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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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1 Title

2 Variation in SARS-CoV-2 seroprevalence in primary and secondary school children across
3 districts, schools and classes 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 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 $\geq 50\%$ 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.

90

91 **Article summary**

92

93 **Strengths and limitations of this study**

- 94
- 95 • This study presents the results of a regionally representative cohort of children,
96 randomized on school and class levels, and thus, allowing the analysis of clustering of
97 cases within schools.
 - 98 • This cross-sectional analysis estimates the seroprevalence in children in June-July in
99 Switzerland, from a period when there is very little evidence about SARS-CoV-2
100 seroprevalence in children globally.
 - 101 • Serological test with high sensitivity and specificity was used, and Bayesian
102 hierarchical models were additionally applied to estimate seroprevalence, adjusting
103 for test accuracy parameters.
 - 104 • Self-reported symptoms might be subject to recall-bias, particularly when reporting
105 retrospectively for a period of over six months.
- 106

107 INTRODUCTION

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

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

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

126
127

128 METHODS

129 The study was registered on ClinicalTrials.gov (identifier number NCT04448717). The
130 protocol for this longitudinal cohort study was reported elsewhere [8]. The study is part of a
131 large nationally coordinated research network *Corona Immunitas* in Switzerland [9,10].
132 The study took place from June 16 to July 9, 2020, in the canton of Zurich, Switzerland. The
133 canton of Zurich comprises 1.5 million residents, roughly 18% of the Swiss population, and
134 includes both urban and rural settings, as well as an ethnically and linguistically diverse
135 population. The first preventive measures in schools were introduced on March 16, 2020,
136 when physical attendance of schools was stopped. Schools were partly reopened on May
137 10, with a combination of online and on-site teaching with preventive measures (e.g.,
138 teaching in smaller groups, school attendance every second day, sports and large group
139 activities limited). Schools were fully reopened on June 7, with minimal preventive measures
140 (e.g., recommended social distancing for teachers, reduction of group events) and otherwise
141 regular operation (e.g., full classrooms with desks mostly facing forward) until the end of the
142 school year on July 17. The prevention measures implemented in schools after May 10 were
143 school-specific and based on the federal and cantonal guidelines [11].

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

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

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

165 In total, 55 out of 156 invited primary and secondary schools agreed to participate, and
166 2585 children in 273 out of 274 invited classes (no children participated in one class).

167 Venous blood samples were collected from 2484 children (a sufficient amount of blood
168 could not be obtained from 101 children). An online questionnaire, containing socio-
169 demographic, health, symptoms and quality of life information for the children, and socio-
170 demographic and symptoms information for the household members, was completed for
171 2288 of all enrolled children. Questionnaires were not filled for 297 children, after several
172 email and phone call reminders.

173 Blood samples were analyzed with ABCORA 2.0 binding assay of the Institute of
174 Medical Virology (IMV) of the University of Zurich based on the Luminex technology. The
175 test analyzes immunoglobulins G (IgG), M (IgM) and A (IgA) against four SARS-CoV-2 targets
176 (receptor binding domain (RBD), spike proteins S1 and S2, and the nucleocapsid protein (N),
177 yielding 12 different measurements. Cut-off values were established against pre-pandemic
178 plasma allowing a high sensitivity (93.3%) and specificity (99.6%). Samples were defined as
179 seropositive for SARS-CoV-2 if at least two of the 12 parameters were above the cut-off.
180 SARS-CoV-2 seroprevalence in children was compared to that estimated in a random sample
181 from the general population, adjusting for age group and sex, in the same region in June-
182 July 2020. The adult study, like all studies of the Swiss-wide research program *Corona*
183 *Immunitas* [9,10], used the test SenASTrIS (Sensitive Anti-SARS-CoV-2 Spike Trimer
184 Immunoglobulin Serological) developed by the Centre Hospitalier Universitaire Vaudois
185 (CHUV), the Swiss Federal Institute of Technology in Lausanne (EPFL) and the Swiss Vaccine
186 Center [12]. The test also uses Luminex technology to detect IgG and IgA antibodies binding
187 to the entire trimeric S protein of SARS-CoV-2 and with demonstrated 98.3% sensitivity and
188 98.4% specificity for the combined testing of IgG and IgA (result declared as positive when
189 either or both were positive) [12]. In order to compare the seroprevalence estimates in
190 children and adult cohorts, a sample of 2476 collected children blood samples was also
191 analyzed with the SenASTrIS test (samples were insufficient for analysis with the second test
192 for 8 children) and compared to a random population sample of 857 adults who took part in
193 the second phase of the Switzerland-wide *Corona Immunitas* research program [10].

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

1 196 and children, based on official statistics up to the beginning of June [13]. Statistical analysis
2 197 included descriptive statistics and Bayesian hierarchical modelling to estimate
3
4 198 seroprevalence, accounting for the sensitivity and specificity of the SARS-CoV-2 antibody
5
6 199 test, the hierarchical structure of cohort (individual and school levels), and post-
7
8 200 stratification weights, which adjusted for the population-level grade level and geographic
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10 201 district [14]. The factor of confirmed to total infections (dark figure) was calculated as the
11
12 202 ratio of RT-PCR-confirmed cumulative incidence by the end of June 2020 and the estimated
13
14 203 seroprevalence.
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204

205 **Patient and public involvement**

18 206 Several school principals were consulted during the development of the protocol to ensure
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20 207 feasibility of the planned study procedures. Early feedback was collected from invited
21
22 208 children and parents, in order to adapt the communication strategies and channels.
23
24 209 Numerous online informational sessions, encouraging open exchange and feedback, were
25
26 210 organized for invited and enrolled school principals, personnel and parents of the children.
27
28 211 Results of individual tests were communicated to the participants, and overall study results
29
30 212 disseminated to participating schools. Findings will be disseminated in lay language in the
31
32 213 national and local press, to the national and regional educational and public health
33
34 214 departments and on the website of the study (www.ciao-corona.ch).
35

215

216 RESULTS

217 In total, 55 schools and 2585 children were recruited (1339 (52%) girls, median age 11, age
218 range 6-16 years), 754 (29%) in the lower level, 899 (35%) in the middle, and 932 (36%) in
219 the upper school level. Mean participation rate was 50% of the invited children within
220 invited classes (range from 0% to 94% (0 to 21 children), interquartile range 32% to 63%).
221 Venous blood was collected and analyzed for 2484 children (1278 (51%) girls, median age
222 11, age range 6 to 16 years).

223 74 children had SARS-CoV-2 antibodies, resulting in overall weighted seroprevalence
224 of 2.8 % (95% credible interval (CrI) 1.5 to 4.1%), ranging from 1.0% to 4.5% in districts
225 (Figure 1). Seroprevalence was 3.8% (95% CrI 2.0 to 6.1%) in grades one to two, 2.4% (95%
226 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
227 eight (Figure 1).

228 Seroprevalence of children, as measured with the SenASTrIS test of IgG and IgA
229 combined, was very similar to the seroprevalence of randomly selected adults in the same
230 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
231 estimates of seroprevalence in the children cohort, as measured with the SenASTrIS test, in
232 different school levels and districts were similar to those estimated with the primary
233 ABCORA 2.0 test (Figure 1).

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

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

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

244 SenASTrIS – Estimate based on the SenASTrIS test.

246 At least one seropositive child was present in 36/55 (65%) of tested schools. Within the
247 levels of the schools, at least one seropositive child was present in the lower level of 17
248 (59%) out of 29 tested schools, in the middle level of 14 (50%) out of 28 tested schools, and
249 in the upper level of 16 (64%) out of 25 tested schools (Figure 2).

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

1 252 with at least one seropositive child, 38 (86%) had only one, 4 (9%) had two and 2 (5%) had
2 253 three seropositive children. When considering all classes regardless of participation rate,
3
4 254 24% of classes had at least one seropositive child; whereas when considering higher
5
6 255 inclusion threshold of ≥ 15 children and $\geq 60\%$ of the class tested, 45% of classes had at least
7
8 256 one seropositive child.

9 257

10
11 258 **Figure 2** Seropositive children in tested schools and school levels

12
13 259 Each square illustrates an invited child. Each block of squares illustrates a school level in a school. Lower
14 260 and middle levels are both taught in the primary schools; however, lower and middle levels of the same
15 261 school are not matched in this graph due to protection of participant privacy. The distribution of the
16 262 invited, tested and seropositive children is depicted only on the school level in the figures of this
17 263 manuscript to preserve deidentification and privacy of the participants.

18 264

19 265 **Figure 3** Clustering of seropositive children in classes: number and proportion of

20 266 seropositive children in the tested classes

21
22 267 Only classes where at least 5 children and at least 50% of the class were tested are shown. The
23 268 distribution of seropositive children in the enrolled classes is only presented in an aggregated form to
24 269 preserve the deidentification and privacy of the participants.

25 270

26 271 No sex differences in seroprevalence were noted (2.8% (95% CrI 1.6 to 4.1%) in girls and
27 272 2.7% (95% CrI 1.5 to 4.0%) in boys). 73% of children reported any SARS-CoV-2 compatible
28 273 symptoms, such as cough, fever, fatigue or diarrhea (see Figure 4 for the full list), between
29 274 January and June 2020. None of the symptoms was more frequent in seropositive than in
30 275 seronegative children (Figure 4).

31 276

32 277 **Figure 4** Self-reported symptoms in seropositive and seronegative children in January-June
33 278 2020

34 279 Point estimates and 95% confidence intervals are shown.

35 280

282 DISCUSSION

1
2 283 In this study of randomly sampled schools and classes by July 2020, we found variation in
3
4 284 seroprevalence in 6 to 16-year-old children across districts, schools and classes, but no
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6 285 indication of major transmission and outbreaks within schools. The overall seroprevalence
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8 286 was not different from a randomly selected adult population living in the same region –
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10 287 pointing to striking underdiagnosis of SARS-CoV-2 infection in children, with only one in 89
11
12 288 cases diagnosed. Such a high proportion of seropositive cases not previously diagnosed with
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14 289 RT-PCR is likely explained by our observation that symptoms do not seem to be suggestive
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16 290 of a SARS-CoV-2 infection in children, contrary to more specific symptoms in adults [15], and
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18 291 by cautious testing indications by health authorities, with similar indications for children and
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20 292 adults during the first half of 2020. Contrary to studies of symptomatic infections [16] and
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22 293 some other population-based studies of seroprevalence [17], there was a trend of higher
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24 294 seroprevalence in younger children. Although no outbreaks were reported in schools at the
25
26 295 time of testing in the canton of Zurich (comprising 18% of the Swiss population),
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28 296 seropositive children were detected in more than half of the tested schools and a third of all
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30 297 tested classes. However, the vast majority of classes with seropositive children had only a
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32 298 single seropositive child among the tested children, reflecting low prevalence and no
33
34 299 significant clustering within classes after the re-opening of the schools. The presence of
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36 300 symptoms was very common (three of four children reported one or several symptoms
37
38 301 compatible with a COVID-19 infection) and importantly not specific to the seropositive
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40 302 children.

41
42 303 Currently, there are few studies focusing on SARS-CoV-2 spread in schools [17–19].
43
44 304 Most of the reported evidence consists of cases studies of outbreaks in specific schools or
45
46 305 reports of contact tracing of index cases in educational settings. Mostly low secondary
47
48 306 attack rates in schools are reported [4,20], but there are also conflicting observations of
49
50 307 outbreaks [2,21]. Although some studies of seroprevalence have included children
51
52 308 [14,22,23], they mostly focused on households and the general population. The
53
54 309 management of SARS-CoV-2 transmission in schools is therefore highly debated [24,25]. The
55
56 310 present study is thus unique as one of the first major studies reporting variation in
57
58 311 seroprevalence in children from randomly selected schools in a country where the general
59
60 312 lock-down on a population level was mild and short (one month), and school closure lasted
313 only for two months.

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

1 316 compared to adults [17], our results indicate very similar seroprevalence in adults and
2 317 children. Intriguingly, we observed a not statistically significant trend of younger children
3
4 318 having higher seroprevalence than older children, when measured with the comprehensive
5
6 319 ABCORA 2.0 test. Infections with circulating human coronaviruses (hCoVs) are common in
7
8 320 childhood and antibodies to hCoVs 229E, NL63, OC43 and HKU1A are prevalent in the
9
10 321 human population and particularly children [27,28]. Cross-reactivity with hCoVs was thus
11
12 322 considered in the development of both serology tests used in this study and both tests
13
14 323 detect SARS-CoV-2 with high specificity (99.6% for ABCORA 2.0 and 98.4% for SenASTriS).
15
16 324 Importantly, to adjust for the possibility of a few false-positive results, we employed a
17
18 325 Bayesian hierarchical model, which adjusts for the accuracy parameters of the tests to
19
20 326 estimate population-level seroprevalence. Higher seroprevalence in younger children could
21
22 327 be related to virtually impossible social distancing behaviour, but also to a possibly more
23
24 328 vigorous immune response to the virus in early age that will be interesting to explore in
25
26 329 forthcoming studies.

27 330 The frequency of both more specific (e.g., fever, cough) and less specific (rhinorrhea,
28 331 headache, nausea) symptoms [29] was not different among seropositive and seronegative
29 332 children. In general, symptoms, particularly rhinorrhea, cough, headache and sore throat,
30 333 were reported frequently, with three of four children reporting any symptoms within the
31 334 last 6 months in both groups. The specificity of COVID-19 compatible symptoms, therefore,
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33 335 seems to be lower in children than in adults. Moreover, the range of symptoms reported in
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35 336 children has shown to be different compared to adults [30].

36
37 337 Particular strengths of the design of this study are school-based random sampling,
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39 338 hierarchical data structure, and large sample size, allowing to identify clusters within
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41 339 district, school, grade and class levels. Testing was done in schools and study information
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43 340 presented in multiple formats, including videos in multiple languages, to minimize selection
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45 341 bias within enrolled children. The participation rate of 50% can be considered rather high
46
47 342 for a study in children involving venous blood sampling, and additional children from the
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49 343 invited classes will have the opportunity to be enrolled in subsequent testing phases
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51 344 (October/November 2020, March/April 2021), further increasing participation rate and the
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53 345 size of the cohort.

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55 346 The study has a few limitations. First, the retrospective evaluation of symptoms over
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57 347 six months could have been subject to recall bias. Second, the study enrolled only 35%
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59 348 (55/156) of the invited schools. Commonly stated reasons for non-participation on school
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349 level were constraints in time and human resources and competing participation in other

1 350 studies. Participation rate varied significantly depending on the district, and for a few
2 351 districts, the schools had to be invited with a maximum of three invitation rounds to have a
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4 352 regionally representative sample. Third, although the schools were open for one to two
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6 353 months directly before the study, they were closed for the two months of the highest
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8 354 community transmission of SARS-CoV-2 in the canton of Zurich [13]. Therefore, the
9
10 355 measured seroprevalence in children might be dominated by infections in households rather
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12 356 than school. The follow up of this study will shed more light on transmission in schools.

13 357 In conclusion, clustering of SARS-CoV-2 seropositive children within schools and
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15 358 grades was not prominent shortly after reopening of schools in this large population-based
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17 359 study. Seroprevalence was similar to adults and higher in lower grade compared to higher
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19 360 grade children, resulting in a strikingly higher ratio of diagnosed to seropositive cases than
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21 361 in adults, in particular in the very young children. Considering the time window required for
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23 362 SARS-CoV-2 antibodies to form, this study reflects infection of SARS-CoV-2 until
24
25 363 approximately end of May 2020, covering four months of SARS-CoV-2 infection in the
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27 364 community, with two months of school closure and mild lock-down policy. The subsequent
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29 365 testing of parents and school personnel and the follow-up of the children cohort in fall 2020
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31 366 will yield further evidence of the spread of SARS-CoV-2 within and outside schools.

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368 Author Contributions

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2 369 SK and MAP initiated the project and preliminary design, with support of JF. SK, MAP, CB,
3
4 370 TR, RJ, JB, AF and AU developed the design and methodology. SK, RJ, AU, TR, JB, AF and CC
5
6 371 recruited study participants, collected and managed the data. SRH performed statistical
7
8 372 analysis. AT, MH, MaSch, MeSch and IA developed the serology analysis plan, supervised,
9
10 373 conducted and evaluated the serology tests. AU wrote the first draft of the manuscript. All
11
12 374 authors contributed to the design of the study and interpretation of its results, and revised
13
14 375 and approved the manuscript for intellectual content. SK is the guarantor and accepts full
15
16 376 responsibility for the work and the conduct of the study, had access to the data, and
17
18 377 controlled the decision to publish. The corresponding author SK attests that all listed
19
20 378 authors meet authorship criteria and that no others meeting the criteria have been omitted.
21
22 379

380 Competing interests

23
24 381 The authors declare no competing interests to disclose.
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26 382

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27
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29
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31
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33
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37
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39
40 390 role in the design and conduct of the study; collection, management, analysis, and
41
42 391 interpretation of the data; preparation, review, or approval of the manuscript; and decision
43
44 392 to submit the manuscript for publication. All authors had full access to all data analysis
45
46 393 outputs (reports and tables) and take responsibility for their integrity and accuracy
47
48 394

395 Data sharing statement

49
50 396 Data is still being collected for the cohort study *Ciao Corona*. Deidentified participant data
51
52 397 might be available on reasonable request by email to the corresponding author at later
53
54 398 stages of the study.
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400 Ethics approval

1 401 The study was approved by the Ethics Committee of the Canton of Zurich, Switzerland
2 402 (2020-01336). All participants provided written informed consent before being enrolled in
3
4 403 the study.

5 404

7 405 **Transparency declaration**

9 406 The lead authors affirm that the manuscript is an honest, accurate, and transparent account
10
11 407 of the study being reported, no important aspects of the study have been omitted, and any
12
13 408 discrepancies from the study as originally planned and registered have been explained.

14 409

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504 Figure legends

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

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

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

512

513 **Figure 2** Seropositive children in tested schools and school levels

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

520

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

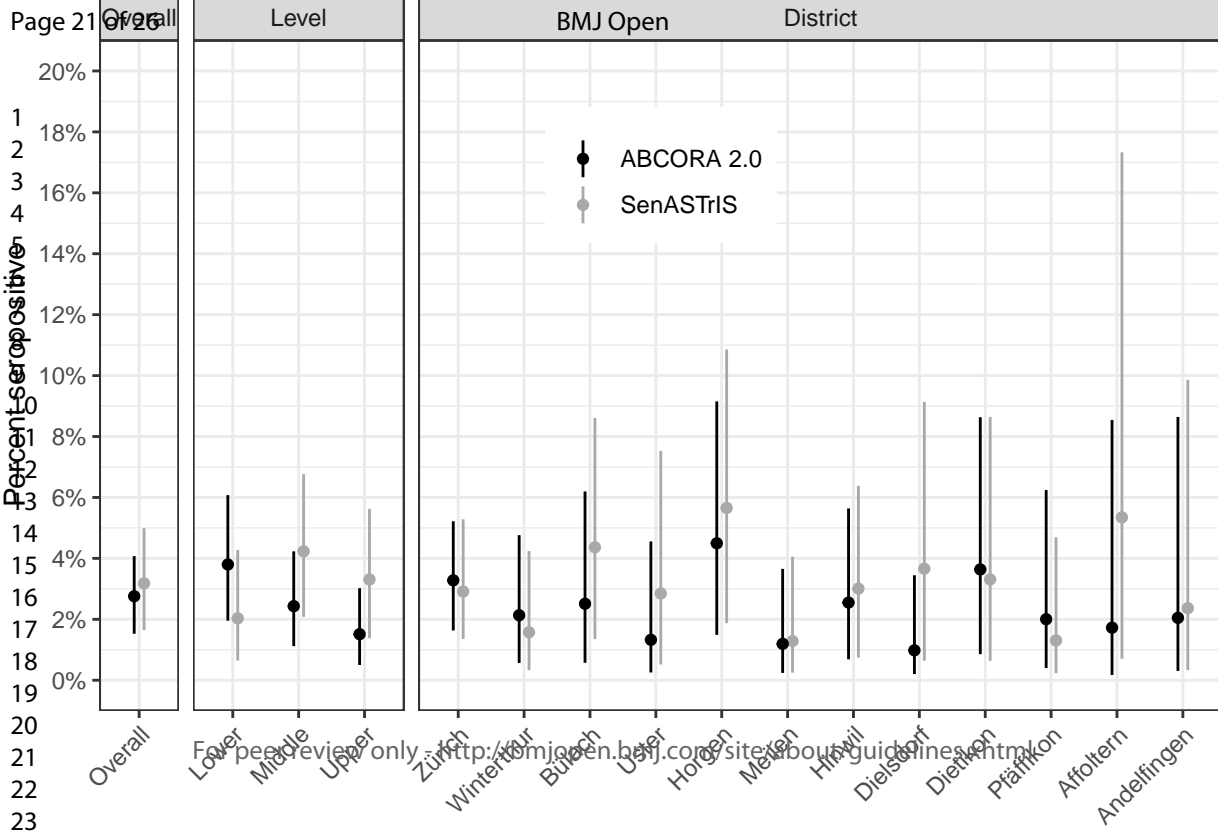
523 Only classes where at least 5 children and at least 50% of the class were tested are shown.
524 The distribution of seropositive children in the enrolled classes is only presented in an
525 aggregated form to preserve the deidentification and privacy of the participants.

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527 **Figure 4** Self-reported symptoms in seropositive and seronegative children in January-June
528 2020

529 Point estimates and 95% confidence intervals are shown.

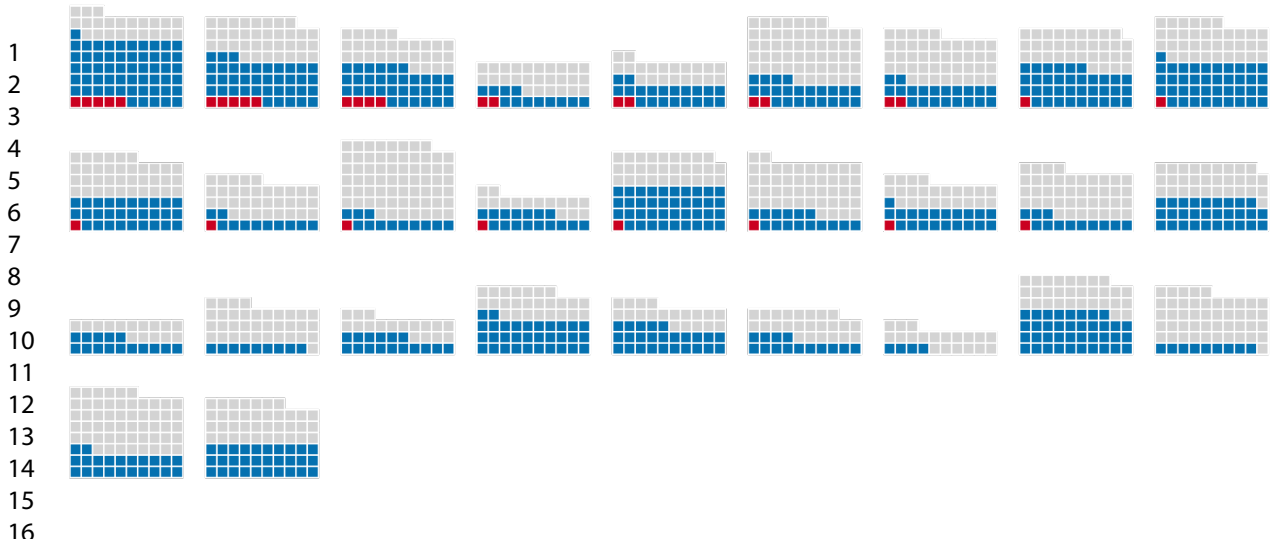
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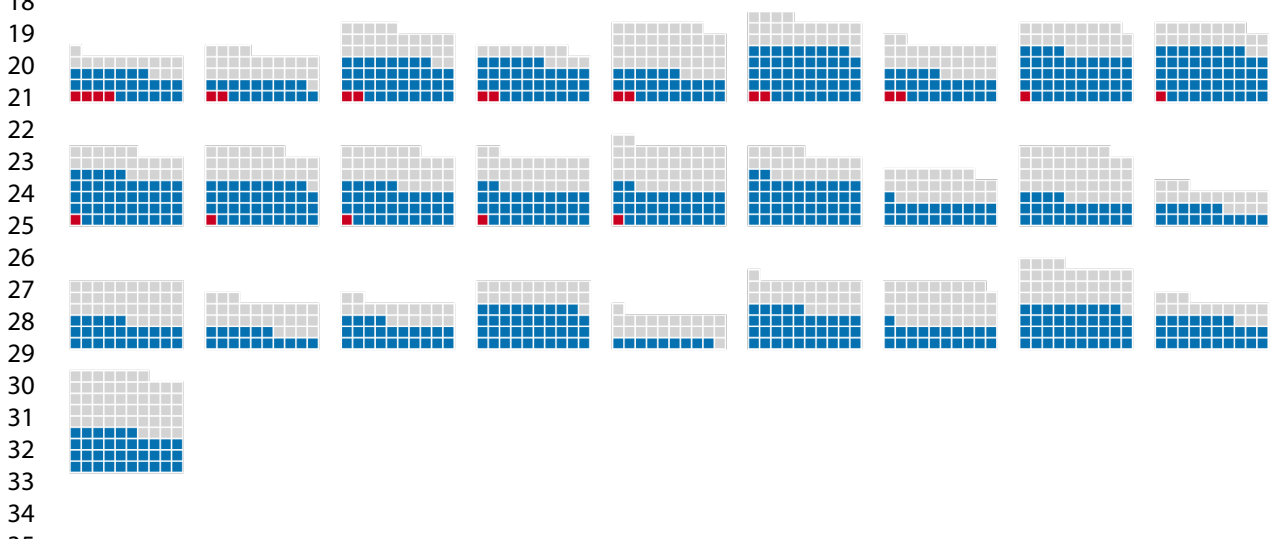
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Lower level

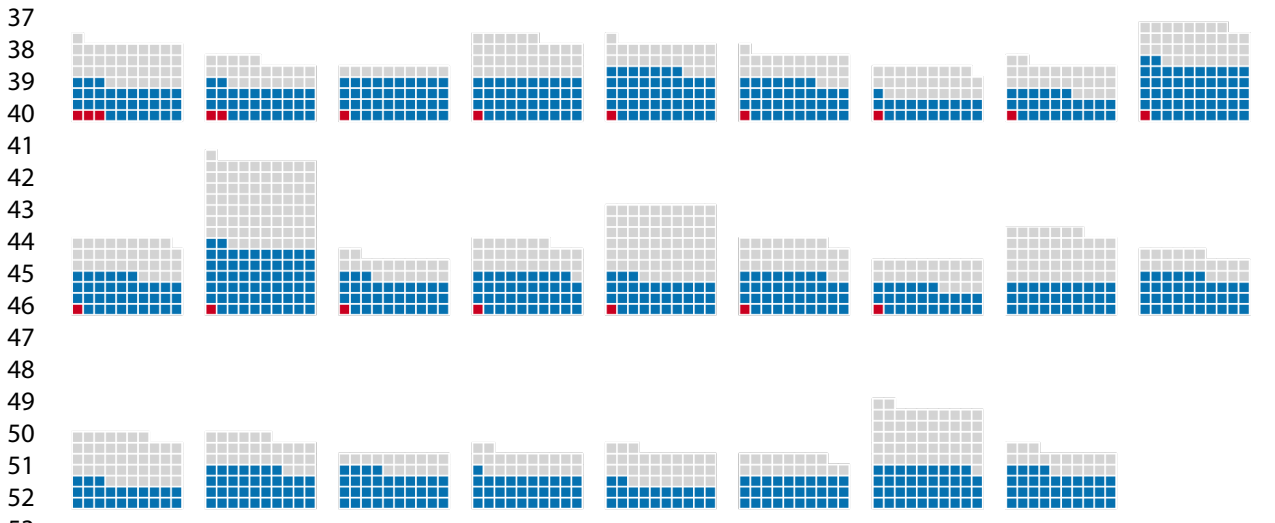
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Middle level

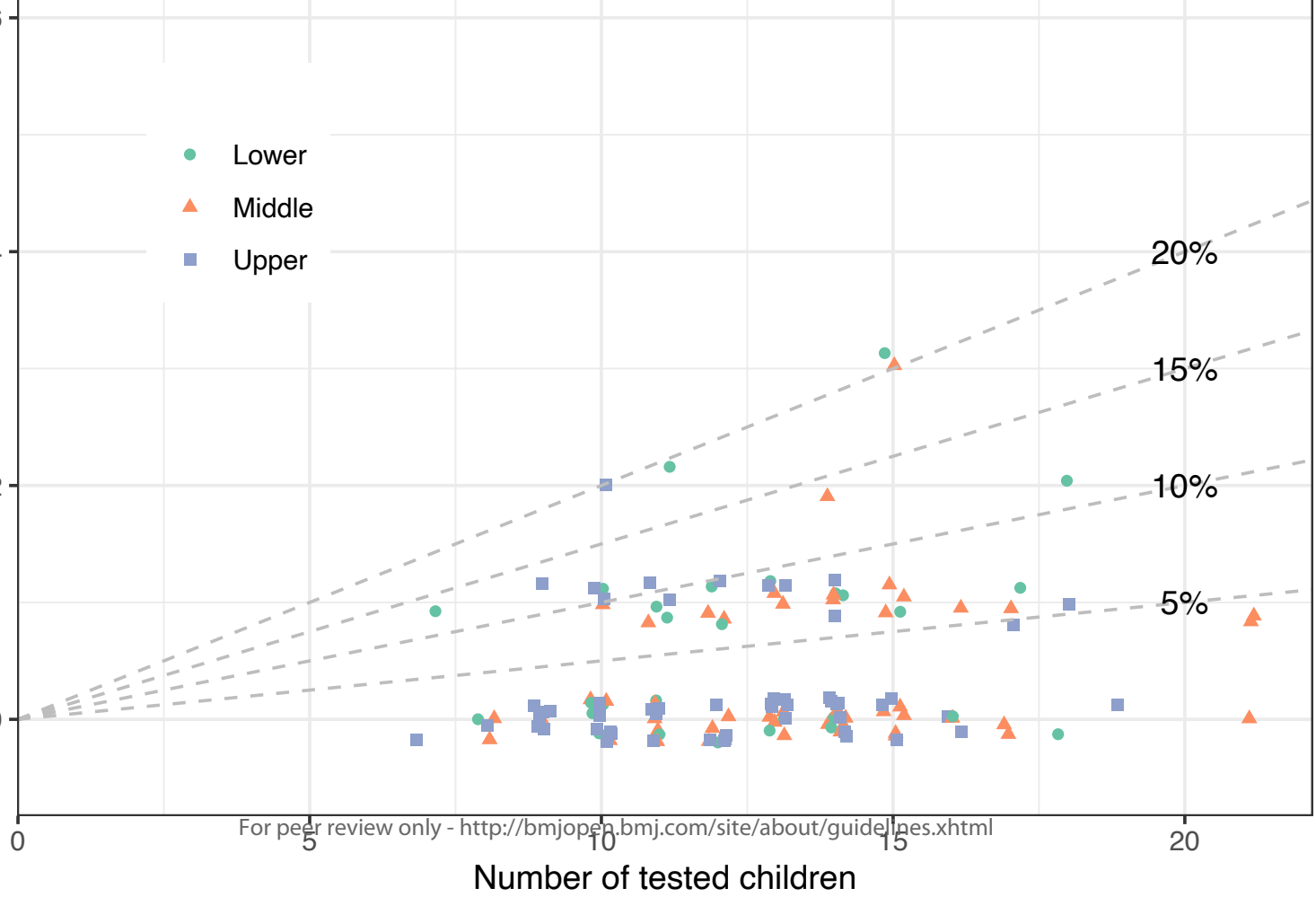


Upper level

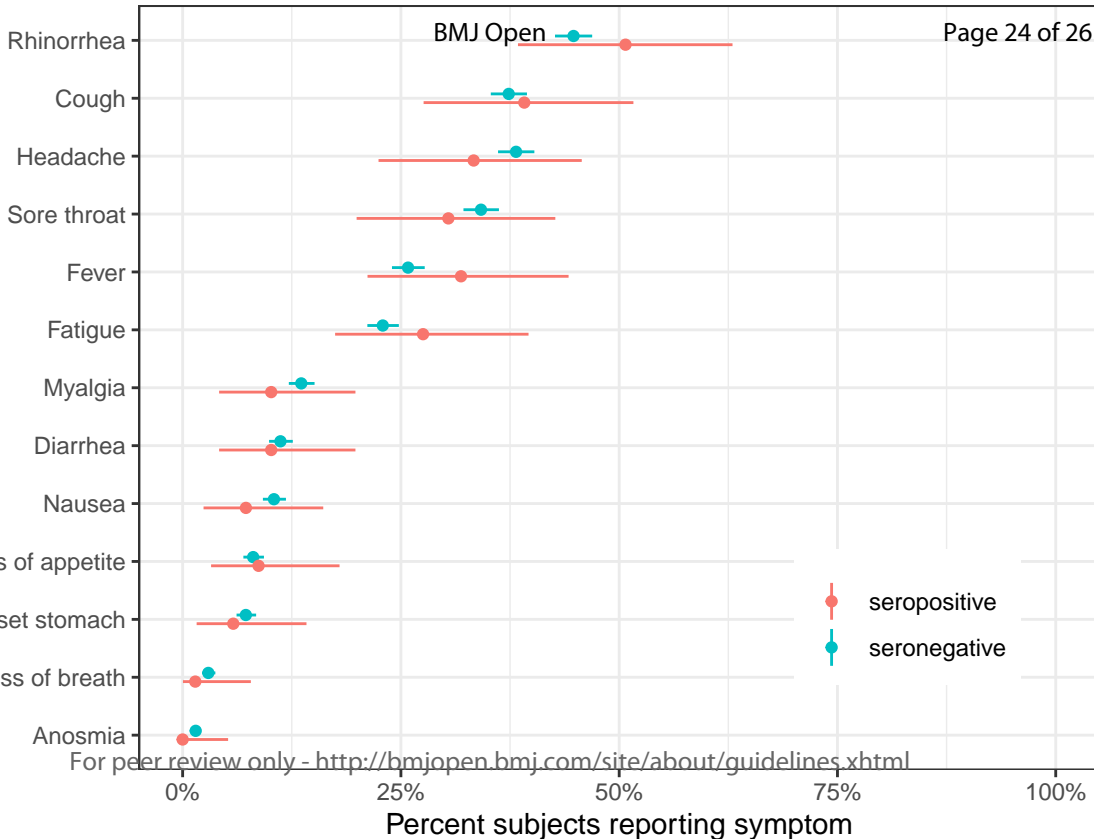


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- Lower
- ▲ Middle
- Upper



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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) <i>Cohort study</i> —Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up <i>Case-control study</i> —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 <i>Cross-sectional study</i> —Give the eligibility criteria, and the sources and methods of selection of participants	6	
		(b) <i>Cohort study</i> —For matched studies, give matching criteria and number of exposed and unexposed		

		<i>Case-control study</i> —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) <i>Cohort study</i> —If applicable, explain how loss to follow-up was addressed	7-8
		<i>Case-control study</i> —If applicable, explain how matching of cases and controls was addressed <i>Cross-sectional study</i> —If applicable, describe analytical methods taking account of sampling strategy	
		(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) <i>Cohort study</i> —Summarise follow-up time (eg, average and total amount)	
Outcome data	15*	<i>Cohort study</i> —Report numbers of outcome events or summary measures over time	
		<i>Case-control study</i> —Report numbers in each exposure category, or summary measures of exposure	
		<i>Cross-sectional study</i> —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

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2020-047483.R1
Article Type:	Original research
Date Submitted by the Author:	27-Feb-2021
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Primary Subject Heading:	Epidemiology
Secondary Subject Heading:	Infectious diseases, Public health
Keywords:	Paediatric infectious disease & immunisation < PAEDIATRICS, Infection control < INFECTIOUS DISEASES, Epidemiology < INFECTIOUS DISEASES, VIROLOGY, Community child health < PAEDIATRICS

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1 Title

2 Variation in SARS-CoV-2 seroprevalence across districts, schools and classes: baseline
3 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 $\geq 50\%$ 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.

90

91 **Article summary**

92

93 **Strengths and limitations of this study**

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

105

106

107 INTRODUCTION

108 The transmission of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in the
109 school setting is not well understood [1], partly as schools were closed in many countries
110 during the peaks of the pandemic, partly due to lack of representative studies with random
111 sampling. Anecdotal evidence and case studies suggest that outbreaks can happen in
112 schools [2–4], but it is not clear if they represent outlier events or widely underdiagnosed
113 spread of the infection. The effect of school closures on the community transmission of
114 SARS-CoV-2 ranges from minimal to substantial [5], with some modelling studies even
115 predicting an increase of the total number of deaths [6]. Currently existing or planned
116 population-based studies focusing on SARS-CoV-2 spread in schools are few and small-sized
117 [7,8].

118 In this study, we present the results of the first cross-sectional analysis of a large
119 cohort of children from randomly selected schools and classes in the canton of Zurich,
120 Switzerland. The cohort study follows the seroprevalence, symptoms, socio-demographic
121 and lifestyle factors of enrolled children from June 2020 to April 2021. The participating
122 children were enrolled from June 16 to July 9, 2020. Schools in Switzerland were closed for a
123 relatively short period (March 16 to May 10) compared to other countries, and lock-down
124 measures were mild. Restaurants, bars and non-essential shops and services were closed on
125 March 17 and events or meetings with over 5 people prohibited on March 20, but no strict
126 confinement at home implemented. These measures were gradually lifted in April-May
127 2020.

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

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134 METHODS

135 *Study setting*

136 The study was registered on ClinicalTrials.gov (identifier number NCT04448717). The
137 protocol for this longitudinal cohort study was reported elsewhere [9]. The study is part of a
138 large nationally coordinated research network *Corona Immunitas* in Switzerland [10,11].
139 The study took place from June 16 to July 9, 2020, in the canton of Zurich, Switzerland. The
140 canton of Zurich comprises 1.5 million residents, roughly 18% of the Swiss population, and
141 includes both urban and rural settings, as well as an ethnically and linguistically diverse
142 population. The first preventive measures in schools were introduced on March 16, 2020,
143 when physical attendance of schools was stopped. Schools were partly reopened on May
144 10, with a combination of online and on-site teaching with preventive measures (e.g.,
145 teaching in smaller groups, school attendance every second day, sports and large group
146 activities limited). Schools were fully reopened on June 7, with minimal preventive measures
147 (e.g., recommended social distancing for teachers, reduction of group events) and otherwise
148 regular operation (e.g., full classrooms with desks mostly facing forward) until the end of the
149 school year on July 17. The prevention measures implemented in schools after May 10 were
150 school-specific and based on the federal and cantonal guidelines [12].

151

152 *Study procedures*

153 School were selected based on a full list of schools provided by the Educational Department
154 of the canton. Primary schools were randomly selected by a computer program from the list
155 of all primary schools in the respective district. The closest secondary school (often in the
156 same building or area) was then selected. The random sample of primary and secondary
157 schools was stratified by geographic district. Within schools, randomly selected classes in
158 lower school level (grades one to two, attended by 6 to 9-year-old children), middle (grades
159 four to five, 9 to 13-year-old children), and upper school level (grades seven to eight, 12 to
160 16-year-old children) were invited. Invited grades and classes were selected to ensure that
161 the same cohort of children within the class can be followed until April 2021. Therefore,
162 grades 1-2, 4-5 and 7-8 (but not grades 3, 6, and 9) were included, as they normally stay in
163 the same school and class for the next school-year. We aimed to enroll at least three classes
164 and 40 children per school level. As we were only able to test the children at schools, a
165 major exclusion criterion was a suspected or confirmed infection with SARS-CoV-2 in the
166 given child on the testing date, precluding attendance of school.

1 167 Study information, link to study website (www.ciao-corona.ch), and informational
2 168 videos in multiple languages for schools, parents, and children were sent to school principals
3
4 169 and further to families of children from the selected classes. Children were enrolled and
5
6 170 venous blood samples taken in schools between June 16 and July 9, 2020. Questionnaires
7
8 171 with information on socio-demographics and symptoms compatible with SARS-CoV-2
9
10 172 infection from January to June, 2020, were completed online for the majority of children by
11
12 173 their parents in June-July, 2020 (for 3% of children in August-September).

13 174 In total, 55 out of 156 invited primary and secondary schools agreed to participate,
14
15 175 and 2585 children in 273 out of 274 invited classes (no children participated in one class).
16
17 176 Venous blood samples were collected from 2484 children (a sufficient amount of blood
18
19 177 could not be obtained from 101 children). An online questionnaire, containing socio-
20
21 178 demographic, health, symptoms and quality of life information for the children, and socio-
22
23 179 demographic and symptoms information for the household members, was completed for
24
25 180 2288 of all enrolled children. Questionnaires were not filled for 297 children, after several
26
27 181 email and phone call reminders.

28 182

29 183 **Serological tests**

30
31 184 The primary outcome of the study was the serological results of blood serum samples
32
33 185 analyzed with ABCORA 2.0 binding assay of the Institute of Medical Virology (IMV) of the
34
35 186 University of Zurich based on the Luminex technology [13]. The test analyzes
36
37 187 immunoglobulins G (IgG), M (IgM) and A (IgA) against four SARS-CoV-2 targets (receptor
38
39 188 binding domain (RBD), spike proteins S1 and S2, and the nucleocapsid protein (N), yielding
40
41 189 12 different measurements. Cut-off values were established against pre-pandemic plasma
42
43 190 allowing a high sensitivity (93.3%) and specificity (99.6%). Samples were defined as
44
45 191 seropositive for SARS-CoV-2 if at least two of the 12 parameters were above the cut-off.

46 192 SARS-CoV-2 seroprevalence in children was compared to that estimated in a random
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48 193 sample from the general population, adjusting for age group and sex, in the same region in
49
50 194 June-July 2020. The adult study, like all studies of the Swiss-wide research program *Corona*
51
52 195 *Immunitas* [10,11], used the test SenASTrIS (Sensitive Anti-SARS-CoV-2 Spike Trimer
53
54 196 Immunoglobulin Serological) developed by the Centre Hospitalier Universitaire Vaudois
55
56 197 (CHUV), the Swiss Federal Institute of Technology in Lausanne (EPFL) and the Swiss Vaccine
57
58 198 Center [14]. The test also uses Luminex technology to detect IgG and IgA antibodies binding
59
60 199 to the entire trimeric S protein of SARS-CoV-2 and with demonstrated 98.3% sensitivity and
200 98.4% specificity for the combined testing of IgG and IgA (result declared as positive when

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

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

209

210 **Statistical analysis**

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

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

227

228 **Patient and public involvement**

229 Several school principals were consulted during the development of the protocol to ensure
230 feasibility of the planned study procedures. Early feedback was collected from invited
231 children and parents, in order to adapt the communication strategies and channels.

232 Numerous online informational sessions, encouraging open exchange and feedback, were
233 organized for invited and enrolled school principals, personnel and parents of the children.

234 Results of individual tests were communicated to the participants, and overall study results

1 235 disseminated to participating schools. Findings will be disseminated in lay language in the
2 236 national and local press, to the national and regional educational and public health
3
4 237 departments and on the website of the study (www.ciao-corona.ch).
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239 RESULTS

240 In total, 55 schools and 2585 children were recruited (1339 (52%) girls, median age 11, age
241 range 6-16 years), 754 (29%) in the lower level, 899 (35%) in the middle, and 932 (36%) in
242 the upper school level. Mean participation rate was 50% of the invited children within
243 invited classes (range from 0% to 94% (0 to 21 children), interquartile range 32% to 63%).
244 Venous blood was collected and analyzed for 2484 children (1278 (51%) girls, median age
245 11, age range 6 to 16 years).

246 74 children had SARS-CoV-2 antibodies, resulting in overall weighted seroprevalence
247 of 2.8 % (95% credible interval (CrI) 1.5 to 4.1%), ranging from 1.0% to 4.5% in districts, as
248 measured with ABCORA test (Figure 1). Seroprevalence was 3.8% (95% CrI 2.0 to 6.1%) in
249 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
250 3.0%) in grades seven to eight (Figure 1).

251 Seroprevalence of children, as measured with the SenASTriS test of IgG and IgA
252 combined, was very similar to the seroprevalence of randomly selected adults in the same
253 region in June-July 2020 (children 3.2%, 95% CrI 1.7 to 5.0%, versus adults 3.6%, 95% CrI 1.7
254 to 5.4%). The estimates of seroprevalence in different school levels and districts were
255 similar to those estimated with the primary ABCORA 2.0 test (Figure 1). Seroprevalence
256 measured with SenASTriS test was 2.0% (95% CrI 0.6 to 4.3%) in grades one to two, 4.2%
257 (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
258 to eight.

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

263

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

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

269 SenASTriS – Estimate based on the SenASTriS test.

270

271 At least one seropositive child was present in 36/55 (65%) of tested schools. Within the
272 levels of the schools, at least one seropositive child was present in the lower level of 17/29
273 (59%) schools, in the middle level of 14/28 (50%) schools, and in the upper level of 16/25
274 (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 $\geq 50\%$ 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

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

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

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

1 341 not statistically significant trend of younger children having higher seroprevalence than
2 342 older children, when measured with the comprehensive ABCORA 2.0 test. Infections with
3 343 circulating human coronaviruses (hCoVs) are common in childhood and antibodies to hCoVs
4 344 229E, NL63, OC43 and HKU1A are prevalent in the human population and particularly
5 345 children [30,31]. Cross-reactivity with hCoVs was thus considered in the development of
6 346 both serology tests used in this study and both tests detect SARS-CoV-2 with high specificity
7 347 (99.6% for ABCORA 2.0 and 98.4% for SenASTriS). Importantly, to adjust for the possibility of
8 348 a few false-positive results, we employed a Bayesian hierarchical model, which adjusts for
9 349 the accuracy parameters of the tests to estimate population-level seroprevalence. Higher
10 350 seroprevalence in younger children could be compatible with less feasible social distancing
11 351 behavior and possibly more vigorous immune response to the virus in early age. The trend
12 352 could also reflect a chance finding, as substantial proportion of false positives is expected in
13 353 the low seroprevalence setting, and it was not observed in the SenASTriS test results.
14 354 Further testing of the cohort and forthcoming studies will show if this trend is observed in
15 355 the future.

16 356 The frequency of both more specific (e.g., fever, cough) and less specific (rhinorrhea,
17 357 headache, nausea) symptoms [32] was not different among seropositive and seronegative
18 358 children. In general, symptoms, particularly rhinorrhea, cough, headache and sore throat,
19 359 were reported frequently, with three of four children reporting any symptoms within the
20 360 last 6 months in both groups. Anosmia was not reported by any of the seropositive children.
21 361 The specificity of COVID-19 compatible symptoms, therefore, seems to be lower in children
22 362 than in adults. Moreover, the range of symptoms reported in children was shown to be
23 363 different than in adults [33]. Somewhat unspecific symptoms in children, contrary to more
24 364 specific symptoms in adults [34], could partly explain the high proportion of seropositive
25 365 children who were not previously diagnosed with RT-PCR. Furthermore, testing indications
26 366 by health authorities were cautious both for children and adults during the first half of 2020.
27 367 Testing was recommended only for children with acute upper respiratory tract infection
28 368 symptoms or acute anosmia, and not recommended in children with rhinitis only or without
29 369 symptoms [35].

30 370 Particular strengths of the design of this study are school-based random sampling,
31 371 hierarchical data structure, and large sample size, allowing to identify clusters within
32 372 district, school, grade and class levels. Testing was done in schools and study information
33 373 presented in multiple formats, including videos in multiple languages, to minimize selection
34 374 bias within enrolled children. The participation rate of 50% can be considered rather high

1 375 for a study in children involving venous blood sampling, and additional children from the
2 376 invited classes will have the opportunity to be enrolled in subsequent testing phases
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4 377 (October/November 2020, March/April 2021), further increasing participation rate and the
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6 378 size of the cohort.

7 379 The study has a few limitations. First, the retrospective evaluation of symptoms over
8
9 380 six months could have been subject to recall bias. Second, the study enrolled only 35%
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11 381 (55/156) of the invited schools. Commonly stated reasons for non-participation on school
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13 382 level were constraints in time and human resources and competing participation in other
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15 383 studies. Participation rate on school level varied significantly depending on the district, and
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17 384 for a few districts, a maximum of three invitation rounds was needed to recruit a regionally
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19 385 representative sample. Third, although the schools were open for one to two months
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21 386 directly before the study, they were closed for the two months of the highest community
22
23 387 transmission of SARS-CoV-2 in the canton of Zurich [15]. Therefore, the measured
24
25 388 seroprevalence in children might be dominated by infections in households rather than
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27 389 school. The follow up of this study will shed more light on transmission in schools.

28 390 In conclusion, clustering of SARS-CoV-2 seropositive children within classes and
29
30 391 schools was not prominent shortly after reopening of schools in this large population-based
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32 392 study. Seroprevalence was similar to adults, resulting in strikingly fewer diagnosed
33
34 393 infections in comparison to the seropositive cases in children than in adults. Considering the
35
36 394 time window required for SARS-CoV-2 antibodies to form, this study reflects infection of
37
38 395 SARS-CoV-2 until approximately end of May 2020, covering four months of SARS-CoV-2
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40 396 infection in the community, with two months of school closure and mild lock-down policy.
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42 397 The subsequent testing of parents and school personnel and the follow-up of the children
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44 398 cohort in fall 2020 will yield further evidence on the observed trends and of the spread of
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46 399 SARS-CoV-2 within and outside schools.

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401 Author Contributions

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2 402 SK and MAP initiated the project and preliminary design, with support of JF. SK, MAP, CB,
3
4 403 TR, RJ, JB, AF and AU developed the design and methodology. SK, RJ, AU, TR, JB, AF and CC
5
6 404 recruited study participants, collected and managed the data. SRH performed statistical
7
8 405 analysis. AT, MH, MaSch, MeSch and IA developed the serology analysis plan, supervised,
9
10 406 conducted and evaluated the serology tests. AU wrote the first draft of the manuscript. All
11
12 407 authors contributed to the design of the study and interpretation of its results, and revised
13
14 408 and approved the manuscript for intellectual content. SK is the guarantor and accepts full
15
16 409 responsibility for the work and the conduct of the study, had access to the data, and
17
18 410 controlled the decision to publish. The corresponding author SK attests that all listed
19
20 411 authors meet authorship criteria and that no others meeting the criteria have been omitted.

21 412

413 Competing interests

22
23
24 414 The authors declare no competing interests to disclose.

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28
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30
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34
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40
41 423 role in the design and conduct of the study; collection, management, analysis, and
42
43 424 interpretation of the data; preparation, review, or approval of the manuscript; and decision
44
45 425 to submit the manuscript for publication. All authors had full access to all data analysis
46
47 426 outputs (reports and tables) and take responsibility for their integrity and accuracy

48 427

428 Data sharing statement

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51 429 Data is still being collected for the cohort study *Ciao Corona*. Deidentified participant data
52
53 430 might be available on reasonable request by email to the corresponding author at later
54
55 431 stages of the study.

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433 Ethics approval

434 The study was approved by the Ethics Committee of the Canton of Zurich, Switzerland
 435 (2020-01336). All participants provided written informed consent before being enrolled in
 436 the study.

437

438 **Transparency declaration**

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

442

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1 **551 Figure legends**

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4 **553 Figure 1** Overall, school level and district estimates of SARS-CoV-2 seroprevalence in
5 554 children

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7 555 Weighted point estimates and 95% credible intervals are shown. Districts are ordered by
8 556 population size.

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10 557 ABCORA 2.0 – Primary estimate based on the ABCORA 2.0 of the Institute of Medical
11 558 Virology, University of Zurich test. SenASTrIS – Estimate based on the SenASTrIS test.

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14 **560 Figure 2** Seropositive children in tested schools and school levels

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16 561 Each square illustrates an invited child. Each block of squares illustrates a school level in a
17 562 school. Lower and middle levels are both taught in the primary schools; however, lower and
18 563 middle levels of the same school are not matched in this graph due to protection of
19 564 participant privacy. The distribution of the invited, tested and seropositive children is
20 565 depicted only on the school level in the figures of this manuscript to preserve
21 566 deidentification and privacy of the participants.

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23
24 **568 Figure 3** Self-reported symptoms in seropositive and seronegative children in January-June
25 569 2020

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27 570 Point estimates and 95% confidence intervals are shown.

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30 **572 Figure 4** Clustering of seropositive children in classes: number and proportion of

31 573 seropositive children in the tested classes

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33 574 Each dot represents a class. Diagonal lines partition the figure into classes with 0-5% of
34 575 tested seropositive children (below 5% line), 5-10% of tested seropositive children (between
35 576 5% and 10% lines), etc. Only classes where at least 5 children and at least 50% of the class
36 577 were tested are shown. The distribution of seropositive children in the enrolled classes is
37 578 only presented in an aggregated form rather than clustered within schools to preserve the
38 579 deidentification and privacy of the participants.

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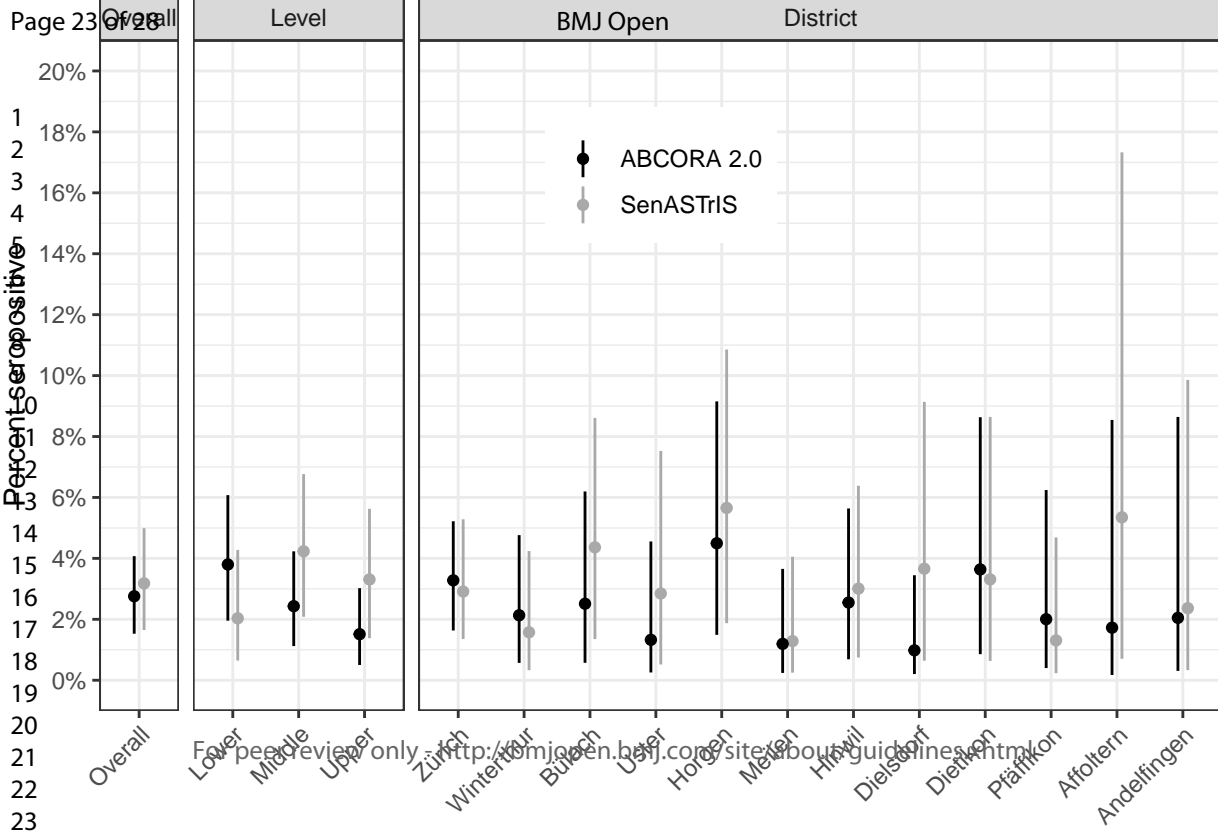
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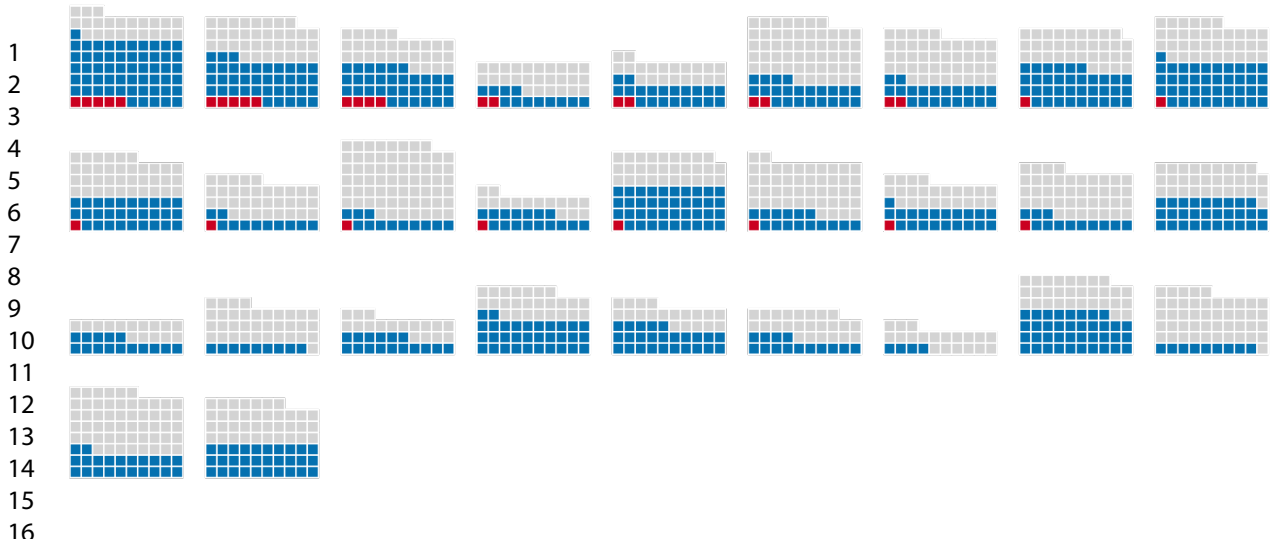
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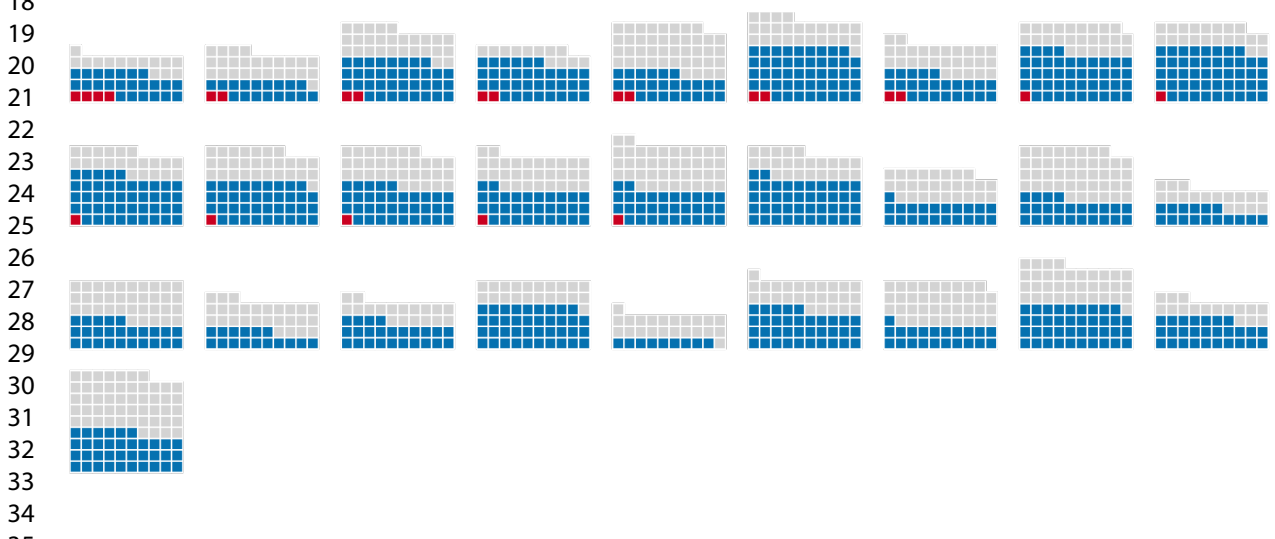
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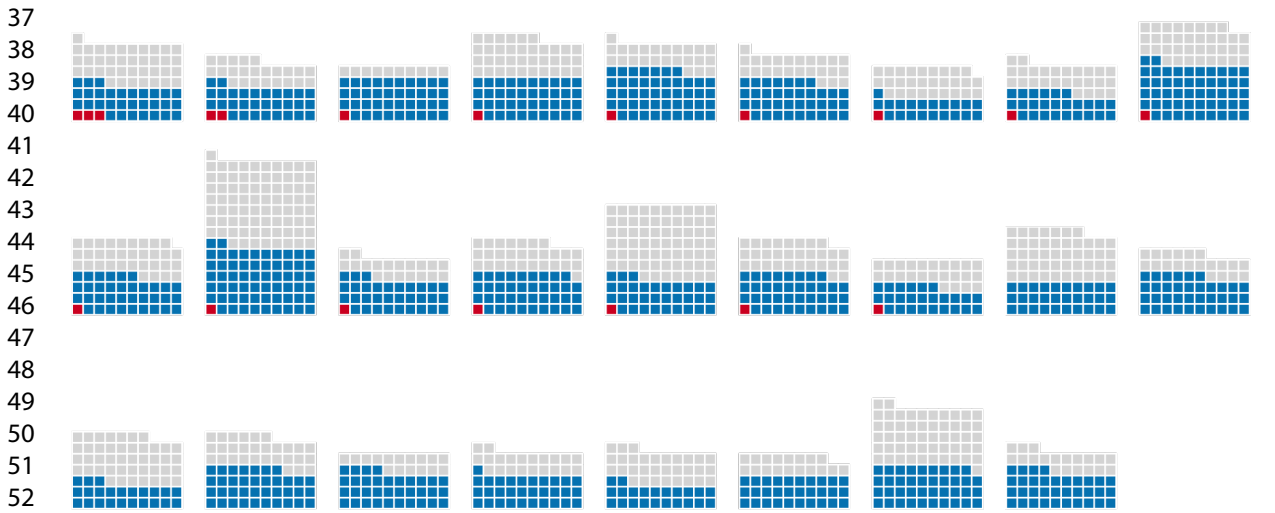


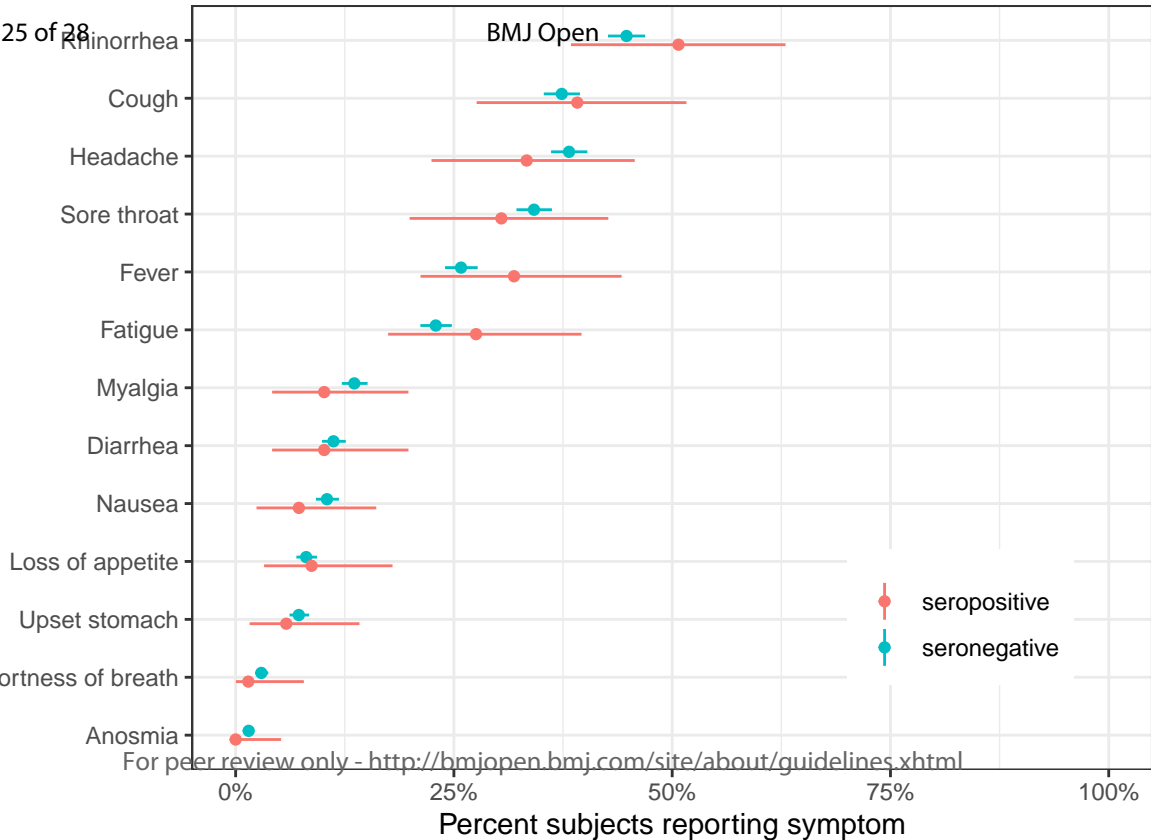


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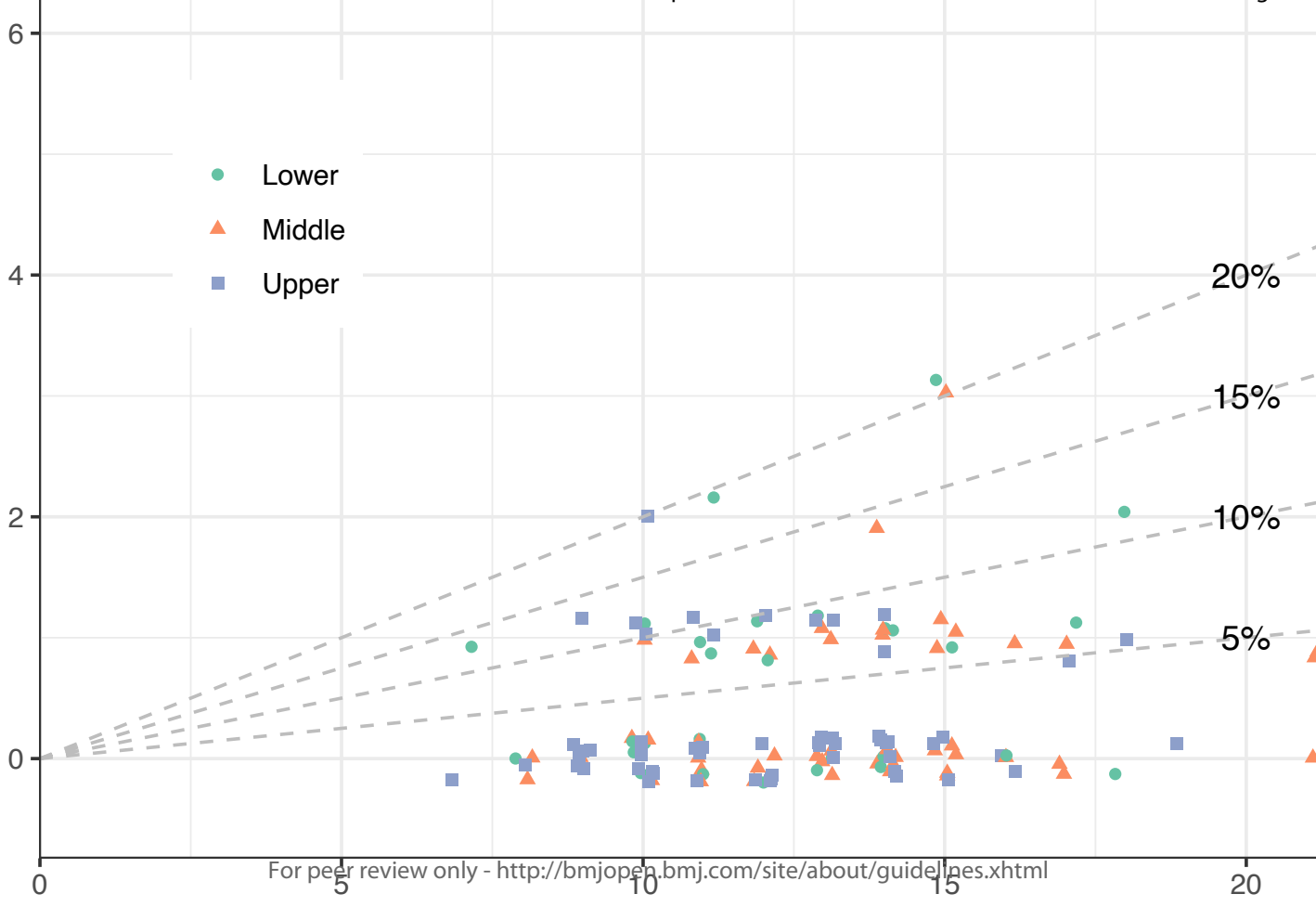
Upper level



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- Lower
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- Upper



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) <i>Cohort study</i> —Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up <i>Case-control study</i> —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 <i>Cross-sectional study</i> —Give the eligibility criteria, and the sources and methods of selection of participants	6	
		(b) <i>Cohort study</i> —For matched studies, give matching criteria and number of exposed and unexposed		

		<i>Case-control study</i> —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) <i>Cohort study</i> —If applicable, explain how loss to follow-up was addressed	7-8
		<i>Case-control study</i> —If applicable, explain how matching of cases and controls was addressed <i>Cross-sectional study</i> —If applicable, describe analytical methods taking account of sampling strategy	
		(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) <i>Cohort study</i> —Summarise follow-up time (eg, average and total amount)	
Outcome data	15*	<i>Cohort study</i> —Report numbers of outcome events or summary measures over time	
		<i>Case-control study</i> —Report numbers in each exposure category, or summary measures of exposure	
		<i>Cross-sectional study</i> —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

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2020-047483.R2
Article Type:	Original research
Date Submitted by the Author:	27-Apr-2021
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Primary Subject Heading:	Epidemiology
Secondary Subject Heading:	Infectious diseases, Public health, Paediatrics
Keywords:	Paediatric infectious disease & immunisation < PAEDIATRICS, Infection control < INFECTIOUS DISEASES, Epidemiology < INFECTIOUS DISEASES, VIROLOGY, Community child health < PAEDIATRICS

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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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Word count: 3200

62 Abstract

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

65 **Design:** Baseline measurements of a longitudinal cohort study (*Ciao Corona*) from June-July
66 2020.

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

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

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

75 **Results:** Overall seroprevalence was 2.8% (95% CI 1.5 to 4.1%), ranging from 1.0% to 4.5%
76 across districts. Seroprevalence in grades 1-2 was 3.8% (2.0 to 6.1%), in grades 4-5 – 2.4%
77 (1.1 to 4.2%), and in grades 7-8 – 1.5% (0.5 to 3.0%). At least one seropositive child was
78 present in 36 of 55 (65%) schools and in 44 (34%) of 131 classes where ≥ 5 children and $\geq 50\%$
79 of children within the class were tested. 73% of children reported COVID-19-compatible
80 symptoms since January 2020, with the same frequency in seropositive and seronegative
81 children for all symptoms. Seroprevalence of children and adults was similar (3.2%, 95% CrI
82 1.7-5.0%, versus 3.6%, 95% CrI 1.7-5.4%). The ratio of confirmed SARS-CoV-2 cumulative
83 incidence to seropositive cases was 1:89 in children and 1:12 in adults.

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

88 **Trial registration:** ClinicalTrials.gov NCT04448717.
89 <https://clinicaltrials.gov/ct2/show/NCT04448717>

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

91

92 **Article summary**

93

94 **Strengths and limitations of this study**

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

106

107

108 INTRODUCTION

109 The transmission of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in the
110 school setting is not well understood [1], partly as schools were closed in many countries
111 during the peaks of the pandemic, partly due to lack of representative studies with random
112 sampling. Anecdotal evidence and case studies suggest that outbreaks can happen in
113 schools [2–4], but it is not clear if they represent outlier events or widely underdiagnosed
114 spread of the infection. The effect of school closures on the community transmission of
115 SARS-CoV-2 ranges from minimal to substantial [5], with some modelling studies even
116 predicting an increase of the total number of deaths [6]. Currently existing or planned
117 population-based studies focusing on SARS-CoV-2 spread in schools are few and small-sized
118 [7,8].

119 In this study, we present the results of the first cross-sectional analysis of a large
120 cohort of children from randomly selected schools and classes in the canton of Zurich,
121 Switzerland. The cohort study follows the seroprevalence, symptoms, socio-demographic
122 and lifestyle factors of enrolled children from June 2020 to April 2021. The participating
123 children were enrolled from June 16 to July 9, 2020. Schools in Switzerland were closed for a
124 relatively short period (March 16 to May 10) compared to other countries, and lock-down
125 measures were mild. Restaurants, bars and non-essential shops and services were closed on
126 March 17 and events or meetings with over 5 people prohibited on March 20, but no strict
127 confinement at home implemented. These measures were gradually lifted in April-May
128 2020.

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

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135 METHODS

136 *Study setting*

137 The study was registered on ClinicalTrials.gov (identifier number NCT04448717). The
138 protocol for this longitudinal cohort study was reported elsewhere [9]. The study is part of a
139 large nationally coordinated research network *Corona Immunitas* in Switzerland [10,11].
140 The study took place from June 16 to July 9, 2020, in the canton of Zurich, Switzerland. The
141 canton of Zurich comprises 1.5 million residents, roughly 18% of the Swiss population, and
142 includes both urban and rural settings, as well as an ethnically and linguistically diverse
143 population. The first preventive measures in schools were introduced on March 16, 2020,
144 when physical attendance of schools was stopped. Schools were partly reopened on May
145 10, with a combination of online and on-site teaching with preventive measures (e.g.,
146 teaching in smaller groups, school attendance every second day, sports and large group
147 activities limited). Schools were fully reopened on June 7, with minimal preventive measures
148 (e.g., recommended social distancing for teachers, reduction of group events) and otherwise
149 regular operation (e.g., full classrooms with desks mostly facing forward) until the end of the
150 school year on July 17. The prevention measures implemented in schools after May 10 were
151 school-specific and based on the federal and cantonal guidelines [12].

152

153 *Study procedures*

154 School were selected based on a full list of schools provided by the Educational Department
155 of the canton. Primary schools were randomly selected by a computer program from the list
156 of all primary schools in the respective district. The closest secondary school (often in the
157 same building or area) was then selected. The random sample of primary and secondary
158 schools was stratified by geographic district. Within schools, randomly selected classes in
159 lower school level (grades one to two, attended by 6 to 9-year-old children), middle (grades
160 four to five, 9 to 13-year-old children), and upper school level (grades seven to eight, 12 to
161 16-year-old children) were invited. Invited grades and classes were selected to ensure that
162 the same cohort of children within the class can be followed until April 2021. Therefore,
163 grades 1-2, 4-5 and 7-8 (but not grades 3, 6, and 9) were included, as they normally stay in
164 the same school and class for the next school-year. We aimed to enroll at least three classes
165 and 40 children per school level. As we were only able to test the children at schools, a
166 major exclusion criterion was a suspected or confirmed infection with SARS-CoV-2 in the
167 given child on the testing date, precluding attendance of school.

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

175 In total, 55 out of 156 invited primary and secondary schools agreed to participate,
176 and 2585 children in 273 out of 274 invited classes (no children participated in one class).
177 Venous blood samples were collected from 2484 children (a sufficient amount of blood
178 could not be obtained from 101 children). An online questionnaire, containing socio-
179 demographic, health, symptoms and quality of life information for the children, and socio-
180 demographic and symptoms information for the household members, was completed for
181 2288 of all enrolled children. Questionnaires were not filled for 297 children, after several
182 email and phone call reminders.

183

184 **Serological tests**

185 The primary outcome of the study was the serological results of blood serum samples
186 analyzed with ABCORA 2.0 binding assay of the Institute of Medical Virology (IMV) of the
187 University of Zurich based on the Luminex technology [13]. The test analyzes
188 immunoglobulins G (IgG), M (IgM) and A (IgA) against four SARS-CoV-2 targets (receptor
189 binding domain (RBD), spike proteins S1 and S2, and the nucleocapsid protein (N), yielding
190 12 different measurements. Cut-off values were established against pre-pandemic plasma
191 allowing a high sensitivity (93.3%) and specificity (99.6%). Samples were defined as
192 seropositive for SARS-CoV-2 if at least two of the 12 parameters were above the cut-off.

193 SARS-CoV-2 seroprevalence in children was compared to that estimated in a random
194 sample from the general population, adjusting for age group and sex, in the same region in
195 June-July 2020. The adult study, like all studies of the Swiss-wide research program *Corona*
196 *Immunitas* [10,11], used the test SenASTrIS (Sensitive Anti-SARS-CoV-2 Spike Trimer
197 Immunoglobulin Serological) developed by the Centre Hospitalier Universitaire Vaudois
198 (CHUV), the Swiss Federal Institute of Technology in Lausanne (EPFL) and the Swiss Vaccine
199 Center [14]. The test also uses Luminex technology to detect IgG and IgA antibodies binding
200 to the entire trimeric S protein of SARS-CoV-2 and with demonstrated 98.3% sensitivity and
201 98.4% specificity for the combined testing of IgG and IgA (result declared as positive when

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

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

210

211 **Statistical analysis**

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

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

229

230 **Patient and public involvement**

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

1 236 Results of individual tests were communicated to the participants, and overall study results
2 237 disseminated to participating schools. Findings will be disseminated in lay language in the
3
4 238 national and local press, to the national and regional educational and public health
5
6 239 departments and on the website of the study (www.ciao-corona.ch).
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241 RESULTS

242 In total, 55 schools and 2585 children were recruited (1339 (52%) girls, median age 11, age
243 range 6-16 years), 754 (29%) in the lower level, 899 (35%) in the middle, and 932 (36%) in
244 the upper school level. Mean participation rate was 50% of the invited children within
245 invited classes (range from 0% to 94% (0 to 21 children), interquartile range 32% to 63%).
246 Venous blood was collected and analyzed for 2484 children (1278 (51%) girls, median age
247 11, age range 6 to 16 years).

248 74 children had SARS-CoV-2 antibodies, resulting in overall weighted seroprevalence
249 of 2.8 % (95% credible interval (CrI) 1.5 to 4.1%), ranging from 1.0% to 4.5% in districts, as
250 measured with ABCORA test (Figure 1). Seroprevalence was 3.8% (95% CrI 2.0 to 6.1%) in
251 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
252 3.0%) in grades seven to eight (Figure 1).

253 Seroprevalence of children, as measured with the SenASTriS test of IgG and IgA
254 combined, was very similar to the seroprevalence of randomly selected adults in the same
255 region in June-July 2020 (children 3.2%, 95% CrI 1.7 to 5.0%, versus adults 3.6%, 95% CrI 1.7
256 to 5.4%). The estimates of seroprevalence in different school levels and districts were
257 somewhat different from those estimated with the primary ABCORA 2.0 test (Figure 1);
258 however, the credible intervals of the estimates overlapped. Seroprevalence measured with
259 SenASTriS test was 2.0% (95% CrI 0.6 to 4.3%) in grades one to two, 4.2% (95% CrI 2.1 to
260 6.8%) in grades four to five, and 3.3% (95% CrI 1.4 to 5.6%) in grades seven to eight.

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

265

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

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

271 SenASTriS – Estimate based on the SenASTriS test.

272

273 At least one seropositive child was present in 36/55 (65%) of tested schools. Within the
274 levels of the schools, at least one seropositive child was present in the lower level of 17/29
275 (59%) schools, in the middle level of 14/28 (50%) schools, and in the upper level of 16/25
276 (64%) schools (Figure 2).

277 **Figure 2** Seropositive children in tested schools and school levels

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

283

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

289

290 **Figure 3** Self-reported symptoms in seropositive and seronegative children in January-June
291 2020

292 Point estimates and 95% confidence intervals are shown.

293

294 At least one seropositive child was present in 34% (44/131) of classes where ≥ 5
295 children and $\geq 50\%$ of children within the class were tested (Figure 4). Among the classes
296 with at least one seropositive child, 38 (86%) had only one, 4 (9%) had two and 2 (5%) had
297 three seropositive children. When considering all classes regardless of participation rate,
298 24% (65/273) of classes had at least one seropositive child.

299

300 **Figure 4** Clustering of seropositive children in classes: number and proportion of

301 seropositive children in the tested classes

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

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DISCUSSION

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

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

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

1 343 not statistically significant trend of younger children having higher seroprevalence than
2 344 older children, when measured with the comprehensive ABCORA 2.0 test. Infections with
3 345 circulating human coronaviruses (hCoVs) are common in childhood and antibodies to hCoVs
4 346 229E, NL63, OC43 and HKU1A are prevalent in the human population and particularly
5 347 children [30,31]. Cross-reactivity with hCoVs was thus considered in the development of
6 348 both serology tests used in this study and both tests detect SARS-CoV-2 with high specificity
7 349 (99.6% for ABCORA 2.0 and 98.4% for SenASTriS). Importantly, to adjust for the possibility of
8 350 a few false-positive results, we employed a Bayesian hierarchical model, which adjusts for
9 351 the accuracy parameters of the tests to estimate population-level seroprevalence. Higher
10 352 seroprevalence in younger children could be compatible with less feasible social distancing
11 353 behavior and possibly more vigorous immune response to the virus in early age. The trend
12 354 could also reflect a chance finding, as substantial proportion of false positives is expected in
13 355 the low seroprevalence setting, and it was not observed in the SenASTriS test results.
14 356 Further testing of the cohort and forthcoming studies will show if this trend is observed in
15 357 the future.

16 358 The frequency of both more specific (e.g., fever, cough) and less specific (rhinorrhea,
17 359 headache, nausea) symptoms [32] was not different among seropositive and seronegative
18 360 children. In general, symptoms, particularly rhinorrhea, cough, headache and sore throat,
19 361 were reported frequently, with three of four children reporting any symptoms within the
20 362 last 6 months in both groups. Anosmia was not reported by any of the seropositive children.
21 363 The specificity of COVID-19 compatible symptoms, therefore, seems to be lower in children
22 364 than in adults. Moreover, the range of symptoms reported in children was shown to be
23 365 different than in adults [33]. Somewhat unspecific symptoms in children, contrary to more
24 366 specific symptoms in adults [34], could partly explain the high proportion of seropositive
25 367 children who were not previously diagnosed with RT-PCR. Furthermore, testing indications
26 368 by health authorities were cautious both for children and adults during the first half of 2020.
27 369 Testing was recommended only for children with acute upper respiratory tract infection
28 370 symptoms or acute anosmia, and not recommended in children with rhinitis only or without
29 371 symptoms [35].

30 372 Particular strengths of the design of this study are school-based random sampling,
31 373 hierarchical data structure, and large sample size, allowing to identify clusters within
32 374 district, school, grade and class levels. Testing was done in schools and study information
33 375 presented in multiple formats, including videos in multiple languages, to minimize selection
34 376 bias within enrolled children. The participation rate of 50% can be considered rather high

1 377 for a study in children involving venous blood sampling, and additional children from the
2 378 invited classes will have the opportunity to be enrolled in subsequent testing phases
3
4 379 (October/November 2020, March/April 2021), further increasing participation rate and the
5
6 380 size of the cohort.

7 381 The study has a few limitations. First, the retrospective evaluation of symptoms over
8
9 382 six months could have been subject to recall bias. Second, the study enrolled only 35%
10
11 383 (55/156) of the invited schools. Commonly stated reasons for non-participation on school
12
13 384 level were constraints in time and human resources and competing participation in other
14
15 385 studies. Participation rate on school level varied significantly depending on the district, and
16
17 386 for a few districts, a maximum of three invitation rounds was needed to recruit a regionally
18
19 387 representative sample. Third, although the schools were open for one to two months
20
21 388 directly before the study, they were closed for the two months of the highest community
22
23 389 transmission of SARS-CoV-2 in the canton of Zurich [15]. Therefore, the measured
24
25 390 seroprevalence in children might be dominated by infections in households rather than
26
27 391 school. The follow up of this study will shed more light on transmission in schools. Finally,
28
29 392 we do not have information on how many eligible children did not attend the testing due to
30
31 393 acute infection with SARS-CoV-2. We did not provide testing for such children at home or an
32
33 394 alternative date due to limited resources. However, reported total weekly incidence of
34
35 395 SARS-CoV-2 infections in the canton of Zurich ranged from 42 to 185 cases (among 1.5
36
37 396 million residents), and 7 to 15 cases among people under 20 years old during the testing
38
39 397 period. Considering that, in comparison, over 700 total weekly cases were reported in
40
41 398 March-April 2020, we believe that excluding acutely infected children during the testing
42
43 399 period could not lead to substantial underestimation of the seroprevalence.

44 400 In conclusion, clustering of SARS-CoV-2 seropositive children within classes and
45
46 401 schools was not prominent shortly after reopening of schools in this large population-based
47
48 402 study. Seroprevalence was similar to adults, resulting in strikingly fewer diagnosed
49
50 403 infections in comparison to the seropositive cases in children than in adults. Considering the
51
52 404 time window required for SARS-CoV-2 antibodies to form, this study reflects infection of
53
54 405 SARS-CoV-2 until approximately end of May 2020, covering four months of SARS-CoV-2
55
56 406 infection in the community, with two months of school closure and mild lock-down policy.
57
58 407 The subsequent testing of parents and school personnel and the follow-up of the children
59
60 408 cohort in fall 2020 will yield further evidence on the observed trends and of the spread of
409 SARS-CoV-2 within and outside schools.

410

411 Author Contributions

1
2 412 SK and MAP initiated the project and preliminary design, with support of JF. SK, MAP, CB,
3
4 413 TR, RJ, JB, AF and AU developed the design and methodology. SK, RJ, AU, TR, JB, AF and CC
5
6 414 recruited study participants, collected and managed the data. SRH performed statistical
7
8 415 analysis. AT, MH, MaSch, MeSch and IA developed the serology analysis plan, supervised,
9
10 416 conducted and evaluated the serology tests. AU wrote the first draft of the manuscript. All
11
12 417 authors contributed to the design of the study and interpretation of its results, and revised
13
14 418 and approved the manuscript for intellectual content. SK is the guarantor and accepts full
15
16 419 responsibility for the work and the conduct of the study, had access to the data, and
17
18 420 controlled the decision to publish. The corresponding author SK attests that all listed
19
20 421 authors meet authorship criteria and that no others meeting the criteria have been omitted.

21 422

423 Competing interests

24 424 The authors declare no competing interests to disclose.

25 425

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40 433 role in the design and conduct of the study; collection, management, analysis, and
41
42 434 interpretation of the data; preparation, review, or approval of the manuscript; and decision
43
44 435 to submit the manuscript for publication. All authors had full access to all data analysis
45
46 436 outputs (reports and tables) and take responsibility for their integrity and accuracy

47 437

438 Data sharing statement

49
50 439 Data is still being collected for the cohort study *Ciao Corona*. Deidentified participant data
51
52 440 might be available on reasonable request by email to the corresponding author at later
53
54 441 stages of the study.

55 442

443 Ethics approval

444 The study was approved by the Ethics Committee of the Canton of Zurich, Switzerland
 445 (2020-01336). All participants provided written informed consent before being enrolled in
 446 the study.

447

448 **Transparency declaration**

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

452

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1 **Figure legends**

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3
4 **Figure 1** Overall, school level and district estimates of SARS-CoV-2 seroprevalence in
5 children

6
7 Weighted point estimates and 95% credible intervals are shown. Districts are ordered by
8 population size.

9
10 ABCORA 2.0 – Primary estimate based on the ABCORA 2.0 of the Institute of Medical
11 Virology, University of Zurich test. SenASTrIS – Estimate based on the SenASTrIS test.

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13
14 **Figure 2** Seropositive children in tested schools and school levels

15
16 Each square illustrates an invited child. Each block of squares illustrates a school level in a
17 school. Lower and middle levels are both taught in the primary schools; however, lower and
18 middle levels of the same school are not matched in this graph due to protection of
19 participant privacy. The distribution of the invited, tested and seropositive children is
20 depicted only on the school level in the figures of this manuscript to preserve
21 deidentification and privacy of the participants.
22
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25
26 **Figure 3** Self-reported symptoms in seropositive and seronegative children in January-June
27 2020

28
29 Point estimates and 95% confidence intervals are shown.

30 581

31 **Figure 4** Clustering of seropositive children in classes: number and proportion of

32 seropositive children in the tested classes

33
34 Each dot represents a class. Diagonal lines partition the figure into classes with 0-5% of
35 tested seropositive children (below 5% line), 5-10% of tested seropositive children (between
36 5% and 10% lines), etc. Only classes where at least 5 children and at least 50% of the class
37 were tested are shown. The distribution of seropositive children in the enrolled classes is
38 only presented in an aggregated form rather than clustered within schools to preserve the
39 deidentification and privacy of the participants.
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47 **Supplemental material**

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51 **Appendix 1** Description of Bayesian model

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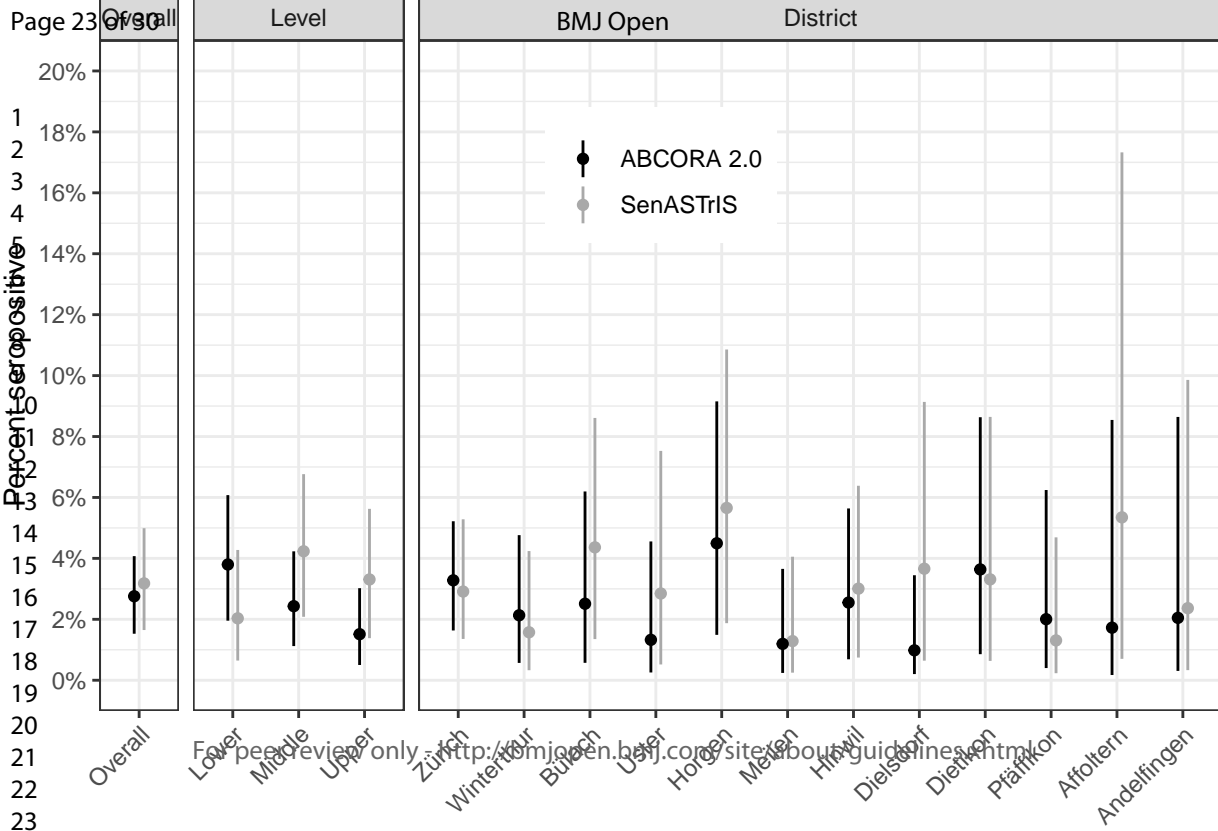
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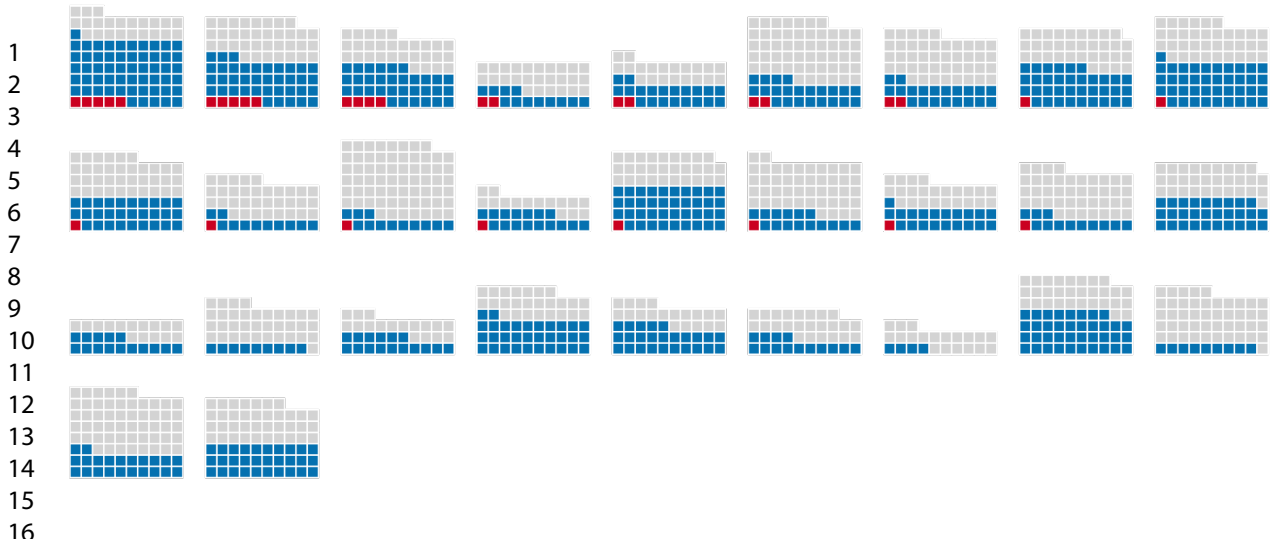
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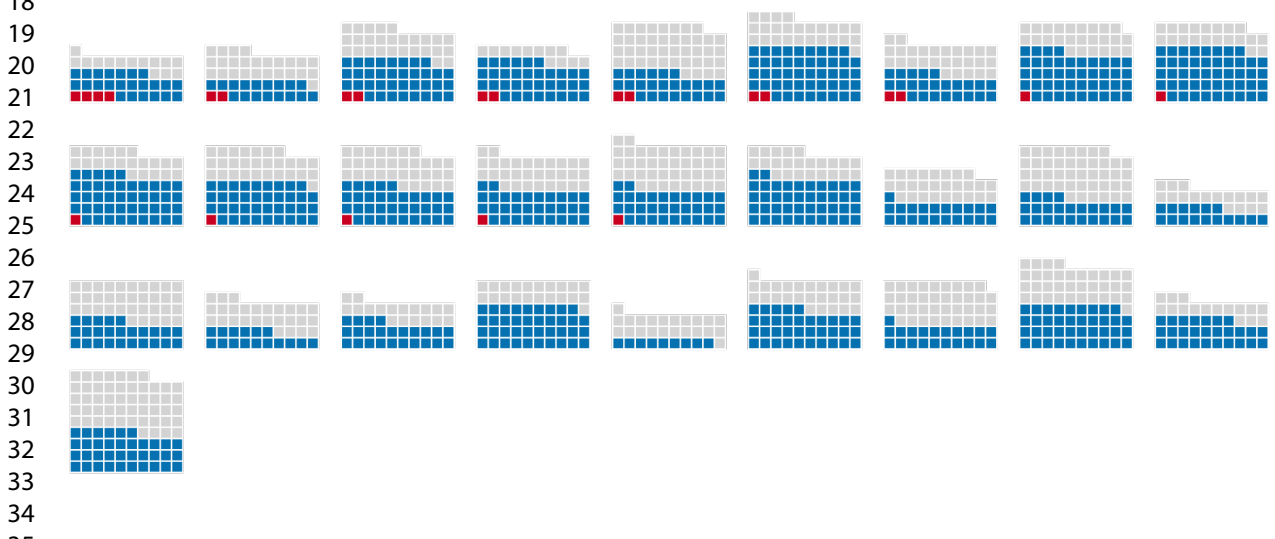


Lower level

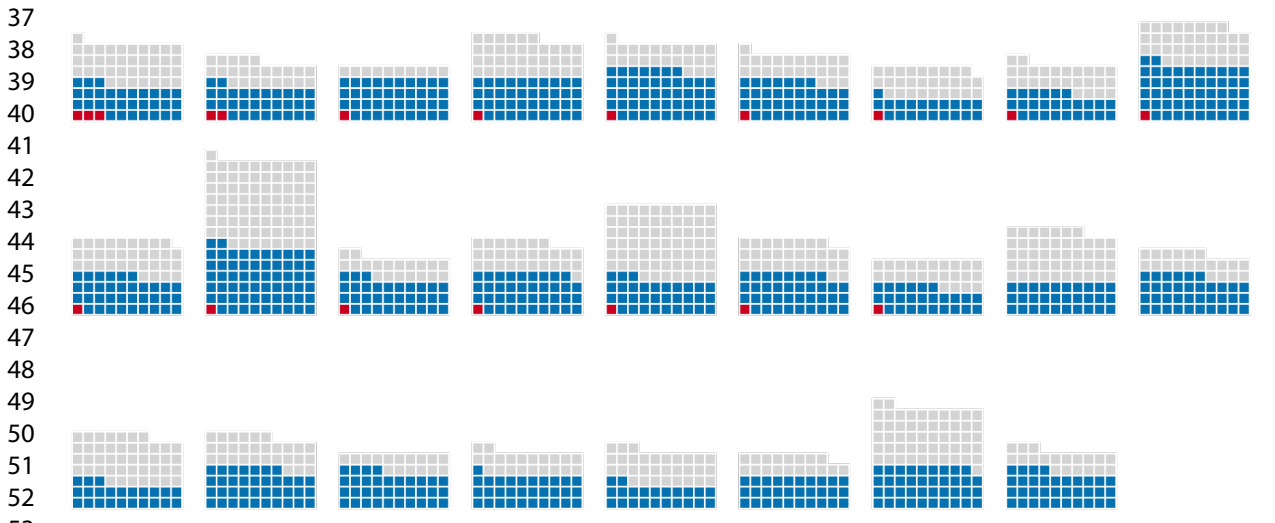
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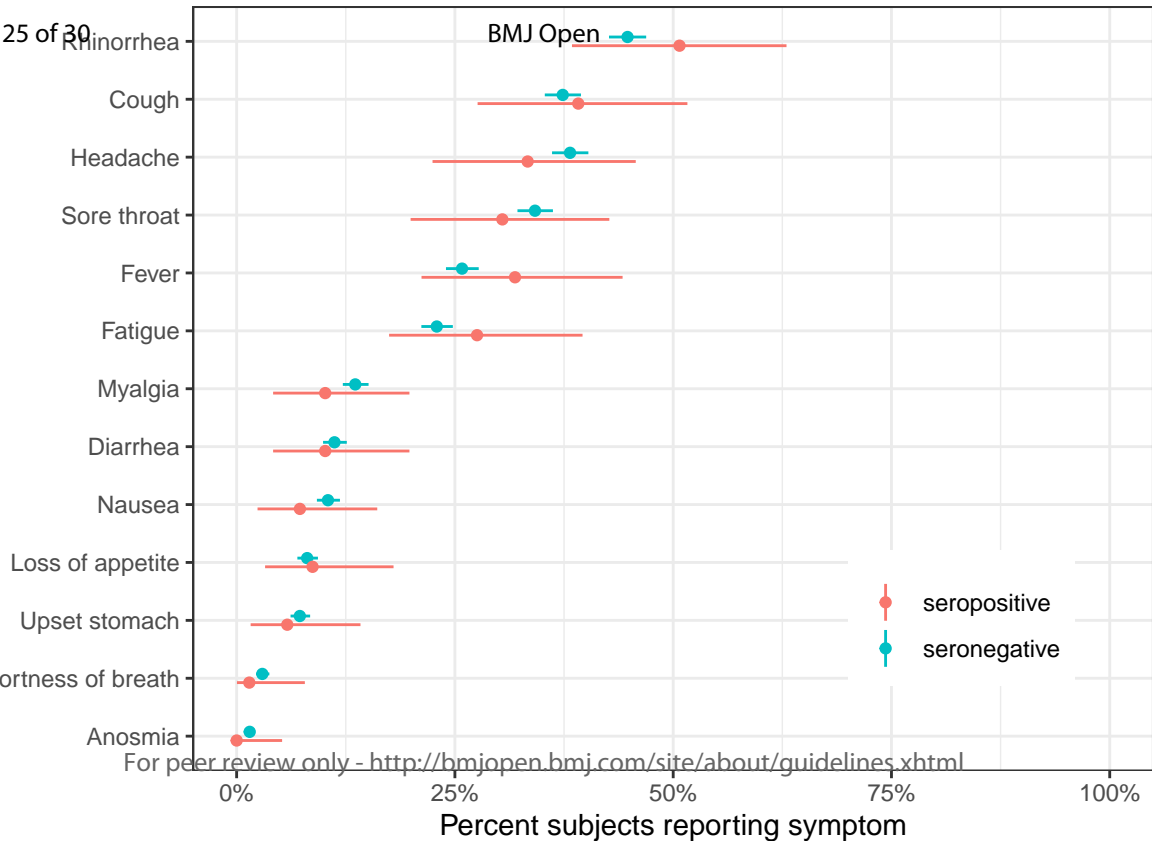
Middle level



Upper level

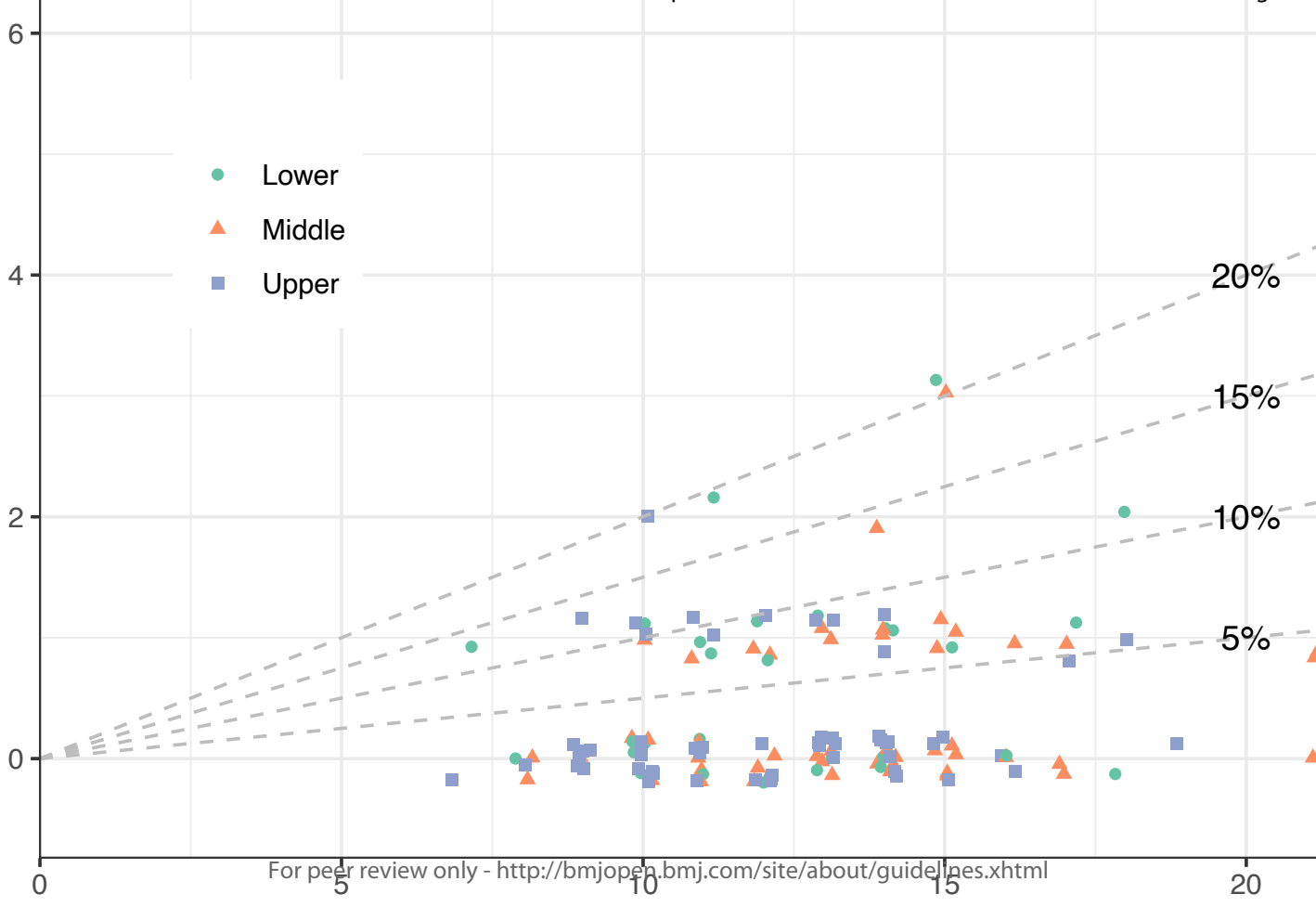


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- Lower
- ▲ Middle
- Upper



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Number of tested children

20%

15%

10%

5%

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 Schwarzmüller, A Trkola, J Fehr, MA Puhon 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:

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

where x_i is the result of the antibody test (in primary analyses) for the i th person ($i = 1, \dots, N$) 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 β , and a random effect for school, α_h ($h = 1, \dots, H$), 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 β . 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:

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

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

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) <i>Cohort study</i> —Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	6	
		<i>Case-control study</i> —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		
		<i>Cross-sectional study</i> —Give the eligibility criteria, and the sources and methods of selection of participants		
		(b) <i>Cohort study</i> —For matched studies, give matching criteria and number of exposed and unexposed		

		<i>Case-control study</i> —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) <i>Cohort study</i> —If applicable, explain how loss to follow-up was addressed	7-8
		<i>Case-control study</i> —If applicable, explain how matching of cases and controls was addressed <i>Cross-sectional study</i> —If applicable, describe analytical methods taking account of sampling strategy	
		(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) <i>Cohort study</i> —Summarise follow-up time (eg, average and total amount)	
Outcome data	15*	<i>Cohort study</i> —Report numbers of outcome events or summary measures over time	
		<i>Case-control study</i> —Report numbers in each exposure category, or summary measures of exposure	
		<i>Cross-sectional study</i> —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.