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Evolution of SARS-CoV-2 seroprevalence and clusters in school children from June 2020 to April 2021: prospective cohort study Ciao Corona

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Summary

BACKGROUND: Few studies have explored the spread of SARS-CoV-2 in schools in 2021, with the advent of variants of concern. We aimed to examine the evolution of the proportion of seropositive children at schools from June-July 2020 to March-April 2021. We also examined symptoms, under-detection of infections, potential preventive effect of face masks, and reasons for non-participation in the study.

METHODS: Children in lower (7–10 years), middle (8–13 years) and upper (12–17 years) school levels in randomly selected schools and classes in the canton of Zurich, Switzerland, were invited to participate in the prospective cohort study *Ciao Corona*. Three testing rounds were completed in June-July 2020, October-November 2020 and March-April 2021. From 5230 invited, 2974 children from 275 classes in in 55 schools participated in at least one testing round. We measured SARS-CoV-2 serology in venous blood, and parents filled in questionnaires on sociodemographic information and symptoms.

RESULTS: The proportion of children seropositive for SARS-CoV-2 increased from 1.5% (95% credible interval [CrI] 0.6–2.6%) by June-July 2020, to 6.6% (4.0–8.9%) by October-November, and to 16.4% (12.1–19.5%) by March-April 2021. By March-April 2021, children in upper school level (12.4%; 7.3–16.7%) were less likely to be seropositive than those in middle (19.5%; 14.2–24.4%) or lower school levels (16.0%; 11.0–20.4%). The ratio of PCR-diagnosed to all seropositive children changed from one to 21.7 (by June-July 2020) to one to 3.5 (by March-April 2021). Potential clusters of three or more newly seropositive children were detected in 24 of 119 (20%) classes, 17 from which could be expected by chance. Clustering was not higher than expected by chance in middle and upper school levels. Children in the upper school level, who were wearing face masks at school from November 2020, had a 5.1% (95% confidence interval 9.4% to 0.7%) lower than expected seroprevalence by March-April 2021 than those in middle school level, based on difference-in-differences analysis. Symptoms were reported by 37% of newly seropositive and 16% seronegative children. Fear of blood sampling (64%) was the most frequently reported reason for non-participation.

CONCLUSIONS: Although the proportion of seropositive children increased from 1.5% in June-July 2020 to 16.4% in March-April 2021, few infections were likely associated with potential spread within schools. In March-April 2021, significant clustering of seropositive children within classes was observed only in the lower school level.

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Introduction

In response to the high incidence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infections and emerging variants of concern in the autumn and winter of 2020/2021 [1] attendance of schools has been disrupted in many countries. Half of the countries worldwide and in Europe interrupted physical attendance of schools for at least 30 weeks from March 2020 to June 2021 [2], but this was only for 7 weeks in Switzerland. Nevertheless, by November 2020, only minimal clustering of seropositive children was observed in the canton of Zurich, Switzerland, after 2–3 months of school attendance including 2–6 weeks of high community incidence [3]. Other studies showed that the spread of SARS-CoV-2 infection within schools was not larger than in the surrounding community in 2020, when variants of concern such as Alpha (B.1.1.7) and Delta (B.1.617.2) were not prevalent in most of the countries, and the rates of secondary attack and outbreaks low [4–7]. In contrast, the expected damage caused by school closures could result in worse mental health in children, reduced learning and subsequent income losses, and amplify

gender and socioeconomic inequalities within and between countries [8].

However, only scarce information is available yet on SARS-CoV-2 infection in schools since December 2020, when alpha and subsequently delta variants of SARS-CoV-2 started dominating in Europe and other countries. In Switzerland, approximately 80% of SARS-CoV-2 infections were due to the alpha variant in March 2021 (see appendix 1) [9]. Children below the age of 12 will be the last group to be offered vaccination. Preventive measures in schools will likely need to be adjusted as new variants spread while more people, especially vulnerable populations, become vaccinated. Thus, monitoring the evolution of seroprevalence and clustering of infections within schools remains relevant.

The Ciao Corona study uniquely examines SARS-CoV-2 seroprevalence on the class, school, and district level. The objectives of this study were to assess longitudinally, with measurements in June-July and October-November 2020, and March-April 2021, the proportion of seropositive children and adolescents within school levels, cantonal districts and the region (canton of Zurich), the association of seropositivity with reported symptoms, and the frequency and evolution of clustering of seropositive children within classes in schools. In addition, we examined the potential effect of face masks on the evolution of seroprevalence in upper school level children and reasons for participation and non-participation in this cohort study, in order to address potential participation bias.

Materials and methods

The protocol [10] and previous results of this longitudinal study [3, 11] are reported elsewhere. This study is part of the nationally coordinated research network Corona Immunitas [12]. The study follows a cohort of randomly selected schools and classes in the canton of Zurich, Switzerland. The canton has a population of 1.5 million linguistically and ethnically diverse inhabitants in both rural and urban settings, and comprises 18% of the Swiss population [13].

During the COVID-19 pandemic in Switzerland, physical attendance of schools was interrupted only in March-May 2020. Preventive measures, such as distancing and reduced mixing of classes, were implemented with some variation between schools. All schools required ill children to stay home unless with very mild symptoms. Adults at school were required to wear masks from October 2020, secondary school children from November 2020, and primary school children in the middle school level grades from late January 2021.

School-specific contact tracing was implemented in school year 2020/2021. Testing and quarantine recommendations depended on the specific situation. As a general rule, the whole class was quarantined when two or more infected children were detected in the class simultaneously. If children were wearing masks, only close contacts were quarantined. Daily incidence of diagnosed SARS-CoV-2 cases between October 2020 and April 2021 in the canton of Zurich and Switzerland and the proportion of variants of concern is shown in appendix 1.

Ethics 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.

Population

As described previously [3, 10, 11], in May-June 2020 we randomly selected primary schools in the canton of Zurich and matched the geographically closest secondary school. The number of schools invited in the 12 districts of the canton was proportional to population size.

We randomly selected classes within participating schools, stratified by school level: grades 1 to 2 in lower level (attended by 6-to 9-year-old-children), grades 4 to 5 in middle level (9- to 13-year-old children) and grades 7 to 8 in upper school level (12- to 16-year-old-children); grades were selected from the eligible grades in the school randomly. We aimed to invite at least three classes or at least 40 children in each invited school level of a school (i.e., ensuring that at least 40 children were invited if fewer than three classes were eligible within smaller schools, and a sufficient number of classes so that a total of at least 40 children are invited in schools with small classes). The random invitation of schools and classes ensured that the invited population is approximately representative for the school-aged children within the districts of the canton of Zurich.

Eligible children and adolescents (hereafter referred to as children) of the selected classes could participate in any of the testing rounds. Major exclusion criterion was suspected or confirmed SARS-CoV-2 infection during the testing (precluding child's attendance of the testing at school).

Serological testing and questionnaire information

Venous blood samples were collected at schools from 16 June to 9 July 2020 (T1), 26 October to 19 November 2020 (T2); and 15 March to 16 April 2021 (T3). Blood samples were analysed with the ABCORA binding assay of the Institute of Medical Virology (IMV) of the University of Zurich, which is based on Luminex technology [14]. Binary results of the ABCORA 2.3 algorithm showed 98.2% sensitivity and 99.4% specificity [14]. Figure 1 shows the flowchart of participants, and appendix 2 includes counts of children with serological results at each testing round, and further details of the test.

We defined a composite (cumulative) serological outcome as the proportion of children who tested seropositive in at least one of the testing rounds (ever-seropositive children). The composite outcome allowed the proportion of all children who had SARS-CoV-2 antibodies at any time by a specific testing to be estimated, and was measured for T1, T2 and T3 separately (see detailed definitions of outcomes T1, T12, and T123 in appendix 2). To examine the clustering of new cases within tested classes, we defined the outcome of newly seropositive children at T3 (seropositive at T3 but not in the previous testing rounds), in a way similar to the approach in the previous publication [3].

Parents of participants filled in online questionnaires on sociodemographic and health information on the child and household. In March-April 2021, parents of eligible non-

participating children were invited to complete an anonymous questionnaire on the reasons for non-participation (details in appendix 3). After T3 testing, we interviewed the principals of schools in which at least two classes with new clusters of seropositive children were detected (details in appendix 4).

We obtained official statistics of SARS-CoV-2 infections in the canton of Zurich [15] in order to calculate the cumulative incidence of diagnosed SARS-CoV-2 cases by T1, T2 and T3 testing, in children aged 7–17 years, and compare with the proportion of children who were seropositive by 30 June 2020 (median time point of T1), 6 November 2020 (T2), and 29 March 2021 (T3).

Statistical analysis

We report key characteristics of participants (age, school level, sex) as well as their reported symptoms summarised as median (range) or count (%).

To estimate the proportion of ever-seropositive children by T1, T2 and T3 testing, we employed Bayesian logistic regression [16], which was adjusted for participants' grade at school, sex and geographic district of the school, and contained random effects for school levels (lower, middle and upper). The Bayesian approach permitted adjustment for the accuracy of serological test and the hierarchical structure of the cohort (individual, school and district levels). To compute estimates representative for the canton of Zurich even in the case of differential participation rates of children within districts or grades, we post-stratified our results according to the total population size at the school level and geographic district. We compared the estimates across school levels and districts.

Masks were introduced for upper school level children at the end of October 2020. Masks were also obligatory for middle school level children from late January 2021; however, the majority of SARS-CoV-2 infections between T2 and T3 happened earlier, in November 2020 to January 2021. We employed difference-in-differences analysis (DD) [17] to examine whether upper school level had a different from expected seroprevalence at T3, in regard to the previous trend at T1-T2 and in comparison with middle and lower school levels. Briefly, assuming that the outcome (seroprevalence) would develop in parallel over time in the compared groups (different school levels), DD allows potential deviation from the parallel trend in the intervention group to be identified . We modelled DD and examined the parallel trends assumption with a linear probability model [18]. The model included children who were tested three times (n = 1965). The binary outcome was ever-testing-positive by T1, T2 and T3. The models controlled for the measurement time point (T1, T2 or T3) and class of the child (thus, implicitly also for district and school grade). Robust cluster-corrected errors at the child level were used, to adjust for the heteroscedasticity of residuals and autocorrelation of outcomes at the three times points in the same child [19]. We applied the model to compare upper school level with lower, middle, and combined lower and middle school levels.

We compared the cumulative incidence of SARS-CoV-2 cases confirmed with reverse transcriptase polymerase chain-reaction testing (RT-PCR) with the proportion of ever-seropositive children, in order to estimate the ratio of undiagnosed to all SARS-CoV-2 cases.

Data analysis was performed with R version 4.0.3 [20]. Bayesian hierarchical modelling was performed using the R package rstan [21].

Analysis of potential clusters

We examined potential clusters (at least three newly T3 seropositive children) in classes in which at least five children were eligible and at least 50% of the children were tested. We compared the observed distribution of clusters with the distribution expected if SARS-CoV-2 infections were distributed among children across classes independently (randomly), as described previously [3]. In a simulation, we created 2500 hypothetical populations of children, corresponding to the study population in terms of the observed overall seroprevalence, number of classes and tested children within them. We further ran the simulation separately for classes within different school levels separately. By comparing the expected and the observed distributions, we could estimate whether the observed number of classes with clusters is compatible with the hypothesis of no association of SARS-CoV-2 infections within classes. We further compared the results of the simulation with the information from school principal interviews.

Results

In total, 2487 children from 275 classes in 55 schools in the canton of Zurich were tested between 15 March and 16 April 2021 (T3). Of these, 2237 (90%) participated in October-November 2020, and 2176 (87%) in June-July 2020. Retention rate from T1 to T2 was 84% (2176/2601) and 88% (2237/2552) from T2 to T3. The flowchart of participants is shown in figure 1.

Serological results were available at T3 for 768 children in the lower school level (median age 8, age range 7–10 years), 845 children in the middle school level (median age 12, age range 8–13 years) and 837 children in the upper

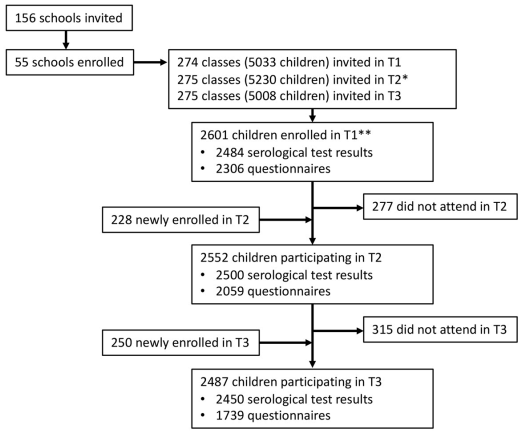


Figure 1: Flowchart of study participants. T1 – testing in June-July 2020, T2 – testing in October-November 2020, T3 – testing in March-April 2021. *Newly enrolled* children were not tested in the previous round; *did not attend* means they did not attend the subsequent round. * Some classes were split or rearranged after the summer break. ** 16 of these children were enrolled from late August to early September 2020 (10 serological results, 16 questionnaires available).

school level (median age 14, age range 12–17 years). Of these, 1279 children reported female, 1165 male and 6 other gender. The median participation rate within classes was 50% (interquartile range 35–63%; median 45% in lower, 54% in middle, and 50% in upper school levels).

Serological results

Table 1 presents the distribution of serological results at T1, T2 and T3 of children who were tested in all three testing rounds. Among children with serological results in the respective two rounds of testing, 33/52 (63%) of those seropositive at T1 were seropositive after 4 months at T2, 32/46 (70%) after 9 months at T3, and 101/115 (88%) of those seropositive at T2 were seropositive after 5 months at T3 (appendix 2).

The proportion of ever seropositive children was 1.5% (95% credible interval [CrI] 0.6–2.6%) at T1, 6.6% (95% CrI 4.0–8.9%) at T2 and 16.4% (95% CrI 12.1–19.5%) at T3 (fig. 2). The proportion of ever seropositive children at T3 was 16.0% (11.0–20.4%) in lower, 19.5% (14.2–24.4%) in middle, and 12.4% (7.3–16.7%) in upper school levels. Seroprevalence was not statistically significantly different between lower and middle ($p = 0.26$) or lower and upper levels ($p = 0.18$), but different between middle and upper school levels ($p = 0.02$). The proportion of ever-seropositive children ranged from 9.2% to 25.7% in the districts of Zurich at T3. The proportion did not differ between boys and girls, although the estimates were slightly higher for boys (T1: 1.8% vs 1.1%; T2: 7.1% vs 6.1%; T3: 17.2% vs 15.6%).

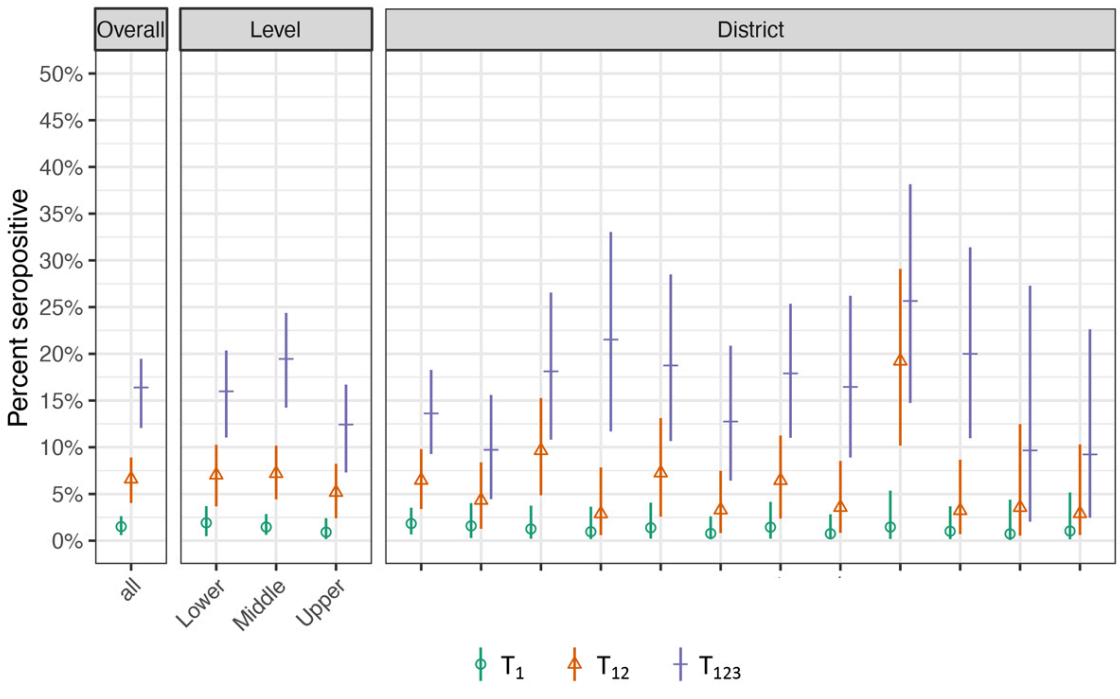
In the difference-in-differences analysis, the parallel trends assumption between T1 and T2 was valid based on visual inspection (fig. 3A) and formal examination with the linear probability models (fig. 3B). The proportion of ever-seropositive children in the upper school level was lower than expected at T3 in comparison with lower and middle

Table 1:
Longitudinal serological results of children tested at all three testing rounds (n = 1965).

T1 result		T2 result		T3 result	
Positive	44	Positive	29	Positive	26
				Negative	3
		Negative	15	Positive	5
				Negative	10
Negative	1921	Positive	73	Positive	65
				Negative	8
		Negative	1848	Positive	209
				Negative	1639

T1 – 16 June to 9 July 2020; T2 – 26 October to 19 November 2020; T3 – 15 March to 16 April 2021. The total number of serological results at each of T1, T2, and T3 was 1965. The table depicts all possible combinations of test results at the three testing rounds. For example, from 44 children who tested positive at T1, 29 tested positive at T2 (from them – 26 tested positive and 3 tested negative at T3) and 15 tested negative at T2 (from them – 5 positive and 10 negative at T3).

Figure 2: Proportion of ever-seropositive children in June-July 2020 (T1), October-November 2020 (T12) and March-April 2021 (T123). T1 – proportion of children testing seropositive by June-July 2020, T12 – by October-November 2020, T123 – by March-April 2021. Overall and school level specific estimates (lower school level: grades 1–2, children aged 6–10 years,; middle school level: grades 4–5, children aged 9–13 years; upper school level: grades 7–8, children aged 12–16 years; grades and age range are reported for time point of T1), and district specific estimates for the canton of Zurich, Switzerland. Districts are ranked by their population size, from largest to smallest.



school levels (absolute difference of 3.2%, 95% confidence interval [CI] –7.0% to 0.6%; p = 0.098, relative difference of 19%). The proportion was lower by 5.1% (95% CI –9.4% to –0.7%, p = 0.022, relative difference of 27%) when compared only to the middle school level, and by 0.8% (95% CI –5.4% to 3.8%, p = 0.718, relative difference of 6%) when compared only to the lower school level.

The ratio of diagnosed to seropositive children was 1 to 21.7 by June-July 2020, 1 to 5.8 by October-November 2020, and 1 to 3.5 by March-April 2021.

Symptoms

Symptoms between T2 and T3 were reported in 67/182 (37%) of newly seropositive and 235/1443 (16%) of seronegative children (fig. 4). Symptoms most commonly reported by newly seropositive children were fatigue (30/182, 17%), sore throat (30/182, 17%), headache (27/182, 15%), fever (26/182, 14% for fever ≥38°C and 25/182, 14% for subjective fever) and runny or congested nose (22/182, 12%). Fatigue, sore throat, headache, fever, stomach ache, muscle or joint pain, and loss of smell or taste were reported more frequently by seropositive than seronegative children. Similar proportions of seropositive children reported symptoms in lower (35%, 22/63), middle (40%, 32/81) and upper (37%, 14/38) school levels. None of the participating children reported hospitalisation between T2 and T3 testing.

Figure 3: Difference-in-differences model of the change in ever-seroprevalence between T2 and T3 in upper school level. A – Raw proportion of children, included in the models, ever testing seropositive by T1, T2 and T3 time points B – Difference-in-differences estimates of linear probability models, with T2 as the reference. The parallel trends assumption is valid, as the estimates at T1 are not different from the T2 reference. Average treatment (effect) on the treated denotes the change in seroprevalence, potentially attributable to the effect of mask wearing by upper school level children.

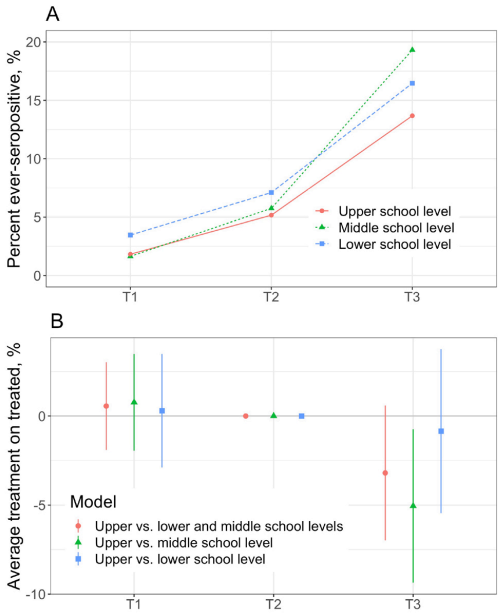
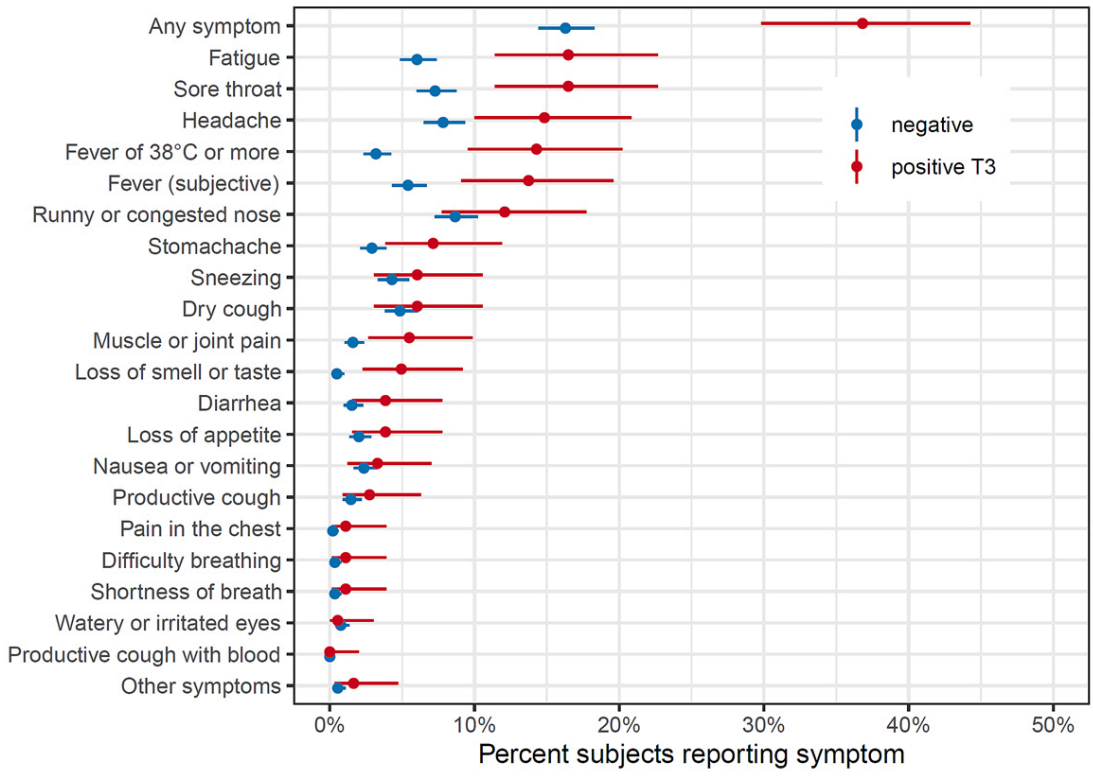


Figure 4: Symptoms reported between October-November 2020 and March-April 2021 in children who were seronegative and newly seropositive at T3 (March-April 2021).



Cluster analysis

At least one child newly seropositive at T3 was detected in 53 of 55 schools and 151 of 275 (55%) classes (76 of 119 [64%] classes with high participation rate). At least one child who was ever-seropositive at T3 was detected in all 55 schools and 184 of 275 (67%) classes (or in 95 of 119 [80%] classes with high participation rate). Figure 5 shows the distribution of seropositive children within classes with high participation rate.

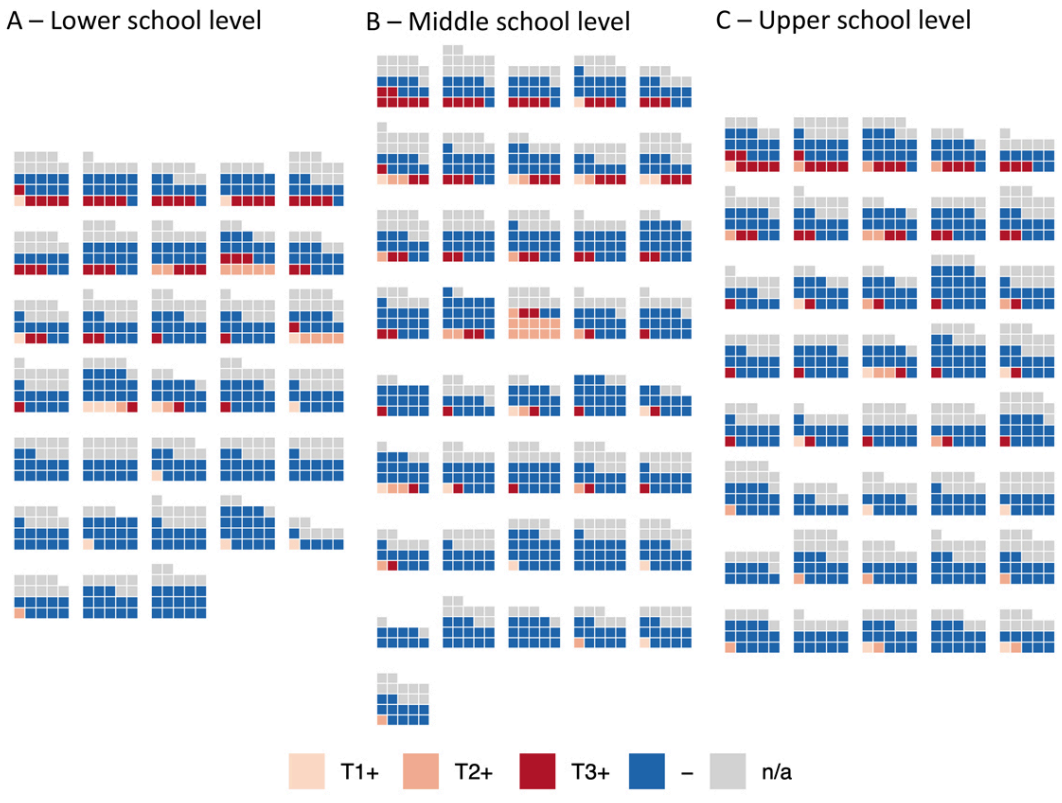
At T3, 39 of 275 (14%) classes had potential clusters of newly seropositive children. Twenty-four of 119 (20%) classes with high participation had clusters: 9 of 33 (27%) in the lower school level, 10 of 41 (24%) in the middle, and 5 of 45 (11%) in upper school level. Based on the interviews with school principals, intra-class transmission could have happened in 12 classes with potential clusters (63%), was improbable in 7 (37%), and could not be assessed because of insufficient information in 6 classes. The majority of RT-PCR-diagnosed infections and exposure resulting in quarantine of children originated from children's households. Detailed results of the interviews are presented in appendix 4.

Assuming a uniform 10.9% rate of newly seropositive children among those not previously tested positive (158/1422 within classes with high participation), we would expect to observe a median of 17 (95% CrI 12–22) clusters within 119 classes with high participation. In comparison, we observed 24/119 (20%) such classes, making the hypothesis of completely independent distribution of seropositive children unlikely ($p = 0.0052$). The simulated distributions are shown in appendix 5. The expected and observed distributions of clusters were different in the lower school level (median of 5/33 expected vs 9/33 observed; $p = 0.001$) but not in middle (9/41 expected vs 10/41 observed; $p = 0.27$) and upper school levels (4/45 expected vs 5/45 observed; $p = 0.29$).

Reasons for participation and non-participation

On the day of testing at T3, 10 of 5517 (0.2%) children in the invited classes were not at school because of diagnosed or suspected SARS-CoV-2 infection, 47 (0.9%) because of quarantine, and 164 (3.0%) for other reasons. Parents of 712 of 2659 (27%) children provided reasons for non-participation; child's fear of the blood sampling was reported the most often (358/564, 64% of children; 189/276, 69% of girls and 158/259, 61% of boys), especially in lower school levels (191/264, 72%). Among the participants, 28% (374/1331) reported participating for personal and 90% (1192/1331) for societal reasons. Detailed results are provided in appendix 3.

Figure 5: Distribution of children who were seropositive at T1, T2 and T3 in classes with high participation rate at T3. Each block of squares represents a class. High participation rate means that at least 5 children were eligible and at least 50% children were tested in the class. T1+ (light pink) – children who tested seropositive at T1 (June-July 2020), T2+ (middle orange) – children who tested seropositive at T2 (October-November 2020) but not T1, T3+ (dark red) – children who tested seropositive at T3 (March-April 2021) but not at T1 or T2, - (dark blue) – children who tested seronegative at T3 and were not test seropositive previously, n/a (grey) – children without a serological result at T3.



Discussion

In this cohort study of more than 2500 children in 55 schools, the proportion of children seropositive for SARS-CoV-2 increased from 1.5% in June-July 2020 to 6.6% in October-November and to 16.4% in March-April 2021. In March-April 2021, seroprevalence was lower in the upper (12.4%) than in middle (19.5%) or lower (16.0%) school levels. Although potential clusters of seropositive children were detected in approximately 20% of classes, the majority could be explained by infections not associated within a class, particularly in the middle and upper school levels. Seropositive children reported a history of acute symptoms more frequently than seronegative children, although 63% did not report any symptoms. Eighty-eight percent of seropositive children retained their antibodies for at least 5 months.

The canton of Zurich experienced an early second wave of the SARS-CoV-2 pandemic in 2020. Daily incidence of RT-PCR cases peaked at 88 in 100,000 inhabitants in late October 2020 [15], comparable to the peaks of 75 and 90 new cases in 100,000 inhabitants in the US and UK in early January 2021 [1]. Our study suggests an increasingly higher proportion of children diagnosed with RT-PCR, reflecting the revised indications for testing in children. Initially restrictive, the indications for testing children over 12 years matched those for adults in September 2020, and extended to children over 6 years from March 2021 [22]. However, the persisting high number of undiagnosed infections and thus the total spread of SARS-CoV-2 in children should be considered while planning preventive measures, such as masking in indoor spaces.

The increase of seropositive children by March-April 2021 was the smallest (and potential clusters least frequent) in upper school level. In contrast to our findings, SARS-CoV-2 has been observed to spread more among older children and adolescents [23, 24], partly explained by different patterns of contacts [25, 26] and susceptibility [27]. SARS-CoV-2 spread and secondary attack rates were associated with children's age in a school contact tracing study in late 2020 [28]. In Switzerland, adolescents from grade 7 (approximately age 14) have been required to wear masks at school since November 2020. Potentially, consistent masking during the second wave of SARS-CoV-2 infections in November 2020 to January 2021, as well as different distancing behaviour of teenagers towards their parents compared with young children, could have contributed to fewer infections. The preventive effect of masking is supported by the results of the difference-in-differences analysis. In comparison with the middle school level, where masks were introduced 3 months later, the proportion of children ever testing seropositive in the upper school levels was lower by 5.1% (relative reduction of 27%) than could be expected if seroprevalence developed in the same trend as in the middle school level between T2 and T3. However, no significant difference was found in the development of seroprevalence between T2 and T3 between upper and lower school levels, potentially, as the age (and thus, susceptibility and behavioural patterns) are more different. Indeed, school contact tracing studies showing the positive association of SARS-CoV-2 infections with age were often conducted in countries with all school-aged children wearing masks (e.g., Italy [29], Catalonia [28, 30]), or no consistent mandate of masking for children at school (e.g., UK [31]). Although there are randomised studies on the effectiveness of masking to prevent SARS-CoV-2 spread for adult populations [32] and correlation studies of masking and SARS-CoV-2 infections at schools [33, 34], our study was unique in studying the effect of masking on SARS-CoV-2 infections in school-aged children.

Our results suggest that only a small part of potential clusters of seropositive children within classes were likely associated with intraclass transmission in 2020–2021. Clustering in 2021 was the most prevalent in lower school level, where masks for children were not mandated at any time point. Based on interviews with school principals, at least 33% of the clusters were unlikely to be due to intraclass transmission. These findings are supported by prospective studies of school contact tracing in late 2020, showing that the majority of SARS-CoV-2 infections identified in a school setting were not associated with further secondary cases within the school [29]. Furthermore, the secondary attack rates within families with a child index case were lower during school time as compared with the summer holiday [30].

In contrast to previous measurements in summer and autumn of 2020, three symptoms were reported more frequently in seropositive than seronegative children. The retrospective recall of symptoms could have been influenced by the knowledge of and increased attention to the symptoms, population incidence, and personal diagnosis of SARS-CoV-2. The influence of variants of concern on the symptoms of SARS-CoV-2 infection in children cannot be ruled out. However, the majority of symptom episodes were reported in December 2020 to February 2021 and variants of concern became predominant in Switzerland from March 2021 (see appendix 1). Although previously, 20–30% of child cases were estimated to be asymptomatic [35], a recent meta-analysis found almost half of SARS-CoV-2 infections in children, significantly more than in adults, to be asymptomatic [37].

The predominant reason for non-participation in the study was fear of blood sampling (not likely to be associated with particularly biased selection into the study), followed by lack of interest in participating in a research study. The non-participation rate was higher than the attrition rate (50% vs 16%), meaning that once enrolled, few children dropped out.

Most of the studies of SARS-CoV-2 spread in schools rely on contact tracing data [5, 6]. At the start of the 2020/2021 school year, such studies, a large online survey in the US [34], and the few available seroprevalence studies of school children [3, 38] pointed to the spread being low in schools with implemented protective measures. Prevalence of acute SARS-CoV-2 infections in school children and the general population tend to correlate [7, 11, 39]. Less evidence exists about how SARS-CoV-2 spread within schools might change owing to vaccination of adults and variants of concern with higher infectiousness [36], or in the case of implemented PCR-based screening of school children, which could substantially increase the proportion of diagnosed SARS-CoV-2 infections.

Strengths and limitations

The Ciao Corona cohort study is a population-based cohort of children within randomly selected schools and classes, with repeated measurements of SARS-CoV-2 serology and a high retention rate. Ascertainment of SARS-CoV-2 infections via serological testing means that the asymptomatic, previously not diagnosed children are also detected. It captures the whole spectrum of SARS-CoV-2 infections in children, which is important as under-detection remains significant.

The study has limitations. First, the exact timing of infection could not be ascertained. We could examine associations of infections within a class only indirectly (e.g., simulation study of distribution of clusters) or retrospectively (e.g., interviews with school principals). Second, although we used a highly accurate serological test and adjusted for accuracy in the Bayesian models, false positive and negative results cannot be avoided on an individual level. Low prevalence at T1 and T2 resulted in relatively lower positive predictive value of the serological test (approximately 0.75 at T1 and 0.90 at T2); however, it reached 0.97 at T3. Retention was slightly lower between T1 and T2 than between T2 and T3, potentially as more false positives were expected among T1 participants. Third, although 50% participation rate in our study is rather high for a study with venous blood sampling in children [40], it could still cause selection bias.

Conclusion

In Switzerland, an increase of SARS-CoV-2 seroprevalence in school children from 1.5% in June-July 2020 to 16.4% in March-April 2020 was accompanied by an increase of potential clusters of seropositive children in classes. Despite schools remaining open since May 2020, the majority of clusters in classes could be explained by unrelated, independent infections, likely stemming from household or community transmission. The increase in seroprevalence and clustering was lower in the upper school level, where masks were introduced in November 2020. Preventive measures in schools and improved detection of infections possibly contributed to mitigate the spread of SARS-CoV-2 within schools.

Data sharing statement

Data is still being collected for the longitudinal cohort study Ciao Corona. Upon study completion in 2022, de-identified and potentially aggregated participant data, together with required data dictionaries, will be available on reasonable request by email to the corresponding author. The purpose and methods of data analysis will be evaluated by the study team first to ensure that it complies with the ethics approval.

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Contributors: SK and MAP initiated the project and preliminary design, with support of JF. SK, MAP, CB, TR and AU developed the design and methodology, with contribution of SRH. SK, AU, TR, PA recruited study participants, collected and managed the data. SRH performed statistical analysis. AT and IA 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, AU, TR and SRH had access to and verified all underlying data. 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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Competing interests

All authors have completed and submitted the International Committee of Medical Journal Editors form for disclosure of potential conflicts of interest. No potential conflicts of interest was disclosed..

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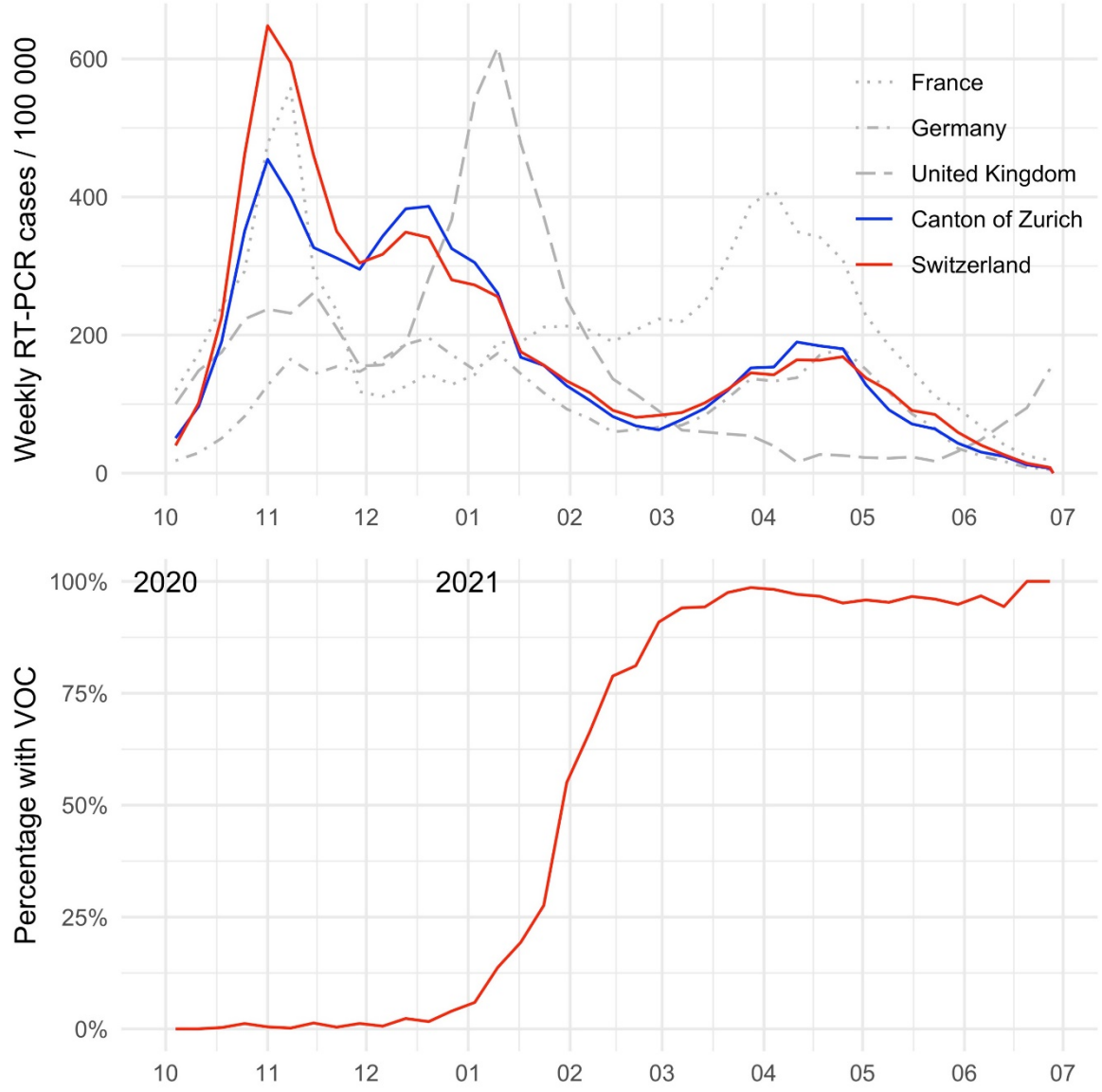
Appendix 1

Weekly incidence of SARS-CoV-2 detected cases and the proportion of the variants of concern (VOC) among them in September 2020 – April 2021



Percentage with VOC was measured for Switzerland, based on a representative sample of approximately 2000 positive samples per week.
Data source: Swiss Federal Office of Public Health, https://opendata.swiss/de/dataset/covid-19-schweiz (accessed July 2, 2021), Our World In Data, https://github.com/owid/covid-19-data/tree/master/public/data (accessed July 15, 2021)

Appendix 2

Combinations of longitudinal serological results, at three testing time points, and serological outcomes

Blood samples were analysed with ABCORA binding assay of the Institute of Medical Virology (IMV) of the University of Zurich, which is based on Luminex technology. The test analyses immunoglobulins G (IgG), M (IgM) and A (IgA) against four SARS-CoV-2 targets (receptor binding domain (RBD), spike proteins S1 and S2, and the nucleocapsid protein (N), as well as S1 protein of human coronavirus HKU1 (HCoV-HKU1)), yielding 15 different parameters. The primary serological outcome was the binary result of ABCORA 2.3 algorithm, which showed 98.2% sensitivity and 99.4% specificity (see Abela IA, Pasin C, Schwarzmüller M, et al. Multifactorial SARS-CoV-2 seroprofiling dissects interdependencies with human coronaviruses and predicts neutralization activity. medRxiv. April 2021:2021.04.21.21255410. doi:10.1101/2021.04.21.21255410)

Serology test results				**Serological outcomes**		
T1	**T2**	**T3**	**N**	**T_1**	**T_{12}**	**T_{123}**
+	+	+	26	+	+	+
+	+	-	3	+	+	+
+	+	NA	4	+	+	+
+	-	+	5	+	+	+
+	-	-	10	+	+	+
+	-	NA	4	+	+	+
+	NA	+	1	+	+	+
+	NA	-	1	+	+	+
+	NA	NA	2	+	+	+
-	+	+	65	-	+	+
-	+	-	8	-	+	+
-	+	NA	12	-	+	+
NA	+	+	10		+	+
NA	+	-	3		+	+
NA	+	NA	7		+	+
-	-	+	209	-	-	+
NA	-	+	22		-	+
-	NA	+	27	-		+
NA	NA	+	28			+
-	-	-	1639	-	-	-
NA	-	-	179		-	-

-	NA	-	89	-		-
NA	NA	-	129			-
-	-	NA	225	-	-	
NA	-	NA	69		-	
-	NA	NA	154	-		
NA	NA	NA	47			
Number of positive results (red)				56	161	447
Total number included in the analysis of this outcome (coloured)				2484	2504	2483
Raw proportion of seropositive results				2.3%	6.4%	18.0%

Results in the coloured cells are included in the analysis of the specific outcome (column). T_{12} and T_{123} outcomes are binary (red – ever-seropositive and blue – seronegative), but the seropositive can be further considered by the time they first tested seropositive (different hues of red for T1, T2, and T3). For T_{12} and T_{123} outcomes all negative results of the current relevant testing and positive results of current and previous testing rounds are included. The colours of the cells correspond to the colours used in Figure 4 of the manuscript (light orange – newly seropositive at T1, middle red – newly seropositive at T2, dark red – newly seropositive at T3, blue – negative).

Appendix 3

Detailed results of survey for participants and non-participants on reasons for participation and non-participation

Methods

The first two testing rounds of the prospective cohort study Ciao Corona took place in June-July 2020 (T1) and in October-November 2020 (T2). Testing rounds included blood sampling in participating children and adolescents aged 6-16 years and questionnaires completed by their parents. From conception of the study to the start of the recruitment of schools and children, we had a tight two months' window. The details of the study design are provided in the study protocol 1.

During the third round of testing between March 15 – April 16, 2021 (T3) parents of participants of the Ciao Corona study responded to an open question about reasons of participation, integrated in the regular follow-up questionnaire. Likewise, we assessed reasons for non-participation in the study by distributing a questionnaire to non-participating children and adolescents in the classes invited to participate in Ciao Corona study during T3. In the following sections we report findings from these two separate assessments to document and interpret reasons for participation (Section 1) and non-participation (Section 2) in our study.

1. Survey among participants of the Ciao Corona study – Reasons for participation

At the time of the third testing round in March 15 – April 16, 2021 (T3), we distributed a questionnaire that contained an open question about reasons for participation in the *Ciao Corona* study: "Why did you and your child decide to participate in the Ciao Corona study? Please give the most important reasons." Two researchers (PA, SK) independently categorized the answers into categories related to "support of society and/or research", "personal/family reasons", or both. Agreement for discordant ratings was obtained by discussion.

Results

Overall, parents of 1356 from 2978 children participating in any of the testing rounds (46%) responded to the open question about reasons for participation in the study, of which 1331 (98%) were categorizable. As documented in Figure S1, the majority of survey respondents stated that they wanted to contribute to a better understanding of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic and help society to overcome the pandemic and/or support research (n=1192, 90%). 374 (28 %) expressed personal or family interests to learn if they had SARS-CoV-2 antibodies, but only in a minority (n=139, 10%) reported this as the only reason.

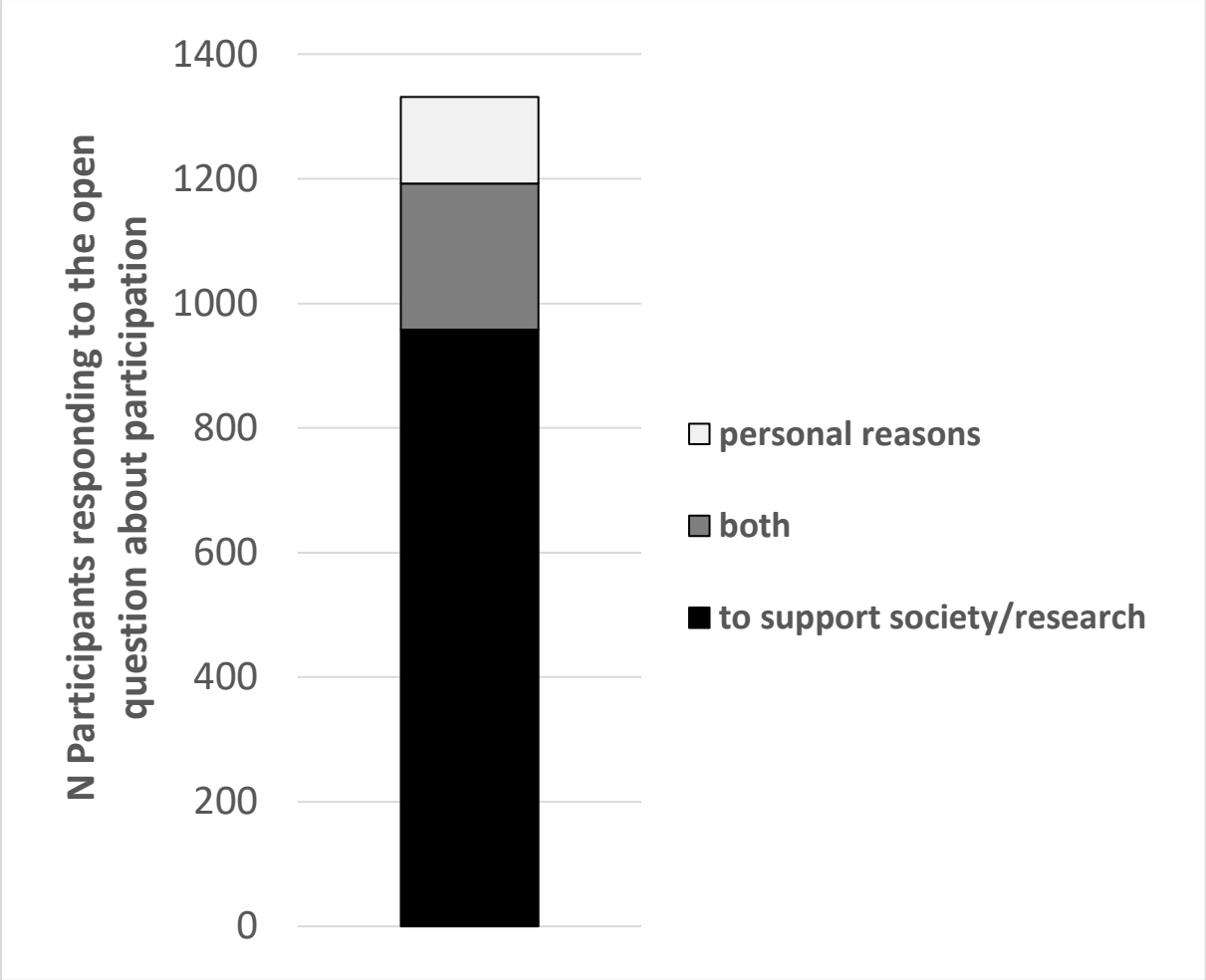


Figure 1S Reasons for participation in the Ciao Corona study among 1331 (45% of all) participants assessed at the time of the third round of testing in March/April 2021. Individuals who provided a categorizable comment to the open question why they took part in the study are included in this analysis.

Examples of individual comments are provided in the table below:

Society and/or research related reasons
• "Without studies on Corona, there is no basis for fighting the virus" • "We want to support research" • "Because it is important that as many as possible participate so that the study is representative" • "We think it is important to explore the role of children in the Corona pandemic" • "That the virus will go away quickly" • "The more data and studies we have, the better we will come out of this situation and the better policy decisions will be supported" • "I think the study makes sense. My son asked me. I am convinced that we make better progress with facts than with assumptions" • "That we can contribute to scientific research on the virus, which hopefully will help to find a solution in the near future" • "Important for the general public" • "We wanted to help" • "To use all channels together to fight the pandemic. To know if we have gone through the disease unnoticed"
Personal/family reasons
• "Personal curiosity about the development of the situation. The promised goodies were also quite motivating for my child" • "My daughter wanted to take part" • "To protect the grandparents, and because this study is important" • "That we can get tested for free" • "Because there is a gift!"

Interpretation of individual reasons for participation in *Ciao Corona*

90% of families reported an "altruistic reason" of participation in the Ciao Corona study, for example, the wish to support research to learn more about the novel coronavirus (SARS-CoV-2) or to help society to overcome the pandemic. Participation because of personal reasons and benefit, such as getting the individual SARS-CoV-2 antibody result, was not a dominant reason.

Data on participation rates for school-based interventions and prevention programmes are generally scarce and often not reported in the original studies.2 A literature review including 481 school-based studies revealed that only 11.5% of studies reported both consent procedures and participation rates. In studies using active consent procedures by parents, like in the Ciao Corona study, the mean participation rate was 65.5% (range: 11–100%), but most of these studies focused on questionnaires or lifestyle changes and did not include blood sampling.2

We achieved a comparable participation rate (of about 50%) of children and adolescents to that of school-based intervention study including blood sampling, reported by Group et al.3 However, this large study had a much higher attrition rate (defined as the proportion of enrolled participants not attending the last follow-up) than our study (30% versus 16%). This randomised controlled trial in school-aged children included only grade 6 children (on average older than in our study) predominantly from deprived populations. Study investigators paid 50-60 $ for children and 35$ for parents as an incentive for their participation in each of the two health assessments rounds over 2 years. Recruitment and retention rates reported in other school-based studies were much lower than in our study, despite the fact that financial incentives to schools and parents and children were provided.4

School-based studies benefit from being conducted in a location familiar to the child and with precious peer support, which may mitigate mistrust in research and reduce barriers of research in hospital settings.5-7 On the other hand, they are complex in many other respects (e.g., convincing school principals, language barriers, additional time investment for school staff, and potential conflicts with compulsory school lessons). Many hurdles need to be overcome by a whole chain of agreements with cantonal authorities, ethical committees, school authorities, individual school principals and teachers, children and families – until the written parental consent is obtained. Despite the urgency of setting-up our study within two months, the team invested substantially in communicating with school principals, preparing multi-facetted study materials including a website (www.ciao-corona.ch) with child-friendly pictures and videoclips explaining the testing procedures to children and their parents. Further, we organised online meetings for school personnel and parents to explain the rationale of the study, goals, testing procedures, and offered time to discuss individual questions. We also gave children, parents and school staff the possibility to get into contact with us any time by telephone hotline and email.

It is important to highlight that retention of study participants remained very high (89 % at T2, 84% at T3) over the course of the study, including three testing phases over 10 months. The course of the SARS-CoV-2 pandemic itself with high infection rates at the community level, the report of individual and school test results to study participants after each testing phase, involvement of various stakeholders (e.g., school principals, department of education of the canton of Zurich), and the investment in different communication channels (e.g., study website, social media). Also, the frequent media presence, often in collaboration with the cantonal school authorities, may have contributed to the high participation rate in the Ciao Corona study.

2. Survey among non-participants of the Ciao Corona study – Reasons for non-participation

During the third testing round at schools (T3), we distributed an anonymous paper questionnaire to children of the invited classes that did not participate in the T3 Ciao Corona assessment to elucidate reasons for non-participation. Parents of non-participating children were given the opportunity to either complete the questionnaire online (via a public survey link using REDCap, Research Electronic Data Capture, Vanderbilt University, US), or on paper (to be sent back with a pre-paid mailing envelope).

The questionnaire was available in 10 languages (German, English, French, Italian, Spanish, Portuguese, Tamil, Turkey, Croatian, and Albanian) and contained six questions: 1) sex (male, female, prefer not to say), 2) name of the school, 3) school level (lower, middle, upper school), 4) whether the parents were contacted in the past to participate in the Ciao Corona study (yes, no), 5) a list of predefined reasons for non-participation followed by a free text field for comments (Of note, multiple answers could be given for 12 pre-defined reasons and one additional field for "other reasons") , and 6) who in the family made the decision not to participate (parents, child, both together).

We then categorized individual comments by their general emotional connotation towards the study into a more "negative", "positive", or "neutral" response, and whether non-participation reasons were potentially modifiable by the study team. Two members of the study team screened and categorized individual responses independently (SK, TR). Discrepancies categorizations were discussed and consensus achieved though discussion.

Results

50/55 schools agreed to the distribution of the questionnaire within participating classes. At the testing day, 5001/5205 (96%) children in the classes invited to the study in these schools were present, and 2342 of them (47%) participated in T3 testing round. From the remaining 2659 children eligible for the non-participation survey, parents of 712 (27 %) children responded. Of those, 328/695 (47%), 192/695 (28%), and 175/695 (25%) of parents responded for children from lower, middle, and upper school level (17 did not report school level). Among survey participants, 673 (94.5%) responded in German, 12 (1.7%) in English, 7 (1%) in Albanian, 6 (0.8%) in Italian, 5 (0.7%) in Turkish, 4 (0.6) in Portuguese, and 2 (0.3%) in Spanish. 591/657 (90%) participants reported that they received an invitation to take part in the study and 66 (10%) denied that they received an invitation (55 did not respond to this question). Those who reported an individual comment that their child participated in T1 or T2 were removed from the analysis (n=28, 4%).

The final decision not to take part in the study was made by the parent(s) alone for 64/544 children (12%), parent(s) and child together for 205 (38%), or the child alone for 275 children (51%) (20 parents did not respond to this question). Figure 2S (also presented in the main manuscript) provides an overview about reasons for non-participation according to school level. The most frequently reported reason for non-participation was fear of blood sampling (358/564, 64%), which was reported more frequently among girls (189/276, 69%) compared to boys (158/259, 61%), and in children from lower school level (191/264, 72%) compared to middle (85/153, 56%) and upper school level (79/141, 56%), respectively.

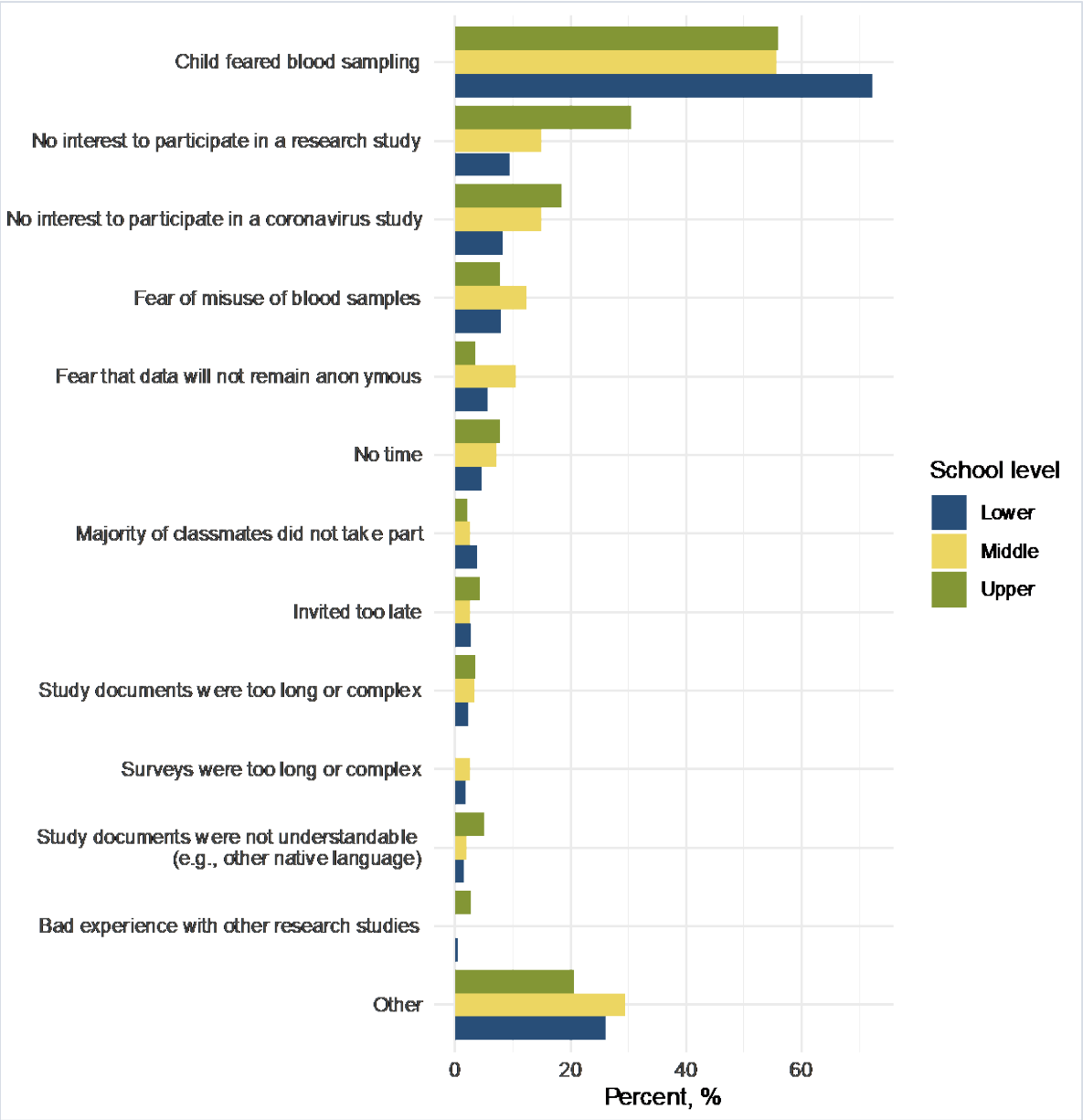


Figure 2S Reasons for non-participation in the Ciao Corona study assessed during the third round of assessment in March/April 2021 among 567 eligible participants according to the school level (e.g. lower=grades 2-3, middle=grades 5-6, upper=grades 8-9). Multiple answers were allowed for the 13 pre-defined reasons including a field for "other reasons"

341/684 survey participants (50%) provided individual comments that we categorized into “overall negative”, “overall positive”, or “neutral” responses, and modifiable versus non-modifiable (**Table 1S**)

Table 1S Emotional connotation and modifiability of reasons for non-participation among 341/684 survey respondents providing specific comments

	N (%)
Emotional connotation of comment	**N = 341**
Overall negative	101 (30)
Overall positive	92 (27)
Neutral	146 (43)
Unclear	2 (0)
Modifiability of reasons	
Non-modifiable	266 (78)
Modifiable	53 (16)
Unclear	22 (6)

Individual comments from survey participants to the question “What could we do differently so that you would participate in the Ciao Corona study?” are given in the table below:

Overall negative – non-modifiable
• "Why don't you do such studies on adults rather than on children/adolescents? Especially if no one in the class has had Corona, an antibody study is probably not that useful". • “I am absolutely against the whole testing thing. If we stopped, the whole Corona craze would be over. Tests have been proven to be unreliable (see WHO)”. • “Because we feel that the current situation and the way in which research is being carried out is not honest. The Federal Council is not interested in scientific facts anyway!” • “Because we didn't understand the point of starting a study with children. We felt it was pointless. We do not support this "scaremongering" and want to be left alone with studies and everything else that has to do with Corona”. • “Not at all – Corona lie” • “Don't write in such a complicated way”
Overall negative – potentially modifiable
• “It was unclear what happens to the data and what is done with the blood. Pass on/sell to third parties?!” • “With better communication. Clear formulation of hypotheses. Unfortunately, it gave the impression in the first test that people simply want to make a name for themselves with the topic and that the children at the schools are the easiest to access in order to have test results. That bothered me a lot”. • “A positive test result would have even more consequences for our self-employment. Fear of existence, shop closure, loss of work”. • “No! Many studies are funded by pharmaceutical companies, and I don't want to participate to support the pharmaceutical lobby”.
Overall positive – non-modifiable
• “With a miracle cure for fainting during blood sampling” • “Our son collapses when blood is taken, cannot be mobilised (stand up) for up to four hours afterwards and needs medical supervision each time. Therefore, he could unfortunately not participate”
Overall positive – potentially modifiable
• “Better information about blood collection”

• "No needles - difficult to implement. The anaesthetic patch should have been promoted more" • "Participation of parent during blood collection" • Eventually my child would have taken part if we parents could have been present. But this was not possible due to Corona"
Neutral – non-modifiable
• "Fear of being quarantined as an extended family" • "Taking blood from the finger, finger prick"
Neutral – potentially modifiable
• "Anonymized data (not just encrypted, with the explicit option to de-encrypt on demand)" • "The teacher could have explained the study to the non-speaking German parents"

Interpretation of individual reasons for non-participation in *Ciao Corona*

Overall, 27% of eligible respondents participated in this anonymous survey and provided reasons for non-participation in the Ciao Corona study. This information is helpful to better understand the huge problem of non-participation and selection bias in virtually every population-based study. The most frequent reason for non-participation was fear of blood sampling (64%), which is understandable given the fact that we invited as young as 6 years old children. Even with a videoclip available explaining the procedures and the use of a plaster with an anesthetic cream to numb the skin over the blood sampling place, this fear was still prominent. Yet, we do not know how many of these "anxious" children did indeed watch the videoclip although a lot of teachers reported that they did so even in class. We could possibly have put even more effort into explaining the blood sampling procedures, for example by preparing more precise video-clips with each step included and explained (meeting in the classroom, application of patches with an anaesthetic cream on the location of venipucture, blood drawing procedures, selection of a plaster, get a sweet and a present) that could be watched in the classroom or at home. We could have found a way to always allowing a parent to be present for the blood sampling (which was not always the case due to school-based mitigation strategies), especially in the younger age group, e.g., by testing the child with a present parent in a bus outside the school. At least, we could have demonstrated the blood sampling on the classroom teachers. From a logistic perspective, most of these adaptations would have been a major challenge, if not even an unrealistic hurdle to take.

Other reasons included lack of interest in research studies in general or SARS-CoV-2-related research (16% and 13%). About 16% reported concerns about ethical issues, e.g., "experimenting" with the child's blood or lack of anonymity. Although a minority, parents with such concerns have to be taken seriously. Although we aimed to provide clear statements in the information and consent sheets that all data got fully anonymised and the blood was used for corona-related research, more effort might have been needed.

Finally, about half of the survey participants provided individual comments and reasons for non-participation in the study. Of those, 70% were either positive or neutral, whereas 30% were categorized as negative. The vast majority of reported individual reasons for non-participation were non-modifiable due to study design (e.g., need for venous blood sampling). About 16% of responses were categorized as modifiable. Most of those comments were very useful for future study planning: they addressed the communication with parents to rule out misinformation/misinterpretation of study contents, the wish for more and repeated information, more simplified explanations of the study objectives and planned testing procedures. Yet, the latter is often interfering with the requirements of the ethical committees requesting an extensive and detailed study information regarding the aim of the study, eligibility criteria, general information about the study, detailed procedures, benefit for participants, rights and obligations, measures taken, confidentiality of data and probes, compensation, insurance coverage, funding, etc. Future collaborative work with ethical committees, schools, children and parents themselves are needed to improve communication strategies that could further increase participation.

Despite a high participation rate of almost 30%, selection bias among eligible survey participants is still expected and may hamper generalisability of the survey. We did not collect socioeconomic characteristics with this anonymous survey, but the fact that fewer than expected parents responded in other languages than German suggest selection into the study. This could signal differential study participation with less disadvantaged families participating more frequently, as was observed in other studies focusing on children[8 9] which could

limit the generalisability of our study findings. Although likely not possible to remove complete, this challenge could be addressed by optimising communication by establishing emotional bonds to the eligible population, simplifying and visualising study information, providing repeated information and communicating the value of each individual participant beyond simply providing material and financial incentives.

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Appendix 4

Detailed information of classes with potential clusters (3 or more newly seropositive children at T3) in schools with 2 or more classes with clusters

After T3 testing, we performed semi-structured telephone interviews with school principals of schools in which 2 or more classes with new clusters of seropositive children were detected. We collected information on diagnosed and quarantined children and their main teachers, and potential index and secondary cases. Intraclass transmission was assessed if at least two confirmed or suspected SARS-CoV-2 infections among class children were reported and deemed plausible if time window between diagnosed or quarantined children was within 2 weeks.

Detailed results of the interviews are presented below.

School	School level	N of pupils: T1+/T2+/T3+ negative missing*	RT-PCR positive in T2-T3		Quarantine/ isolation in T2-T3		Information**	Possible index cases of individual pupils***			Transmission in class plausible ****	Comments
			Teacher	Child	Teacher	Child		Teacher	Child	House-hold		
1	lower	1/0/4 10 3	0	0	0	3	3 pupils in quarantine: n=1 Jan 4-14 due to PCR+ sport coach not related to school, n=2 March 8-12 both due to PCR+ mother of one of the 2 pupils that both had close contact	0	0	1	1	
	lower	1/0/3 5 11	1	0	1	7	7 pupils in quarantine: n=5 due to PCR+ parents or n=2 for unknown reasons; n=2 Nov 1-14, n=1 Nov 15-27, n=2 Dec 13-25, n=1 Dec 20-30, n=1 Dec 28-Jan 8, n=1 Feb 1-13	0	0	1	1	
	lower	0/0/4 8 8	0	0	0	4	4 pupils in quarantine: n=1 Oct 19-30 due to PCR+ household member, n=1 Dec 14-24 due to PCR+ sport teacher outside school, n=1 Jan 4-14 PCR+ family friend, n=1 Feb 6-15 for unknown reasons	0	0	1	0	

	lower	1/0/4 10 3	0	1	1	4	1 pupil in isolation: n=1 PCR+ Jan 18 for unknown reasons; 3 pupils in quarantine: n=1 Nov 1-16 due to PCR+ family member, n=1 Dec 21-Jan 3 for unknown reason, n=1 Dec 27-Jan 3 due to PCR+ family member	0	0	1	0	
2	lower	0/0/4 8 11	0	2	0	3	2 pupils in isolation: n=1 PCR+ Feb 3 prior in quarantine due to PCR+ family members, n=1 PCR+ due to symptoms Feb 5, 2 pupils in quarantine: n=1 Nov 2-7 due to PCR+ father, n=1 Dec 2-12 due to PCR+ parents	0	0	1	1	Outbreak in grade 5 class non-participating in this study, Jan 20-25: 8 pupils PCR+, not clear where Index came from, family of 1 pupil and mother of another pupil were tested PCR+
	middle	0/0/4 10 6	0	0	0	1	1 pupil in quarantine: Jan 12-21 due to PCR+ family member	0	0	1	?	
	middle	0/0/3 3 16	0	0	0	2	2 pupils in quarantine: n=1 Jan 7-16 due to PCR+ father, n=1 Feb 2-12 due to PCR+ sister	0	0	1	0	
3	lower	0/0/3 7 9	1	1	1	whole class	Outbreak in a parallel class due to PCR+ child Jan 25 (probably infected by his mother; mother with severe symptoms but not tested). Then teacher and several children PCR+ in this and the parallel classes, the remaining children not tested; both classes (including this one) quarantined	0	0	1	1	Transmission of virus among pupils in the lower school level class probable, primary Index case most probably mother of a child.
	middle	1/1/3 12 5	0	0	0	1	1 pupil in quarantine due to travel abroad after fall vacation Oct 25-Nov 7	0	0	0	?	
	middle	1/1/3 7 8	1	3	1	3	1 teacher in isolation PCR+ Nov 4, 3 pupils in isolation: n=1 Nov 16 PCR+ due to PCR+ family member, n=1 Dec 17 PCR+ due to PCR+ father, n=1 Jan 25 PCR+ due to PCR+ brother	?	0	1	1	

4	lower	1/0/3 2 15	1	3	whole school	whole school	3 pupils in isolation: n=1 Jan 15 PCR+ for unknown reason; n=1 Jan 18 due to PCR+ mother; n=1 PCR+ Jan 18 with symptoms	0	?	1	1	School outbreak with the whole school in quarantine
	middle	1/1/3 7 8	1	7			1 teacher PCR+, 3-6 days later (Jan 18) PCR+ pupil tested because of loss of taste. Testing of the whole class 2 days later - 6 further pupils PCR+, none with symptoms, 5 pupils PCR-	1	0	0	1	
	middle	0/1/3 7 12	1	6			6 pupils in isolation: n=1 in quarantine Jan 4 due to PCR+ father, then tested PCR+ in the outbreak testing Jan 22 together with 1 teacher and 5 further pupils PCR+	?	?	1	1	
5	lower	1/1/3 3 13	0	0	0	0	No diagnosed or suspected SARS-CoV-2 infections known	?	?	?	?	
	middle	0/0/4 6 11	0	0	0	0	No diagnosed or suspected SARS-CoV-2 infections known	?	?	?	?	
6	middle	0/0/3 13 8	0	8	1	whole class	First a few children PCR+, then 8 PCR+ shortly afterwards. Subsequently class in quarantine, teacher then tested PCR- but later tested seropositive	0	1	?	1	
	middle	1/1/3 3 15	0	3	0	3	3 pupils in quarantine: at unrelated time points due to PCR+ family members	0	0	1	0	

7	upper	0/1/3 10 3	0	2	0	2	2 pupils in isolation: n=1 Jan 4, n=1 March 13, for unknown reasons	0	?	?	0	A class not participating in this study in quarantine during Christmas break Dec 20-Jan 4 due to several PCR+ pupils
	upper	0/0/3 7 5	0	1	0	1	1 pupil in isolation March 16 for unknown reasons	0	?	?	?	
8	lower	0/0/3 4 15	0	1	0	1	1 pupil in isolation: infected by music teacher outside school, whole class tested PCR-	0	0	0	?	
	middle	1/0/3 12 8	0	1	0	4	1 pupil in isolation: infected by music teacher outside school. 3 pupils in quarantine: n=1 due to PCR+ family member, n=2 of for unknown reasons, at unrelated time points	0	0	1	0	
9	middle	2/0/3 8 11	0	1	0	6	1 pupil in isolation: PCR+ during quarantine due to a PCR+ brother. 6 pupils in quarantine: n=2 Nov 26-Dec 6; n=1 Dec 9-18, n=1 Dec 11-20; n=1 Dec 18-27, n=1 Dec 8-15, all due to PCR+ family members	0	0	1	1	Parallel class not participating in this study in quarantine due to infected teacher and class assistant, end of November 2020
	middle	0/1/4 1 18	0	0	0	2	2 pupils in quarantine: n=1 Nov 28-Dec 6; n=1 Jan 1-10 due to PCR+ family members	0	0	1	0	
10	lower	1/0/5 9 9	0	0	0	5	1 pupil in isolation: n=1 Dec 24-Jan 6 due to PCR+ father; 4 pupils in quarantine: n=1 Oct 20-30 due to PCR+ father, n=2 due to PCR+ parents Dec 24-Jan 6, n=1 April 15-25 due to PCR+ contact	0	0	1	1	

	lower	0/0/3 12 8	0	0	0	3	3 pupils in quarantine: n=1 Jan 4-14 due to PCR+ sport coach not related to school, n=2 March 8-12 both due to PCR+ mother of one of the 2 pupils that both had close contact	0	0	1	1	

* T1+ denotes the number of seropositive pupils at T1 (June-July 2020); T2+ denotes the number of newly seropositive pupils at round T2 (October-November 2020); T3+ denotes the number of newly seropositive pupils at T3 (March-April 2021); *negative* denotes the number of seronegative pupils at round T3; *missing* denotes previously non-seropositive pupils who were not tested at T3. Total class size can be calculated by the sum of T1+, T2+, T3+, negative, and missing.

** Dates reported denote month and day(s). September to December refer to the year 2020, January to April – to the year 2021.

*** Possible index cases of individual diagnosed or suspected (RT-PCR+ or quarantined) children in the class.

**** Transmission among pupils/teachers in class was considered possible (1) when the time between RT-PCR diagnoses or quarantine start dates was 2 weeks or below, and not probable (0) if longer. Transmission was defined as unclear (?) if no or only a single child was diagnosed or quarantined in the class according to the interview information.

Appendix 5

Distribution of expected number of classes with clusters (3 or more newly seropositive children), standardised to 100 classes with high participation rate

Vertical line signifies the number of clusters observed in this study, as standardised to 100 classes (all school levels – 20 (corresponding to 24/119), upper school level 11 (5/45), middle level 24 (10/41), and lower school level 27 (9/33)).