#### ORIGINAL ARTICLE

# Lessons from low seroprevalence of SARS-CoV-2 antibodies in schoolchildren: A cross-sectional study

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#### Abstract

**Background:** Children are discussed as hidden SARS-CoV-2 virus reservoir because of predominantly mild or even asymptomatic course of disease. The objective of this cross-sectional study in May-July 2020 was to assess the prevalence of SARS-CoV-2 antibodies and virus RNA in schoolchildren, consistent with previous infection by contact tracing.

**Methods:** School authorities approached parents for voluntary participation. Interested families were contacted by the study team. A nasal and oropharyngeal swab, a blood sample, and a questionnaire were employed. Primary endpoint was the frequency of SARS-CoV-2 real-time PCR (RT-PCR) and antibody-positive children. Antibody positivity was assessed by a highly sensitive first-line ELISA, and a neutralization assay and two other immunoassays as confirmatory assays.

**Results:** Of 2069 children (median age 13 years, IQR 10-15), 2 cases (0.1%) tested positive for SARS-CoV-2 RNA and 26 cases (1.3%) tested positive for specific antibodies. SARS-CoV-2-specific antibodies exhibited detectable virus-neutralizing activity in 92% (24 of 26 samples). Seropositivity was associated with a history of mild clinical symptoms in 14 children (53.8%), while 12 children (46.2%) remained asymptomatic. Among 13 seropositive children being tested concomitantly with their siblings, only one pair of siblings was seropositive. Contact tracing revealed adult family members and school teachers as potential index cases.

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**Conclusion:** In schoolchildren, the infection rate with SARS-CoV-2 is low and associated with a mild or asymptomatic course of disease. Virus spreading seemed to occur more likely in intergenerational contacts than among siblings in the same household. The presence of neutralizing SARS-CoV-2 antibodies in children may reflect protective adaptive immunity.

#### KEYWORDS

immunity, prevalence, SARS-CoV-2, schoolchildren, seroprevalence

#### 1 | BACKGROUND

The outbreak of coronavirus disease 2019 (COVID-19) in mainland China in December 2019, attributed to a newly detected zoonotic virus called severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2), marked the start of an unprecedented pandemic, to date causing over 89 million infections and 1.9 million deaths worldwide. At present, there are insufficient data to draw conclusions about the significance of children regarding SARS-CoV-2 susceptibility and transmission. While infection happens throughout all age groups, children are less likely to develop severe courses of the disease compared to adults, and a large proportion of children seem to stay asymptomatic.<sup>1,2</sup> However, a small percentage, especially children with underlying medical conditions, do suffer from severe COVID-19 or its sequelae, such as a recently described hyperinflammatory syndrome (pediatric inflammatory multisystem syndrome temporarily associated with SARS-CoV-2/ PIMS-TS).<sup>3,4</sup> Even though data are still scarce, there is growing evidence that at least young children are less likely to pass on the infection, and that schools and nurseries are not likely to become silent hotspots of SARS-CoV-2 transmission.<sup>5,6</sup> Without extensive PCR testing and serosurveillance combined with contact tracing, the question regarding the role of children in this pandemic is likely to remain unanswered. In particular, specific data on household transmission are lacking.<sup>7,8</sup> The aim of this study was to shed light on how many school-aged children and adolescents were infected with SARS-CoV-2 during the early phase of the COVID-19 pandemic in Austria, at which a local outbreak revealed 42% and 27% SARS-CoV-2 antibody seroprevalence in adults and children, respectively.<sup>9</sup> The overall estimated SARS-CoV-2 seroprevalence in adults based on data from 27 regional areas in Austria was 4.7% (95% Cl 1.3%-7.9%) in April 2020. The SARS-CoV-2 infection rate in the same time period was estimated at 0.33% (www.bmbwf.gv.at).

### 2 | METHODS

#### 2.1 | Population screening

This academic study was approved by the Institutional Review Boards. Parents were approached by school authorities of the city of Vienna and invited to participate voluntarily. Interested families

#### Key Message

There are concerns about undetected, asymptomatic or mildly symptomatic pediatric cases and SARS-CoV-2 being unknowingly transmitted in the household, schools, and the community. Based on this concern, most countries worldwide have enforced school closures at the beginning of the pandemic. The objective of this cross-sectional study in May-July 2020 was to assess the prevalence of SARS-CoV-2 antibodies and virus RNA in schoolchildren, consistent with previous infection. This study shows that the seroprevalence of SARS-CoV-2 antibodies in our cohort of children was low (1.3%). All affected pediatric cases were either mildly affected or asymptomatic. Contact tracing of seropositive cases revealed intergenerational index cases (parents, teachers). Due to the high number of siblings in the study population, we could provide insight into transmission patterns among children living in the same household. Virus spreading from child-to-child in the same household seems to occur rarely. With the use of 3 ELISAs and a virus neutralization assay, we detected the neutralizing capabilities of antibodies in 92% of seropositive children. In conclusion, children can acquire SARS-CoV-2, mostly develop mild disease or stay asymptomatic, mount correlates of a protective immune response, but seem to transmit the virus infrequently. These observations might have important implications on school- and childcarerelated public health measures during the pandemic.

were contacted by the study team. Symptomatic children and adolescents were excluded at date of visit. Testing was started on May 18, 2020, which coincided with slowly easing of measurements after 9 weeks of lockdown, and finished on July 2, 2020. Schools opening started in stepwise manner on May 18, 2020. The questionnaires regarding gender, age, symptoms compatible with a SARS-CoV-2 infection and contacts with infected individuals were collected at the day of screening.

#### 2.2 | Detection OF SARS-CoV-2 Nucleic acid

Oropharyngeal and nasopharyngeal samples were analyzed by the BD SARS-CoV-2-Reagents for BD MAX<sup>™</sup> System (Becton, Dickinson and Company, Sparks), which comprises a real-time RT-PCR targeting the N1 and N2 regions of the SARS-CoV-2 N gene.

## 2.3 | SARS-CoV-2-Specific antibodies and antibody neutralizing test (NT)

First-line testing for SARS-CoV-2-specific antibodies was performed with the Wantai SARS-CoV-2 total Ab ELISA (Beijing Wantai Biological Pharmacy Enterprise). This ELISA, with the virus-specific Receptor-Binding-Domain as target antigen, had shown excellent performance and high sensitivity in detecting antibodies in different stages and disease severity in previous studies, with a sensitivity of 90.87%-96.81% and a specificity of 98.80%-100.00% (95% Cl, respectively).<sup>10</sup>

For samples with positive or borderline results from first-line testing, an in-house neutralization test (NT) was performed as described.<sup>11</sup> Briefly, duplicates of serial 2-fold dilutions of heatinactivated serum samples were incubated with 50-100 TCID50 (50% tissue culture infective dose) of SARS-CoV-2 for 1 h at 37 C. The mixture was then added to Vero E6 cells, and incubation was continued for 2-3 days. The presence of infectious (non-neutralized) virus was assessed by microscopic evaluation of cytopathic effects. Samples with a positive or borderline test result from first-line testing, also confirmed by a positive NT result, were finally considered as antibody-positive.

In case of a positive or borderline first-line test result but a negative NT result, a combination of two additional immunoassays were applied as confirmatory tests: the EUROIMMUN SARS-CoV-2 IgG and the SARS-CoV-2-NCP-IgG ELISA both (EUROIMMUN, Lübeck, Germany) using the recombinant structural protein (S1 domain) of the spike protein (IgG) and the nucleoprotein (IgG) as antigens. When these additional confirmatory ELISAs unanimously gave a positive test result, a sample was also considered to be antibody-positive (indicating that the level of neutralizing antibodies was probably too low to be detected by the NT). All ELISAs were performed using the protocols and cutoffs provided by the manufacturer.

#### 2.4 | Statistical analysis

Statistical analyses were performed using descriptive statistics presenting numbers and corresponding percentages. To analyze the association between SARS-COV-2 seropositivity and the covariates, univariable and multivariable generalized estimating equations (GEE) were calculated with the R-package mmmgee<sup>12</sup> and the r-function geem2.

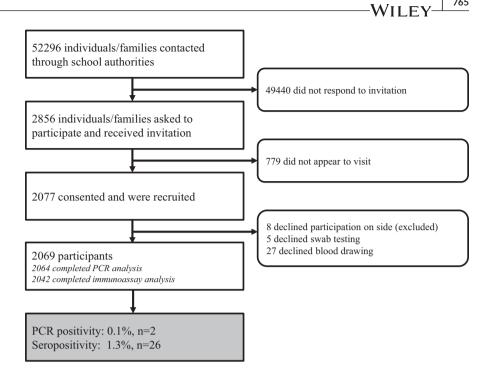
#### 3 | RESULTS

Our target population were children between the ages of 5 and 21 years, encompassing all school grades (n = 52 296 children and adolescents). Schools were approached by mail invitation through the school authorities of the city of Vienna, Austria. Schools which showed interest invited families to participate. The study team received e-mail contacts from the board of schools in Vienna (n = 2856). Details of school characteristics are described in the supplementary appendix, Table S1-S4. The families were asked to participate and received a date for sampling (Figure 1). In total, 2077 out of 2856 (72.7%) children and adolescents were recruited and 2069 participants were included as eight children declined participation on-site. Of 2069 children (52.4% female), 600 were aged 5-10 years (44.7% female), 825 were aged 11-14 years (50.1% female), and 644 were aged 15-21 years (62.7% female). Some children refused swab (n = 5) or blood testing (n = 27), resulting in 2064 PCR analyses and 2042 immunoassays (Figure 1). Of these, 295 were tested concomitantly with 1 other sibling, 42 were tested with 2, and 6 with 3 other siblings, respectively (Table 1). Ages ranged from 5 to 21 years (median 13, IQR 10-15) (Table 1, Table S0). The sex ratio was balanced (1084 females, 985 males). Participant enrollment took place between May 18 and July 2, 2020.

SARS-CoV-2 RNA was detected in 2 cases by real-time PCR (1 pair of siblings, 0.1%). These two girls were both asymptomatic at sample collection; nevertheless, one of them had presented nasal congestion one week before testing (Table 2). Both tested SARS-CoV-2 antibody-negative (Figure 2). Case history revealed 2 other family members being SARS-CoV-2 RT-PCR positive 2 weeks before study visit. All family members were quarantined, and therefore, the two girls did not attend school after its reopening. This family could be related to a known Austrian hotspot of SARS-CoV-2 PCR-positive cases in a mail delivery center (Figure 2).

First-line testing for SARS-CoV-2-specific antibodies detected 37 reactive samples (positive or borderline results, 1.8%), of which 24 (1.2%) showed positive neutralizing activity, confirming their specificity (considered as confirmed antibody-/seropositive). Unanimously, detection of antibodies by two additional ELISAs revealed 2 more cases of confirmed antibody positivity, making 26 (1.3%) seropositive subjects in total. The sex distribution among antibody-positive cases was balanced (n = 13, 50%), and the median age was 13 years (IQR 12.2-13). Among all seropositive children, 3 (11.5%) were between 5 and 10 years, 18 (69.2%) were between 11 and 14 years, and 5 (19.2%) were between 15 and 21 years. Chronic diseases were reported in 6 out of 26 seropositive individuals (23.1%), comprising mainly allergic diseases, which also predominate in the whole study population. Neither age nor the presence of chronic diseases showed evidence to be a risk factor (Tables S5 and S6). Of the 22 seropositive children living in a household with any other sibling, 13 had 1, 5 had 2, and 4 had 3 siblings, not all of which could be tested in the study. Thirteen out of 26 seropositive children were tested concomitantly with their siblings (7 with 1, 4 with 2, and 2 with 3 siblings),

#### FIGURE 1 Flow chart of study participants



of which only one pair of siblings was SARS-CoV-2-antibody-positive (Figure 2C). All other siblings of seropositive individuals living in the same household tested SARS-CoV-2 antibody-negative and all but one reported no symptoms prior to the study (Figure 2).

Contact tracing of seropositive individuals revealed 16 cases with self-reported contact with confirmed COVID-19 cases: 7 inside the family (Figure 2A), 9 outside the family (Figure 2B), all accounted for intergenerational contacts. For the remaining 10 cases (38.5%), the origin of infection remained unknown (Figure 2C). For cluster B (Figure 2B), a particular school was defined offering ski courses at which teachers tested positive for SARS-CoV-2 infection.

A significant larger probability of seropositivity (n = 11, 42%, P < .001) was found for children whose family members had a contact to a suspected COVID-19 case (Table 3). Among children with a potential contact to a suspected SARS-CoV-2 case, 6.3% showed a seropositive result as compared to 0.8% of children with no SARS-CoV-2-positive contact (Table 3 and Table S6).

In the period between December 2019 and July 2020, 27% of study participants (n = 558) reported having suffered acute symptoms, most commonly sore throat (29.6%), cough (29.2%), fever (28.9%), rhinitis/nasal discharge (27.6%), diarrhea (14.2%), and abdominal pain (12.4%) (Table 2).

Among seropositive children and adolescents, the proportion of children who reported any symptoms was 53.9% (n = 14). The most frequent symptom among seropositive individuals was fever (34.6%), sore throat (15.4%), influenza-like symptoms (15.4%), anosmia (11.5%), cough, headache and ageusia (7.7%, respectively). Among seronegative tested individuals, 1479 (73.4%) reported none of the acute symptoms mentioned above prior to the study investigation (Table 2).

Children who had fever or influenza-like symptoms showed a significantly larger probability to be seropositive (Table S6). Within children who reported fever since December 2019, 5.6% showed a seropositive result as compared to 0.9% of children without fever. Within children with influenza-like symptoms, 7.7% showed a seropositive result as compared to 1.1% of children who did not show influenza-like symptoms prior to investigation (Table 2). No significant influence of age, gender, number of siblings, rhinitis/nasal discharge, sore throat, cough or headache on the probability of a seropositive result was observed.

#### DISCUSSION 4

This cross-sectional survey of 2069 schoolchildren aged 5 to 21 years in Vienna, Austria, in May-July 2020, revealed that (a) SARS-CoV-2 infection prevalence in children was low; (b) children showed a mild clinical course or stayed asymptomatic after SARS-CoV-2 infection and (c) mounted SARS-CoV-2 neutralizing antibodies; and (d) virus spreading among siblings in the same household occurred rarely, whereas (e) virus spreading through intergenerational close contacts was more likely.

Our study represents a large single-center, population-based SARS-CoV-2 prevalence study performed in children and adolescents using schools as inclusion procedure in order to estimate the particular risk of SARS-CoV-2 infection in a school setting and to inform public health decision makers. The study started on the day of school reopening after 9 weeks of its closure. Only children without symptoms were included. Moreover, the study cohort comprised schools of primary, middle and upper grades and accounted for age, sex, and socioeconomic factors. Schools were consecutively contacted, securing sequential replies from interested parents. Since voluntariness is a major ethical issue in studies with children, we had to take potential biases concerning recruitment 
 TABLE 1
 RT-PCR analysis and seroprevalence of SARS-CoV-2 by general characteristics: Absolute Values and Percentages (Row percent) are shown. Null percentages are not presented

|                                   | Number of               | RT-PCR analysi         | s                   | Immunoassay            |                     |
|-----------------------------------|-------------------------|------------------------|---------------------|------------------------|---------------------|
|                                   | participants<br>overall | Number of participants | PCR-positive<br>(%) | Number of participants | Seropositive<br>(%) |
| Overall                           | 2069                    | 2064                   | 2 (0.1)             | 2042                   | 26 (1.3)            |
| Sex                               |                         |                        |                     |                        |                     |
| Female                            | 1084                    | 1083                   | 2 (0.2)             | 1072                   | 13 (1.3)            |
| Male                              | 985                     | 981                    |                     | 970                    | 13 (1.2)            |
| Age (female/male)                 |                         |                        |                     |                        |                     |
| 5-10 (268/332)                    | 600                     | 597                    | 0                   | 582                    | 3 (0.5)             |
| 11-14 (412/413)                   | 825                     | 823                    | 1 (0.1)             | 819                    | 18 (2.2)            |
| 15-21 (404/240)                   | 644                     | 644                    | 1 (0.2)             | 641                    | 5 (0.8)             |
| Families in the study             |                         |                        |                     |                        |                     |
| Total number of families          | 1672                    | 1671                   | 1 (0.1)             | 1657                   | 25 (1.5)            |
| Fam. with 1 child in the study    | 1329                    | 1328                   | 0                   | 1315                   | 13 (1.0)            |
| Fam. with 2 children in the study | 295                     | 295                    | 1 (0.3)             | 294                    | 6 (2.0)             |
| Fam. with 3 children in the study | 42                      | 42                     | 0                   | 42                     | 4 (9.5)             |
| Fam. with 4 children in the study | 6                       | 6                      | 0                   | 6                      | 2 (33.3)            |
| Highest education of father       |                         |                        |                     |                        |                     |
| Mandatory school                  | 76                      | 75                     | 0                   | 76                     | 1 (1.3)             |
| Traineeship                       | 348                     | 347                    | 0                   | 333                    | 3 (0.9)             |
| Graduation                        | 396                     | 395                    | 0                   | 393                    | 6 (1.5)             |
| University/college                | 1164                    | 1162                   | 2 (0.2)             | 1155                   | 16 (1.4)            |
| N/A                               | 85                      | 85                     | 0                   | 85                     | 0                   |
| Highest Education of mother       |                         |                        |                     |                        |                     |
| Mandatory school                  | 73                      | 73                     | 0                   | 73                     | 0                   |
| Traineeship                       | 217                     | 217                    | 0                   | 210                    | 3 (1.4)             |
| Graduation                        | 430                     | 430                    | 0                   | 422                    | 6 (1.4)             |
| University/college                | 1309                    | 1304                   | 2 (0.2)             | 1297                   | 15 (1.2)            |
| N/A                               | 40                      | 40                     | 0                   | 40                     | 2 (5)               |

into consideration, such as participation of (a) particularly interested subject groups, possibly based on proven or suspected index cases; (b) families seeking testing with more siblings; (c) individuals with access to technology and/or technology proficiency; and (d) families with higher education (Table 1). Nevertheless, with more than 2000 pediatric participants, our study cohort became comparable to previous large combined adult/children studies,<sup>7,13,14</sup> the largest of which included 6527 children aged 0-19 years in a nationwide Spanish seroepidemiological study (ENE-COVID)<sup>7</sup> and 7812 children aged 0-19 years in a nationwide Brazilian seroprevalence study, respectively.<sup>14</sup>

We found a seroprevalence rate of 1.3%. Of note, 92.3% of positive sera also displayed neutralizing capacity, indicating that children produce antibodies, which combat SARS-CoV-2 and might possibly prevent a re-infection.<sup>15</sup> However, at present it is not clear how long this immunity will last. We generally detected low antibody concentrations and NT titers in the children as compared to adults (data not shown), possibly accounting for distinct primary SARS-CoV-2 infection courses and immune responses in children and adults.<sup>16</sup>

Our estimate of 1.3% SARS-CoV-2 seroprevalence in children of school age ranges low in concordance with most pediatric studies and studies including children.<sup>7,13,17</sup> In the nationwide Brazilian seroprevalence study on 25025 and 31165 randomly selected family members at two timepoints, the seroprevalence estimate on 3400 and 4412 children aged 1 to 19 years was 1.3% and 1.8%, respectively<sup>14</sup>. A nationwide French surveillance revealed a seroprevalence in children of 2.7%.<sup>17</sup> In a preliminary report of a German study detecting acute SARS-CoV-2 infections and seroprevalence in 4932 parent-child pairs, a mRNA prevalence of 0.04% and a seroprevalence of 1.3% were found<sup>18</sup>. Focusing only on children (50% in the age of 0-10 years), PCR positivity was 0.02% (1 case), and the seroprevalence was 0.8% (19 cases).<sup>18</sup> In concordance with the German data, our findings suggest that children may be less affected by a SARS-CoV-2 infection compared to adults.<sup>7,14,19,20</sup>

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|                            | Immunoassay            | Immunoassay         |                     |           |  |  |  |  |
|----------------------------|------------------------|---------------------|---------------------|-----------|--|--|--|--|
| Acute symptoms             | Number of participants | Seropositive<br>(%) | Seronegative<br>(%) | P-value   |  |  |  |  |
| Overall                    | 2042                   | 26 (1.3)            | 2016 (98.7)         |           |  |  |  |  |
| With acute<br>symptoms     | 551                    | 14 (2.5)            | 537 (97.5)          |           |  |  |  |  |
| Without acute<br>symptoms  | 1491                   | 12 (0.8)            | 1479 (99.2)         |           |  |  |  |  |
| Rhinitis/nasal disch       | arge                   |                     |                     |           |  |  |  |  |
| Yes                        | 153                    | 1 (0.6)             | 152 (99.3)          | .290      |  |  |  |  |
| No                         | 1889                   | 25 (1.3)            | 1864 (98.7)         |           |  |  |  |  |
| Sore throat                |                        |                     |                     |           |  |  |  |  |
| Yes                        | 161                    | 4 (2.5)             | 157 (97.5)          | .221      |  |  |  |  |
| No                         | 1881                   | 22 (1.2)            | 1859 (98.8)         |           |  |  |  |  |
| Cough                      |                        |                     |                     |           |  |  |  |  |
| Yes                        | 160                    | 2 (1.2)             | 158 (98.7)          | .901      |  |  |  |  |
| No                         | 1882                   | 24 (1.3)            | 1858 (98.7)         |           |  |  |  |  |
| Dyspnea                    |                        |                     |                     |           |  |  |  |  |
| Yes                        | 8                      | 1 (12.5)            | 7 (87.5)            | Not       |  |  |  |  |
| No                         | 2034                   | 25 (1.2)            | 2009 (98.8)         | Predictab |  |  |  |  |
| Thoracic pain              |                        |                     |                     |           |  |  |  |  |
| Yes                        | 4                      | 1 (25)              | 3 (75)              | Not       |  |  |  |  |
| No                         | 2038                   | 25 (1.2)            | 2013 (98.8)         | Predictab |  |  |  |  |
| Fever                      |                        |                     |                     |           |  |  |  |  |
| Yes                        | 161                    | 9 (5.6)             | 152 (94.4)          | .001      |  |  |  |  |
| No                         | 1881                   | 17 (0.9)            | 1864 (99.1)         |           |  |  |  |  |
| Headache                   |                        |                     |                     |           |  |  |  |  |
| Yes                        | 41                     | 2 (4.9)             | 39 (95.1)           | .075      |  |  |  |  |
| No                         | 2001                   | 24 (1.2)            | 1977 (98.8)         |           |  |  |  |  |
| Fatigue                    |                        |                     |                     |           |  |  |  |  |
| Yes                        | 7                      | 1 (14.3)            | 6 (85.7)            | Not       |  |  |  |  |
| No                         | 2035                   | 25 (1.2)            | 2010 (98.8)         | Predictab |  |  |  |  |
| Anosmia                    |                        |                     |                     |           |  |  |  |  |
| Yes                        | 5                      | 3 (60)              | 2 (40)              | Not       |  |  |  |  |
| No                         | 2037                   | 23 (1.1)            | 2014 (98.9)         | Predictab |  |  |  |  |
| Ageusia                    |                        |                     |                     |           |  |  |  |  |
| Yes                        | 8                      | 2 (25)              | 6 (75)              | Not       |  |  |  |  |
| No                         | 2034                   | 24 (1.2)            | 2010 (98.8)         | Predictab |  |  |  |  |
| INU                        |                        |                     |                     |           |  |  |  |  |
|                            | toms                   |                     |                     |           |  |  |  |  |
| Influenza-like symp<br>Yes | toms<br>52             | 4 (7.7)             | 48 (92.3)           | <.001     |  |  |  |  |

**TABLE 2**Seroprevalence of SARS-CoV-2 by recent acute symptoms of thechild: Absolute Values and Percentages(Row percent) are shown

We could not associate seroprevalence with sex but with age. In accordance with other large cohorts<sup>7,13,19</sup>, seroprevalence was higher in children older than 10 years and peaked at the age of 13 years with 13 cases, accounting for 50% of all seropositive cases. Still, age did not manifest as a significant risk factor in the applied statistical models.

Understanding the role of children in the transmission of SARS-CoV-2 is of global interest given its policy implications regarding school reopening and intergenerational contacts. Our study started on May 18, 2020, after 9 weeks of school closure and lockdown. Because SARS-CoV-2-specific antibodies were found in the absence of any detectable viral RNA and acute symptoms were mostly

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|   | 1    | 2   | 3   | 4   | 5   | 6   | 7   | 8   | 9   | 10  | 11  | 12  | 13  | 14  | 15  | 16  | 17  | 18  | 19  | 20  | 21  | 22  | 23  | 24  | 25  | 26  |
|---|------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Study subjects and their siblings       | 1    | 2   | 5   | 4   | 5   | 0   | /   | 0   | 9   | 10  | 11  | 12  | 15  | 14  | 15  | 10  | 17  | 10  | 19  | 20  | 21  | 22  | 25  | 24  | 25  | 20  |
| sero+ and PCR+ subjects                 | PCR+ | ab+ |
| sibling 1                               | PCR+ | х   | х   | х   |     | ab- |     |     | х   | ab- | ab- | х   | х   | х   | х   | х   | ab+ |     |
| sibling 2                               |      |     |     |     | -   | х   | ab- | ab- |     |     | ab- | ab- | ab- | ab- |     |     |     |     |     |     |     |     |     |     | х   |     |
| sibling 3                               | х    | 1   |     |     |     |     |     |     |     |     |     |     | ab- | ab- |     |     |     |     |     |     |     |     |     |     | х   |     |
| SARS-CoV-2 affected family members      |      |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| mother                                  |      |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| father                                  |      |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| other family members                    |      |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Contact with SARS-CoV-2 affected person |      |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| teacher                                 |      |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Cluster                                 |      |     |     |     | A   |     |     |     |     |     |     |     | В   |     |     |     |     | с   |     |     |     |     |     |     |     |     |

| seropositive tested within the study   |     |  |  |  |  |
|--|-----|--|--|--|--|
| seronegative tested siblings wtihin the study  | ab- |  |  |  |  |
| PCR positive/seronegative tested within the study<br>PCR positive or seropositive by history |     |  |  |  |  |
|  |     |  |  |  |  |

**FIGURE 2** Contact tracing of SARS-COV-2 PCR-positive and seropositive individuals. Columns display 26 families of positive-tested study participants and their contacts inside and outside the family. Among the 26 seropositive individuals (ab+), 16 had self-reported contact with confirmed COVID-19 cases: 7 inside the family (A), 9 outside the family (B), all accounted for by intergenerational contacts. In the residual 10 cases (38.5%), the origin of infection remained unknown (C). Siblings of seropositive individuals were mainly tested negative for antibodies against SARS-CoV-2 within the study (ab-), and only 1 pair of siblings was tested seropositive concomitantly. Two SARS-CoV-2 PCR-positive individuals were siblings and tested seronegative (#1). Nine children were assigned to a ski course at a known hotspot area in Austria (B) [Colour figure can be viewed at wileyonlinelibrary.com]

|                  | <b>RT-PCR</b> analysis    |                  | Immunoassay            |                     |
|------------------|---------------------------|------------------|------------------------|---------------------|
|                  | Number of<br>participants | PCR-positive (%) | Number of participants | Seropositive<br>(%) |
|                  | n = 2                     |                  | n = 26                 |                     |
| Participants wi  | th symptomatic famil      | y members        |                        |                     |
| Overall          | 2063                      | 2 (0.1)          | 2041                   | 26 (1.3)            |
| Mother           | 466                       | 0                | 459                    | 11 (2.4)            |
| Father           | 282                       | 2 (0.7)          | 278                    | 13 (4.7)            |
| Siblings         | 367                       | 1 (0.3)          | 362                    | 5 (1.4)             |
| Contact of a fai | mily member with a s      | suspected case   |                        |                     |
| Overall          | 2046                      | 2 (0.1)          | 2024                   | 26 (1.3)            |
| Yes              | 179                       | 2 (1.1)          | 175                    | 11 (6.3)            |
| No               | 1867                      | 0                | 1849                   | 15 (0.8)            |
| Family member    | in quarantine             |                  |                        |                     |
| Overall          | 2057                      | 2 (0.1)          | 2037                   | 25 (1.2)            |
| Yes              | 137                       | 2 (1.5)          | 135                    | 16 (11.8)           |
| No               | 1920                      | 0                | 1902                   | 9 (0.5)             |
| Family member    | diseased with COVI        | D-19             |                        |                     |
| Overall          | 2056                      | 2 (0.1)          | 2035                   | 24 (1.2)            |
| Yes              | 47                        | 2 (4.3)          | 45                     | 9 (20)              |
| No               | 2009                      | 0                | 1990                   | 15 (0.8)            |

TABLE 3RT-PCR analysis andseroprevalence of SARS-CoV-2 by self-reported clinical characteristics: AbsoluteValues and Percentages (Row percent)are shown. Null percentages are notpresented

reported before or at the beginning of the lockdown period by March 16, 2020, we speculate that most cases had been infected before or at the beginning of the lockdown period. In addition, we were in the privileged situation of having high numbers of individuals with 1 (n = 295), 2 (n = 42), or 3 (n = 6) siblings tested in our cohort. Among these, 13 children were seropositive but only 1 pair of siblings was

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tested positive concomitantly, suggesting that virus transmission between children in the same household might be low. In the contrary, we recorded 11 out of 26 seropositive cases (42.3%) originating from one particular lower-grade high school. Nine pupils of these 26 cases and some teachers of this school had attended ski courses in an Austrian COVID-19 hotspot area.<sup>9</sup> The same school accounted for a high number of participants in the study, likely resulting from proven or suspected SARS-CoV-2-positive cases among the schoolteachers or ski-school teachers. Seven out of the 26 seropositive cases and both PCR-positive cases reported adult family members being tested positive for SARS-CoV-2. Based on these observations, we speculate that SARS-CoV-2 spreading is more likely to have occurred through adult family members or intergenerational contacts rather than through child-to-child household contacts (Figure 2).

All SARS-CoV-2-infected individuals in our study developed mild symptoms or remained asymptomatic. This observation is in line with several previous reports on children from China<sup>21-23</sup> and Europe.<sup>3,5,19</sup> Despite the lower number of cases reported in children, there are concerns about asymptomatic or mildly symptomatic pediatric cases remaining undetected resulting in an unknown transmission of SARS-CoV-2 in the community or in schools. Our results do not provide evidence for the hypothesis that children are a prominent reservoir of SARS-CoV-2 transmission, neither to siblings in the same household nor to adults from the same family or through intergenerational contacts. This is further substantiated by the fact that children, even after mild disease or without any symptoms, developed neutralizing antibodies which might efficiently block virus transmission. This conclusion partly contradicts the assumption of other studies, particularly those focusing on adult family members within households.<sup>7,24</sup> As yet, evidence for direct SARS-CoV-2 transmission by children has not been directly addressed in studies.<sup>25</sup> However, indirect evidence of the potential for SARS-CoV-2 transmission has been documented, the source of virus transmission being fecal shedding, contact of fomites, or airborne spreading.<sup>25-27</sup> Given the literature published to date, the rate of transmission from a child to others within the same household seems to be rather low.<sup>8,19</sup>

When antibody tests with high sensitivity and specificity are used in combination, serological surveys are a valuable tool to determine the spread of an infectious disease, in particular in the presence of asymptomatic cases.<sup>28</sup> The proportion of asymptomatic SARS-CoV-2 infection varies greatly, ranging from 4% to 41%.<sup>29</sup> In our cohort, 46.2% of all seropositive cases were asymptomatic. This finding reinforces the importance of rapid identification and isolation of people with confirmed SARS-CoV-2 infection and their contacts to prevent the spread of the infection.

In conclusion, our study provides a regional insight into SARS-CoV-2 dissemination in schoolchildren in Austria at the beginning of the pandemic. The at-random recording of two SARS-CoV-2 clusters in our study (one real-time RT-PCR-positive pair of siblings and one school with antibody-positive children) suggests that screening policies in schools are highly recommended, including regular random real-time RT-PCR testing of complete schools of different grades. Social distancing measures, hygiene guidelines, and efforts to identify and isolate new cases of infection and their contacts remain the most important strategies for future epidemic control, enabling a safe school environment for children, teachers, and their relatives, without further rigid lockdown measures.

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#### CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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Zsolt Szepfalusi: Conceptualization (lead); Formal analysis (equal); Funding acquisition (lead); Investigation (equal); Methodology (equal); Project administration (equal); Resources (equal); Software (equal); Supervision (equal); Visualization (equal); Writing-original draft (lead); Writing-review & editing (equal). Klara Schmidthaler: Data curation (equal); Formal analysis (equal); Investigation (equal); Methodology (equal); Project administration (equal); Software (equal); Writing-review & editing (equal). Justyna Sieber: Data curation (equal); Formal analysis (equal); Investigation (equal); Methodology (equal); Project administration (equal); Resources (equal); Software (equal); Visualization (equal); Writing-review & editing (equal). Sonja Kopanja: Formal analysis (supporting); Investigation (equal); Methodology (equal); Project administration (supporting); Resources (equal); Writing-review & editing (equal). Florian Götzinger : Formal analysis (equal); Investigation (equal); Methodology (equal); Validation (equal); Writing-review & editing (equal). Anja Schoof: Data curation (equal); Investigation (equal); Writing-review & editing (equal). Jakub Hoz: Investigation (equal); Methodology (supporting); Writing-review & editing (supporting). Athanasios Makristhatis: Investigation (supporting); Methodology (supporting); Writing-review & editing (equal). Birgit Willinger: Investigation (equal); Methodology (supporting); Project 770 | WILE

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#### SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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