositive by October-November (T1+T2). Overall and school level specific estimates (lower school level: grades 1 and 2, children aged 6-9 years; middle school level: grades 4 and 5, children aged 9-13 years; upper school level: grades 7 and 8, children aged 12-16 years), and district level specific estimates for canton of Zurich. Districts are ranked in order of decreasing population size

at T2. Interview questions covered numbers of SARS-CoV-2 infections in teachers and children of the affected classes confirmed by real time polymerase chain reaction before serological testing, numbers of teachers and children who were quarantined, potential temporal sequence of infections, and other related circumstances. Data analysis was performed with R version 4.0.3.[24] Bayesian hierarchical modelling was performed using the R package rstan.[25]

Patient and public involvement

Several school principals were consulted during the development of the protocol to ensure feasibility of the planned study procedures. Feedback was continuously collected from invited and enrolled children and parents to adapt the communication strategies and channels. Online informational sessions, which encouraged open exchange and feedback, were organised at the onset of the study for invited and enrolled school principals, staff, and parents of the children.

Results

In total, 2831 children from 275 classes within 55 schools in the canton of Zurich were enrolled in the study by October-November 2020. Of these, 2603 participated in T1 summer testing and 2552 participated in T2 autumn testing. Serology results were available for 96% and 98% of participants in summer and autumn, respectively. Figure 1 shows the flowchart of enrolled participants with serological test results and questionnaire information available.

At T2 testing, serological results were available for 731 children from lower school level (median age 8, age range 6-10 years), 863 children from middle school level (median age 11, age range 8-13 years), and 909 children from upper school level (median age 14, age range 11-16 years); 1287 children were female, 1211 were male, and five reported other gender. The median participation rate at T2 within a class was 47% (interquartile range 30-62%; median 39% in lower school level, 52% in middle school level, and 50% in upper school level).

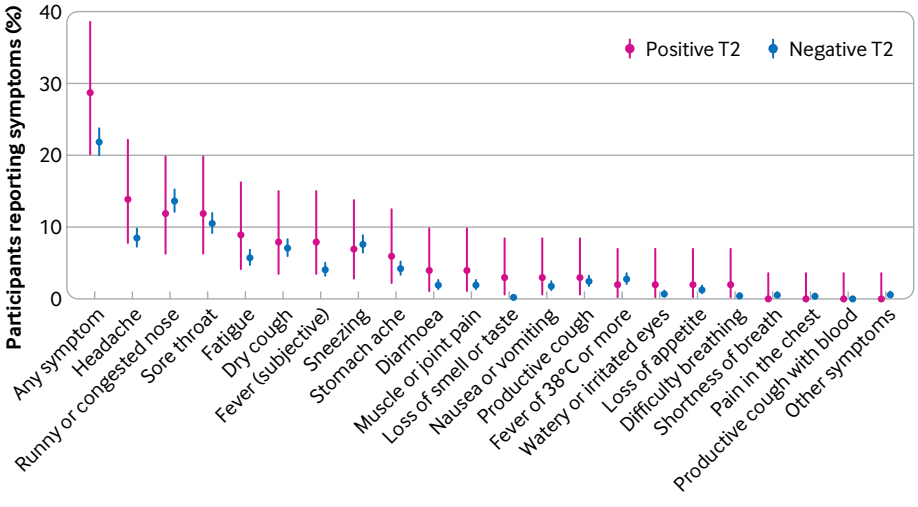

Fig 3 | Symptoms reported between July and November 2020 in children who were seronegative and newly seropositive (at autumn testing—T2)

Table 2 presents the distribution of serological results at T1 and T2. Among children seropositive at T1, 60% (42/70) had a positive serology result at T2. Age distribution and presence of symptoms reported before T1 testing was not different for these 42 children and the 28 children who were previously seropositive and were seronegative at T2.

Figure 2 shows seroprevalence at the two time points in the overall population, at school level, and for districts of Zurich. Estimated SARS-CoV-2 seroprevalence in children at T1 was 2.4% (95% credible interval 1.4% to 3.6%). Seroprevalence in children in the autumn (newly seropositive at T2) was 4.5% (3.2% to 6.0%). The proportion of children who were ever seropositive (T1+T2) by the autumn was 7.8% (6.2% to 9.5%). The proportion of children who were ever seropositive (T1+T2) in the districts of Zurich ranged from 3.5% to 21.2%.

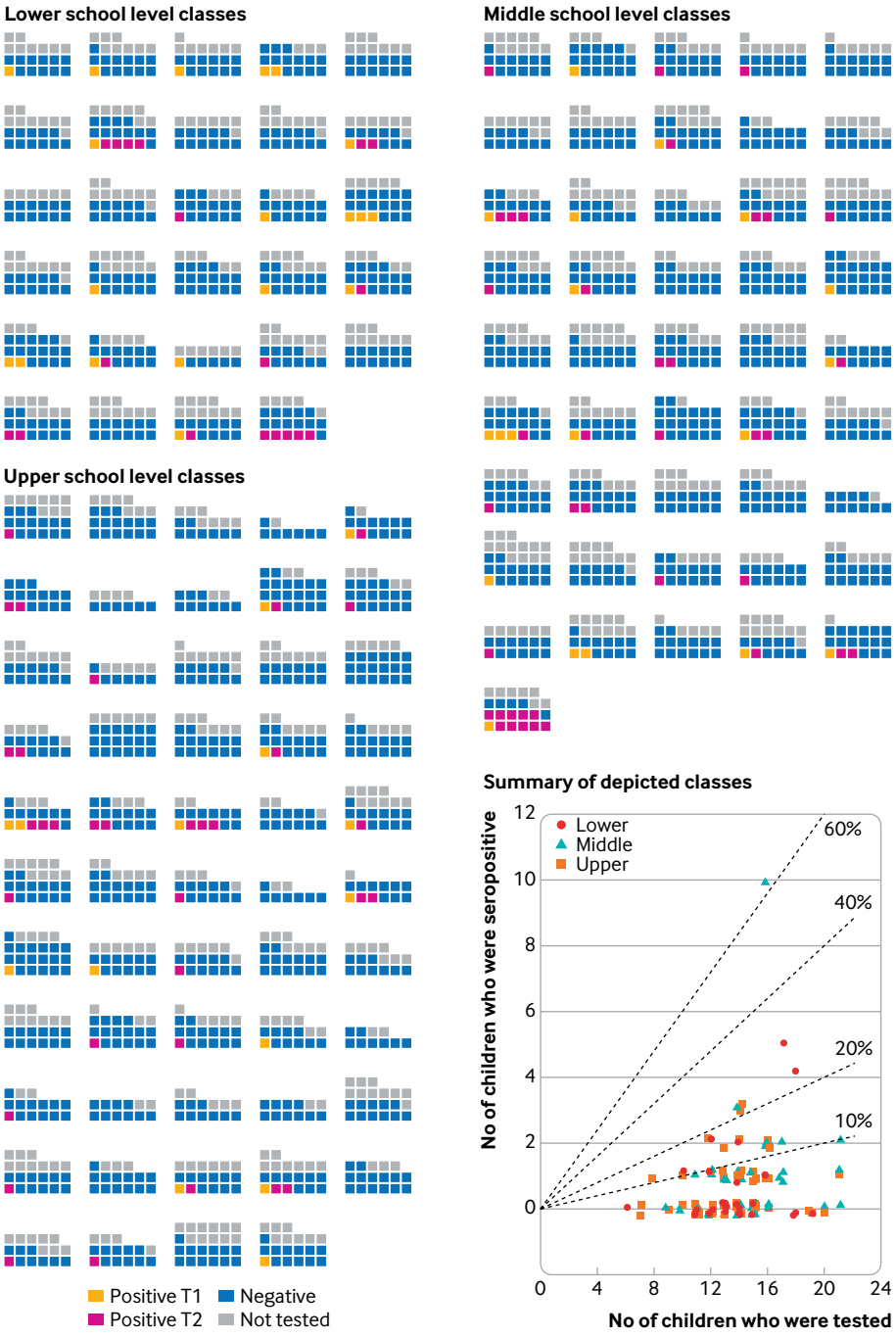

Fig 4 | Distribution of children who were seropositive at T1 and newly seropositive at T2 in classes with at least five children and 50% or more of children tested (29 classes in lower school level, children aged 6-9 years; 46 classes in middle school level, children aged 9-13 years; and 54 classes in upper school level, children aged 12-16 years). Figure depicts the serological status of children in autumn 2020 (T2)

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T2 seroprevalence in lower, middle, and upper school levels was 4.4% (95% credible interval 2.7% to 6.7%), 5.0% (3.0% to 7.4%), and 3.9% (2.1% to 6.2%), respectively; T1+T2 seroprevalence in lower, middle, and upper school levels was 8.5% (6.1% to 11.4%), 8.0% (5.7% to 10.7%), and 6.4% (4.3% to 8.9%), respectively. The estimated seroprevalence did not differ between male and female participants.

Symptoms between the summer break and November 2020 were reported in 21.8% (420/1923) of participants who were seronegative and in 28.7% (29/101) of participants who were newly seropositive (at T2). Figure 3 presents the distribution of individual symptoms. Although reported rarely in general, only loss of smell or taste was more frequent in participants who were seropositive (3.0%, 3/101) than in those who were seronegative (0.2%, 4/1923). The most frequently reported symptoms in participants who were seropositive were headache (13.9%, 14/101), runny or congested nose (11.9%, 12/101), sore throat (11.9%, 12/101), and fatigue (8.9%, 9/101).

Compared with the cumulative incidence of SARS-CoV-2 infections in children aged 4-15 years who lived in the canton of Zurich, the ratio of children diagnosed with SARS-CoV-2 infection to those who were seropositive was 1 to 13 from January to November 2020, and 1 to 8 from July to November.

Cluster analysis

The number of children who were newly seropositive at T2 within a school level ranged from 0 to 12, and within a class from 0 to 10. The number of children who were ever seropositive (T1+T2) ranged from 0 to 14 within a school level, and from 0 to 11 within a class. At least one child who was newly seropositive was detected in 47 of 55 schools and in 90 of 275 classes (56 of 129 classes with at least five children and with 50% or more of children tested).

At least one child who was ever seropositive was detected in 52 of 55 schools and in 125 of 275 classes (75 of 129 classes with at least five children and with 50% or more of children tested). Figure 4 shows the distribution of children who were newly and ever seropositive within tested classes.

Seven classes in five schools had three or more children who were newly seropositive: three classes in the lower school level, two in the middle school level, and two in the upper school level. Table 3 presents detailed information about the clusters. Assuming a uniform 5.4% seropositivity rate across all tested children (corresponding to 131 children who were newly positive among the 2433 tested and eligible for T2 outcome) and numbers of children tested within classes as observed in this study, a simulation study showed that seven or more clusters of at least three children with seropositivity would be expected by chance in 14% of repetitions, with a median of four expected clusters (95% credible interval 1 to 9). Therefore, even if infections within classes are not associated, in a population with a class structure similar to this study and with the same number of children with seropositivity, we would expect to see four clusters of three or more children who are seropositive in a class. Three or more clusters of at least four children would be expected in 1.5% of simulations (median number of clusters 0, 95% credible interval 0 to 2), and two or more clusters of at least five children in 0.2% of simulations (median number of clusters 0, 95% credible interval 0 to 1).

Table 3 | Detailed information on classes with three or more children who were newly seropositive between July and November 2020

School and level	No of children in class			Confirmed infections		Quarantine or isolation			Probable index case		
	Total	Tested	Newly seropositive	Teacher	Children	Teacher	Children	Information	Teacher	Child	Household
1, middle	17	14	3	0	0	0	8	Individual unrelated children in quarantine because of positive household members	—	—	X
2, lower	19	9	3	0	0	0	0	No confirmed infections previously known	?	?	?
3, lower	25	16	4	1	2	1	Whole class	Teacher tested positive; next day two children tested positive	X	—	—
4, middle	23	16	10	1	3	1	Whole class	Initially, a child with symptoms and parent tested positive; next day two other children and teacher tested positive after being identified with contact tracing; many household members (children and adults) of the class infected as well; index case remained unclear	?	?	?
4, lower	22	17	5	0	0	0	2	Two unrelated children in quarantine due to sports camp coach with diagnosed infection and affected household member	—	—	X
5, upper	17	13	3	0	1	0	Whole class	Class quarantined after a mother and subsequently the child (class student) tested positive while the class was at a camp	—	—	X
5, upper	16	12	3	0	0	0	3	Individual, unrelated children quarantined due to exposure to confirmed infection (one due to brother with infection from another class; other two unknown)	—	?	?

Information obtained through semi-structured interviews with school principals, including information about probable index case. One class from school 2 had participation rate of 47% and therefore is not shown in figure 4. Confirmed infections refer to previously diagnosed severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection confirmed by real time polymerase chain reaction test within the class reported by school principals during interviews. Positive test refers to positive real time polymerase chain reaction test for SARS-CoV-2. X refers to a probable index case, and ? to a potential index case, according to the information provided by school principals

In the multilevel logistic regression of individual serology results of the T2 serology model with random effects for school and class, school level (as a proxy for age) and sex were not important predictors. In the intraclass correlation analysis, school accounted for approximately 8% of the total variance, while class accounted for 24% of the variance.

Discussion

Principal findings

In this cohort study of 55 schools and more than 2500 children, seroprevalence increased from 2.4% in June-July to 7.8% in October-November 2020. SARS-CoV-2 antibodies were not detected after four months for 40% of children who were previously seropositive. No difference was found in seroprevalence between school levels, although a trend of lower seroprevalence was observed in older children. We observed only minimal clustering of children who were seropositive within classes and schools between July and November 2020, despite a clear increase in seroprevalence among children and a period of moderate to high SARS-CoV-2 transmission in the community before testing. Some of the clusters could be explained by independent, non-concurrent individual infections among classmates.

In autumn 2020, Switzerland had one of the highest reported incidences of SARS-CoV-2 infections in Europe, peaking at approximately 950 daily infections per million inhabitants in early November.[26] A similarly high incidence was observed in the canton of Zurich where the study was conducted, with approximately 590 daily infections per million inhabitants and around 16% positivity of real time polymerase chain reaction tests in the first half of November.[23] However, schools have been open since the school year started on 17 August 2020. Some, but not extensive preventive measures were implemented, such as masks for school staff and restriction of large group activities, in addition to contact tracing in schools. As the number of infections in the community increased, children in secondary schools (older than 12 years) were required to wear masks from November. Under these circumstances, an increase in seroprevalence after the summer was expected. However, this increase was not accompanied by a high incidence of clusters of children who were seropositive within schools and classes.

Clusters of three or more children who were newly seropositive were observed only in the minority of classes (six out of 129 classes with high participation rate, and in one class with 47% participation rate). In contrast to some other studies, we did not observe higher seroprevalence or clustering in higher school grades. Potentially, behavioural factors and preventive measures (including older children wearing masks and contact tracing strategies in schools) helped to mitigate the potential spread of infection. Diagnosing children with SARS-CoV-2 infection also improved after the summer; the ratio of children diagnosed with SARS-CoV-2 infection to those who were seropositive increased from 1 to 89 (from spring to summer),[1] to 1 to 8 (from summer to autumn).

Observed clustering of children who were seropositive within a class could indicate an outbreak, but does not necessarily mean that an outbreak has occurred. In the seven classes with observed clusters, children who were seropositive were probably not part of the same infection transmission chain in at least two classes. In six classes, at least some of the children who were seropositive had previously been diagnosed with SARS-CoV-2 infection or had been quarantined. The actual number of children with SARS-CoV-2 infection in the autumn (and therefore, the number of clusters) could be even smaller because we assumed that 22 children who were seropositive at T2 but with no serological results at T1 were all infected in the autumn, erring on potentially identifying more clusters. The results of the simulation showed that even if seropositive status was randomly assigned to children of the study population, clusters of three or more children (although not clusters of four or more children) could be observed. Therefore, even if children who were seropositive were not associated within classes (that is, infections were completely independent of each other), some clusters could be reasonably expected to be observed by chance. In comparison, in the canton of Ticino, Switzerland, where community transmission was even higher in November 2020, 1% (14/1410) of classes were affected by quarantine measures in August-November 2020[27] (no corresponding data were publicly available from the canton of Zurich at the time this article was submitted).

Multilevel models suggested that children who were seropositive were associated at class level more often than at school level. This observation could mean that, as expected, infection is more likely to spread within a class rather than within the entire school. Potentially, the random effect of the school would become even smaller once the incidence in the community (district) is controlled for. Therefore, these results support the use of focused, class based quarantine measures to control the spread of SARS-CoV-2 infection within schools.

Comparison with other studies, strengths and limitations

This study offers unique insights into the transmission and prevalence of SARS-CoV-2 infection in schools on a randomly selected, representative, longitudinal, population based cohort. Most other studies of SARS-CoV-2 infections in schools have focused on contact tracing of index cases,[11 28] and so potentially missing unidentified cases. Other studies have relied on the prevalence of diagnosed SARS-CoV-2 infection to assess the frequency of outbreaks and the risk of infection in children while schools are open.[11 16 17] A few studies tried to estimate the overall effect of closing and opening schools on the development of reported SARS-CoV-2 infections and deaths.[29-31] Such ecological, retrospective studies often have major limitations of uncontrolled confounding, high level of aggregation for intervention (eg, pooling school and university closures as one intervention) and outcome

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(eg, outcomes at country level), and potentially measure the outcome in a population not exposed to the intervention. Finally, a stochastic modelling study of infection spread in schools has shown that some, although minimal, clustering of infections is likely even when major prevention and screening strategies are implemented.[32]

In contrast to these retrospective and modelling studies, our study offers a prospective population level view, which corresponds to school structure because of sampling at school and class levels. Additionally, having measured the baseline seroprevalence in June-July 2020, we were able to study the number of children who were newly seropositive and their clustering in classes in the autumn. The study had a high retention rate, with 89% of enrolled children retested in the autumn, and high overall participation rates, especially given the venous blood sampling in children, a process which often triggers anxiety. High participation rates within a large proportion of classes allowed the study of clustering at the class level, which has been lacking in other school based studies on SARS-CoV-2 infection.[33 34] The results of the first two testing rounds, presented in this paper, will be strengthened by subsequent testing in March-April 2021 (which will also include parents and school staff). This further round of testing will capture seroprevalence associated with SARS-CoV-2 infections between November 2020 and March 2021. Subsequent testing will also provide evidence of whether transmission in schools changes as new variants of SARS-CoV-2 emerge and become more prevalent, and the community transmission level changes.

The study has limitations. Firstly, seroprevalence does not necessarily directly correspond to past SARS-CoV-2 infection. Because several days are required for seroconversion after SARS-CoV-2 infection,[35-38] recent infections could be missed. The incubation period of around five days (between 1 and 14 days) also means that clusters might develop in a class over an extended period of time.[39] Serological studies inherently reflect infections that happened at least one week before testing. Ideally, serological testing would take place immediately after rather than during a period of high community transmission. Because testing in the 55 schools had to be scheduled in advance, when the projected development of the pandemic was uncertain, we were not able to move our testing dates to capture a longer period of high community transmission. Additionally, although we could adjust for test accuracy parameters at the population level, some false negatives and positives are expected on an individual level.[40] Although the number of children who were seropositive at T1 and seronegative after four months at T2 (n=28, 40%) is compatible with the expected number of false positives at the low seroprevalence observed at T1, waning of antibodies in the asymptomatic population is also possible. Based on the estimated seroprevalence and test accuracy parameters, among the 131 children who tested positive in the autumn, 20 would be expected to be false positive; and among the 2330 who tested negative, 11 would be expected to be false negative. These data imply that the true rate of clustering could potentially be even lower.

Secondly, measuring seroprevalence rather than acute diagnosis of SARS-CoV-2 infection allows only a retrospective analysis, and prevents full reconstruction of the temporal sequence of infections within classes. We were able to reconstruct some of the temporal information by comparing the serology status of children in the summer and autumn, and so differentiating between children infected in the first (spring) and second (autumn) wave of the pandemic. We also interviewed school principals about the development of infections in classes with clusters.

Thirdly, seroprevalence is a dynamic parameter because some children lose the antibodies and might appear seronegative despite having had the virus. Based on the low reinfection rates in the literature, serological status only tells part of the story about the immunity against SARS-CoV-2 infection. Other unspecific or T cell mediated cellular responses might exist to confer long term immunity.[22] However, the limitation would be even higher with diagnostic testing, within a much smaller temporal window of positivity, and no retrospective information. Finally, although the participation rate was relatively high and analysis of clustering limited to classes with at least 50% participation, we do not have individual level information on reasons for non-participation or sociodemographic characteristics of non-participants for comparison. Although a statistically non-significant negative trend was observed, the socioeconomic context of the region did not differ for non-participating and participating schools, and it was not associated with participation rates within schools.

Conclusions

Clusters of children who were SARS-CoV-2 seropositive occurred in only a few school classes despite an increase in overall seroprevalence in children during a period of moderate to high transmission of SARS-CoV-2 infection in the community. While debate continues about mitigation measures to curb the pandemic and the role schools have in infection transmission, this study provides evidence that clusters of SARS-CoV-2 infection are rare within classes. Future testing rounds of this study will provide insights on transmission within classes over prolonged periods during dynamic levels of community transmission and the spread of new SARS-CoV-2 variants.

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authorship. SK and MAP initiated the project and preliminary design, with support of JF. SK, MAP, CB, TR, and AU developed the design and methods. SK, AU, and TR recruited study participants, collected and managed the data. SRH performed statistical analysis. AT, MH, MSchw, MScha, and IAA developed the serology analysis plan, supervised, conducted, and evaluated the serology tests. AU wrote the first draft of the manuscript. All authors contributed to the design of the study and interpretation of its results, and revised and approved the manuscript for intellectual content. SK is the guarantor and accepts full responsibility for the work and the conduct of the study, had access to the data, and controlled the decision to publish. The corresponding author (SK) attests that all listed authors meet authorship criteria and that no others meeting the criteria have been omitted.

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Ethical approval: The study was approved by the Ethics Committee of the Canton of Zurich, Switzerland (2020-01336). All participants provided written informed consent before being enrolled in the study.

Data sharing: Data are still being collected for the cohort study Ciao Corona. Deidentified participant data might be available on reasonable request by email to the corresponding author at later stages of the study.

The lead authors affirm that the manuscript is an honest, accurate, and transparent account of the study being reported, no important aspects of the study have been omitted, and any discrepancies from the study as originally planned and registered have been explained.

Dissemination to participants and related patient and public communities: Results of individual tests were communicated to the participants. Overall study results were disseminated to participants and the participating schools in a lay language summary factsheet as well as the preprint of the study. Findings were disseminated in lay language in the national and local press, to the national and regional educational and public health departments, and on the website of the study (www.ciao-corona.ch).

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1 Ulyte A, Radtke T, Abela IA, et al. Variation in SARS-CoV-2 seroprevalence in school-children across districts, schools and classes.*medRxiv* 2020;2020.09.18.20191254.
2 Stringhini S, Wisniak A, Piumatti G, et al. Seroprevalence of anti-SARS-CoV-2 IgG antibodies in Geneva, Switzerland (SEROCoV-POP): a population-based study. *Lancet* 2020;396:313-9. doi:10.1016/S0140-6736(20)31304-0
3 Levin AT, Hanage WP, Owusu-Boaitey N, Cochran KB, Walsh SP, Meyerowitz-Katz G. Assessing the age specificity of infection fatality rates for COVID-19: systematic review, meta-analysis, and public policy implications. *Eur J Epidemiol* 2020;35:1123-38. doi:10.1007/s10654-020-00698-1
4 Shekerdemian LS, Mahmood NR, Wolfe KK, et al, International COVID-19 PICU Collaborative. Characteristics and outcomes of children with coronavirus disease 2019 (COVID-19) infection admitted to US and Canadian pediatric intensive care units. *JAMA Pediatr* 2020;174:868-73. doi:10.1001/jamapediatrics.2020.1948
5 Hoang A, Chorath K, Moreira A, et al. COVID-19 in 7780 pediatric patients: a systematic review. *EClinicalMedicine* 2020;24:100433. doi:10.1016/j.eclinm.2020.100433
6 Götzinger F, Santiago-García B, Noguera-Julián A, et al, ptbnet COVID-19 Study Group. COVID-19 in children and adolescents in Europe: a multinational, multicentre cohort study. *Lancet Child Adolesc Health* 2020;4:653-61. doi:10.1016/S2352-4642(20)30177-2
7 UNESCO. School closures caused by coronavirus (Covid-19). 2020. https://en.unesco.org/covid19/educationresponse (accessed 19 Dec 2020).
8 Van Lancker W, Parolin Z. COVID-19, school closures, and child poverty: a social crisis in the making. *Lancet Public Health* 2020;5:e243-4. doi:10.1016/S2468-2667(20)30084-0
9 Norman RE, Byambaa M, De R, Butchart A, Scott J, Vos T. The long-term health consequences of child physical abuse, emotional abuse, and neglect: a systematic review and meta-analysis. *PLoS Med* 2012;9:e1001349. doi:10.1371/journal.pmed.1001349
10 Christakis DA, Van Cleve W, Zimmerman FJ. Estimation of US children's educational attainment and years of life lost associated with primary school closures during the coronavirus disease 2019 pandemic. *JAMA Netw Open* 2020;3:e2028786. doi:10.1001/jamanetworkopen.2020.28786
11 Larosa E, Djuric O, Cassinadri M, et al, Reggio Emilia Covid-19 Working Group. Secondary transmission of COVID-19 in preschool and school settings in northern Italy after their reopening in September 2020: a population-based study. *Euro Surveill* 2020;25:2001911. doi:10.2807/1560-7917.ES.2020.25.49.2001911
12 Zimmerman KO, Akinboyo IC, Brookhart MA, et al, ABC Science Collaborative. Incidence and secondary transmission of SARS-CoV-2 infections in schools. *Pediatrics* 2021;e2020048090. doi:10.1542/peds.2020-048090
13 Brandal LT, Ofitserova TS, Meijerink H, et al. Minimal transmission of SARS-CoV-2 from paediatric COVID-19 cases in primary schools, Norway, August to November 2020. *Euro Surveill* 2021;26:2002011. doi:10.2807/1560-7917.ES.2020.26.1.2002011
14 Stein-Zamir C, Abramson N, Shoob H, et al. A large COVID-19 outbreak in a high school 10 days after schools' reopening, Israel, May 2020. *Euro Surveill* 2020;25:2001352. doi:10.2807/1560-7917.ES.2020.25.29.2001352
15 Szablewski CM, Chang KT, Brown MM, et al. SARS-CoV-2 transmission and infection among attendees of an overnight camp - Georgia, June 2020. *MMWR Morb Mortal Wkly Rep* 2020;69:1023-5. doi:10.15585/mmwr.mm6931e1
16 Otte im Kampe E, Lehfeld A-S, Buda S, et al. Surveillance of COVID-19 school outbreaks, Germany, March to August 2020. *Euro Surveill* 2020;25:2001645. doi:10.2807/1560-7917.ES.2020.25.38.2001645
17 Ismail SA, Saliba V, Lopez Bernal J, Ramsay ME, Ladhani SN. SARS-CoV-2 infection and transmission in educational settings: a prospective, cross-sectional analysis of infection clusters and outbreaks in England. *Lancet Infect Dis* 2021;21:344-53. doi:10.1016/S1473-3099(20)30882-3
18 Buonsenso D, De Rose C, Moroni R, Valentini P. SARS-CoV-2 infections in italian schools: preliminary findings after 1 month of school opening during the second wave of the pandemic. *Front Pediatr* 2021;8:615894. doi:10.3389/fped.2020.615894
19 Ulyte A, Radtke T, Abela IA, et al. Seroprevalence and immunity of SARS-CoV-2 infection in children and adolescents in schools in Switzerland: design for a longitudinal, school-based prospective cohort study. *Int J Public Health* 2020;65:1549-57. doi:10.1007/s00038-020-01495-z
20 West EA, Anker D, Amati R, et al, Corona Immunitas Research Group. Corona Immunitas: study protocol of a nationwide program of SARS-CoV-2 seroprevalence and seroepidemiologic studies in Switzerland. *Int J Public Health* 2020;65:1529-48. doi:10.1007/s00038-020-01494-0
21 Institute of Medical Virology IMV SARS-CoV-2 Antikörper Differenzierung (ABCORA). https://www.virology.uzh.ch/dam/jcr:0f9bbdba-e215-4e42-a877-131a04a2e175/IMVABCORASerologie.pdf (accessed 16 Dec 2020).
22 Dan JM, Mateus J, Kato Y, et al. Immunological memory to SARS-CoV-2 assessed for up to 8 months after infection. *Science* 2021;371:eabf4063.
23 Canton of Zurich. Numbers and facts on COVID-19 [Kanton Zürich. Zahlen & Fakten zu COVID-19]. https://www.zh.ch/de/gesundheit/coronavirus/zahlen-fakten-covid-19.html?keyword=covid19#/home (accessed 13 Nov 2020).
24 R Core Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. 2020. https://www.r-project.org/ (accessed 17 Dec2020).

25 Stan Development Team. RStan: the R interface to Stan. R package version 2.21.2. 2020.
26 Roser M, Ritchie H, Ortiz-Ospina E, Hasell J. Coronavirus pandemic (COVID-19). 2020. https://ourworldindata.org/coronavirus (accessed 17 Dec 2020).
27 Anno scolastico 2020/2021 e Coronavirus - DECS (DECS) - Repubblica e Cantone Ticino [School year 2020/2021 and coronavirus. Canton of Ticino]. https://www4.ti.ch/decs/anno-scolastico-20202021-e-coronavirus/anno-scolastico-20202021-e-coronavirus/ (accessed 17 Feb 2021).
28 Macartney K, Quinn HE, Pillsbury AJ, et al, NSW COVID-19 Schools Study Team. Transmission of SARS-CoV-2 in Australian educational settings: a prospective cohort study. *Lancet Child Adolesc Health* 2020;4:807-16. doi:10.1016/S2352-4642(20)30251-0
29 Haug N, Geyrhofer L, Londei A, et al. Ranking the effectiveness of worldwide COVID-19 government interventions. *Nat Hum Behav* 2020;4:1303-12. doi:10.1038/s41562-020-01009-0
30 Brauner JM, Mindermann S, Sharma M, et al. Inferring the effectiveness of government interventions against COVID-19. *Science* 2021;371:eabd9338.
31 Auger KA, Shah SS, Richardson T, et al. Association between statewide school closure and COVID-19 incidence and mortality in the US. *JAMA* 2020;324:859-70. doi:10.1001/jama.2020.14348
32 Tupper P, Colijn C. COVID-19's unfortunate events in schools: mitigating classroom clusters in the context of variable transmission. *medRxiv* 2020;2020.10.20.20216267.
33 COVID-19 Schools Infection Survey Round 1, England - Office for National Statistics. https://www.ons.gov.uk/peoplepopulationandcommunity/healthandsocialcare/conditionsanddiseases/bulletins/covid19schoolsinfectionsurveyround1england/november2020 (accessed 19 Dec 2020).
34 Vandermeulen C, Boey L, Roelants M, Duysburgh E, Desombere I. Seroprevalence of SARS-CoV-2 antibodies in school aged children in two regions with difference in prevalence of COVID-19 disease. 2020 https://www.sciensano.be/sites/default/files/limburg-validation-sars-cov2_report_20201112_final.pdf (accessed 11 Feb 2021).
35 Wölfel R, Corman VM, Guggemos W, et al. Virological assessment of hospitalized patients with COVID-2019. *Nature* 2020;581:465-9. doi:10.1038/s41586-020-2196-x
36 Okba NMA, Müller MA, Li W, et al. Severe acute respiratory syndrome coronavirus 2-specific antibody responses in coronavirus disease patients. *Emerg Infect Dis* 2020;26:1478-88. doi:10.3201/eid2607.200841
37 Suhandynata RT, Hoffman MA, Kelner MJ, McLawhon RW, Reed SL, Fitzgerald RL. Longitudinal monitoring of SARS-CoV-2 IgM and IgG seropositivity to detect COVID-19. *J Appl Lab Med* 2020;5:908-20. doi:10.1093/jalm/jfaa079
38 Lou B, Li T-D, Zheng S-F, et al. Serology characteristics of SARS-CoV-2 infection after exposure and post-symptom onset. *Eur Respir J* 2020;56:2000763. doi:10.1183/13993003.00763-2020
39 Hu B, Guo H, Zhou P, Shi Z-L. Characteristics of SARS-CoV-2 and COVID-19. *Nat Rev Microbiol* 2021;19:141-54. doi:10.1038/s41579-020-00459-7
40 Watson J, Whiting PF, Brush JE. Interpreting a covid-19 test result. *BMJ* 2020;369:m1808. doi:10.1136/bmj.m1808

Supplementary information: appendix 1