PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Variation in SARS-CoV-2 seroprevalence across districts, schools and classes: baseline measurements from a cohort of primary and secondary school children in Switzerland
AUTHORS	Ulyte, Agne; Radtke, Thomas; Abela, Irene; Haile, Sarah; Blankenberger, Jacob; Jung, Ruedi; Capelli, Celine; Berger, Christoph; Frei, Anja; Huber, Michael; Schanz, Merle; Schwarzmueller, Magdalena; Trkola, Alexandra; Fehr, Jan; Puhan, Milo; Kriemler, Susi

VERSION 1 – REVIEW

REVIEWER	Le Vu, Stéphane Santé publique France, Infectious Diseases
REVIEW RETURNED	05-Jan-2021

GENERAL COMMENTS	Thank you for the opportunity to review "Variation in SARS-CoV-2 seroprevalence in primary and secondary school children across districts, schools and classes in Switzerland". This manuscript reports estimates of seroprevalence in a sample of schools and classes in Switzerland. Such analyses are needed to appreciate the impact of school openings during the pandemic, as the extent of transmission is generally less known in this population category. The major findings are a prevalence in children similar to that of adults but largely under reported as confirmed cases, a possible negative correlation between risk and age and little evidence for transmission within schools, although this was not directly explored. I have a major concern on the exclusion of confirmed or suspected cases. The frequency of such cases is not described and I wonder what the definition of seroprevalence might be with this exclusion rule, and how it would impact the overall prevalence estimation if these infected children were factored in. Aside from this I have few minor comments : - Some details in methods section are missing: A description of the Bayesian model; A reference for assay performance or characteristics of control specimens for assay validation.

	- It is unclear whether a second test was needed to compare prevalence between the children sampled and adults (as performances for the first test were known). - For that matter, it doesn't seem to me that the results from the two serological assays are similar, when considering figure 1 (especially for lower and middle level). - In results section, line 246-256, what is the purpose of the set of proportion in line 246-256 ? This section seems to introduce some arbitrary cut-off in sample sizes that are difficult to interpret. - Also, figure 3 does not seem to provide a clear statement on the relation between sample size per class and prevalence. The authors might consider a simpler plot of proportion positive against sample size. - Please make sure the term "dark figure" is sufficiently explicit and useful to be used in this article.

REVIEWER	Andrey, Diego Geneva University Hospitals
REVIEW RETURNED	11-Jan-2021

GENERAL COMMENTS	This study by Ulyte et al. provides a very interesting picture of SARS-CoV-2 seroprevalence among school children, in Switzerland. Large sampling of venous blood in children represent a significant challenge. The manuscript is well written. Below one major and a few minor comments: Major comment - abstract and line 294: the trend for higher seroprevalence in younger children was observed with the ABCORA 2.0 immunoassay but does not seem to be found within the 2476 blood samples tested by the SenASTrIS immunoassay, based on Figure 1. In addition, this trend was not observed in other countries or Switzerland regions, to the best of my knowledge. This conclusion should thus be toned down in the abstract. These differences across methods should be discussed in the discussion. Minor comments - line 120: please define or describe the Swiss mild lockdown measures. - line 173-179. The authors should provide a reference or link for the ABCORA 2.0 Luminex immunoassay if available, ideally the validation study or report. In addition, was whole blood, plasma or serum tested? Please specify. • - line 288. These data are in line with data reported in another region of Switzerland, (similar children-adult seroprevalence and only 1/100 ratio cumulative incidence to seroprevalence in children). It should be mentioned and the following reference cited

	DOI: 10.1093/cid/ciaa1702 - although the lack of symptoms specifity probably plays a significant role, as stated, the influence of testing guidances in children versus adults and even testing shortage issues (was it the case in Canton Zurich ?) could have influence these results. The authors should clarifiy RT-PCR testing guidances in Switzerland during this period. - line 336. Anosmia, in adults, is considered specific for Covid-19 and possibly less prone to recall biais. The authors could mention that it was not frequently and specifically identified in seropositive children. - line 360, the ratio is lower for children 1 : 89 versus 1 : 12 adults, not higher.

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1
Dr. Stéphane Le Vu, Santé publique France
Comments to the Author:
Thank you for the opportunity to review "Variation in SARS-CoV-2 seroprevalence in primary and secondary school children across districts, schools and classes in Switzerland".

This manuscript reports estimates of seroprevalence in a sample of schools and classes in Switzerland.

Such analyses are needed to appreciate the impact of school openings during the pandemic, as the extent of transmission is generally less known in this population category.

The major findings are a prevalence in children similar to that of adults but largely under reported as confirmed cases, a possible negative correlation between risk and age and little evidence for transmission within schools, although this was not directly explored.

I have a major concern on the exclusion of confirmed or suspected cases. The frequency of such cases is not described and I wonder what the definition of seroprevalence might be with this exclusion rule, and how it would impact the overall prevalence estimation if these infected children were factored in.
Response:
Thank you for your comment. The decision to exclude confirmed or suspected cases was primarily practical, as these children were not present at school and testing at home was not feasible. During the testing period from June 16 to July 9, 2020, the incidence of SARS-CoV-2 infections was low in the canton of Zurich, ranging from 1 to 56 infections diagnosed daily in the total population of 1.5 million people [1]. In 0 to19-year-old children, 7-15 total weekly infections were diagnosed. Even assuming the ratio of underdiagnosis of 1:89, as estimated in our study, the number of children expected to be missing school and thus the opportunity to participate in the study should be minimal.
The majority of SARS-CoV-2 infections in children, leading to seropositive results in June-July 2020, could be expected to have happened in March-May 2020, when the incidence was relatively much higher than in May-July. Thus, the absence of children acutely ill with SARS-CoV-2 infection during the testing should not have influenced the results substantially. In addition, it would be difficult to pool rtPCR-confirmed and seropositive children into a combined new outcome of the study, as rtPCR and serological tests have different accuracy parameters.

Aside from this I have few minor comments :

- Some details in methods section are missing: A description of the Bayesian model; A reference for assay performance or characteristics of control specimens for assay validation.
Response:
Thank you for the comment. We have revised the Methods to describe the Bayesian model in greater detail (lines 218-25). We also added the reference to the detailed description of the ABCORA 2.0 test (line 191). The references for the performance characteristics of the SenASTriS test are provided in the references given in lines 200-3.

- It is unclear whether a second test was needed to compare prevalence between the children sampled and adults (as performances for the first test were known).
Response:
Thank you for the comment. The necessity to analyze the serology of children blood samples with two tests was dictated by the fact that the main test for Ciao Corona study was chosen in May 2020, whereas the test for the other seroprevalence studies in Switzerland (part of Corona Immunitas research network), including the study of adults in the canton of Zurich, was confirmed in July 2020 [2]. As the reviewer notes, if test accuracy parameters are known, it is possible to compare the seroprevalence estimated based on different test results on the population level. However, the comparison is less likely to be systematically biased on the population level if based on the same test (indeed, a single serological test is used in seroprevalence studies in Switzerland for this reason), and is only possible on individual level if using the same test. Indeed, the estimated seroprevalence, even adjusting for testing parameters, is slightly different for the two tests.
- For that matter, it doesn't seem to me that the results from the two serological assays are similar, when considering figure 1 (especially for lower and middle level).
Response:
Thank you for the comment. To clarify the difference between the two tests in different grades, we added the numerical estimates for SenASTriS test to the revised Results (lines 263-5). Although the 95% credible intervals for both tests overlap (and include the estimated values of both tests), they are indeed different. We added a comment to Discussion, to stress that the observed age-trends were not concordant with the two tests (lines 345-9), and removed the statement on the trend in seroprevalence at the school levels from the Conclusion (lines 86, 431-4).

- In results section, line 246-256, what is the purpose of the set of proportion in line 246-256 ? This section seems to introduce some arbitrary cut-off in sample sizes that are difficult to interpret.
Response:
Thank you for the question. The purpose of reporting the proportion of classes with at least one seropositive child in all classes and a subset of classes with higher participation rate was to explore if the distribution of seropositive children is substantially different depending on the participation rate. It could be expected, that if less children are tested within a class (low participation rate), a seropositive child is less likely to be detected. Thus, we also report the proportion of classes with at least one seropositive child among the classes with high participation rate (≥5 and ≥50% children testing within a class). We revised the description of the analysis of seropositive children clusters within classes in the Methods, to specify this purpose (lines 228-33).
Upon reflecting further, we believe that the reporting a third subset of classes with very high participation is indeed somewhat arbitrary and potentially not necessary. Thus, we removed it from the Methods and Results.

- Also, figure 3 does not seem to provide a clear statement on the relation between sample size per class

and prevalence. The authors might consider a simpler plot of proportion positive against sample size.
Response:
Thank you for your suggestion. We have reconsidered Figure 3, and tried plotting the absolute and relative (proportion) numbers of tested children separate (see Figure 1 in the attached response document).

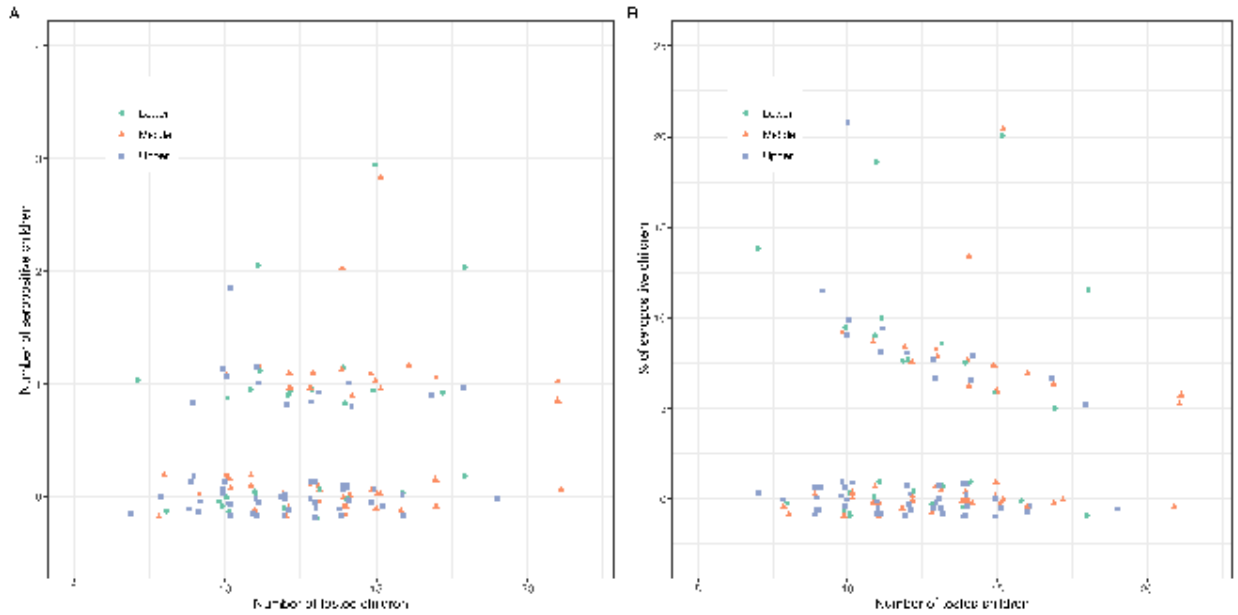

Figure 1 Clustering of seropositive children in classes: number and proportion of seropositive children in the tested classes
Figure 1A shows the underlying plot of the original figure, while Figure 1B plots the percentage (previously marked in categorical 5% lines). However, Figure 1B is rather misleading without confidence intervals added to each dot, because the calculated proportions reflect rather small numbers. Precision of such proportions with sample sizes smaller than 20 as in classes is quite low, so that plotting the observed proportions themselves is not very accurate [3].
This alternative graph might be more informative if populations were aggregated on higher scale – e.g., entire schools. However, since the study focuses on clustering within classes rather than schools, we believe that plotting the proportion of seropositive children in schools is not as important.
Therefore, we opted to keep the original figure. To make it more informative, we added an explanatory comment to its footnote, to explain the meaning of the diagonal (%) lines (lines 3316-8).

- Please make sure the term "dark figure" is sufficiently explicit and useful to be used in this article.
Response:
Thank you for the suggestion. We removed the term “dark figure” from the manuscript, as it is explained more clearly with the more explicit definitions (ratio of the total number of confirmed infections to seropositive cases).
Reviewer: 2
Dr. Diego Andrey, Geneva University Hospitals Comments to the Author:
This study by Ulyte et al. provides a very interesting picture of SARS-CoV-2 seroprevalence among school children, in Switzerland. Large sampling of venous blood in children represent a significant challenge. The manuscript is well written.

Below one major and a few minor comments:

Major comment
- abstract and line 294: the trend for higher seroprevalence in younger children was observed with the ABCORA 2.0 immunoassay but does not seem to be found within the 2476 blood samples tested by the SenASTrIS immunoassay, based on Figure 1. In addition, this trend was not observed in other countries or Switzerland regions, to the best of my knowledge. This conclusion should thus be toned down in the abstract. These differences across methods should be discussed in the discussion.
Response:
Thank you for your suggestion. ABCORA results were the main outcome of the study (we clarified this in the revised Methods, line 189), therefore, we interpreted the group differences primarily based on these results. In contrast, the purpose of SenASTriS test was primarily to compare the overall seroprevalence estimates in children and adults.
To address your comment and report the differences more transparently, we added the numerical estimates for the school levels based on SenASTriS test to Results (lines 263-5). We commented that this trend was not observed with SenASTriS test and in most of the other studies in the Discussion (lines 345-7), added a comment that the trend could as well be a chance finding (lines 388-91), and removed the statement from the conclusion both in Abstract and main text. We amended the conclusion of the Discussion to reflect that the observed trends might be different in the subsequent testing rounds (line 439).

Minor comments
- line 120: please define or describe the Swiss mild lockdown measures.
Response:
Thank you for the suggestion. We have added this description to the Introduction (lines 127-30).

- line 173-179. The authors should provide a reference or link for the ABCORA 2.0 Luminex immunoassay if available, ideally the validation study or report.
Response:
Thank you for the suggestion. We added a reference to the ABCORA 2.0 validation report (line 191).

In addition, was whole blood, plasma or serum tested? Please specify.
Response:
Thank you for the question. Serology was assessed in serum. We specified this in the revised Methods (line 189).

- line 288. in another region of Switzerland, (similar children-adult seroprevalence and only 1/100 ratio cumulative incidence to seroprevalence in children). It should be mentioned and the following reference cited DOI: 10.1093/cid/ciaa1702
Response:
Thank you for the suggestion. The mentioned study describes 40 clinical cases of SARS-CoV-2 infection in children, and the proportion of symptoms reported in the infected children as well as their household contacts. The study did not investigate SARS-CoV-2 serology. We added it to the revised Discussion, to illustrate that the numbers of diagnosed infections in children are significantly lower than those in adults (line 374). We also added further references pointing to similar seroprevalence in children and adults in Geneva in April and November 2020 (line 375-6).
- although the lack of symptoms specificity probably plays a significant role, as stated, the influence of testing guidances in children versus adults and even testing shortage issues (was it the case in Canton Zurich ?) could have influence these results. The authors should clarify RT-PCR testing guidances in Switzerland during this period.
Response:

Thank you for the suggestion. We have added more information on the testing guidance in February-July 2020 in Switzerland to the revised Discussion (lines 403-7).

- line 336. Anosmia, in adults, is considered specific for Covid-19 and possibly less prone to recall biais. The authors could mention that it was not frequently and specifically identified in seropositive children.
Response:
Thank you for the suggestion. We added a sentence in the Discussion to specify that anosmia was not reported by any of the seropositive children (line 397).

- line 360, the ratio is lower for children 1 : 89 versus 1 : 12 adults, not higher.
Response:
Thank you for the comment. We have rewritten the sentence to make it unambiguous (lines 431-4).

VERSION 2 – REVIEW

REVIEWER	Le Vu, Stéphane Santé publique France, Infectious Diseases
REVIEW RETURNED	15-Mar-2021

GENERAL COMMENTS	The authors only partially addressed my comments and especially my main concern on how to account for confirmed cases. " ... the absence of children acutely ill with SARS-CoV-2 infection during the testing should not have influenced the results substantially." I get that infections from March-May probably outweight those from the study period and it would be useful to find this in the article. Of course "rtPCR and serological tests have different accuracy parameters" but I guess accuracy is assessed against the gold standard of confirmed rtPCR. So that it would be appropriate to include any current or past confirmed cases in the seroprevalence estimate, if it means to represent cumulative incidence of the infection. At least, as per my initial comment, the authors could provide in their article the frequency of confirmed or suspected cases during the study period, better explain why they were excluded and discuss the impact on estimates. "Thank you for the comment. We have revised the Methods to describe the Bayesian model in greater detail (lines 218-25)" The added description of the model is still vague and does not allow a proper assessment of the statistical methods. A description containing the regression equation, prior specification, summary of posterior distributions, type of sampling algorithm, etc. would be useful.

	"Although the 95% credible intervals for both tests overlap (and include the estimated values of both tests), they are indeed different." The interpretation of figure 1 still concludes that estimates are similar between the two tests. (line 262)

VERSION 2 – AUTHOR RESPONSE

Reviewer: 1
Dr. Stéphane Le Vu, Santé publique France
Comments to the Author:
The authors only partially addressed my comments and especially my main concern on how to account for confirmed cases.

" ... the absence of children acutely ill with SARS-CoV-2
infection during the testing should not have influenced the results substantially."

I get that infections from March-May probably outweight those from the study period and it would be useful to find this in the article.

Of course "rtPCR and serological tests have different accuracy parameters" but I guess accuracy is assessed against the gold standard of confirmed rtPCR. So that it would be appropriate to include any current or past confirmed cases in the seroprevalence estimate, if it means to represent cumulative incidence of the infection.

At least, as per my initial comment, the authors could provide in their article the frequency of confirmed or suspected cases during the study period, better explain why they were excluded and discuss the impact on estimates.

Response: Thank you for the comment and suggestions. We added to the Discussion that we do not have information on how many children among the eligible could not attend the testing due to acute SARS-CoV-2 infection (lines 392-5). Major reason for their exclusion was inability to test individual children at home rather than at school or at another date (lines 394-5). However, given the low incidence of the reported cases in children and general population during the testing period in the canton of Zurich, and substantially higher incidence in March-April, we believe that such potentially missed cases should not lead to significant underestimation of the seroprevalence. We added this to the Discussion (lines 395-400).
Our results showed that only approximately 1 in 89 infections in children and adolescents were detected with RT-PCR in February-July 2020 (lines 261-4). Selection of symptomatic children or those with better access to testing was likely during this period. In contrast, our study relied on random sampling (of schools and classes). Combining different diagnostic methods applied to different populations to improve the accuracy of the estimated cumulative incidence would require complex modelling and additional assumptions, which would be beyond the scope of this cohort study.

"Thank you for the comment. We have revised the Methods to describe the Bayesian model in greater detail (lines 218-25)"

The added description of the model is still vague and does not allow a proper assessment of the statistical methods. A description containing the regression equation, prior specification, summary of posterior distributions, type of sampling algorithm, etc. would be useful.

Response: Thank you for the suggestion. We have added the detailed description of the model and weighing procedure to estimate seroprevalence to Appendix 1. The same modelling approach is used by all Corona Immunitas research network studies in Switzerland, and the reference to the detailed description is provided in the Methods section of the manuscript (Stringhini et al. 2020: reference 16) as well as in the appendix. The model is described in even greater detail and example code for implementation in the open access repository linked to Stringhini et al. 2020 article; we provide links to it in the Appendix.

"Although the 95% credible intervals for both tests overlap (and include the estimated values of both tests), they are indeed different."

The interpretation of figure 1 still concludes that estimates are similar between the two tests. (line 262)

Response: Thank you for the comment. We have adjusted the text further to stress the difference between the estimates, which is, however, small in absolute terms and with overlapping credibility intervals (lines 257-8).