Prevalence of RT-PCR-detected SARS-CoV-2 infection at schools: First results from the Austrian School-SARS-CoV-2 Study

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Abstract

Background: There is much debate about the role of schools and children in the SARS-CoV-2 pandemic. We aimed to quantify reliably the prevalence of SARS-CoV-2 infections at schools detected with reverse-transcription polymerase-chain-reaction (RT-PCR).

Methods: This nationwide prospective cohort study monitors a representative sample of pupils (grade 1-8) and teachers at Austrian schools throughout the school year 2020/2021. We repeatedly test participants for SARS-CoV-2 infection using a gargling solution and RT-PCR. We herein report on the first two rounds of examinations. We used mixed-effect logistic regression to estimate odds ratios and robust 95% confidence intervals (95% CI).

Findings: We analysed data on 10734 participants from 245 schools (9465 pupils, 1269 teachers). Prevalence of RT-PCR-detected SARS-CoV-2 infection increased from 0.39% at round 1 (95% CI 0.28-0.55%, 29 September-22 October 2020) to 1.42% at round 2 (95% CI 1.06-1.90%, 10-16 November). Odds ratios for SARS-CoV-2 infection were 2.29 (95% CI 1.26-4.17, P=0.007) in regions with >500 vs. ≤500 inhabitants/km^2, 1.69 (95% CI 1.42-2.00, P<0.001) for a two-fold higher regional 7-day incidence, and 2.71 (95% CI 1.68-4.39, P<0.001) in pupils at schools with a high/very high vs. low/moderate social deprivation index. Associations of community incidence and social deprivation persisted in multivariable models. There were no differences between age groups, sexes, pupils vs. teachers, or primary (grade 1-4) vs. secondary schools (grade 5-8).

Interpretation: This monitoring study in Austrian schools revealed SARS-CoV-2 infection in 0.39%-1.42% of participants and identified associations of regional community incidence and social deprivation with higher prevalence.

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Introduction

The SARS-CoV-2 pandemic poses unprecedented challenges on our educational systems.[1] As part of wider strategies to contain the spread of the SARS-CoV-2 virus, many countries have devised measures at schools with the aim of reducing infection risk. These measures include adapted in-person learning (e.g. reduced class sizes, staggered time tables, wearing of masks), complete school closures coupled with virtual learning, or hybrid models.[2] School closures represent a very effective non-pharmaceutical intervention to reduce the transmission of SARS-CoV-2,[3] but have many adverse consequences.[1] Thus, there is extensive debate about the role of schools and children in the SARS-CoV-2 pandemic.[1,4]

Several prior studies have examined representative samples of the general population to assess how frequently SARS-CoV-2 infections occur in children compared to adults. For instance, seroepidemiological studies[5–7] showed a somewhat lower prevalence of SARS-CoV-2 antibodies in children than in adults, but it remains unclear whether this difference arises from reduced exposure related to schools closures, a distinct immune response, or – indeed – reduced susceptibility. Furthermore, screening studies of the general population in the UK demonstrated significant time trends in SARS-CoV-2 infection in children over the past months, with a sharp increase in prevalence in the latest report.[8,9] In contrast to these studies in the general population, evidence from studies with a specific focus on schools is sparse and is largely restricted to contact-tracing and notification-based studies,[10] rather than large-scale screening of schools for SARS-CoV-2 infection. As children are more likely to be asymptomatic than adults after a SARS-CoV-2 infection,[1] large-scale screening studies are particularly needed for comprehensively investigating their role in the SARS-CoV-2 pandemic.

We therefore designed the School-SARS-CoV-2 Study, a nationwide prospective cohort study that screens pupils and teachers at schools in Austria for the occurrence of SARS-CoV-2 infection. We planned to examine study participants repetitively throughout the school year 2020/2021 in 3-5 week intervals. In the present analysis, we analysed data from the first two rounds of examinations with two main aims: (i) to reliably quantify the prevalence of SARS-CoV-2 infection detected with reverse-transcription polymerase-chain-reaction (RT-PCR) and (ii) to determine factors that may be associated with a higher or lower prevalence, thereby informing upcoming public health policies.

Methods

Study population

The School-SARS-CoV-2 Study is a nationwide prospective cohort study that monitors a representative sample of pupils and teachers in Austrian schools for presence of RT-PCR-detectable SARS-CoV-2 infection. Throughout the school year 2020/2021, repeat measurements are conducted every 3-5 weeks. The present analysis reports on the first two rounds of examinations. After excluding schools with less than 20 pupils from the sampling

frame, we randomly selected a total of 250 schools to participate in the study. The selection process was stratified by federal states, employed selection probabilities proportional to the numbers of pupils enrolled at the schools, and involved primary schools (grade 1-4) and secondary schools (grade 5-8).

Within each school, 60 pupils spread across all classes were invited randomly to participate in the study. In small schools with a total number of pupils less than 60, all pupils at the respective school were invited. The study population was supplemented with a random selection of teachers at a target sampling proportion of 1:10, compared to the number of pupils selected at a school.

The study received ethics approvals by the ethics committees of the Medical University of Graz (no. 32-672 ex 19/20), Medical University of Innsbruck (no. 1319/2020), the Johannes Kepler University of Linz (no. 1222/2020), and the University of Vienna (no. 00591/2020). Written informed consent was obtained from (i) teachers, (ii) participants and their legal representative for pupils aged 14 years or older, or (iii) their legal representative only for pupils younger than 14 years, according to the ethical approval.

SARS-CoV-2 detection by RT-PCR

To test participants for presence of RT-PCR-detectable SARS-CoV-2 infection, they were asked to gargle 5 ml of a physiological saline solution or a modified Hank's balanced salt solution ($CaCl_2$ x $2H_2O$ 1.26 mmol, $MgCl_2$ x $6H_2O$ 0.493mM, $MgSO_4$ x $7H_2O$ 0.41 mM, KCl 5.33 mM, KH_2PO_4 0.44 mM, $NaHCO_3$ 4.17 mM, NaCl 137.93 mM, Na_2HPO_4 0.34 mM, D-Glucose 5.56 mM) for a total of 60 seconds. Participants were asked not to eat or drink for at least one hour before gargling. After gargling, the specimen was first transferred to a 50 ml falcon tube and subsequently pipetted into a sample tube (approximately 700 µl), cooled at a temperature of 2-8 °C, and transported by courier to one of the four study laboratories for further analysis within a day. To assure that gargling specimen was collected in a standardised manner, school doctors, their assistants, and participants received access to training videos and printed material with detailed step-by-step instructions. Gargling for sample generation is part of the Austrian test strategy outlined by the Austrian Ministry of Health and is widely applied in Austria also for diagnostic testing. Gargling has been demonstrated to produce comparable sample quality like throat swab samples for other respiratory viruses[11] and has also been applied successfully for the detection of SARS-CoV-2.[12–15]

At the laboratories, sample inactivation, RNA extraction, and RT-qPCR detection of SARS-CoV-2 was performed according to previously established protocols (for details, see **Supplementary Material**). Gargling samples were analysed in pools with a maximal pool size of 10. Positively tested pools were opened and samples were analysed individually. For all positively reported samples at least two viral genes were detected. SARS-CoV-2 PCR results were immediately reported to the study participants and school administrations via text

messaging and/or email. Whenever a positive test result was obtained, the local health authorities were also informed instantaneously according to Austrian law.

Collection of additional data on participants and schools

In addition to the exact time point and the SARS-CoV-2 PCR test result, data were recorded on the participants' age and sex and – for pupils – the grade and class they currently attend. At the school level, we collated information on (i) the type of school (primary vs. secondary school), (ii) the geographical location, (iii) the total number of teachers, pupils, and classes at the school, (iv) population density at the municipality in which the school is located, and (v) an average social deprivation index for the pupils attending the school.

The social deprivation index had been ascertained in 2013 using methods described previously[16]. In brief, it combined information on four distinct domains: (i) highest level of education of the pupil's parents, (ii) current occupation of the pupil's parents, (iii) migration background, defined as both parents born in a foreign country (OECD definition), and (iv) first language other than German. Following the recommendation of Bruneforth *et al.*,[17] we categorised the social deprivation index score, as "low" (score 100-<115), "moderate" (score 115-<125), "high" (score 125-135) and "very high" (score >135). Finally, we obtained data on the regional 7-day incidence of documented COVID-19 cases for all 94 districts in Austria via the publicly available data and dashboard of the Austrian health authority[18] and merged these data with the school datasets according to districts and time points of the gargling tests.

Statistical methods

In planning the sample size for this study, we estimated the widths of 95% confidence intervals of SARS-CoV-2 infection prevalences ranging between 0 and 1%, assuming a design effect of 4.0, and considered a sample size involving 11900 pupils and 1200 teachers recruited across 250 schools to afford sufficient statistical power. For instance, based on the assumed design effect, we expected prevalences of 0.15%, 0.40%, and 0.70% to be associated with 95% confidence intervals of 0.05-0.36%, 0.21-0.68%, and 0.45-1.05%, respectively.

In descriptive analyses, we summarised categorical variables as counts and percentages and continuous variables as means and standard deviations (if approximately normally distributed). We tested whether characteristics of participating schools were associated with each other using χ^2-tests. In analyses of the prevalence of RT-PCR-detectable SARS-CoV-2 infection (ie, overall prevalence, differences in the prevalence across the two rounds of examinations, and differences in the prevalence across subgroups), to take into account the clustering of the data at the school level, we calculated 95% confidence intervals from robust standard errors based on clustered Sandwich estimators[19,20]. Participants with a positive test result at round 1 were censored at round 2, as they are not considered to be at risk after having already experienced the infection. Odds ratios for RT-PCR-detectable SARS-CoV-2 infection were estimated using mixed-effect logistic regression models with random intercepts at the participant level. In a first step, participant and school characteristics were entered in a univariable model. Variables that

were associated with RT-PCR-detectable SARS-CoV-2 infection at a significance level of P ≤0.05 were, in a second step, entered concomitantly in a multivariable model. We conducted analyses with Stata version 14.1 MP. We used two-sided statistical tests and considered P values ≤0.05 as statistically significant.

Role of the funding source

The Federal Ministry of Education, Science and Research of the Republic of Austria funded the study and supported sample selection, logistics, and assessments at the schools, but had no role in data analysis or writing of the report. The corresponding authors had full access to all data in the study and had final responsibility for the decision to submit for publication.

Results

Study population

The flow chart of the schools and participants involved in our study is shown in **Figure 1**. Of the 250 schools selected for our study, 245 schools participated (98%). At these schools, a total of 10957 participants were recruited into our study during the two rounds of examinations. After exclusion of 223 individuals without a valid gargle test result (2.0%, mostly due to transfer of insufficient volumes to the test tubes by the school doctors), 10734 individuals were remained for further analysis.

Key characteristics of schools and participants are provided in **Table 1** and **Figure 2**. The number of participants was distributed equally across primary and secondary schools (**Table 1**). At the schools participating in the study, the median total number of pupils was 230 (interquartile range [IQR] 147-331), the median total number of teachers was 27 (IQR 17-43), and classes consisted – on average – of 21 pupils (IQR 18-23) (**Figure 2**). Schools recruited a median of 40 pupils (IQR 29-50) and 6 teachers (IQR 5-6) into our study. On average, teachers constituted 11.8% of a school's study sample (IQR 10.0-14.6%).

Supplementary Table 1 illustrates pairwise associations between several school characteristics. Higher local population density was associated with a greater social deprivation index (P<0.001) and higher average number of pupils per class (P<0.001). As expected, primary schools had smaller class sizes than secondary schools (P<0.001).

Prevalence of RT-PCR-detectable SARS-CoV-2 infection

Table 3 presents detailed information on round 1 and 2 of our study. The first round was conducted between 29 September 2020 and 22 October 2020 and covered 243 schools spanning across all nine federal states of Austria and resulted in 10156 participants. The median regional 7-day incidence of SARS-CoV-2 cases in the general population at the time of the assessments was 75 per 100.000 inhabitants (IQR 43-125). The second round was conducted between 10 and 16 November 2020, ending early due to the closure of schools as part of a wider lockdown

in Austria employed on 17 November 2020 and resulted in 3745 participants. Round 2 therefore involved 88 schools only across five federal states of Austria. The corresponding median regional 7-day incidence in the general population was 419 per 100.000 inhabitants (IQR 392-641).

At round 1, 40 out of 10156 participants were tested positive, corresponding to a prevalence of 0.39% (95% confidence interval [CI]: 0.28-0.55%) (**Table 2**). At round 2, 53 additional participants out of 3745 participants were tested positive. This corresponded to a prevalence of 1.42% (95% CI 1.06-1.90%) and was significantly higher than at round 1 (odds ratio [OR] 3.63, 95% CI 2.36-5.59, P<0.001). At round 1, 209 schools recorded no cases of SARS-CoV-2 infection, 28 schools recorded one case, and 6 schools recorded two cases. At round 2, 52 schools recorded no cases, 23 schools recorded one case, 9 schools recorded two cases, and 4 schools recorded three cases.

Association of participant and school characteristics with SARS-CoV-2 infection

Figure 3 investigated whether participant and school characteristics were associated with the odds of being tested positive for RT-PCR-detectable SARS-CoV-2 infection. In the unadjusted model (**Figure 3A**), significant positive associations were detected for local population density, regional 7-day incidence in the general population, and the social deprivation index. The ORs for RT-PCR-detectable SARS-CoV-2 infection were 2.29 for schools located in regions with >500 vs. ≤500 inhabitants per km^2 (95% CI 1.26-4.17, P=0.007), 1.69 for a two-fold higher regional 7-day incidence (95% CI 1.42-2.00, P<0.001), and 2.71 in pupils at schools with a high or very high social deprivation index compared to their counterparts (95% CI 1.68-4.39, P<0.001).

In the multivariable adjusted model involving these three parameters (**Figure 3B**), only regional 7-day incidence in the general population and the social deprivation index retained statistical significance, with ORs of 1.66 (95% CI 1.38-1.99, P<0.001) and 2.05 (95% CI 1.23-3.42, P=0.006), respectively. There was no significant association for local population density in the multivariable adjusted model (P=0.719).

Discussion

The present study reports on the prevalence of RT-PCR-detectable SARS-CoV-2 infection at Austrian schools involving a total of 245 schools and 10734 individuals. At the first round of examinations conducted between 28 September and 22 October 2020, we detected SARS-CoV-2 infection in 0.39% of the study participants (95% CI 0.25-0.55%). At the second round conducted between 10 and 16 November, we observed an approximately 3.6-fold higher prevalence with a point estimate of 1.42% (95% CI 1.06-1.90%). Furthermore, among a range of participant and school characteristics, regional 7-day incidence in the general population and social deprivation emerged as relevant factors associated with presence of RT-PCR-detectable SARS-CoV-2 infection at the participating schools. Collectively, the study provides crucial

evidence about the extent and determinants of SARS-CoV-2 infection at schools, thereby informing decision making about in-person education at Austrian schools and elsewhere in upcoming months.

To which extent children are inflicted by SARS-CoV-2 and to which extent opening or closing of schools impacts the dynamics of the SARS-CoV-2 pandemic is much debated.[1,4] One aspect of this debate concerns the occurrence of SARS-CoV-2 infection in children compared to adults. In our study, we detected SARS-CoV-2 infection in 1.42% (95% CI 1.06-1.90%) of study participants at the second round of examinations (10-16 November). This prevalence was somewhat less than the screen-detected prevalence of 2.12% in adults, which was observed by a different nationwide population-based study[21] conducted at a similar time frame (12-14 November, 48 out of 2263 tests positive based on nasopharyngeal swabs). A crude comparison of the two studies yields a prevalence ratio of 0.67 (95% CI 0.45-0.98, P=0.039), although one should interpret this ratio with caution due to different testing methods (e.g. gargling vs. nasopharyngeal swabs, sample pooling only used in the school study) and potential selection biases (e.g. participation rate 28.9% in the latter study[21]).

This prevalence ratio is consistent with several population-based studies that investigated seroprevalence of antibodies to SARS-CoV-2 across the age spectrum.[22] For instance, the seroepidemiological study in Spain ENE-COVID reported a continuous rise in seropositivity from the youngest age groups (1.1% <1 yr, 2.1% 1-4 yrs, 3.1% 5-9 yrs, 4.0% 10-14 yrs) into adulthood with a plateau at around 6% at 45 years of age or older.[5] Differences by age were also observed in regional seroprevalence studies in Switzerland (SEROCoV-POP Study[6]) and Austria (Ischgl Study[7]). While these differences may stem from lower susceptibility to SARS-CoV-2 in children (e.g. due to reduced *ACE2* expression in the nasal epithelium[23,24]), they could also arise from reduced exposure (e.g. due to school closures) or a milder clinical course of the infection coupled with a distinct immune response characterised by absence of anti-nucleocapsid IgG antibodies[25]. Furthermore, in light of changing policies on school-based preventive measures and potential seasonal variation, prevalence estimates of SARS-CoV-2 infection in children may be highly volatile. For instance, the latest round of the UK-based REACT-1 study[8] reported that prevalence in children aged 5-17 years had increased sharply from mid-September to December 2020 and had surpassed the prevalence observed in adults, with similar time trends reported by the UK Office of National Statistics.[9]

Our study has identified several factors associated with higher odds of a SARS-CoV-2 case in the setting of in-person education at schools. The strongest link was observed with regional 7-day incidence in the general population, associated with 1.66-fold odds (95% CI 1.38-1.99, P<0.001) for each doubling of community incidence. This observation is in agreement with a Public Health England report that regional community incidence was significantly associated with the risk of COVID-19 outbreaks at schools.[10] Our study crucially extends the existing evidence to the months in autumn and to a markedly higher community incidence (median 7-day incidence 114 vs. ~6 per 100.000). Another important factor associated with higher odds

of SARS-CoV-2 cases was social deprivation. While this finding highlights the need for additional carefully-targeted support of affected children, the underlying mechanisms remain to be determined. These likely go beyond school-related factors and may include less self-isolation and physical distancing,[26] cramped living conditions,[26] and lack of possibilities for parents to work from home, also coupled with challenges in taking care of a sick child. Of note, ecological analyses in the US[27] and Germany[28] have previously shown that deprived areas are disproportionately affected by SARS-CoV-2. It is also noteworthy that we detected no significant differences in SARS-CoV-2 prevalence between primary vs. secondary schools, smaller vs. larger class sizes, pupils vs. teachers, and females vs. males, nor according to the participants' age.

Another key aspect in assessing the role of schools in the SARS-CoV-2 pandemic is the infectiousness of children. It is well established that symptomatic children have viral nucleic acids in their nasopharynx at levels comparable with adults.[29,30] SARS-CoV-2 viruses from children can be cultured in vitro, suggesting that transmission from them is plausible.[31] Actual infectiousness is investigated best in contact tracing studies conducted within schools and/or households. Some of these studies indicate that children may be somewhat less infectious than adults.[1] Analyses based on notifications of SARS-CoV-2 cases showed low transmission rates in Ireland before school closures[32] and in Australian schools operating at reduced physical attendance[33], but these analyses did not include asymptomatically infected children. In Germany, after reopening of schools, health authorities identified 2.2 outbreaks per week with four cases per outbreak on average.[34] In England, outbreak analyses largely based on symptomatic cases showed that staff members were more frequently the seeding case in schools than students.[10] In Austria, health authorities linked 4.3% of the SARS-CoV-2 infections recorded between 5 October and 15 November 2020 with a traceable source of infection (42%) to educational settings[35], but it should be kept in mind that infected children are often asymptomatic and that children as contact persons were not systematically tested. A large-scale household study in South Korea suggested infected children aged 6-11 years were less contagious than infected older children that were even more contagious than adults.[36] It is important to stress that these reduced transmission rates have been observed against the backdrop of extensive preventive measures implemented in schools, including reduced class sizes, staggered time tables, frequent ventilation, wearing of masks, and staying home even with minimal symptoms.[2] In contrast, a report from a US summer camp illustrated the potential efficient spread in children even if they were younger than 10 years.[37] After a 10-day camp involving various indoor and outdoor activities, including singing and cheering, a staggering attack rate of 44% was observed among 597 participants and 51% of the children <10 years were infected. Consistent with this observation, a prospective household study from the US reported substantial transmission also when the index case was a child.[38]

The study we presented herein has several major strengths. It evaluated prevalence of SARS-CoV-2 detection at a time during which in-person education at school was in place nationwide (for pupils in grade 1-8). Furthermore, it involved a large-scale sample of schools across

Austria, thereby enhancing generalisability of our findings. It also has several limitations. Prevalence of SARS-CoV-2 infections detected in this study likely underestimate the true burden, since symptomatic individuals (pupils and teachers) or those retained in quarantine were not present at the time of testing. Also, the second repeat assessment of study participants was incomplete due to the decision of the Austrian government to close schools for in-person learning on 17 November 2020. We anticipate restarting repeated screening of the cohort mid-January and continue assessments until the end of June.

In conclusion, in a large-scale study involving 245 schools in Austria, prevalence of RT-PCR detected SARS-CoV-2 increased from 0.39% to 1.42% within a period of one month. Higher community incidence (quantified as the 7-day incidence in the school district) and social deprivation were associated with higher odds of SARS-CoV-2 at the schools. By determining SARS-CoV-2 prevalence and identifying potential contributing factors, our study provides evidence relevant to the decision making about in-person education at Austrian schools and elsewhere.

Contributors

PW, BL, RK, and MW designed the study. RK, JZ, AK, DvL, HS, IS and MW planned the logistics and/or laboratory measurements for the study. BH, ES, HS, RH, DB, WB, CD, and JP performed laboratory measurements. PW and AB performed the statistical analysis. PW, BL, RK, and MW drafted the manuscript. All authors critically revised the manuscript and agreed to be accountable for all aspects of the work.

Declaration of interest

The authors report no conflicts of interest in relation to this study.

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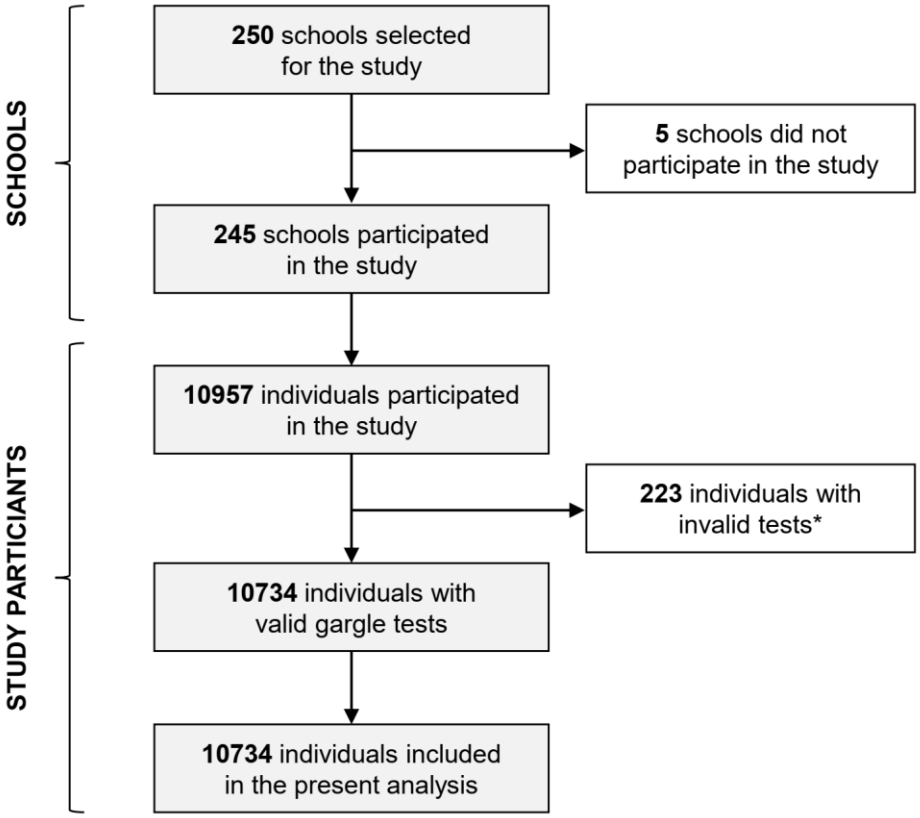
Figure 1. Flow-chart of the recruitment into the study.



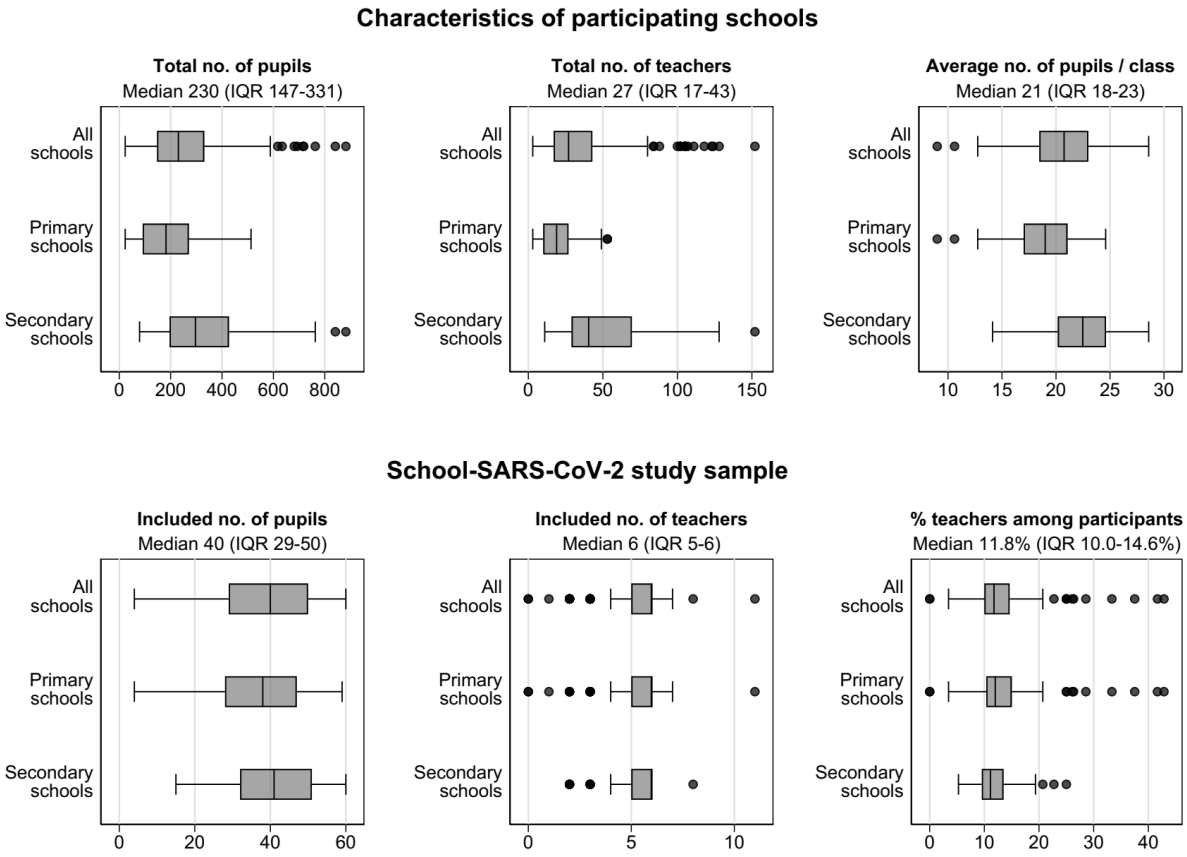
*Mostly due to transfer of insufficient volumes to the test tubes by the school doctors.

Table 1. Descriptive summary of schools and participants investigated as part of the School-SARS-CoV-2 Study.

	No. of schools (%)	No. of participants (%)
Total	245 (100.0%)	10734 (100.0%)
Type of school		
Primary school	129 (52.7%)	5367 (50.0%)
Secondary school	116 (47.3%)	5367 (50.0%)
Geographical location by federal state*		
Burgenland	15 (6.1%)	669 (6.2%)
Carinthia	15 (6.1%)	665 (6.2%)
Lower Austria	46 (18.8%)	2072 (19.3%)
Upper Austria	42 (17.1%)	1716 (16.0%)
Salzburg	15 (6.1%)	610 (5.7%)
Styria	29 (11.8%)	1287 (12.0%)
Tyrol	18 (7.3%)	583 (5.4%)
Vorarlberg	15 (6.1%)	579 (5.4%)
Vienna	50 (20.4%)	2553 (23.8%)
Local population density		
≤100 inhabitants/km^2	23 (9.4%)	893 (8.3%)
>100-250 inhabitants/km^2	47 (19.2%)	1863 (17.4%)
>250-500 inhabitants/km^2	34 (13.9%)	1396 (13.0%)
>500-10000 inhabitants/km^2	126 (51.4%)	5781 (53.9%)
>10000 inhabitants/km^2	15 (6.1%)	801 (7.5%)
Social deprivation index		
High/very high	183 (75.6%)	7833 (73.9%)
Low/moderate	59 (24.4%)	2762 (26.1%)
Average no. of pupils/class		
≤20 pupils/class	104 (42.4%)	4087 (38.1%)
>20 pupils/class	141 (57.6%)	6647 (61.9%)

*In comparison, the overall number of pupils per federal state were 21056 (3.1%) in Burgenland, 41322 (6.1%) in Carinthia, 128962 (19.0%) in Lower Austria, 120603 (17.8%) in Upper Austria, 42863 (6.3%) in Salzburg, 89844 (13.2%) in Styria, 57570 (8.5%) in Tyrol, 33285(4.9%) in Vorarlberg, and 143617 (21.1%) in Vienna.

Figure 2. Total no. of teachers and pupils at participating schools and no. of teachers and pupils selected to be included in the School-SARS-CoV-2 Study.



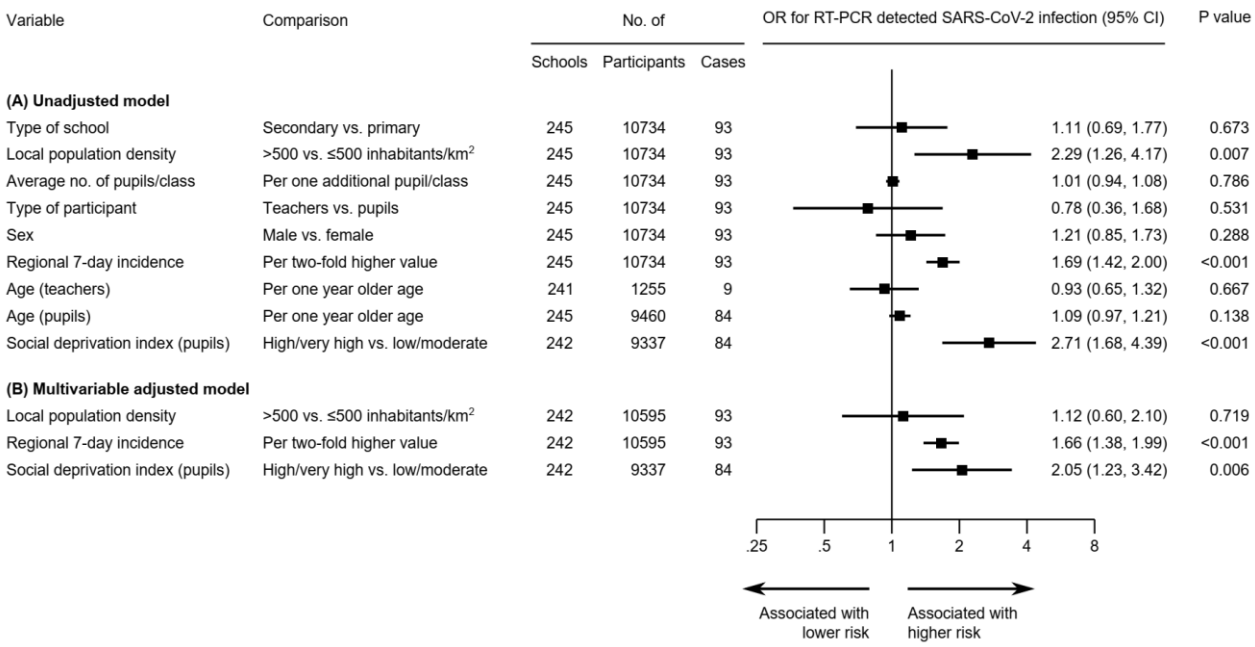
IQR denotes interquartile range. Medians and interquartile ranges included in the figure subtitles refer to the overall study sample (all schools).

Table 2. Participant characteristics and prevalence of RT-PCR-detectable SARS-CoV-2 infection at the two rounds of examinations conducted between 28 September and 16 November 2020 within the School-SARS-CoV-2 Study.

	Round 1	Round 2	Overall
Assessment of participants			
No. of schools	243	88	245
Federal states involved in assessment	All	Burgenland, Lower Austria, Upper Austria, Vorarlberg, Vienna	All
Median date of assessment in the year 2020 (range)	12.10. (28.09.-22.10.)	11.11. (10.11.-16.11.)	14.10. (28.09.-16.11.)
Regional 7-day incidence in the general population (per 100,000 inhabitants), median (IQR)	75 (43-125)	419 (392-641)	114 (53-357)
No. of participants	10156	3745	10734
Participants by type of school			
No. at primary school (%)	5029 (50%)	2046 (55%)	5367 (50%)
No. at secondary school (%)	5127 (50%)	1699 (45%)	5367 (50%)
Teachers			
No. of teachers (%)	1222 (12%)	450 (12%)	1269 (12%)
Age (years), mean (SD)	43.9 (11.8)	44.3 (11.9)	44.0 (11.8)
No. of females (%)	986 (81%)	372 (83%)	1026 (81%)
Pupils			
No. of pupils (%)	8934 (88%)	3295 (88%)	9465 (88%)
Age (years), mean (SD)	9.9 (2.4)	9.8 (2.4)	9.8 (2.4)
No. of females (%)	4291 (48%)	1592 (48%)	4541 (48%)
RT-PCR-detectable SARS-CoV-2 infection			
No. of cases	40	53	93
Period prevalence (95% CI)	0.39% (0.28-0.55%)	1.42% (1.06-1.90%)	0.87% (0.68-1.11%)

CI denotes confidence interval, SD denotes standard deviation. 95% CI were calculated from robust standard errors estimated based on clustered Sandwich estimators.

Figure 3. Odds ratios for RT-PCR-detectable SARS-CoV-2 infection at the two rounds of examinations according to participant and school characteristics in an unadjusted model (Panel A) and a multivariable adjusted model (Panel B).



CI denotes confidence interval, OR odds ratio, and RT-PCR real-time polymerase chain reaction. The analysis involved data from round 1 and 2 of the School-SARS-CoV-2 Study. Odds ratios were estimated using mixed-effect logistic regression models with random intercepts at the participant level. 95% confidence intervals were calculated from robust standard errors estimated from clustered Sandwich estimators. Variables that were associated with RT-PCR-detectable SARS-CoV-2 infection at a significant level of ≤0.05 in the unadjusted model (Panel A) were included in the multivariable adjusted model (Panel B).

Supplementary material

Supplementary methods: RNA extraction and RT-qPCR

At the laboratories, gargle samples in 1 ml FluidX tubes with externally threaded caps (Brooks Life Sciences, Chelmsford, MA) were organized into 96-well racks and tube barcodes were scanned (Perception HD, Brooks Life Sciences, Chelmsford, MA). On full racks, positions A1-A4 and E1 were left empty for RNA extraction and RT-qPCR controls. The outside surface of sample tubes was disinfected by briefly dipping the filled racks into 70% ethanol. Subsequently, the racks were centrifuged (500 X *g* for 1 min at room temperature to remove residual ethanol. Thereafter, sample racks were introduced into a laminar flow hood and sample tubes were decapped using a FluidX IntelliXcap 96-format Decapper/Capper (Brooks Life Sciences, Chelmsford). Using a semi-automated Viaflo 96-tip pipettor (Integra Biosciences AG, Zizers Switzerland) and sterile, 300 µl wide-bore filter tips (Integra Biosciences AG, Zizers Switzerland), 12.5 µl of freshly prepared 2 M 1,4-dithiothreitol (DTT, 5-35 mM final concentration, Carl Roth, GmbH, Karlsruhe, Germany) were added to each tube and mixed via pipetting to reduce viscosity. Tubes were recapped and incubated for 10 min at room temperature, followed by centrifugation for 1 min at 500 X *g* at room temperature to enrich cellular material on the tube bottom. Sample tubes were again decapped and surplus sample material was removed into a 96 deep-well "waste" plate, until 200 µl of the cell enriched sample fraction remained in each sample tube. The remaining sample material was gently mixed by pipetting up and down (10 x 180 µl). For pooling of two or more 96-well racks, 50 µl of sample was transferred to a 96 V-bottom deep-well plate (Ritter, Germany) using the Viaflo pipettor. Additional samples were added as 50 µl aliquots. Pools sizes varied from 3 to 10. Pooling has been used for the diagnosis of influenza[1] and has been adapted and employed successfully in the field of SARS-CoV diagnostics[2,3]. Pooled samples were homogenized using mechanical mixing in a KingFisher Flex (ThermoFisher Scientific) with a 96-tip comb for 65 s at fast speed. One hundred µl of the mixed pool was transferred to a 96 deep-well V-bottom plate containing 350 µl of lysis buffer (80 mM Tris pH 6.4, 5.7 M guanidinium isothiocyante, 35 mM EDTA, 2% Triton X-100, 56 mM DTT freshly added) and incubated for 10 min at room temperature. At this step, 10 µl of RNA extracted from previous positively tested samples were added in duplicate as sample processing controls to test for RNA degradation and PCR inhibition (positions A4 and E1; leading to approx. Ct 35 in subsequent RT-qPCR). Automated RNA extraction was performed using the KingFisher Flex Magnetic Particle Processor System (ThermoFisher Scientific) or the CyBio Felix System (Analytik Jena) based on carboxylated magnetic bead separation[4] according to the instruction of the manufacturers.

RT-qPCR was performed with 7 µl of extracted RNA using the Luna Universal Probe One-Step RT-qPCR Kit with the Luna WarmStart RT Enzyme Mix (New England Biolabs, Ipswich, MA) or the BioRad OnestepRT qPCR Kit according to the manufacturers protocol in a final reaction volume of 20 µl. RNA extracts from pooled samples were analyzed by duplexing primers and probes for RP2 (human target; AGATTTGGACCTGCGAGCG, GCAACAACTGAATAGCCAAGGT, HEX-TTCTGACCTGAAGGCTCTGCGCG–BHQ1) with orf1b (viral target, TGGGGTTTTACAGGTAACCT,

AACACGCTTAACAAAGCACTC, TexasRed-TAGTTGTGATGCAATCATGACTAG-BHQ1) and UTR (viral target, AGTGTACAGTGAACAATGCT, ATCACATGGGGATAGCACTA HEX-AGCTGCCTATATGGAAGAGCCCT-BHQ1) with N2 (viral target, TTACAAACATTGGCCGCAAA, GCGCGACATTCCGAAGAA, FAM-ACAATTTGCCCCCAGCGCTTCAG-BHQ1) or by triplex PCR (CDC-N2, CDC-ORF1b, CDC-RP2. Three RT-qPCR reaction controls included: (i) an RNA extract from a previously tested positive sample (position A1) with a target Ct value of 25 or Twist Synthetic SARS-CoV-2 RNA Control 2 (10E4 molecules per reaction); (ii) an RNA extract from a previously tested negative samples (A2); and (iii) a no template control (A3. RT-qPCR reactions were run on a CFX96 Touch Real-Time PCR Detection System (Bio-Rad, Hercules, CA) with cycling conditions of 55°C for 10 min, 95°C for 1 min and 45 cycles of 95°C for 10 s, and 55°C for 30 s for both duplex PCRs. Fluorescence signal was recorded at the 55°C annealing and elongation step of each cycle. Resultant fluorescent curves were analyzed and Ct values were calculated using the CFX Maestro software (Bio-Rad, Hercules, CA). RNA was extracted from individual samples from positive pools using 100 µl of remaining DTT-treated original gargle sample as described above. RNA extracts of unpooled samples were examined using a triplex RT-qPCR targeting N2, orf1b (viral targets), and RP2 (human target) in 20 µl reactions using the PCR conditions described above and the Ct values of positive samples were recorded. In some analyses, unpooled samples were additionally analysed by a duplex PCR (CDC-N1, ORF10).

At the first survey, presence of SARS-CoV-2 RNA of samples send to the Molecular Diagnostics Laboratory, Diagnostic & Research Institute of Hygiene, Microbiology and Environmental Medicine at the Medical University of Graz was determined by real-time PCR (qPCR) using the in vitro diagnostics/Conformité Européenne (IVD/CE)-labeled cobas® SARS-CoV-2 test (Roche Molecular Systems, Branchburg, NJ, USA) for use on the cobas® 6800/8800 system (Roche Molecular Diagnostics, Rotkreuz, Switzerland) according to the manufacturer's instructions [1,2].[5,6] Selective amplification of target nucleic acid from each sample was achieved by the use of target-specific forward and reverse primers for ORF1a/b nonstructural region that is unique to SARS-CoV-2. In addition, a conserved region in the structural protein envelope E-gene was chosen for pan-Sarbecovirus detection. The pan-Sarbecovirus detection set also detect SARS-CoV-2 virus. An RNA Internal Control, used to monitor the entire preparation and PCR amplification process, was introduced into each specimen during sample processing. Results showing inhibition were repeat tested.

At the second survey, a test system consisting of extraction on the KingFisher™ Flex (ThermoFisher Scientific) instrument using the MagMaX™ Viral/Pathogen Nucleic Acid Isolation Kit (Life Technologies Corporation, Austin, TX, USA) according to the manufacturer's instructions and amplification on the CFX96 Touch Real-time PCR Detection System (Bio-Rad) for detection of SARS-CoV-2 RNA according to the protocol described above (triplex PCR for individual samples and duplex PCRs for pools) was used. For extraction, an input volume of 200 µl and an elution volume of 50 µl were chosen.

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Supplementary Table 1. Associations of characteristics of the schools participating in the School-SARS-CoV-2 Study.

No. of schools within categories (column %)	School type		Social deprivation index		Average class size	
	Primary school	Secondary school	Low/ moderate	High/very high	≤20 pupils/ class	>20 pupils/ class
Local population density						
≤100 inhabitants / km^2	17 (13%)	6 (5%)	23 (13%)	0 (0%)	19 (18%)	4 (3%)
>100-250 inhabitants / km^2	25 (19%)	22 (19%)	47 (26%)	0 (0%)	30 (30%)	17 (12%)
>250-500 inhabitants / km^2	19 (15%)	15 (13%)	30 (16%)	2 (3%)	21 (21%)	13 (9%)
>500-10000 inhabitants / km^2	60 (47%)	66 (57%)	80 (44%)	45 (76%)	31 (31%)	95 (67%)
>10000 inhabitants / km^2	8 (6%)	7 (6%)	3 (2%)	12 (20%)	3 (3%)	12 (9%)
	P=0.231		P<0.001		P<0.001	
School type						
Primary school			93 (51%)	33 (56%)	77 (74%)	52 (37%)
Secondary school			90 (49%)	26 (44%)	27 (26%)	89 (63%)
			P=0.494		P<0.001	
Social deprivation index						
Low/moderate					85 (83%)	98 (70%)
High/very high					17 (17%)	42 (30%)
					P=0.017	

P values are from χ^2-tests.