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Variation in SARS-CoV-2 seroprevalence in primary and secondary school children across districts, schools and classes in Switzerland

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Title

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Abstract

Objectives: To determine the variation in SARS-CoV-2 seroprevalence in school children, and the relationship with self-reported symptoms.

Design: Baseline measurements of a longitudinal cohort study *(Ciao Corona)* from June-July 2020.

Setting: 55 schools stratified by district in the canton of Zurich, Switzerland.

Participants: 2585 children (1339 girls, median age 11, age range 6-16 years), attending grades 1-2, 4-5 and 7-8.

Main outcome measures: Variation in seroprevalence of SARS-CoV-2 in children across 12 cantonal districts, schools, and grades, assessed with Luminex-based test of four epitopes for each of IgG, IgA and IgM. Clustering of cases within classes. Association of seropositivity and symptoms. Comparison with seroprevalence in adult population, assessed with Luminex-based test of combining IgG and IgA.

Results: Overall seroprevalence was 2.8 % (95% CI 1.5 to 4.1%), ranging from 1.0% to 4.5% across districts. Seroprevalence in grades 1-2 was 3.8% (2.0 to 6.1%), in grades 4-5 – 2.4% (1.1 to 4.2%), and in grades 7-8 – 1.5% (0.5 to 3.0%). At least one seropositive child was present in 36 of 55 (65%) schools and in 44 (34%) of 131 classes where ≥5 children and ≥50% of children within the class were tested. 73% of children reported COVID-19-compatible symptoms since January 2020, with the same frequency in seropositive and seronegative children for all symptoms. Seroprevalence of children and adults was similar (3.2%, 95% CrI 1.7-5.0%, versus 3.6%, 95% CrI 1.7-5.4%). The ratio of confirmed cumulative incidence to seropositive cases was 1:89 in children and 1:12 in adults.

Conclusions: Seroprevalence was inversely related to age and revealed a ratio of diagnosed to seropositive cases of around one to 90 in children by the end of June. We did not detect clustering of SARS-CoV-2 seropositive children, but the follow-up of this study will shed more light on transmission within schools.

Trial registration: ClinicalTrials.gov NCT04448717. https://clinicaltrials.gov/ct2/show/NCT04448717

Key words: SARS-CoV-2, COVID-19, children, adolescents, school.

Article summary

Strengths and limitations of this study

- This study presents the results of a regionally representative cohort of children, randomized on school and class levels, and thus, allowing the analysis of clustering of cases within schools.
- This cross-sectional analysis estimates the seroprevalence in children in June-July in Switzerland, from a period when there is very little evidence about SARS-CoV-2 seroprevalence in children globally.
- Serological test with high sensitivity and specificity was used, and Bayesian hierarchical models were additionally applied to estimate seroprevalence, adjusting for test accuracy parameters.
- Self-reported symptoms might be subject to recall-bias, particularly when reporting retrospectively for a period of over six months.

INTRODUCTION

The transmission of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in the school setting is not well understood [1], partly as schools were closed in many countries during the peaks of the pandemic, partly due to lack of representative studies with random sampling. Anecdotal evidence and case studies suggest that outbreaks can happen in schools [2–4], but it is not clear if they represent outlier events or widely underdiagnosed spread of the infection. School closures are even predicted to increase the total number of deaths by some modelling studies [5]. Currently existing or planned population-based studies focusing on SARS-CoV-2 spread in schools are few and small-sized [6,7].

In this study, we present the results of the first cross-sectional analysis of a large cohort of children from randomly selected schools and classes in the canton of Zurich, Switzerland. The participating children were enrolled from June 16 to July 9, 2020. Schools in Switzerland were closed for a relatively short period (March 16 to May 10) compared to other countries, and lock-down measures were mild. The cohort study follows the seroprevalence, symptoms, socio-demographic and lifestyle factors of enrolled children from June 2020 to April 2021.

The aim of this analysis is to present the overall estimate of seroprevalence and its variation across districts, schools, grades and classes, and the association of seroprevalence with self-reported symptoms.

METHODS

The study was registered on ClinicalTrials.gov (identifier number NCT04448717). The protocol for this longitudinal cohort study was reported elsewhere [8]. The study is part of a large nationally coordinated research network *Corona Immunitas* in Switzerland [9,10]. The study took place from June 16 to July 9, 2020, in the canton of Zurich, Switzerland. The canton of Zurich comprises 1.5 million residents, roughly 18% of the Swiss population, and includes both urban and rural settings, as well as an ethnically and linguistically diverse population. The first preventive measures in schools were introduced on March 16, 2020, when physical attendance of schools was stopped. Schools were partly reopened on May 10, with a combination of online and on-site teaching with preventive measures (e.g., teaching in smaller groups, school attendance every second day, sports and large group activities limited). Schools were fully reopened on June 7, with minimal preventive measures (e.g., recommended social distancing for teachers, reduction of group events) and otherwise regular operation (e.g., full classrooms with desks mostly facing forward) until the end of the school year on July 17. The prevention measures implemented in schools after May 10 were school-specific and based on the federal and cantonal guidelines [11].

School were selected based on a full list of schools provided by the Educational Department of the canton. Primary schools were randomly selected by a computer program from the list of all primary schools in the respective district. The closest secondary school (often in the same building or area) was then selected. The random sample of primary and secondary schools was stratified by geographic district. Within schools, randomly selected classes in lower school level (grades one to two, attended by 6 to 9-year-old children), middle (grades four to five, 9 to 13-year-old children), and upper school level (grades seven to eight, 12 to 16-year-old children) were invited. Invited grades and classes were selected to ensure that the same cohort of children within the class can be followed until April 2021. Therefore, grades 1-2, 4-5 and 7-8 (but not grades 3, 6, and 9) were included, as they normally stay in the same school and class for the next school-year. We aimed to enroll at least three classes and 40 children per school level. Major exclusion criterion for the invited children was a suspected or confirmed infection with SARS-CoV-2 in the given child during the testing at schools.

Study information, link to study website (www.ciao-corona.ch), and informational videos in multiple languages for schools, parents, and children were sent to school principals and further to families of children from the selected classes. Children were enrolled and venous blood samples taken in schools between June 16 and July 9, 2020. Questionnaires

with information on socio-demographics and symptoms compatible with SARS-CoV-2 infection from January to June, 2020, were completed online for the majority of children by their parents in June-July, 2020 (for 3% of children in August-September).

In total, 55 out of 156 invited primary and secondary schools agreed to participate, and 2585 children in 273 out of 274 invited classes (no children participated in one class). Venous blood samples were collected from 2484 children (a sufficient amount of blood could not be obtained from 101 children). An online questionnaire, containing socio-demographic, health, symptoms and quality of life information for the children, and socio-demographic and symptoms information for the household members, was completed for 2288 of all enrolled children. Questionnaires were not filled for 297 children, after several email and phone call reminders.

Blood samples were analyzed with ABCORA 2.0 binding assay of the Institute of Medical Virology (IMV) of the University of Zurich based on the Luminex technology. The test analyzes 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), yielding 12 different measurements. Cut-off values were established against pre-pandemic plasma allowing a high sensitivity (93.3%) and specificity (99.6%). Samples were defined as seropositive for SARS-CoV-2 if at least two of the 12 parameters were above the cut-off. SARS-CoV-2 seroprevalence in children was compared to that estimated in a random sample from the general population, adjusting for age group and sex, in the same region in June-July 2020. The adult study, like all studies of the Swiss-wide research program *Corona Immunitas* [9,10], used the test SenASTrIS (Sensitive Anti-SARS-CoV-2 Spike Trimer Immunoglobulin Serological) developed by the Centre Hospitalier Universitaire Vaudois (CHUV), the Swiss Federal Institute of Technology in Lausanne (EPFL) and the Swiss Vaccine Center [12]. The test also uses Luminex technology to detect IgG and IgA antibodies binding to the entire trimeric S protein of SARS-CoV-2 and with demonstrated 98.3% sensitivity and 98.4% specificity for the combined testing of IgG and IgA (result declared as positive when either or both were positive) [12]. In order to compare the seroprevalence estimates in children and adult cohorts, a sample of 2476 collected children blood samples was also analyzed with the SenASTrIS test (samples were insufficient for analysis with the second test for 8 children) and compared to a random population sample of 857 adults who took part in the second phase of the Switzerland-wide *Corona Immunitas* research program [10].

Seroprevalence was also compared to the cumulative incidence of reverse transcription polymerase chain reaction (RT-PCR)-confirmed SARS-CoV-2 infections in adults

and children, based on official statistics up to the beginning of June [13]. Statistical analysis included descriptive statistics and Bayesian hierarchical modelling to estimate seroprevalence, accounting for the sensitivity and specificity of the SARS-CoV-2 antibody test, the hierarchical structure of cohort (individual and school levels), and post-stratification weights, which adjusted for the population-level grade level and geographic district [14]. The factor of confirmed to total infections (dark figure) was calculated as the ratio of RT-PCR-confirmed cumulative incidence by the end of June 2020 and the estimated seroprevalence.

Patient and public involvement

Several school principals were consulted during the development of the protocol to ensure feasibility of the planned study procedures. Early feedback was collected from invited children and parents, in order to adapt the communication strategies and channels. Numerous online informational sessions, encouraging open exchange and feedback, were organized for invited and enrolled school principals, personnel and parents of the children. Results of individual tests were communicated to the participants, and overall study results disseminated to participating schools. Findings will be 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).

RESULTS

In total, 55 schools and 2585 children were recruited (1339 (52%) girls, median age 11, age range 6-16 years), 754 (29%) in the lower level, 899 (35%) in the middle, and 932 (36%) in the upper school level. Mean participation rate was 50% of the invited children within invited classes (range from 0% to 94% (0 to 21 children), interquartile range 32% to 63%). Venous blood was collected and analyzed for 2484 children (1278 (51%) girls, median age 11, age range 6 to 16 years).

74 children had SARS-CoV-2 antibodies, resulting in overall weighted seroprevalence of 2.8 % (95% credible interval (CrI) 1.5 to 4.1%), ranging from 1.0% to 4.5% in districts (Figure 1). Seroprevalence was 3.8% (95% CrI 2.0 to 6.1%) in grades one to two, 2.4% (95% CrI 1.1 to 4.2%) in grades four to five, and 1.5% (95% CrI 0.5 to 3.0%) in grades seven to eight (Figure 1).

Seroprevalence of children, as measured with the SenASTrIS test of IgG and IgA combined, was very similar to the seroprevalence of randomly selected adults in the same region in June-July 2020 (3.2%, 95% CrI 1.7 to 5.0%, versus 3.6%, 95% CrI 1.7 to 5.4%). The estimates of seroprevalence in the children cohort, as measured with the SenASTrIS test, in different school levels and districts were similar to those estimated with the primary ABCORA 2.0 test (Figure 1).

Based on the cumulative incidence of SARS-CoV-2 RT-PCR-confirmed cases by the end of June (0.03% for children and 0.24% for adult populations), the ratio of confirmed infections and seropositive cases (dark figure) in children was one to 89, compared to a ratio of one to 12 in the adult Swiss population.

Figure 1 Overall, school level and district estimates of SARS-CoV-2 seroprevalence in children

Weighted point estimates and 95% credible intervals are shown. Districts are ordered by population size.
ABCORA 2.0 – Primary estimate based on the ABCORA 2.0 of the Institute of Medical Virology, University of Zurich test.
SenASTrIS – Estimate based on the SenASTrIS test.

At least one seropositive child was present in 36/55 (65%) of tested schools. Within the levels of the schools, at least one seropositive child was present in the lower level of 17 (59%) out of 29 tested schools, in the middle level of 14 (50%) out of 28 tested schools, and in the upper level of 16 (64%) out of 25 tested schools (Figure 2).

At least one seropositive child was present in 34% (44/131) of classes where ≥5 children and ≥50% of children within the class were tested (Figure 3). Among the classes

with at least one seropositive child, 38 (86%) had only one, 4 (9%) had two and 2 (5%) had three seropositive children. When considering all classes regardless of participation rate, 24% of classes had at least one seropositive child; whereas when considering higher inclusion threshold of ≥15 children and ≥60% of the class tested, 45% of classes had at least one seropositive child.

Figure 2 Seropositive children in tested schools and school levels

Each square illustrates an invited child. Each block of squares illustrates a school level in a school. Lower and middle levels are both taught in the primary schools; however, lower and middle levels of the same school are not matched in this graph due to protection of participant privacy. The distribution of the invited, tested and seropositive children is depicted only on the school level in the figures of this manuscript to preserve deidentification and privacy of the participants.

Figure 3 Clustering of seropositive children in classes: number and proportion of seropositive children in the tested classes

Only classes where at least 5 children and at least 50% of the class were tested are shown. The distribution of seropositive children in the enrolled classes is only presented in an aggregated form to preserve the deidentification and privacy of the participants.

No sex differences in seroprevalence were noted (2.8% (95% CrI 1.6 to 4.1%) in girls and 2.7% (95% CrI 1.5 to 4.0%) in boys). 73% of children reported any SARS-CoV-2 compatible symptoms, such as cough, fever, fatigue or diarrhea (see Figure 4 for the full list), between January and June 2020. None of the symptoms was more frequent in seropositive than in seronegative children (Figure 4).

Figure 4 Self-reported symptoms in seropositive and seronegative children in January-June 2020

Point estimates and 95% confidence intervals are shown.

DISCUSSION

In this study of randomly sampled schools and classes by July 2020, we found variation in seroprevalence in 6 to 16-year-old children across districts, schools and classes, but no indication of major transmission and outbreaks within schools. The overall seroprevalence was not different from a randomly selected adult population living in the same region – pointing to striking underdiagnosis of SARS-CoV-2 infection in children, with only one in 89 cases diagnosed. Such a high proportion of seropositive cases not previously diagnosed with RT-PCR is likely explained by our observation that symptoms do not seem to be suggestive of a SARS-CoV-2 infection in children, contrary to more specific symptoms in adults [15], and by cautious testing indications by health authorities, with similar indications for children and adults during the first half of 2020. Contrary to studies of symptomatic infections [16] and some other population-based studies of seroprevalence [17], there was a trend of higher seroprevalence in younger children. Although no outbreaks were reported in schools at the time of testing in the canton of Zurich (comprising 18% of the Swiss population), seropositive children were detected in more than half of the tested schools and a third of all tested classes. However, the vast majority of classes with seropositive children had only a single seropositive child among the tested children, reflecting low prevalence and no significant clustering within classes after the re-opening of the schools. The presence of symptoms was very common (three of four children reported one or several symptoms compatible with a COVID-19 infection) and importantly not specific to the seropositive children.

Currently, there are few studies focusing on SARS-CoV-2 spread in schools [17–19]. Most of the reported evidence consists of cases studies of outbreaks in specific schools or reports of contact tracing of index cases in educational settings. Mostly low secondary attack rates in schools are reported [4,20], but there are also conflicting observations of outbreaks [2,21]. Although some studies of seroprevalence have included children [14,22,23], they mostly focused on households and the general population. The management of SARS-CoV-2 transmission in schools is therefore highly debated [24,25]. The present study is thus unique as one of the first major studies reporting variation in seroprevalence in children from randomly selected schools in a country where the general lock-down on a population level was mild and short (one month), and school closure lasted only for two months.

Although manifest clinical disease of COVID-19 is much less prevalent in children than in adults [16,26] and preliminary evidence points to lower susceptibility of children

compared to adults [17], our results indicate very similar seroprevalence in adults and children. Intriguingly, we observed a not statistically significant trend of younger children having higher seroprevalence than older children, when measured with the comprehensive ABCORA 2.0 test. Infections with circulating human coronaviruses (hCoVs) are common in childhood and antibodies to hCoVs 229E, NL63, OC43 and HKU1A are prevalent in the human population and particularly children [27,28]. Cross-reactivity with hCoVs was thus considered in the development of both serology tests used in this study and both tests detect SARS-CoV-2 with high specificity (99.6% for ABCORA 2.0 and 98.4% for SenASTrIS). Importantly, to adjust for the possibility of a few false-positive results, we employed a Bayesian hierarchical model, which adjusts for the accuracy parameters of the tests to estimate population-level seroprevalence. Higher seroprevalence in younger children could be related to virtually impossible social distancing behaviour, but also to a possibly more vigorous immune response to the virus in early age that will be interesting to explore in forthcoming studies.

The frequency of both more specific (e.g., fever, cough) and less specific (rhinorrhea, headache, nausea) symptoms [29] was not different among seropositive and seronegative children. In general, symptoms, particularly rhinorrhea, cough, headache and sore throat, were reported frequently, with three of four children reporting any symptoms within the last 6 months in both groups. The specificity of COVID-19 compatible symptoms, therefore, seems to be lower in children than in adults. Moreover, the range of symptoms reported in children has shown to be different compared to adults [30].

Particular strengths of the design of this study are school-based random sampling, hierarchical data structure, and large sample size, allowing to identify clusters within district, school, grade and class levels. Testing was done in schools and study information presented in multiple formats, including videos in multiple languages, to minimize selection bias within enrolled children. The participation rate of 50% can be considered rather high for a study in children involving venous blood sampling, and additional children from the invited classes will have the opportunity to be enrolled in subsequent testing phases (October/November 2020, March/April 2021), further increasing participation rate and the size of the cohort.

The study has a few limitations. First, the retrospective evaluation of symptoms over six months could have been subject to recall bias. Second, the study enrolled only 35% (55/156) of the invited schools. Commonly stated reasons for non-participation on school level were constraints in time and human resources and competing participation in other

studies. Participation rate varied significantly depending on the district, and for a few districts, the schools had to be invited with a maximum of three invitation rounds to have a regionally representative sample. Third, although the schools were open for one to two months directly before the study, they were closed for the two months of the highest community transmission of SARS-CoV-2 in the canton of Zurich [13]. Therefore, the measured seroprevalence in children might be dominated by infections in households rather than school. The follow up of this study will shed more light on transmission in schools.

In conclusion, clustering of SARS-CoV-2 seropositive children within schools and grades was not prominent shortly after reopening of schools in this large population-based study. Seroprevalence was similar to adults and higher in lower grade compared to higher grade children, resulting in a strikingly higher ratio of diagnosed to seropositive cases than in adults, in particular in the very young children. Considering the time window required for SARS-CoV-2 antibodies to form, this study reflects infection of SARS-CoV-2 until approximately end of May 2020, covering four months of SARS-CoV-2 infection in the community, with two months of school closure and mild lock-down policy. The subsequent testing of parents and school personnel and the follow-up of the children cohort in fall 2020 will yield further evidence of the spread of SARS-CoV-2 within and outside schools.

Author Contributions

SK and MAP initiated the project and preliminary design, with support of JF. SK, MAP, CB, TR, RJ, JB, AF and AU developed the design and methodology. SK, RJ, AU, TR, JB, AF and CC recruited study participants, collected and managed the data. SRH performed statistical analysis. AT, MH, MaSch, MeSch 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 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.

Competing interests

The authors declare no competing interests to disclose.

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Data sharing statement

Data is 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.

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.

Transparency declaration

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.

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Figure legends

Figure 1 Overall, school level and district estimates of SARS-CoV-2 seroprevalence in children

Weighted point estimates and 95% credible intervals are shown. Districts are ordered by population size.

ABCORA 2.0 – Primary estimate based on the ABCORA 2.0 of the Institute of Medical Virology, University of Zurich test. SenASTrIS – Estimate based on the SenASTrIS test.

Figure 2 Seropositive children in tested schools and school levels

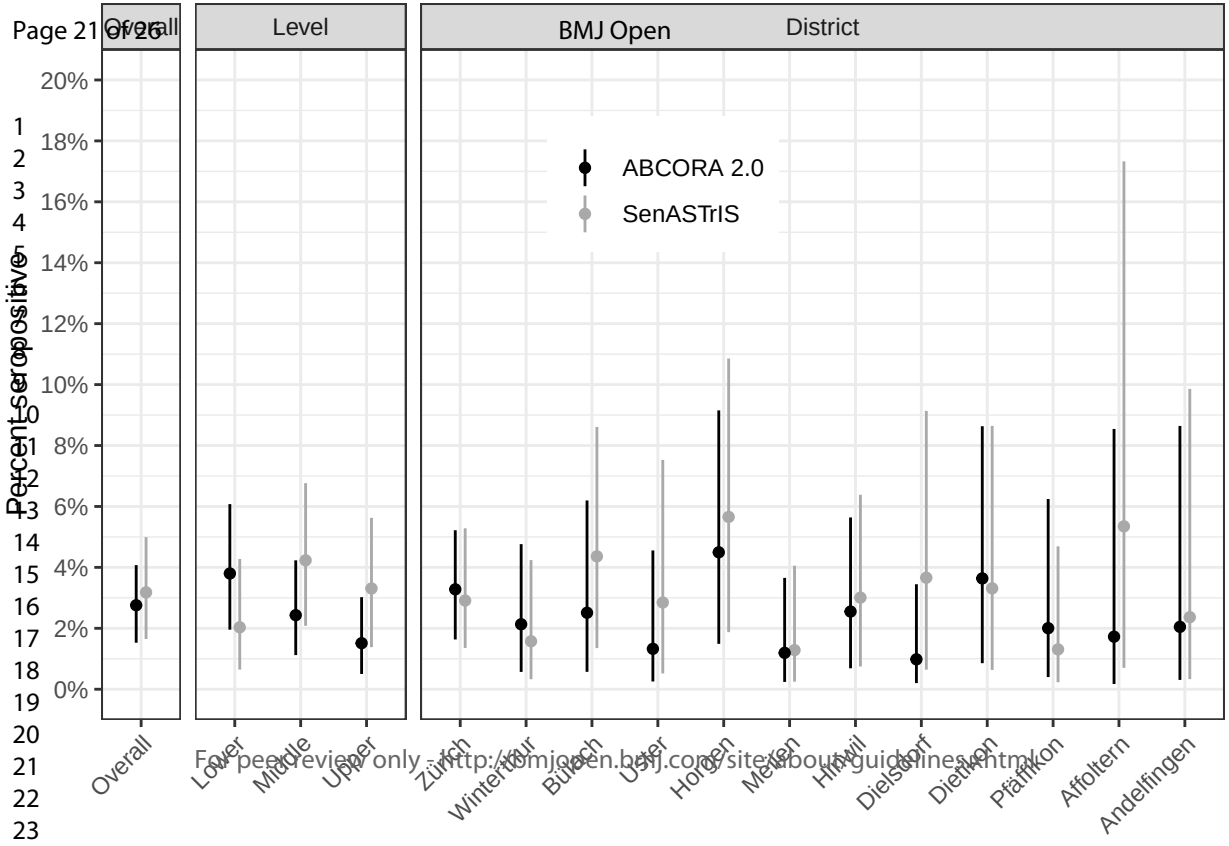
Each square illustrates an invited child. Each block of squares illustrates a school level in a school. Lower and middle levels are both taught in the primary schools; however, lower and middle levels of the same school are not matched in this graph due to protection of participant privacy. The distribution of the invited, tested and seropositive children is depicted only on the school level in the figures of this manuscript to preserve deidentification and privacy of the participants.

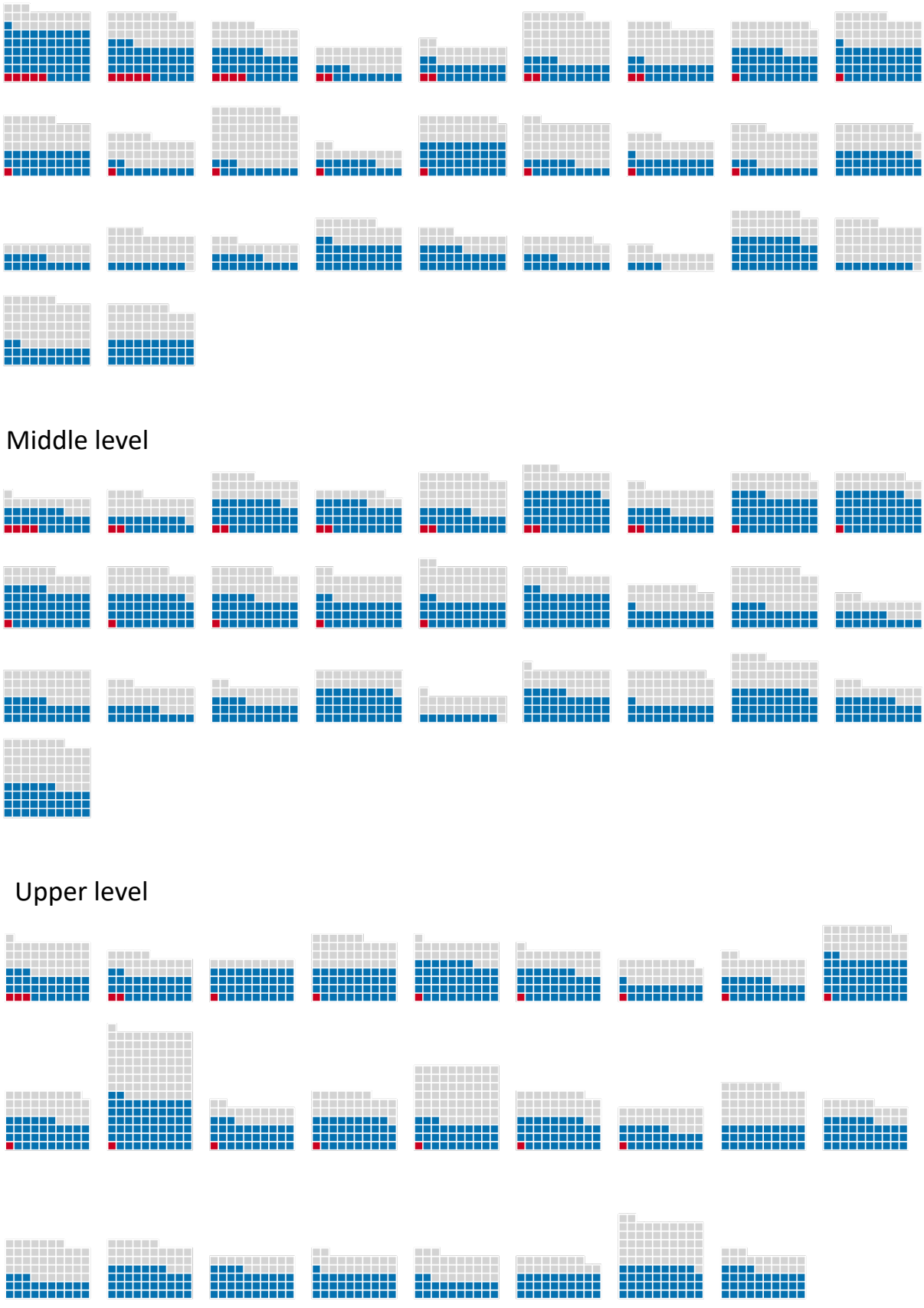
Figure 3 Clustering of seropositive children in classes: number and proportion of seropositive children in the tested classes

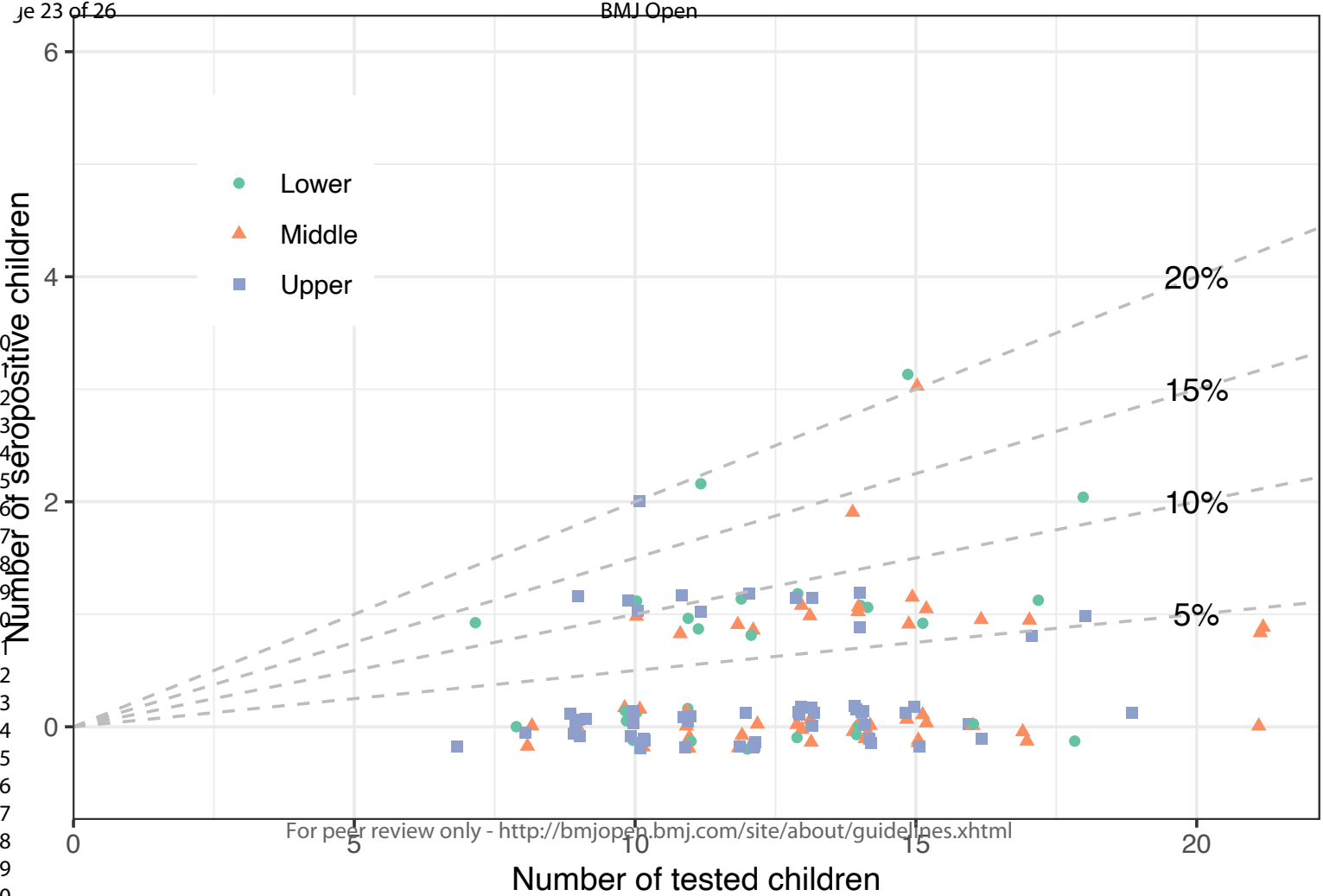
Only classes where at least 5 children and at least 50% of the class were tested are shown. The distribution of seropositive children in the enrolled classes is only presented in an aggregated form to preserve the deidentification and privacy of the participants.

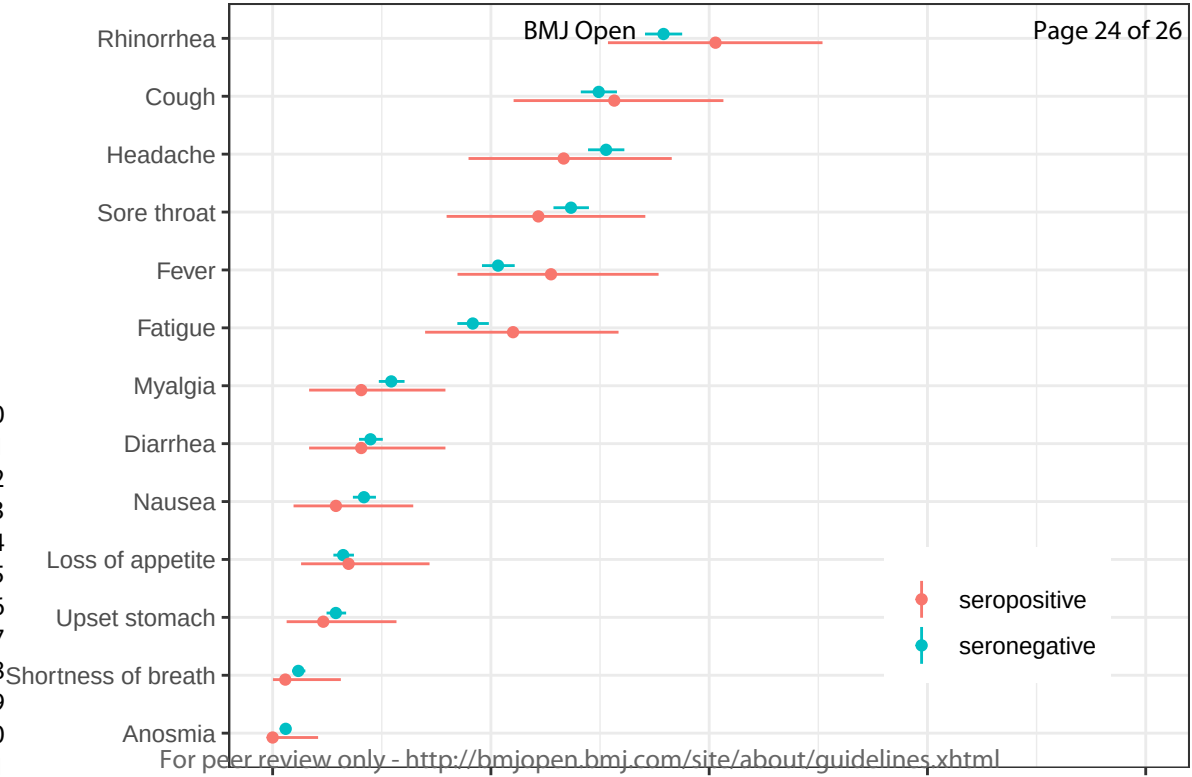
Figure 4 Self-reported symptoms in seropositive and seronegative children in January-June 2020

Point estimates and 95% confidence intervals are shown.









STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No.	Recommendation	Page No.	Relevant text from manuscript
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	5	first cross-sectional analysis of a large cohort of children from randomly selected schools and classes
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	3	
Introduction				
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	5	
Objectives	3	State specific objectives, including any prespecified hypotheses	3, 5	To determine the variation in SARS-CoV-2 seroprevalence in school children across districts, schools, grades, and classes, and the relationship of SARS-CoV-2 seroprevalence with self-reported symptoms.
Methods				
Study design	4	Present key elements of study design early in the paper	6-8	
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	6	
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	6	
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed		

		Case-control study—For matched studies, give matching criteria and the number of controls per case		
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	7-8	
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	7-8	
Bias	9	Describe any efforts to address potential sources of bias	7	
Study size	10	Explain how the study size was arrived at	6	Details are provided in the study protocol.

Continued on next page

Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	7-8
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	7-8
		(*b*) Describe any methods used to examine subgroups and interactions	NA
		(*c*) Explain how missing data were addressed	NA
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	7-8
		(*e*) Describe any sensitivity analyses	NA
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	6
		(b) Give reasons for non-participation at each stage	6
		(c) Consider use of a flow diagram	NA
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	6, 9
		(b) Indicate number of participants with missing data for each variable of interest	6-7
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	
		Cross-sectional study—Report numbers of outcome events or summary measures	9
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	9
		(*b*) Report category boundaries when continuous variables were categorized	NA
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	NA

Continued on next page

Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	9
Discussion			
Key results	18	Summarise key results with reference to study objectives	11
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	12-13
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	13
Generalisability	21	Discuss the generalisability (external validity) of the study results	13
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	14

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ Open

Variation in SARS-CoV-2 seroprevalence across districts, schools and classes: baseline measurements of primary and secondary school children cohort in Switzerland

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Title

Variation in SARS-CoV-2 seroprevalence across districts, schools and classes: baseline measurements of primary and secondary school children cohort in Switzerland

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Abstract

Objectives: To determine the variation in SARS-CoV-2 seroprevalence in school children, and the relationship with self-reported symptoms.

Design: Baseline measurements of a longitudinal cohort study *(Ciao Corona)* from June-July 2020.

Setting: 55 schools stratified by district in the canton of Zurich, Switzerland.

Participants: 2585 children (1339 girls, median age 11, age range 6-16 years), attending grades 1-2, 4-5 and 7-8.

Main outcome measures: Variation in seroprevalence of SARS-CoV-2 in children across 12 cantonal districts, schools, and grades, assessed with Luminex-based test of four epitopes for IgG, IgA and IgM (ABCORA 2.0). Clustering of cases within classes. Association of seropositivity and symptoms. Comparison with seroprevalence in adult population, assessed with Luminex-based test of IgG and IgA (SenASTriS).

Results: Overall seroprevalence was 2.8% (95% CI 1.5 to 4.1%), ranging from 1.0% to 4.5% across districts. Seroprevalence in grades 1-2 was 3.8% (2.0 to 6.1%), in grades 4-5 – 2.4% (1.1 to 4.2%), and in grades 7-8 – 1.5% (0.5 to 3.0%). At least one seropositive child was present in 36 of 55 (65%) schools and in 44 (34%) of 131 classes where ≥5 children and ≥50% of children within the class were tested. 73% of children reported COVID-19-compatible symptoms since January 2020, with the same frequency in seropositive and seronegative children for all symptoms. Seroprevalence of children and adults was similar (3.2%, 95% CrI 1.7-5.0%, versus 3.6%, 95% CrI 1.7-5.4%). The ratio of confirmed SARS-CoV-2 cumulative incidence to seropositive cases was 1:89 in children and 1:12 in adults.

Conclusions: SARS-CoV-2 seroprevalence was low in children and similar to that in adults by the end of June 2020. Very low ratio of diagnosed to seropositive children was observed. We did not detect clustering of SARS-CoV-2 seropositive children within classes, but the follow-up of this study will shed more light on transmission within schools.

Trial registration: ClinicalTrials.gov NCT04448717. https://clinicaltrials.gov/ct2/show/NCT04448717

Key words: SARS-CoV-2, COVID-19, children, adolescents, school.

Article summary

Strengths and limitations of this study

- This study presents the results of a regionally representative cohort of children, randomly selected on school and class levels, and thus, allowing the analysis of clustering of cases within classes and schools.
- This cross-sectional analysis estimates the seroprevalence in children in June-July in Switzerland, from a period when there is very little evidence about SARS-CoV-2 seroprevalence in children globally.
- Serological test with high sensitivity and specificity was used, and Bayesian hierarchical models were applied to estimate seroprevalence, adjusting for test accuracy parameters.
- Self-reported symptoms might be subject to recall-bias, particularly when reporting retrospectively for a period of over six months.

INTRODUCTION

The transmission of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in the school setting is not well understood [1], partly as schools were closed in many countries during the peaks of the pandemic, partly due to lack of representative studies with random sampling. Anecdotal evidence and case studies suggest that outbreaks can happen in schools [2–4], but it is not clear if they represent outlier events or widely underdiagnosed spread of the infection. The effect of school closures on the community transmission of SARS-CoV-2 ranges from minimal to substantial [5], with some modelling studies even predicting an increase of the total number of deaths [6]. Currently existing or planned population-based studies focusing on SARS-CoV-2 spread in schools are few and small-sized [7,8].

In this study, we present the results of the first cross-sectional analysis of a large cohort of children from randomly selected schools and classes in the canton of Zurich, Switzerland. The cohort study follows the seroprevalence, symptoms, socio-demographic and lifestyle factors of enrolled children from June 2020 to April 2021. The participating children were enrolled from June 16 to July 9, 2020. Schools in Switzerland were closed for a relatively short period (March 16 to May 10) compared to other countries, and lock-down measures were mild. Restaurants, bars and non-essential shops and services were closed on March 17 and events or meetings with over 5 people prohibited on March 20, but no strict confinement at home implemented. These measures were gradually lifted in April-May 2020.

The aim of this analysis is to present the overall estimate of seroprevalence in children and its variation across districts, schools, grades and classes, the association of seroprevalence with self-reported symptoms, and the clustering of seropositive children within classes.

METHODS

Study setting

The study was registered on ClinicalTrials.gov (identifier number NCT04448717). The protocol for this longitudinal cohort study was reported elsewhere [9]. The study is part of a large nationally coordinated research network *Corona Immunitas* in Switzerland [10,11]. The study took place from June 16 to July 9, 2020, in the canton of Zurich, Switzerland. The canton of Zurich comprises 1.5 million residents, roughly 18% of the Swiss population, and includes both urban and rural settings, as well as an ethnically and linguistically diverse population. The first preventive measures in schools were introduced on March 16, 2020, when physical attendance of schools was stopped. Schools were partly reopened on May 10, with a combination of online and on-site teaching with preventive measures (e.g., teaching in smaller groups, school attendance every second day, sports and large group activities limited). Schools were fully reopened on June 7, with minimal preventive measures (e.g., recommended social distancing for teachers, reduction of group events) and otherwise regular operation (e.g., full classrooms with desks mostly facing forward) until the end of the school year on July 17. The prevention measures implemented in schools after May 10 were school-specific and based on the federal and cantonal guidelines [12].

Study procedures

School were selected based on a full list of schools provided by the Educational Department of the canton. Primary schools were randomly selected by a computer program from the list of all primary schools in the respective district. The closest secondary school (often in the same building or area) was then selected. The random sample of primary and secondary schools was stratified by geographic district. Within schools, randomly selected classes in lower school level (grades one to two, attended by 6 to 9-year-old children), middle (grades four to five, 9 to 13-year-old children), and upper school level (grades seven to eight, 12 to 16-year-old children) were invited. Invited grades and classes were selected to ensure that the same cohort of children within the class can be followed until April 2021. Therefore, grades 1-2, 4-5 and 7-8 (but not grades 3, 6, and 9) were included, as they normally stay in the same school and class for the next school-year. We aimed to enroll at least three classes and 40 children per school level. As we were only able to test the children at schools, a major exclusion criterion was a suspected or confirmed infection with SARS-CoV-2 in the given child on the testing date, precluding attendance of school.

Study information, link to study website (www.ciao-corona.ch), and informational videos in multiple languages for schools, parents, and children were sent to school principals and further to families of children from the selected classes. Children were enrolled and venous blood samples taken in schools between June 16 and July 9, 2020. Questionnaires with information on socio-demographics and symptoms compatible with SARS-CoV-2 infection from January to June, 2020, were completed online for the majority of children by their parents in June-July, 2020 (for 3% of children in August-September).

In total, 55 out of 156 invited primary and secondary schools agreed to participate, and 2585 children in 273 out of 274 invited classes (no children participated in one class). Venous blood samples were collected from 2484 children (a sufficient amount of blood could not be obtained from 101 children). An online questionnaire, containing socio-demographic, health, symptoms and quality of life information for the children, and socio-demographic and symptoms information for the household members, was completed for 2288 of all enrolled children. Questionnaires were not filled for 297 children, after several email and phone call reminders.

Serological tests

The primary outcome of the study was the serological results of blood serum samples analyzed with ABCORA 2.0 binding assay of the Institute of Medical Virology (IMV) of the University of Zurich based on the Luminex technology [13]. The test analyzes 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), yielding 12 different measurements. Cut-off values were established against pre-pandemic plasma allowing a high sensitivity (93.3%) and specificity (99.6%). Samples were defined as seropositive for SARS-CoV-2 if at least two of the 12 parameters were above the cut-off.

SARS-CoV-2 seroprevalence in children was compared to that estimated in a random sample from the general population, adjusting for age group and sex, in the same region in June-July 2020. The adult study, like all studies of the Swiss-wide research program *Corona Immunitas* [10,11], used the test SenASTrIS (Sensitive Anti-SARS-CoV-2 Spike Trimer Immunoglobulin Serological) developed by the Centre Hospitalier Universitaire Vaudois (CHUV), the Swiss Federal Institute of Technology in Lausanne (EPFL) and the Swiss Vaccine Center [14]. The test also uses Luminex technology to detect IgG and IgA antibodies binding to the entire trimeric S protein of SARS-CoV-2 and with demonstrated 98.3% sensitivity and 98.4% specificity for the combined testing of IgG and IgA (result declared as positive when

either or both were positive) [14]. In order to compare the seroprevalence estimates in children and adult cohorts, blood serum samples of 2476 children were also analyzed with the SenASTrIS test (samples were insufficient for analysis with the second test for 8 children) and compared to a random population sample of 857 adults who took part in the second phase of the Switzerland-wide *Corona Immunitas* research program [11].

Seroprevalence was also compared to the cumulative incidence of reverse transcription polymerase chain reaction (RT-PCR)-confirmed SARS-CoV-2 infections in adults and children, based on official statistics up to the beginning of June [15].

Statistical analysis

Statistical analysis included descriptive statistics and Bayesian hierarchical modelling to estimate seroprevalence [16]. The Bayesian approach allowed to account for the sensitivity and specificity of the SARS-CoV-2 antibody test and the hierarchical structure of cohort (individual and school levels). The model (Bayesian logistic regression) was adjusted for participants' sex, grade, and geographic district of the school, and included random effects for school levels (lower, middle and upper). In order to compute an estimate representative for the population of the canton of Zurich, we applied post-stratification weights, which adjusted for the total population size of the specific school level and the geographic district.

The factor of confirmed to total infections was calculated as the ratio of RT-PCR-confirmed cumulative incidence by the end of June 2020 and the estimated seroprevalence. We assessed the clustering of seropositive children within classes, school levels, and schools by studying the distribution of classes with zero, one, or more seropositive children. As the probability of detecting a seropositive child increases with more children tested, we separately assessed the proportion of classes with at least one seropositive child among all class and among classes with ≥5 participating children and ≥50% of children participating from the class.

Patient and public involvement

Several school principals were consulted during the development of the protocol to ensure feasibility of the planned study procedures. Early feedback was collected from invited children and parents, in order to adapt the communication strategies and channels. Numerous online informational sessions, encouraging open exchange and feedback, were organized for invited and enrolled school principals, personnel and parents of the children. Results of individual tests were communicated to the participants, and overall study results

disseminated to participating schools. Findings will be 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).

RESULTS

In total, 55 schools and 2585 children were recruited (1339 (52%) girls, median age 11, age range 6-16 years), 754 (29%) in the lower level, 899 (35%) in the middle, and 932 (36%) in the upper school level. Mean participation rate was 50% of the invited children within invited classes (range from 0% to 94% (0 to 21 children), interquartile range 32% to 63%). Venous blood was collected and analyzed for 2484 children (1278 (51%) girls, median age 11, age range 6 to 16 years).

74 children had SARS-CoV-2 antibodies, resulting in overall weighted seroprevalence of 2.8 % (95% credible interval (CrI) 1.5 to 4.1%), ranging from 1.0% to 4.5% in districts, as measured with ABCORA test (Figure 1). Seroprevalence was 3.8% (95% CrI 2.0 to 6.1%) in grades one to two, 2.4% (95% CrI 1.1 to 4.2%) in grades four to five, and 1.5% (95% CrI 0.5 to 3.0%) in grades seven to eight (Figure 1).

Seroprevalence of children, as measured with the SenASTrIS test of IgG and IgA combined, was very similar to the seroprevalence of randomly selected adults in the same region in June-July 2020 (children 3.2%, 95% CrI 1.7 to 5.0%, versus adults 3.6%, 95% CrI 1.7 to 5.4%). The estimates of seroprevalence in different school levels and districts were similar to those estimated with the primary ABCORA 2.0 test (Figure 1). Seroprevalence measured with SenASTriS test was 2.0% (95% CrI 0.6 to 4.3%) in grades one to two, 4.2% (95% CrI 2.1 to 6.8%) in grades four to five, and 3.3% (95% CrI 1.4 to 5.6%) in grades seven to eight.

Based on the cumulative incidence of SARS-CoV-2 RT-PCR-confirmed cases by the end of June (0.03% for children and 0.24% for adult populations), the ratio of confirmed infections to seropositive cases in children was one to 89, compared to a ratio of one to 12 in the adult population.

Figure 1 Overall, school level and district estimates of SARS-CoV-2 seroprevalence in children

Weighted point estimates and 95% credible intervals are shown. Districts are ordered by population size.
ABCORA 2.0 – Primary estimate based on the ABCORA 2.0 of the Institute of Medical Virology, University of Zurich test.
SenASTrIS – Estimate based on the SenASTrIS test.

At least one seropositive child was present in 36/55 (65%) of tested schools. Within the levels of the schools, at least one seropositive child was present in the lower level of 17/29 (59%) schools, in the middle level of 14/28 (50%) schools, and in the upper level of 16/25 (64%) schools (Figure 2).

Figure 2 Seropositive children in tested schools and school levels

Each square illustrates an invited child. Each block of squares illustrates a school level in a school. Lower and middle levels are both taught in the primary schools; however, lower and middle levels of the same school are not matched in this graph due to protection of participant privacy. The distribution of the invited, tested and seropositive children is depicted only on the school level in the figures of this manuscript to preserve deidentification and privacy of the participants.

No sex differences in seroprevalence were noted (2.8% (95% CrI 1.6 to 4.1%) in girls and 2.7% (95% CrI 1.5 to 4.0%) in boys). 73% of children reported any SARS-CoV-2 compatible symptoms, such as cough, fever, fatigue or diarrhea (see Figure 3 for the full list), between January and June 2020. None of the symptoms was more frequent in seropositive than in seronegative children (Figure 3).

Figure 3 Self-reported symptoms in seropositive and seronegative children in January-June 2020

Point estimates and 95% confidence intervals are shown.

At least one seropositive child was present in 34% (44/131) of classes where ≥5 children and ≥50% of children within the class were tested (Figure 4). Among the classes with at least one seropositive child, 38 (86%) had only one, 4 (9%) had two and 2 (5%) had three seropositive children. When considering all classes regardless of participation rate, 24% (65/273) of classes had at least one seropositive child.

Figure 4 Clustering of seropositive children in classes: number and proportion of seropositive children in the tested classes

Each dot represents a class. Diagonal lines partition the figure into classes with 0-5% of tested seropositive children (below 5% line), 5-10% of tested seropositive children (between 5% and 10% lines), etc. Only classes where at least 5 children and at least 50% of the class were tested are shown. The distribution of seropositive children in the enrolled classes is only presented in an aggregated form rather than clustered within schools to preserve the deidentification and privacy of the participants.

DISCUSSION

In this study of randomly sampled schools and classes, we found variation in seroprevalence in 6 to 16-year-old children across districts, schools and classes by July 2020, but no indication of major transmission and outbreaks within classes and schools. The overall seroprevalence was not different from a randomly selected adult population living in the same region – pointing to striking underdiagnosis of SARS-CoV-2 infection in children, with only one in 89 cases diagnosed. Contrary to studies of symptomatic infections [17] and some other population-based studies of seroprevalence [16,18], there was a trend of higher seroprevalence in younger children as measured with the main ABCORA test (the trend was not present in seroprevalence estimated based on SenASTriS test). The presence of symptoms was very common (three of four children reported one or several symptoms compatible with a COVID-19 infection) and importantly not specific to the seropositive children. Although no outbreaks were reported in schools at the time of testing in the canton of Zurich (comprising 18% of the Swiss population), seropositive children were detected in more than half of the tested schools and a third of all tested classes. However, the vast majority of classes with seropositive children had only a single seropositive child among the tested children, reflecting low prevalence and no significant clustering within classes after the re-opening of the schools.

By the time of conducting this study, there were few studies focusing on SARS-CoV-2 spread in schools [18–20]. Most of the reported evidence consisted of cases studies of outbreaks in specific schools or reports of contact tracing of index cases in educational settings. Most of the studies reported low secondary attack rates in schools [4,21] but also some conflicting observations of outbreaks [2,22]. Although some studies of seroprevalence had included children [16,23,24], they mostly focused on households and the general population. The management of SARS-CoV-2 transmission in schools was therefore highly debated [25,26]. The present study is unique as one of the first major studies reporting variation in seroprevalence in children from randomly selected schools in a country where the general lock-down on a population level was mild and short (one month), and school closure lasted only for two months.

Although manifest clinical disease of COVID-19 is much less prevalent in children than in adults [17,27,28] and preliminary evidence points to lower susceptibility of children compared to adults [18], our results indicate very similar seroprevalence in adults and children. Similar seroprevalence in adults and school-aged children was found in another region in Switzerland in April [16] and in November 2020 [29]. Intriguingly, we observed a

not statistically significant trend of younger children having higher seroprevalence than older children, when measured with the comprehensive ABCORA 2.0 test. Infections with circulating human coronaviruses (hCoVs) are common in childhood and antibodies to hCoVs 229E, NL63, OC43 and HKU1A are prevalent in the human population and particularly children [30,31]. Cross-reactivity with hCoVs was thus considered in the development of both serology tests used in this study and both tests detect SARS-CoV-2 with high specificity (99.6% for ABCORA 2.0 and 98.4% for SenASTrIS). Importantly, to adjust for the possibility of a few false-positive results, we employed a Bayesian hierarchical model, which adjusts for the accuracy parameters of the tests to estimate population-level seroprevalence. Higher seroprevalence in younger children could be compatible with less feasible social distancing behavior and possibly more vigorous immune response to the virus in early age. The trend could also reflect a chance finding, as substantial proportion of false positives is expected in the low seroprevalence setting, and it was not observed in the SenASTriS test results. Further testing of the cohort and forthcoming studies will show if this trend is observed in the future.

The frequency of both more specific (e.g., fever, cough) and less specific (rhinorrhea, headache, nausea) symptoms [32] was not different among seropositive and seronegative children. In general, symptoms, particularly rhinorrhea, cough, headache and sore throat, were reported frequently, with three of four children reporting any symptoms within the last 6 months in both groups. Anosmia was not reported by any of the seropositive children. The specificity of COVID-19 compatible symptoms, therefore, seems to be lower in children than in adults. Moreover, the range of symptoms reported in children was shown to be different than in adults [33]. Somewhat unspecific symptoms in children, contrary to more specific symptoms in adults [34], could partly explain the high proportion of seropositive children who were not previously diagnosed with RT-PCR. Furthermore, testing indications by health authorities were cautious both for children and adults during the first half of 2020. Testing was recommended only for children with acute upper respiratory tract infection symptoms or acute anosmia, and not recommended in children with rhinitis only or without symptoms [35].

Particular strengths of the design of this study are school-based random sampling, hierarchical data structure, and large sample size, allowing to identify clusters within district, school, grade and class levels. Testing was done in schools and study information presented in multiple formats, including videos in multiple languages, to minimize selection bias within enrolled children. The participation rate of 50% can be considered rather high

for a study in children involving venous blood sampling, and additional children from the invited classes will have the opportunity to be enrolled in subsequent testing phases (October/November 2020, March/April 2021), further increasing participation rate and the size of the cohort.

The study has a few limitations. First, the retrospective evaluation of symptoms over six months could have been subject to recall bias. Second, the study enrolled only 35% (55/156) of the invited schools. Commonly stated reasons for non-participation on school level were constraints in time and human resources and competing participation in other studies. Participation rate on school level varied significantly depending on the district, and for a few districts, a maximum of three invitation rounds was needed to recruit a regionally representative sample. Third, although the schools were open for one to two months directly before the study, they were closed for the two months of the highest community transmission of SARS-CoV-2 in the canton of Zurich [15]. Therefore, the measured seroprevalence in children might be dominated by infections in households rather than school. The follow up of this study will shed more light on transmission in schools.

In conclusion, clustering of SARS-CoV-2 seropositive children within classes and schools was not prominent shortly after reopening of schools in this large population-based study. Seroprevalence was similar to adults, resulting in strikingly fewer diagnosed infections in comparison to the seropositive cases in children than in adults. Considering the time window required for SARS-CoV-2 antibodies to form, this study reflects infection of SARS-CoV-2 until approximately end of May 2020, covering four months of SARS-CoV-2 infection in the community, with two months of school closure and mild lock-down policy. The subsequent testing of parents and school personnel and the follow-up of the children cohort in fall 2020 will yield further evidence on the observed trends and of the spread of SARS-CoV-2 within and outside schools.

Author Contributions

SK and MAP initiated the project and preliminary design, with support of JF. SK, MAP, CB, TR, RJ, JB, AF and AU developed the design and methodology. SK, RJ, AU, TR, JB, AF and CC recruited study participants, collected and managed the data. SRH performed statistical analysis. AT, MH, MaSch, MeSch 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 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.

Competing interests

The authors declare no competing interests to disclose.

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Data sharing statement

Data is 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.

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.

Transparency declaration

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.

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Figure legends

Figure 1 Overall, school level and district estimates of SARS-CoV-2 seroprevalence in children

Weighted point estimates and 95% credible intervals are shown. Districts are ordered by population size.

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Figure 2 Seropositive children in tested schools and school levels

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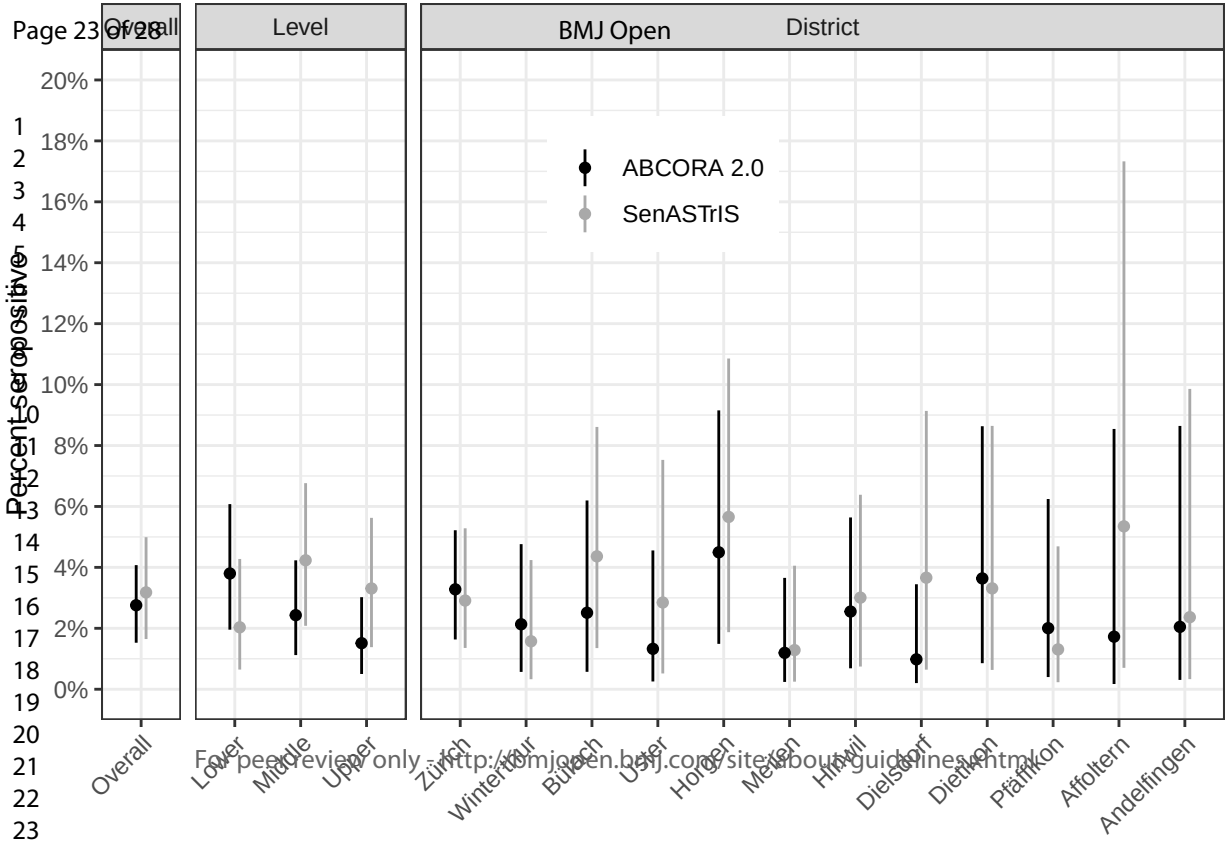
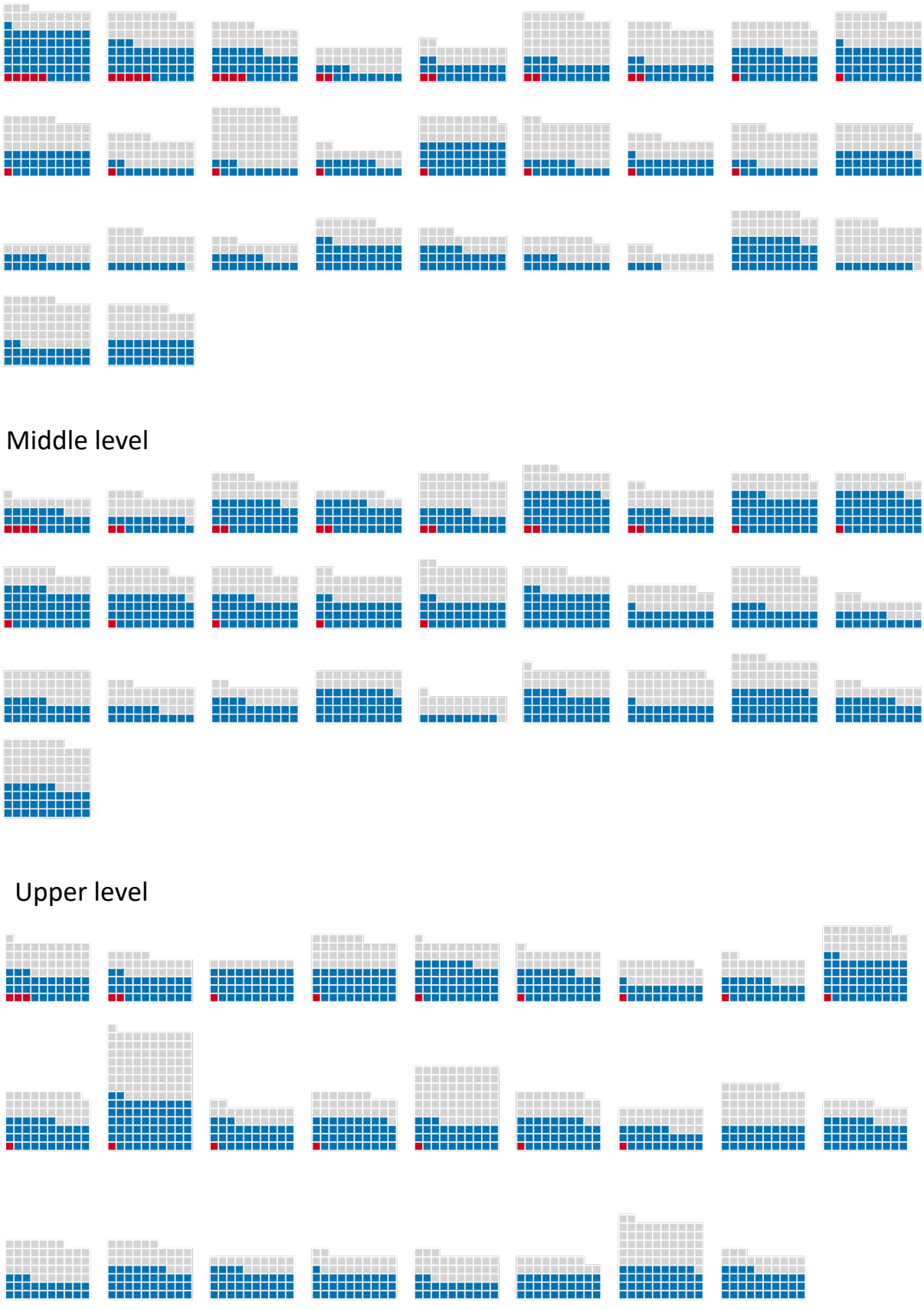
Figure 3 Self-reported symptoms in seropositive and seronegative children in January-June 2020

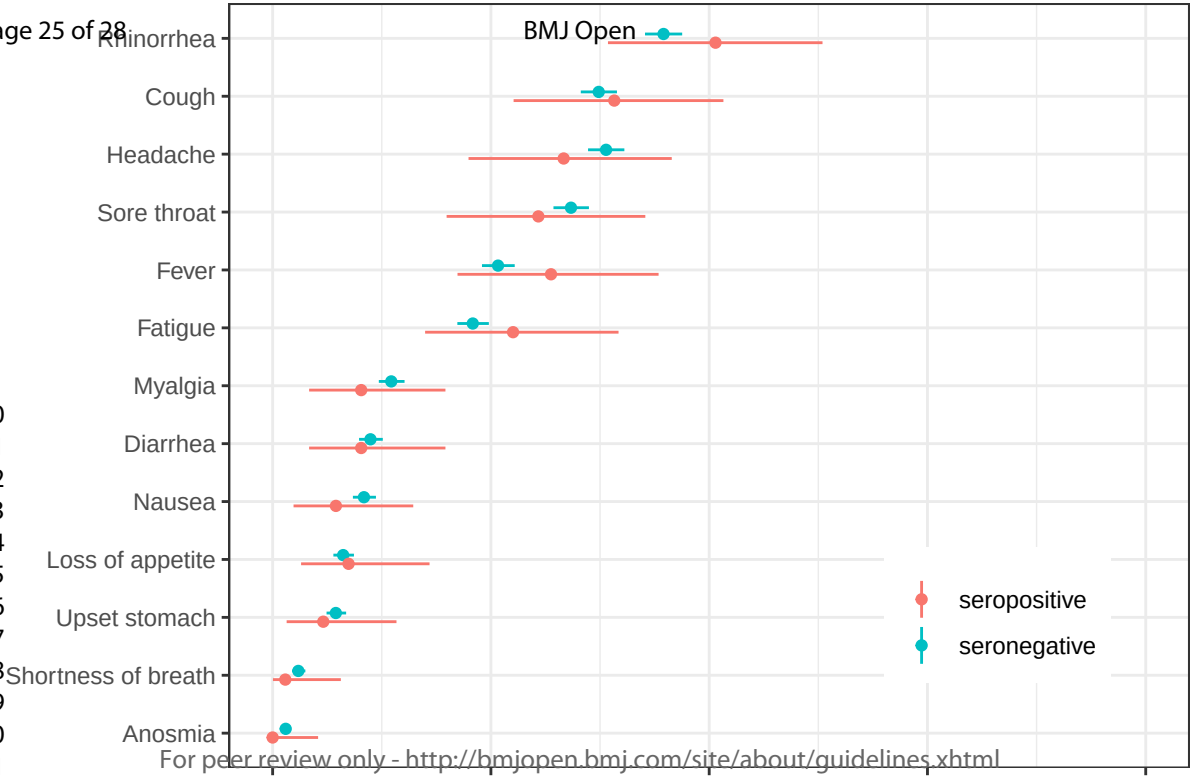
Point estimates and 95% confidence intervals are shown.

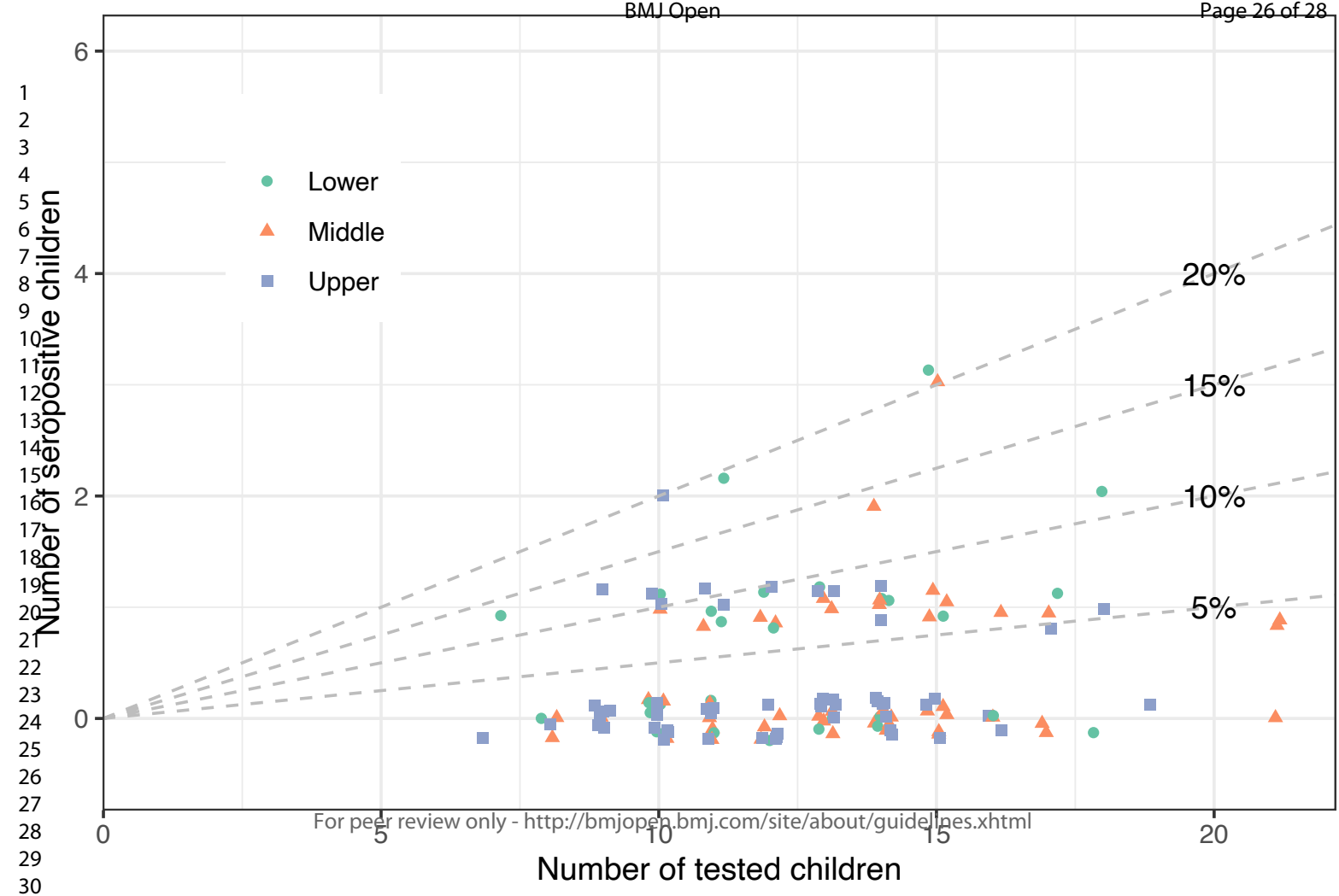
Figure 4 Clustering of seropositive children in classes: number and proportion of seropositive children in the tested classes

Each dot represents a class. Diagonal lines partition the figure into classes with 0-5% of tested seropositive children (below 5% line), 5-10% of tested seropositive children (between 5% and 10% lines), etc. Only classes where at least 5 children and at least 50% of the class were tested are shown. The distribution of seropositive children in the enrolled classes is only presented in an aggregated form rather than clustered within schools to preserve the deidentification and privacy of the participants.









STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No.	Recommendation	Page No.	Relevant text from manuscript
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	5	first cross-sectional analysis of a large cohort of children from randomly selected schools and classes
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	3	
Introduction				
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	5	
Objectives	3	State specific objectives, including any prespecified hypotheses	3, 5	To determine the variation in SARS-CoV-2 seroprevalence in school children across districts, schools, grades, and classes, and the relationship of SARS-CoV-2 seroprevalence with self-reported symptoms.
Methods				
Study design	4	Present key elements of study design early in the paper	6-8	
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	6	
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	6	
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed		

		Case-control study—For matched studies, give matching criteria and the number of controls per case		
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	7-8	
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	7-8	
Bias	9	Describe any efforts to address potential sources of bias	7	
Study size	10	Explain how the study size was arrived at	6	Details are provided in the study protocol.

Continued on next page

Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	7-8
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	7-8
		(*b*) Describe any methods used to examine subgroups and interactions	NA
		(*c*) Explain how missing data were addressed	NA
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	7-8
		(*e*) Describe any sensitivity analyses	NA
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	6
		(b) Give reasons for non-participation at each stage	6
		(c) Consider use of a flow diagram	NA
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	6, 9
		(b) Indicate number of participants with missing data for each variable of interest	6-7
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	
		Cross-sectional study—Report numbers of outcome events or summary measures	9
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	9
		(*b*) Report category boundaries when continuous variables were categorized	NA
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	NA

Continued on next page

Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	9
Discussion			
Key results	18	Summarise key results with reference to study objectives	11
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	12-13
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	13
Generalisability	21	Discuss the generalisability (external validity) of the study results	13
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	14

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ Open

Variation in SARS-CoV-2 seroprevalence across districts, schools and classes: baseline measurements from a cohort of primary and secondary school children in Switzerland

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Title

Variation in SARS-CoV-2 seroprevalence across districts, schools and classes: baseline measurements from a cohort of primary and secondary school children in Switzerland

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Abstract

Objectives: To determine the variation in SARS-CoV-2 seroprevalence in school children, and the relationship with self-reported symptoms.

Design: Baseline measurements of a longitudinal cohort study *(Ciao Corona)* from June-July 2020.

Setting: 55 schools stratified by district in the canton of Zurich, Switzerland.

Participants: 2585 children (1339 girls, median age 11, age range 6-16 years), attending grades 1-2, 4-5 and 7-8.

Main outcome measures: Variation in seroprevalence of SARS-CoV-2 in children across 12 cantonal districts, schools, and grades, assessed with Luminex-based test of four epitopes for IgG, IgA and IgM (ABCORA 2.0). Clustering of cases within classes. Association of seropositivity and symptoms. Comparison with seroprevalence in adult population, assessed with Luminex-based test of IgG and IgA (SenASTriS).

Results: Overall seroprevalence was 2.8% (95% CI 1.5 to 4.1%), ranging from 1.0% to 4.5% across districts. Seroprevalence in grades 1-2 was 3.8% (2.0 to 6.1%), in grades 4-5 – 2.4% (1.1 to 4.2%), and in grades 7-8 – 1.5% (0.5 to 3.0%). At least one seropositive child was present in 36 of 55 (65%) schools and in 44 (34%) of 131 classes where ≥5 children and ≥50% of children within the class were tested. 73% of children reported COVID-19-compatible symptoms since January 2020, with the same frequency in seropositive and seronegative children for all symptoms. Seroprevalence of children and adults was similar (3.2%, 95% CrI 1.7-5.0%, versus 3.6%, 95% CrI 1.7-5.4%). The ratio of confirmed SARS-CoV-2 cumulative incidence to seropositive cases was 1:89 in children and 1:12 in adults.

Conclusions: SARS-CoV-2 seroprevalence was low in children and similar to that in adults by the end of June 2020. Very low ratio of diagnosed to seropositive children was observed. We did not detect clustering of SARS-CoV-2 seropositive children within classes, but the follow-up of this study will shed more light on transmission within schools.

Trial registration: ClinicalTrials.gov NCT04448717. https://clinicaltrials.gov/ct2/show/NCT04448717

Key words: SARS-CoV-2, COVID-19, children, adolescents, school.

Article summary

Strengths and limitations of this study

- This study presents the results of a regionally representative cohort of children, randomly selected on school and class levels, and thus, allowing the analysis of clustering of cases within classes and schools.
- This cross-sectional analysis estimates the seroprevalence in children in June-July in Switzerland, from a period when there is very little evidence about SARS-CoV-2 seroprevalence in children globally.
- Serological test with high sensitivity and specificity was used, and Bayesian hierarchical models were applied to estimate seroprevalence, adjusting for test accuracy parameters.
- Self-reported symptoms might be subject to recall-bias, particularly when reporting retrospectively for a period of over six months.

INTRODUCTION

The transmission of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in the school setting is not well understood [1], partly as schools were closed in many countries during the peaks of the pandemic, partly due to lack of representative studies with random sampling. Anecdotal evidence and case studies suggest that outbreaks can happen in schools [2–4], but it is not clear if they represent outlier events or widely underdiagnosed spread of the infection. The effect of school closures on the community transmission of SARS-CoV-2 ranges from minimal to substantial [5], with some modelling studies even predicting an increase of the total number of deaths [6]. Currently existing or planned population-based studies focusing on SARS-CoV-2 spread in schools are few and small-sized [7,8].

In this study, we present the results of the first cross-sectional analysis of a large cohort of children from randomly selected schools and classes in the canton of Zurich, Switzerland. The cohort study follows the seroprevalence, symptoms, socio-demographic and lifestyle factors of enrolled children from June 2020 to April 2021. The participating children were enrolled from June 16 to July 9, 2020. Schools in Switzerland were closed for a relatively short period (March 16 to May 10) compared to other countries, and lock-down measures were mild. Restaurants, bars and non-essential shops and services were closed on March 17 and events or meetings with over 5 people prohibited on March 20, but no strict confinement at home implemented. These measures were gradually lifted in April-May 2020.

The aim of this analysis is to present the overall estimate of seroprevalence in children and its variation across districts, schools, grades and classes, the association of seroprevalence with self-reported symptoms, and the clustering of seropositive children within classes.

METHODS

Study setting

The study was registered on ClinicalTrials.gov (identifier number NCT04448717). The protocol for this longitudinal cohort study was reported elsewhere [9]. The study is part of a large nationally coordinated research network *Corona Immunitas* in Switzerland [10,11]. The study took place from June 16 to July 9, 2020, in the canton of Zurich, Switzerland. The canton of Zurich comprises 1.5 million residents, roughly 18% of the Swiss population, and includes both urban and rural settings, as well as an ethnically and linguistically diverse population. The first preventive measures in schools were introduced on March 16, 2020, when physical attendance of schools was stopped. Schools were partly reopened on May 10, with a combination of online and on-site teaching with preventive measures (e.g., teaching in smaller groups, school attendance every second day, sports and large group activities limited). Schools were fully reopened on June 7, with minimal preventive measures (e.g., recommended social distancing for teachers, reduction of group events) and otherwise regular operation (e.g., full classrooms with desks mostly facing forward) until the end of the school year on July 17. The prevention measures implemented in schools after May 10 were school-specific and based on the federal and cantonal guidelines [12].

Study procedures

School were selected based on a full list of schools provided by the Educational Department of the canton. Primary schools were randomly selected by a computer program from the list of all primary schools in the respective district. The closest secondary school (often in the same building or area) was then selected. The random sample of primary and secondary schools was stratified by geographic district. Within schools, randomly selected classes in lower school level (grades one to two, attended by 6 to 9-year-old children), middle (grades four to five, 9 to 13-year-old children), and upper school level (grades seven to eight, 12 to 16-year-old children) were invited. Invited grades and classes were selected to ensure that the same cohort of children within the class can be followed until April 2021. Therefore, grades 1-2, 4-5 and 7-8 (but not grades 3, 6, and 9) were included, as they normally stay in the same school and class for the next school-year. We aimed to enroll at least three classes and 40 children per school level. As we were only able to test the children at schools, a major exclusion criterion was a suspected or confirmed infection with SARS-CoV-2 in the given child on the testing date, precluding attendance of school.

Study information, link to study website (www.ciao-corona.ch), and informational videos in multiple languages for schools, parents, and children were sent to school principals and further to families of children from the selected classes. Children were enrolled and venous blood samples taken in schools between June 16 and July 9, 2020. Questionnaires with information on socio-demographics and symptoms compatible with SARS-CoV-2 infection from January to June, 2020, were completed online for the majority of children by their parents in June-July, 2020 (for 3% of children in August-September).

In total, 55 out of 156 invited primary and secondary schools agreed to participate, and 2585 children in 273 out of 274 invited classes (no children participated in one class). Venous blood samples were collected from 2484 children (a sufficient amount of blood could not be obtained from 101 children). An online questionnaire, containing socio-demographic, health, symptoms and quality of life information for the children, and socio-demographic and symptoms information for the household members, was completed for 2288 of all enrolled children. Questionnaires were not filled for 297 children, after several email and phone call reminders.

Serological tests

The primary outcome of the study was the serological results of blood serum samples analyzed with ABCORA 2.0 binding assay of the Institute of Medical Virology (IMV) of the University of Zurich based on the Luminex technology [13]. The test analyzes 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), yielding 12 different measurements. Cut-off values were established against pre-pandemic plasma allowing a high sensitivity (93.3%) and specificity (99.6%). Samples were defined as seropositive for SARS-CoV-2 if at least two of the 12 parameters were above the cut-off.

SARS-CoV-2 seroprevalence in children was compared to that estimated in a random sample from the general population, adjusting for age group and sex, in the same region in June-July 2020. The adult study, like all studies of the Swiss-wide research program *Corona Immunitas* [10,11], used the test SenASTrIS (Sensitive Anti-SARS-CoV-2 Spike Trimer Immunoglobulin Serological) developed by the Centre Hospitalier Universitaire Vaudois (CHUV), the Swiss Federal Institute of Technology in Lausanne (EPFL) and the Swiss Vaccine Center [14]. The test also uses Luminex technology to detect IgG and IgA antibodies binding to the entire trimeric S protein of SARS-CoV-2 and with demonstrated 98.3% sensitivity and 98.4% specificity for the combined testing of IgG and IgA (result declared as positive when

either or both were positive) [14]. In order to compare the seroprevalence estimates in children and adult cohorts, blood serum samples of 2476 children were also analyzed with the SenASTrIS test (samples were insufficient for analysis with the second test for 8 children) and compared to a random population sample of 857 adults who took part in the second phase of the Switzerland-wide *Corona Immunitas* research program [11].

Seroprevalence was also compared to the cumulative incidence of reverse transcription polymerase chain reaction (RT-PCR)-confirmed SARS-CoV-2 infections in adults and children, based on official statistics up to the beginning of June [15].

Statistical analysis

Statistical analysis included descriptive statistics and Bayesian hierarchical modelling to estimate seroprevalence [16]. The Bayesian approach allowed to account for the sensitivity and specificity of the SARS-CoV-2 antibody test and the hierarchical structure of cohort (individual and school levels). The model (Bayesian logistic regression) was adjusted for participants' sex, grade, and geographic district of the school, and included random effects for school levels (lower, middle and upper). In order to compute an estimate representative for the population of the canton of Zurich, we applied post-stratification weights, which adjusted for the total population size of the specific school level and the geographic district. The model and weighing procedure are described in detail in Appendix 1.

The factor of confirmed to total infections was calculated as the ratio of RT-PCR-confirmed cumulative incidence by the end of June 2020 and the estimated seroprevalence. We assessed the clustering of seropositive children within classes, school levels, and schools by studying the distribution of classes with zero, one, or more seropositive children. As the probability of detecting a seropositive child increases with more children tested, we separately assessed the proportion of classes with at least one seropositive child among all class and among classes with ≥5 participating children and ≥50% of children participating from the class.

Patient and public involvement

Several school principals were consulted during the development of the protocol to ensure feasibility of the planned study procedures. Early feedback was collected from invited children and parents, in order to adapt the communication strategies and channels. Numerous online informational sessions, encouraging open exchange and feedback, were organized for invited and enrolled school principals, personnel and parents of the children.

Results of individual tests were communicated to the participants, and overall study results disseminated to participating schools. Findings will be 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).

RESULTS

In total, 55 schools and 2585 children were recruited (1339 (52%) girls, median age 11, age range 6-16 years), 754 (29%) in the lower level, 899 (35%) in the middle, and 932 (36%) in the upper school level. Mean participation rate was 50% of the invited children within invited classes (range from 0% to 94% (0 to 21 children), interquartile range 32% to 63%). Venous blood was collected and analyzed for 2484 children (1278 (51%) girls, median age 11, age range 6 to 16 years).

74 children had SARS-CoV-2 antibodies, resulting in overall weighted seroprevalence of 2.8 % (95% credible interval (CrI) 1.5 to 4.1%), ranging from 1.0% to 4.5% in districts, as measured with ABCORA test (Figure 1). Seroprevalence was 3.8% (95% CrI 2.0 to 6.1%) in grades one to two, 2.4% (95% CrI 1.1 to 4.2%) in grades four to five, and 1.5% (95% CrI 0.5 to 3.0%) in grades seven to eight (Figure 1).

Seroprevalence of children, as measured with the SenASTrIS test of IgG and IgA combined, was very similar to the seroprevalence of randomly selected adults in the same region in June-July 2020 (children 3.2%, 95% CrI 1.7 to 5.0%, versus adults 3.6%, 95% CrI 1.7 to 5.4%). The estimates of seroprevalence in different school levels and districts were somewhat different from those estimated with the primary ABCORA 2.0 test (Figure 1); however, the credible intervals of the estimates overlapped. Seroprevalence measured with SenASTriS test was 2.0% (95% CrI 0.6 to 4.3%) in grades one to two, 4.2% (95% CrI 2.1 to 6.8%) in grades four to five, and 3.3% (95% CrI 1.4 to 5.6%) in grades seven to eight.

Based on the cumulative incidence of SARS-CoV-2 RT-PCR-confirmed cases by the end of June (0.03% for children and 0.24% for adult populations), the ratio of confirmed infections to seropositive cases in children was one to 89, compared to a ratio of one to 12 in the adult population.

Figure 1 Overall, school level and district estimates of SARS-CoV-2 seroprevalence in children

Weighted point estimates and 95% credible intervals are shown. Districts are ordered by population size.
ABCORA 2.0 – Primary estimate based on the ABCORA 2.0 of the Institute of Medical Virology, University of Zurich test.
SenASTrIS – Estimate based on the SenASTrIS test.

At least one seropositive child was present in 36/55 (65%) of tested schools. Within the levels of the schools, at least one seropositive child was present in the lower level of 17/29 (59%) schools, in the middle level of 14/28 (50%) schools, and in the upper level of 16/25 (64%) schools (Figure 2).

Figure 2 Seropositive children in tested schools and school levels

Each square illustrates an invited child. Each block of squares illustrates a school level in a school. Lower and middle levels are both taught in the primary schools; however, lower and middle levels of the same school are not matched in this graph due to protection of participant privacy. The distribution of the invited, tested and seropositive children is depicted only on the school level in the figures of this manuscript to preserve deidentification and privacy of the participants.

No sex differences in seroprevalence were noted (2.8% (95% CrI 1.6 to 4.1%) in girls and 2.7% (95% CrI 1.5 to 4.0%) in boys). 73% of children reported any SARS-CoV-2 compatible symptoms, such as cough, fever, fatigue or diarrhea (see Figure 3 for the full list), between January and June 2020. None of the symptoms was more frequent in seropositive than in seronegative children (Figure 3).

Figure 3 Self-reported symptoms in seropositive and seronegative children in January-June 2020

Point estimates and 95% confidence intervals are shown.

At least one seropositive child was present in 34% (44/131) of classes where ≥5 children and ≥50% of children within the class were tested (Figure 4). Among the classes with at least one seropositive child, 38 (86%) had only one, 4 (9%) had two and 2 (5%) had three seropositive children. When considering all classes regardless of participation rate, 24% (65/273) of classes had at least one seropositive child.

Figure 4 Clustering of seropositive children in classes: number and proportion of seropositive children in the tested classes

Each dot represents a class. Diagonal lines partition the figure into classes with 0-5% of tested seropositive children (below 5% line), 5-10% of tested seropositive children (between 5% and 10% lines), etc. Only classes where at least 5 children and at least 50% of the class were tested are shown. The distribution of seropositive children in the enrolled classes is only presented in an aggregated form rather than clustered within schools to preserve the deidentification and privacy of the participants.

DISCUSSION

In this study of randomly sampled schools and classes, we found variation in seroprevalence in 6 to 16-year-old children across districts, schools and classes by July 2020, but no indication of major transmission and outbreaks within classes and schools. The overall seroprevalence was not different from a randomly selected adult population living in the same region – pointing to striking underdiagnosis of SARS-CoV-2 infection in children, with only one in 89 cases diagnosed. Contrary to studies of symptomatic infections [17] and some other population-based studies of seroprevalence [16,18], there was a trend of higher seroprevalence in younger children as measured with the main ABCORA test (the trend was not present in seroprevalence estimated based on SenASTriS test). The presence of symptoms was very common (three of four children reported one or several symptoms compatible with a COVID-19 infection) and importantly not specific to the seropositive children. Although no outbreaks were reported in schools at the time of testing in the canton of Zurich (comprising 18% of the Swiss population), seropositive children were detected in more than half of the tested schools and a third of all tested classes. However, the vast majority of classes with seropositive children had only a single seropositive child among the tested children, reflecting low prevalence and no significant clustering within classes after the re-opening of the schools.

By the time of conducting this study, there were few studies focusing on SARS-CoV-2 spread in schools [18–20]. Most of the reported evidence consisted of cases studies of outbreaks in specific schools or reports of contact tracing of index cases in educational settings. Most of the studies reported low secondary attack rates in schools [4,21] but also some conflicting observations of outbreaks [2,22]. Although some studies of seroprevalence had included children [16,23,24], they mostly focused on households and the general population. The management of SARS-CoV-2 transmission in schools was therefore highly debated [25,26]. The present study is unique as one of the first major studies reporting variation in seroprevalence in children from randomly selected schools in a country where the general lock-down on a population level was mild and short (one month), and school closure lasted only for two months.

Although manifest clinical disease of COVID-19 is much less prevalent in children than in adults [17,27,28] and preliminary evidence points to lower susceptibility of children compared to adults [18], our results indicate very similar seroprevalence in adults and children. Similar seroprevalence in adults and school-aged children was found in another region in Switzerland in April [16] and in November 2020 [29]. Intriguingly, we observed a

not statistically significant trend of younger children having higher seroprevalence than older children, when measured with the comprehensive ABCORA 2.0 test. Infections with circulating human coronaviruses (hCoVs) are common in childhood and antibodies to hCoVs 229E, NL63, OC43 and HKU1A are prevalent in the human population and particularly children [30,31]. Cross-reactivity with hCoVs was thus considered in the development of both serology tests used in this study and both tests detect SARS-CoV-2 with high specificity (99.6% for ABCORA 2.0 and 98.4% for SenASTrIS). Importantly, to adjust for the possibility of a few false-positive results, we employed a Bayesian hierarchical model, which adjusts for the accuracy parameters of the tests to estimate population-level seroprevalence. Higher seroprevalence in younger children could be compatible with less feasible social distancing behavior and possibly more vigorous immune response to the virus in early age. The trend could also reflect a chance finding, as substantial proportion of false positives is expected in the low seroprevalence setting, and it was not observed in the SenASTriS test results. Further testing of the cohort and forthcoming studies will show if this trend is observed in the future.

The frequency of both more specific (e.g., fever, cough) and less specific (rhinorrhea, headache, nausea) symptoms [32] was not different among seropositive and seronegative children. In general, symptoms, particularly rhinorrhea, cough, headache and sore throat, were reported frequently, with three of four children reporting any symptoms within the last 6 months in both groups. Anosmia was not reported by any of the seropositive children. The specificity of COVID-19 compatible symptoms, therefore, seems to be lower in children than in adults. Moreover, the range of symptoms reported in children was shown to be different than in adults [33]. Somewhat unspecific symptoms in children, contrary to more specific symptoms in adults [34], could partly explain the high proportion of seropositive children who were not previously diagnosed with RT-PCR. Furthermore, testing indications by health authorities were cautious both for children and adults during the first half of 2020. Testing was recommended only for children with acute upper respiratory tract infection symptoms or acute anosmia, and not recommended in children with rhinitis only or without symptoms [35].

Particular strengths of the design of this study are school-based random sampling, hierarchical data structure, and large sample size, allowing to identify clusters within district, school, grade and class levels. Testing was done in schools and study information presented in multiple formats, including videos in multiple languages, to minimize selection bias within enrolled children. The participation rate of 50% can be considered rather high

for a study in children involving venous blood sampling, and additional children from the invited classes will have the opportunity to be enrolled in subsequent testing phases (October/November 2020, March/April 2021), further increasing participation rate and the size of the cohort.

The study has a few limitations. First, the retrospective evaluation of symptoms over six months could have been subject to recall bias. Second, the study enrolled only 35% (55/156) of the invited schools. Commonly stated reasons for non-participation on school level were constraints in time and human resources and competing participation in other studies. Participation rate on school level varied significantly depending on the district, and for a few districts, a maximum of three invitation rounds was needed to recruit a regionally representative sample. Third, although the schools were open for one to two months directly before the study, they were closed for the two months of the highest community transmission of SARS-CoV-2 in the canton of Zurich [15]. Therefore, the measured seroprevalence in children might be dominated by infections in households rather than school. The follow up of this study will shed more light on transmission in schools. Finally, we do not have information on how many eligible children did not attend the testing due to acute infection with SARS-CoV-2. We did not provide testing for such children at home or an alternative date due to limited resources. However, reported total weekly incidence of SARS-CoV-2 infections in the canton of Zurich ranged from 42 to 185 cases (among 1.5 million residents), and 7 to 15 cases among people under 20 years old during the testing period. Considering that, in comparison, over 700 total weekly cases were reported in March-April 2020, we believe that excluding acutely infected children during the testing period could not lead to substantial underestimation of the seroprevalence.

In conclusion, clustering of SARS-CoV-2 seropositive children within classes and schools was not prominent shortly after reopening of schools in this large population-based study. Seroprevalence was similar to adults, resulting in strikingly fewer diagnosed infections in comparison to the seropositive cases in children than in adults. Considering the time window required for SARS-CoV-2 antibodies to form, this study reflects infection of SARS-CoV-2 until approximately end of May 2020, covering four months of SARS-CoV-2 infection in the community, with two months of school closure and mild lock-down policy. The subsequent testing of parents and school personnel and the follow-up of the children cohort in fall 2020 will yield further evidence on the observed trends and of the spread of SARS-CoV-2 within and outside schools.

Author Contributions

SK and MAP initiated the project and preliminary design, with support of JF. SK, MAP, CB, TR, RJ, JB, AF and AU developed the design and methodology. SK, RJ, AU, TR, JB, AF and CC recruited study participants, collected and managed the data. SRH performed statistical analysis. AT, MH, MaSch, MeSch 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 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.

Competing interests

The authors declare no competing interests to disclose.

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Data sharing statement

Data is 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.

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.

Transparency declaration

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.

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Figure legends

Figure 1 Overall, school level and district estimates of SARS-CoV-2 seroprevalence in children

Weighted point estimates and 95% credible intervals are shown. Districts are ordered by population size.

ABCORA 2.0 – Primary estimate based on the ABCORA 2.0 of the Institute of Medical Virology, University of Zurich test. SenASTrIS – Estimate based on the SenASTrIS test.

Figure 2 Seropositive children in tested schools and school levels

Each square illustrates an invited child. Each block of squares illustrates a school level in a school. Lower and middle levels are both taught in the primary schools; however, lower and middle levels of the same school are not matched in this graph due to protection of participant privacy. The distribution of the invited, tested and seropositive children is depicted only on the school level in the figures of this manuscript to preserve deidentification and privacy of the participants.

Figure 3 Self-reported symptoms in seropositive and seronegative children in January-June 2020

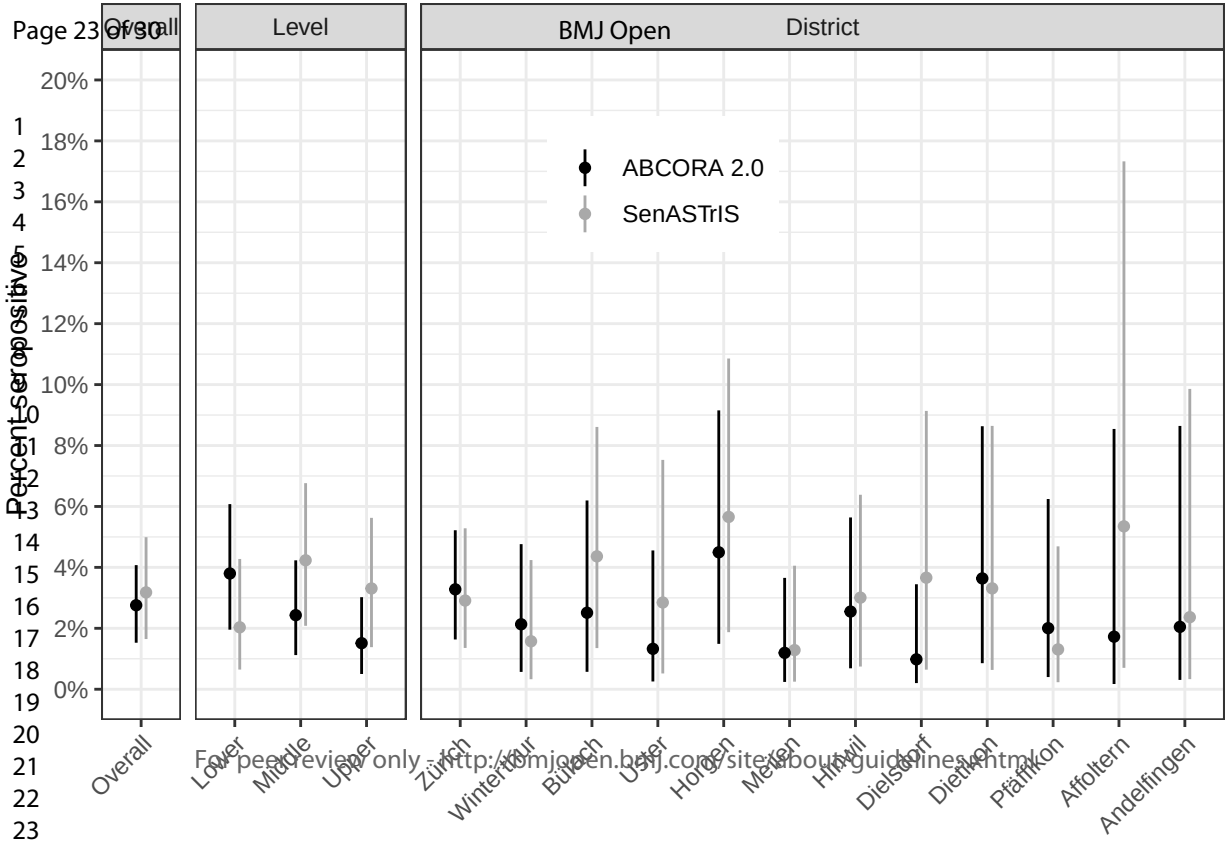
Point estimates and 95% confidence intervals are shown.

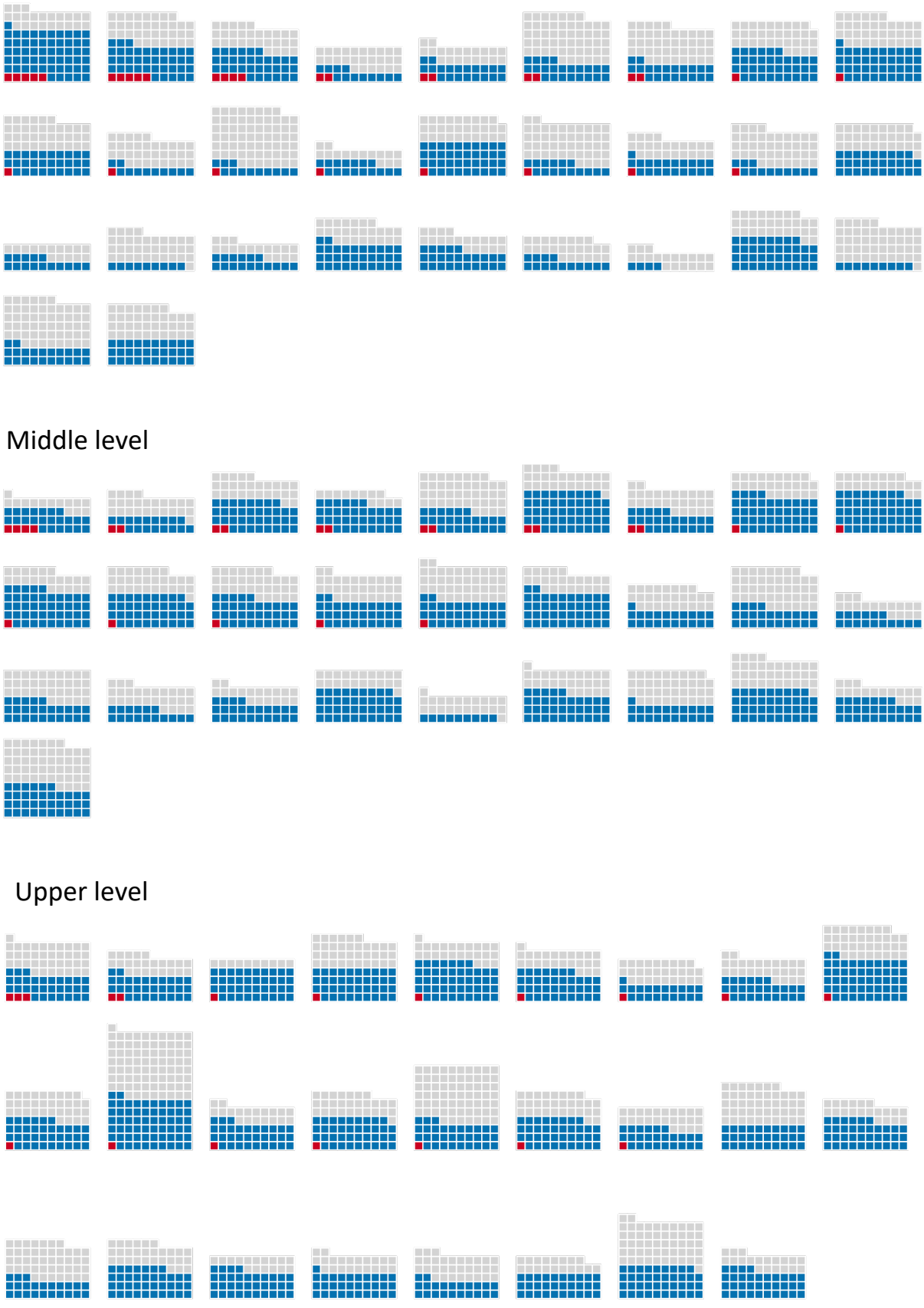
Figure 4 Clustering of seropositive children in classes: number and proportion of seropositive children in the tested classes

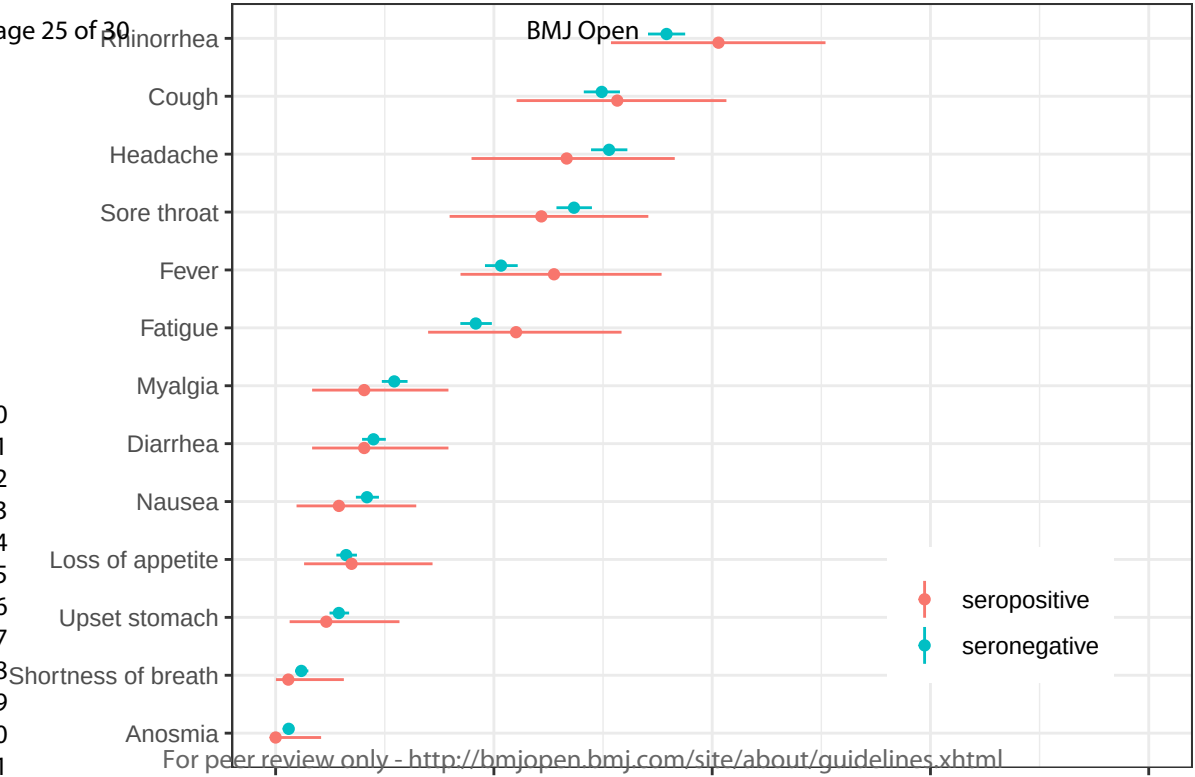
Each dot represents a class. Diagonal lines partition the figure into classes with 0-5% of tested seropositive children (below 5% line), 5-10% of tested seropositive children (between 5% and 10% lines), etc. Only classes where at least 5 children and at least 50% of the class were tested are shown. The distribution of seropositive children in the enrolled classes is only presented in an aggregated form rather than clustered within schools to preserve the deidentification and privacy of the participants.

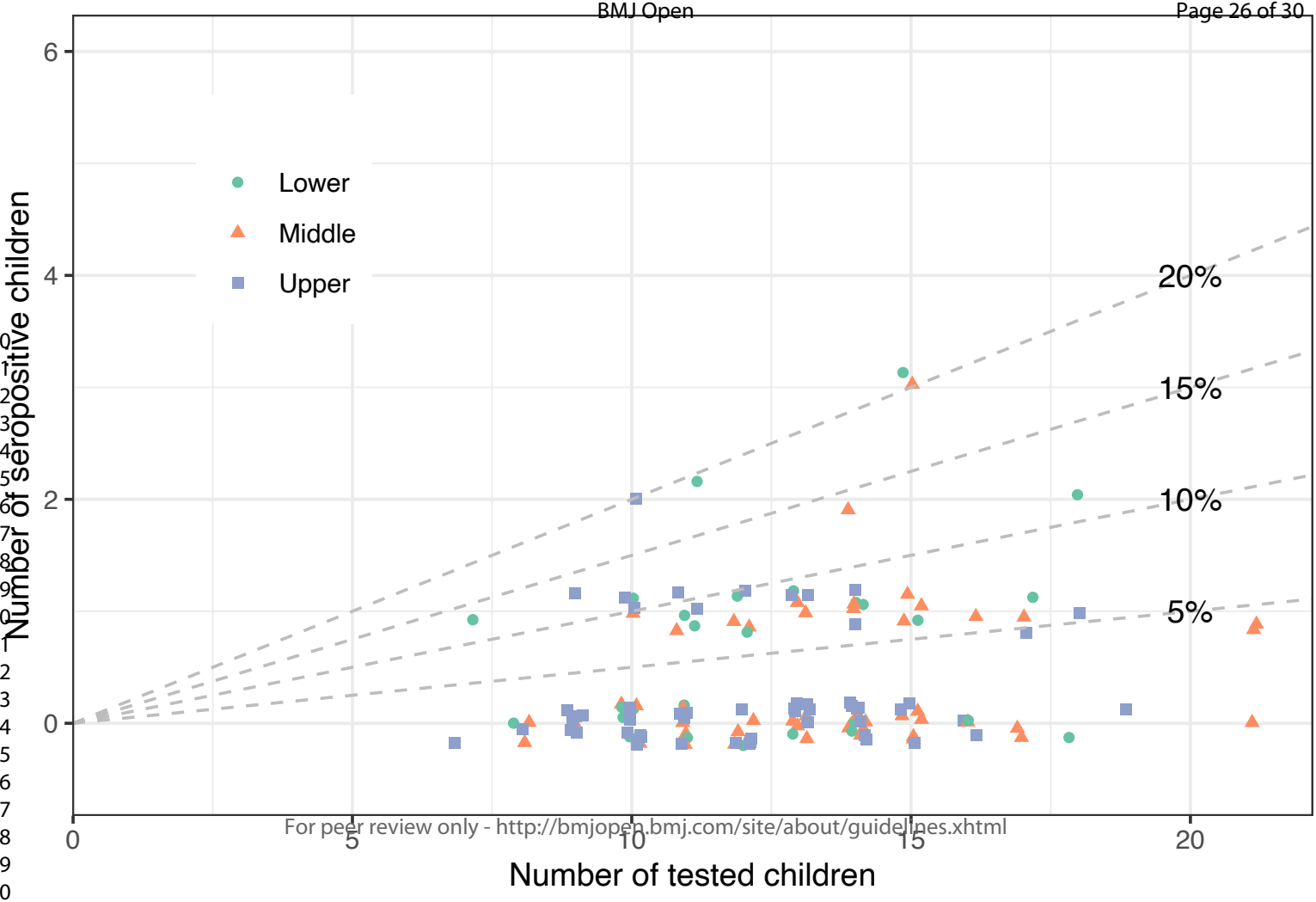
Supplemental material

Appendix 1 Description of Bayesian model









Variation in SARS-CoV-2 seroprevalence across districts, schools and classes: baseline measurements from a cohort of primary and secondary school children in Switzerland

Appendix 1: Description of Bayesian model

A Ulyte, T Radtke, IA Abela, SR Haile, J Blankenberger, R Jung, C Capelli, C Berger, A Frei, M Huber, M Schanz, M Schwarzmueller, A Trkola, J Fehr, MA Puhan and S Kriemler

Methods

The model formulation is essentially that proposed by "Seroprevalence of anti-SARS-CoV-2 IgG antibodies in Geneva, Switzerland (SEROCoV-POP): a population-based study" Stringhini *et al* 2020 (see also the appendix to that paper), with full description and example code in their github repository `HopkinsIDD/serocovpop`. Both *Ciao Corona* and *SEROCoV-POP* are part of the Corona Immunitas program of studies in Switzerland.

Statistical model

In this paper, our goal is to estimate the true underlying seroprevalence of the population of schoolchildren (in grades 1, 2, 4, 5, 7, or 8) as measured in the of the Canton of Zurich, denoted p^*).

We start by estimating the probability that each person in the survey is seropositive using a Bayesian logistic regression model that accounts for school-level clustering, the sensitivity and specificity of the antibody test, each individual's grade level and sex:

$$
\begin{aligned}
x_i &\sim Bernoulli(p_i\theta^+ + (1-p_i) * (1-\theta^-)) \\
logit(p_i) &= \alpha_h + \mathbf{X}_i\boldsymbol{\beta} \\
\alpha_h &\sim Normal(0, \sigma^2) \\
x^+ &\sim Binomial(n^+, \theta^+) \\
x^- &\sim Binomial(n^-, 1-\theta^-)
\end{aligned}
$$

where x_i is the result of the antibody test (in primary analyses) for the ith person ($i = 1, \ldots, N$) in the serosurvey. The sensitivity, θ^+, is determined using n^+ RT-PCR positive controls from the lab validation study, of which x^+ tested positive. The specificity, θ^- , is determined using n^- pre-pandemic negative controls, of which x^- tested positive. The model estimates of the sensitivity and specificity are shown below.

ABCORA 2.0	Cohort	N tested	N positive	N negative
SARS-CoV-2 PCT Positive	104	97	7	93.27% sensitivity
pre-pandemic healthy	251	1	250	99.6% specificity

The probability of observing a diagnostic positive is a function of the true positive rate and the false negative rate with regards to the true underlying probability of seropositivity p_i for that person. This probability itself is a function of covariates $\mathbf{X}$, which consists of sex, grade level, and timepoint of testing, and their coefficients $\boldsymbol{\beta}$, and a random effect for school, α_h $(h = 1, \ldots, H)$, with variance σ^2. We used naive priors on all parameters to allow for an exploration of the parameter space. The priors on the sensitivity and specificity were flat from 0 to 1, equivalent to $Uniform(0, 1)$ or $Beta(1, 1)$. We used weak $Normal(0, 1)$ priors for the logistic regression coefficients $\boldsymbol{\beta}$. The prior on the standard deviation of the school effect, σ, was flat from 0 to infinity.

We implemented this model in the Stan probabilistic programming language and used the RStan package in R to run the model and analyse outputs. We ran 5,000 iterations (4 chains with 1,500 iterations each with 250 for warm-up) and assessed convergence visually.

Seroprevalence estimate

We estimated the seroprevalence p^* by post-stratifying the posterior samples of our parameter estimates to match the demographics of all schoolchildren in grades 1, 2, 4, 5, 7, or 8 in the Canton of Zurich. For every combination of grade level and sex, as well as timepoint of testing, we estimated the probability of seropositivity for each posterior draw of β and σ. We can find estimate the weekly seroprevalence by taking a weighted average of the p, where weights are determined by the demographic distribution of the Canton of Zurich:

$$p = \int_0^1 logit^{-1}(\mathbf{X}\beta + \sigma\Phi^{-1}(t))dt$$

$$p^* = \sum_{sex} \sum_{grade} \frac{pop_{sex,grade} * p_{sex,grade}}{pop}$$

where $\Phi^{-1}(t)$ is the quantile function of a standard normal distribution, $pop_{sex,grade}$ is the population of each demographic "cell" and *pop* is the school age population of the Canton of Zurich. We estimate the average probability of seropositivity for the population in each demographic cell by integrating across all values of a logit-normal distribution with the standard deviation defined by the school random effect σ.

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No.	Recommendation	Page No.	Relevant text from manuscript
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	5	first cross-sectional analysis of a large cohort of children from randomly selected schools and classes
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	3	
Introduction				
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	5	
Objectives	3	State specific objectives, including any prespecified hypotheses	3, 5	To determine the variation in SARS-CoV-2 seroprevalence in school children across districts, schools, grades, and classes, and the relationship of SARS-CoV-2 seroprevalence with self-reported symptoms.
Methods				
Study design	4	Present key elements of study design early in the paper	6-8	
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	6	
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	6	
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed		

		Case-control study—For matched studies, give matching criteria and the number of controls per case		
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	7-8	
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	7-8	
Bias	9	Describe any efforts to address potential sources of bias	7	
Study size	10	Explain how the study size was arrived at	6	Details are provided in the study protocol.

Continued on next page

Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	7-8
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	7-8
		(*b*) Describe any methods used to examine subgroups and interactions	NA
		(*c*) Explain how missing data were addressed	NA
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	7-8
		(*e*) Describe any sensitivity analyses	NA
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	6
		(b) Give reasons for non-participation at each stage	6
		(c) Consider use of a flow diagram	NA
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	6, 9
		(b) Indicate number of participants with missing data for each variable of interest	6-7
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	
		Cross-sectional study—Report numbers of outcome events or summary measures	9
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	9
		(*b*) Report category boundaries when continuous variables were categorized	NA
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	NA

Continued on next page

Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	9
Discussion			
Key results	18	Summarise key results with reference to study objectives	11
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	12-13
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	13
Generalisability	21	Discuss the generalisability (external validity) of the study results	13
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	14

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.