Original Article

SARS-CoV-2 Seroprevalence in Preschool and School-Age Children

Population Screening Findings From January 2020 to June 2022

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Summary

Background: The SARS-CoV-2 pandemic is ongoing in Germany. Children and adolescents are increasingly being infected, and many cases presumably remain undetected and unreported. Sero-epidemiological studies can help estimate the true number of infections.

Methods: From January 2020 to June 2022, 59 786 persons aged 1–17 years were tested for SARS-CoV-2 antibodies as part of a screening program for presymptomatic type 1 diabetes in the German federal state of Bavaria (the Fr1da study).

Results: In June 2022, the seroprevalence in the overall population was 73.5%. The seroprevalence was significantly higher in school-age children (from 5 to 10 years of age) than in preschool children (ages 1–4): 84.4% vs. 66.6%, p <0.001. In contrast, in November 2021, before the appearance of the omicron variant, the overall seroprevalence was 14.7% (16.2% of school-age children, 13.0% of preschool children, p = 0.06). In the overall collective, seroprevalence increased fivefold from the fall of 2021 to June 2022 (by a factor of 5.2 in school-age children and 5.1 in preschool children). Similar seroprevalences, with smaller case numbers, were observed in June 2022 in the corresponding Fr1da studies in Saxony and Northern Germany: 87.8% and 76.7%, respectively.

Conclusion: Monthly case counts reveal a substantial rise in SARS-CoV-2-infections among children and adolescents from late 2021 to mid-2022. The high percentage of preschool and school-age children who have been infected with SARS-CoV-2, in a population that has low vaccination coverage, should be taken into account in the development of health policies.

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Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has been present in Germany since January 2020 and had infected around 28 million persons nationwide by June 2022 (1). Between January 2020 and June 2022, there were six waves of infection with different dominant variants of the virus. The SARS-CoV-2 B.1.617.2 (Delta) variant, predominant since July 2021, was displaced from late 2021 onwards by the rapidly spreading SARS-CoV-2 B.1.1.529 (Omicron) variant, leading to a dramatic increase in case numbers within just a few months (2). This trend affects all age groups and is becoming increasingly evident in children and adolescents (2).

German registration data show that by June 2022 around 890 000 children aged 0–4 years and some 3.9 million in the age group 5–14 years had become infected (3). It is difficult to assess how precise these figures are and how many infections occurred but were not registered. However, the predominantly asymptomatic or mild course of COVID-19 in children and adolescents (4, 5) makes under-recording in these age groups very likely. Studies in Germany have shown that numbers of seropositive children greatly exceed the registered cases. For example, an investigation of the monthly seroprevalence from June 2020 to February 2021 (the CorKid study) revealed that the total of seropositive children was 3 to 4 times higher than the number of cases registered (6). Moreover, previous analyses of the present study cohort showed a 6 times higher number of cases between April and June 2020 (7) and under-recording by a factor of 3–4 at the beginning of 2021 (8).

Given the higher vaccination rates in adults and the suspension of most public measures to protect against infection, e.g., mandatory mask wearing in schools, it may be particularly children and adolescents who are driving infections, especially because members of this age group tend to come into close physical contact with one another. In June 2022, about 28% of 12- to 17-year-olds (around 1.2 million persons) and about 78% of 5- to 11-year-olds (around 4.2 million) had still not had their first vaccination (9). Vaccination of children under the age of 5 years has not yet been recommended by the German Standing Committee on Vaccination (STIKO). Accordingly, although the infections are rarely serious

and seldom lead to hospitalization, children and adolescents represent a vulnerable group. Little is known, however, about the long-term consequences of infection.

Accurate knowledge of the rates of seropositivity in children and adolescents is crucial for future assessment of infection events, development of vaccination strategies, and decisions on public infection protection policies, particularly in day-care centers and schools.

This article describes our analysis of the monthly seroprevalence of SARS-CoV-2 antibodies over the period from January 2020 to June 2022 in children and adolescents participating in the Fr1da study in the German federal state of Bavaria. To permit specific evaluation of the infection process in infants, preschool children, and those of school age, the seroprevalence data were additionally stratified by age (1–4 years and 5–10 years). Data from other Fr1da studies (in Saxony and Lower Saxony/Hamburg) were used for comparison of the prevalence of infection in our cohort with other regions of Germany.

Method

Study cohort

The members of our cohort are participants in the Fr1da study in Bavaria (n ~ 167 000), a public health screening program for presymptomatic type 1 diabetes launched in 2015 (10, 11). All children in Bavaria aged 2–10 years (more precisely 1.75–10.99 years) with no previous diagnosis of diabetes are eligible to take part. The participants' first-degree relatives aged 1–17 years (more precisely 1.0–17.99 years) can also be screened. At the 684 pediatricians' offices that have signed up to the study, a sample of capillary blood is taken from each participant and sent directly to the Institute for Diabetes Research at Helmholtz Munich for analysis. The design of the Fr1da study has been described in detail elsewhere (10, 11). Since January 2020, all Fr1da participants have been offered additional testing for antibodies to SARS-CoV-2 (7, 8, 12). In the period from 9 January 2020 to 30 June 2022, a total of 59 786 blood samples were analyzed for SARS-CoV-2 antibodies: 38 430 samples from 1- to 4-year-olds, 20 534 from 5- to 10-year-olds, and 822 from 11- to 17-year-olds. Pediatric examination included documentation, on a standardized form, of age, sex, and, from January 2022 onwards, vaccination status.

For purposes of comparison, we analyzed data on children and adolescents from the Fr1da studies in Saxony and northern Germany (Lower Saxony and Hamburg), the protocols of which corresponded to the Fr1da study in Bavaria. Altogether, 376 participants from the Fr1da study in Saxony have been investigated for SARS-CoV-2 antibodies since 1 September 2021 and 851 from the Fr1da study in northern Germany since 1 August 2021.

The ethics committee of the Technical University of Munich reviewed the ethical aspects of the Fr1da study and approved its conduct (nos. 70/14 s and 235/20 s). The parents or legal guardians of all children and adolescents included in the study gave their written informed consent.

SARS-CoV-2 antibody detection

Immunoglobulin-G antibodies to the SARS-CoV-2 receptor binding domain (RBD) and the SARS-CoV-2 nucleocapsid protein (NP) were measured in capillary serum, using the highly specific luciferase immunoprecipitation system (LIPS). A detailed description of the methods has been published previously (7), and more information can be found in the *eMethods*.

Statistics

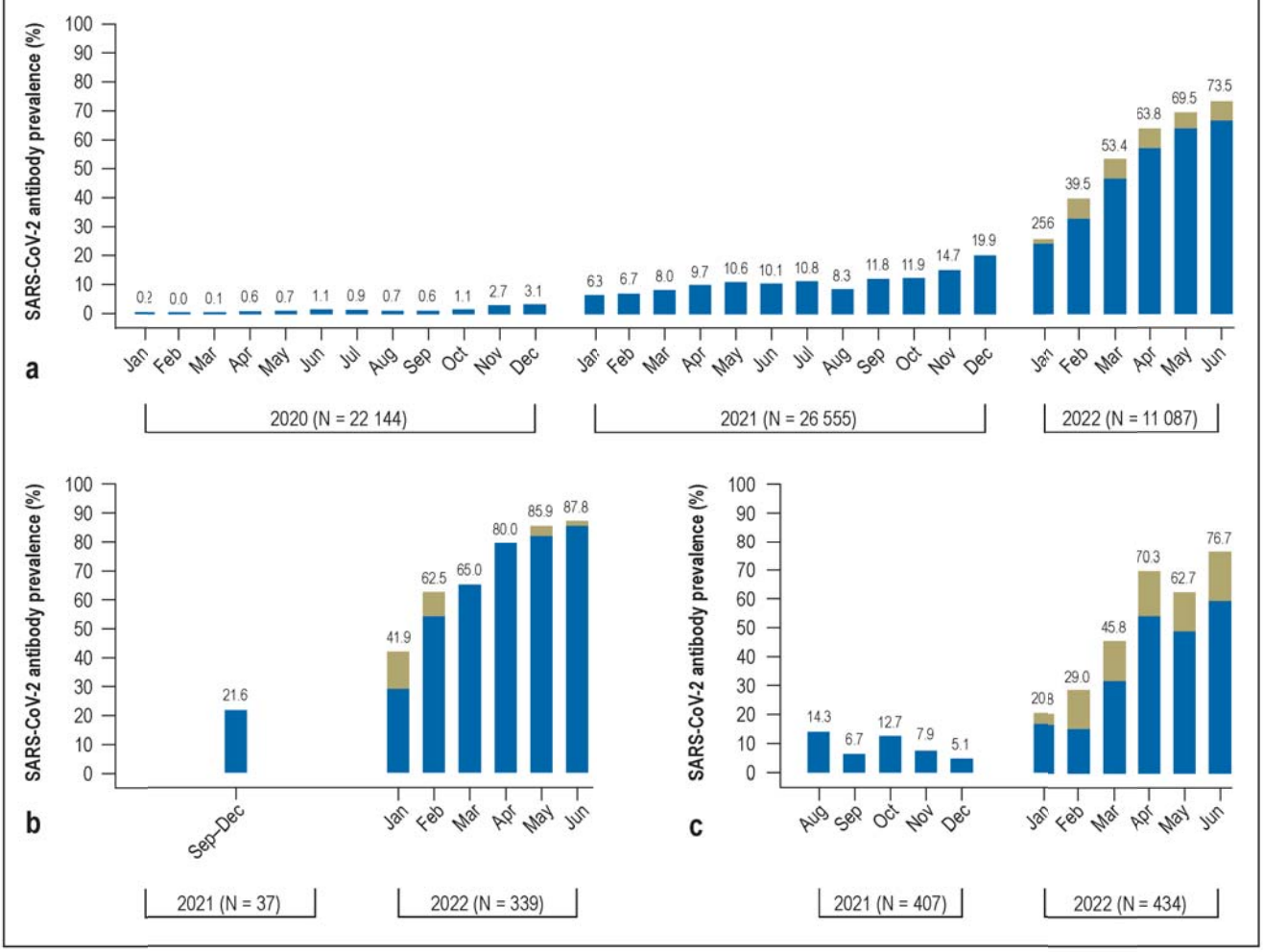
The prevalence of seropositivity in children and adolescents is expressed as the percentage of samples tested in the respective month that were positive for SARS-CoV-2 (RBD positive and NP positive/negative). The data for each month embrace samples from the first to the last day of the month, with the exception of January 2020, when sampling began on the 9th. Because vaccination of 5- to 11-year-old children was recommended from January 2022 onwards, in the period January to June 2022 documentation of overall seroprevalence was accompanied by separate recording of the proportions of participants with vaccination without prior infection or vaccination long after infection. For age-specific evaluation of seroprevalence in 1- to 4-year-olds and 5- to 11-year-olds after waves of infection in Germany, we used the official definitions of the periods concerned (13). Ages were documented in terms of complete years: for instance, all children from 10.0 to 10.99 years of age counted as 10-year-olds. Because there were only a small number of children between 11 and 17 years of age, this group was not analyzed seperately. For comparison across the age groups, monthly seroprevalence is expressed in terms of median (minimum, maximum) for each wave of infection. For each wave, the proportions of samples with a positive result were compared between the two age groups by means of chi-square testing. The SARS-CoV-2 RBD antibody titers in the whole cohort are expressed as median and interquartile range (IQR) and as a box plot (median and IQR) with lines for measurements within 1.5 IQR and dots for measurements exceeding 1.5 IQR. The RBD antibody titers before 1 March 2022 and after that date were statistically compared using the Wilcoxon test. A Wilcoxon test was also used for group comparison of RBD antibody titers between participants who had recovered from COVID-19 and had not been vaccinated (RBD- and NP-positive cases up to July 2021) and vaccinated participants with or without infection (confirmed first vaccination, RBD positive and NP negative or positive). All statistical tests were carried out using the R software (R Core Team, Vienna, Austria). A p-value < 0.05 was defined as showing a statistically significant difference.

Results

Monthly seroprevalence in the entire cohort

Between January 2020 and June 2022 a total of 59 786 children and adolescents were tested for antibodies to SARS-CoV-2, a monthly average of 1993 tests. The prevalence of RBD-positive SARS-CoV-2 cases in

FIGURE 1



Monthly SARS-CoV-2 seroprevalence (proportion of tested samples that were antibody-positive) in children and adolescents aged 1–17 years. a) Fr1da study in Bavaria from January 2020 to June 2022. b) Fr1da study in Saxony from September 2021 to June 2022. c) Fr1da study in northern Germany (Lower Saxony and Hamburg) from August 2021 to June 2022. N is the total number of children and adolescents tested in each separate period. The blue columns show the prevalence of recovered or recovered and vaccinated persons among the participants, while the beige columns (from January 2022, when vaccination of children aged 5–11 years was recommended in Germany) depict the prevalence of vaccinated persons without prior infection or with infection at a much earlier time, based on their antibody profile.
SARS-CoV-2, Severe acute respiratory syndrome coronavirus 2

June 2022 was 73.5 % *(Figure 1a)*. Based on the antibody profile, this figure was made up of 66.7% who had recovered from COVID-19 (some vaccinated, others not) and 6.8% who had been vaccinated, either without previous infection or long after infection. The seroprevalence in June 2022 was the highest yet recorded.

From November 2021 (seroprevalence 14.7%) onwards, the case numbers rose markedly and rapidly until June 2022. As soon as March 2022, more than half of the tested children and adolescents were seropositive (53.4%; *Figure 1a*). From November 2021 to June 2022 the proportion of members of this study cohort who were seropositive increased around fivefold.

From spring 2021 to fall 2021, the seroprevalence had been relatively stable at 10–12% *(Figure 1a)*. Before that, the case numbers had increased steadily from 2.7% in November 2020 to 10.6% in May 2021.

Monthly seroprevalence in the age groups 1–4 years and 5–10 years

The monthly seroprevalence in preschool children (1–4 years) and school-age children (5–10 years) broadly reflected that of the cohort as a whole. In June 2022 the seroprevalence was 66.6% in the 1- to 4-year-olds (number positive: n = 613, number tested: n = 920) and statistically significantly higher, at, 84.4%, in the 5- to 10-year-olds (number positive: n = 416, number tested: n = 493; $p < 0.001$). Of those,

62.1% of the preschool children and 73.8% of the school-age children had recovered from the infection (with or without vaccination). In June 2022, the proportion of children with an antibody profile that corresponded to vaccination either without previous infection or with infection at a much earlier time was 4.5% in the 1- to 4-year-olds and 10.5% in the 5- to 10-year-olds. In November 2021 the two age groups had exhibited similar seroprevalences (preschool children 13.0%, school-age children 16.2%; p = 0.06).

Focused analysis of the seroprevalence over the course of the first five waves of infection showed—independent of age group—a distinct elevation in the fifth wave (January to May 2022) compared with the foregoing periods *(Table)*. In the fifth wave of infection, the median seropositivity rates were 44.6% for the 1- to 4-year-olds and 63% among the 5- to 10-year-olds (p < 0.001). In the second, third, and fourth waves, seroprevalence rates were statistically significantly higher for the 5- to 10-year-olds than for the younger age group *(Table)*. The median seroprevalence during the first wave of infection was around 0.3% in both age groups (p = 0.7904).

Monthly seroprevalence of the studies in Saxony and Lower Saxony/Hamburg

The data from the Fr1da study in Saxony also point to a steep increase in the seroprevalence among children and adolescents since fall/winter 2021 (seroprevalence 21.6%; *Figure 1b*). Because of the low numbers of tests carried out in the period September to December 2021, the case numbers for these months were amalgamated to get a group of similar size to the individual months thereafter. Also in Saxony, the proportion of seropositivity in children and adolescents tested in June 2022 was the highest yet recorded (87.8%; *Figure 1b*). According to the antibody profile, the proportion of children and adolescents who had been vaccinated without having been infected or with infection much earlier was highest in January 2022, at 12.9%, and fell to 1.8% by June 2022 *(Figure 1b)*.

Analysis of the Fr1da study in northern Germany from August 2021 to June 2022 shows a similar pattern *(Figure 1c)*. This cohort from Lower Saxony and Hamburg also displayed a pronounced, rapid rise in case numbers between November/December 2021 (seroprevalence 10.9/7.9 %) and June 2022 (seroprevalence 76.7 %) *(Figure 1c)*. The proportion of children and adolescents in the Fr1da study in northern Germany who had been vaccinated either without infection or with infection much earlier was lowest in January 2022, at 3.9%, and rose to 16.7% by June 2022 *(Figure 1c)*.

SARS-CoV-2 RBD antibody titer of the study in Bavaria

The RBD antibody assay used in this study showed statistically significantly lower individual RBD antibody titers of the RBD-positive samples in the cohort as a whole from March 2022 onwards than in the whole of the preceding period (p < 0.001; *Figure 2*).

This is reflected by the median antibody titers over the course of the five waves of infection. In the fifth wave (January to May 2022), the median titer was 440.1 binding antibody units (BAU; the international standard unit [14]) (IQR 95.7–4030.4), much lower than in the earlier waves of infection. The titers in the fourth wave, from August to December 2021, were similar to those during the second wave, from September 2020 to January 2021 (fourth wave: 2220.9 [615.4–5802.3] BAU; second wave: 2532.4 [454.4–4752.6] BAU). The highest antibody titers observed were those in the third wave of infection (February to May 2021), at 2902.9 (1040.3–5807.4) BAU. In contrast, the median figure during the first wave (March to April 2020) was 509.4 (276.4–1141.9) BAU.

A comparison of recovered persons without vaccination (n = 1532), vaccinated persons without infection (n = 226), and vaccinated persons with infection (n = 206) showed statistically significant increases in antibody titer from group to group *(eFigure 1)*. The median titers were 2589.8 (963.8–5238.2) BAU for recovered persons without vaccination, 17 225.9 (8602.9–29 508.0) BAU for vaccinated persons without infection, and 40 789.9 (18 777.7–70 087.4) BAU for vaccinated persons with infection (all comparisons p < 0.001).

Discussion

Seroepidemiological studies form an important part of the analysis of the infection process and are thus useful for the planning of future (protective) health policies.

In Bavaria the Fr1da study participants have been able to avail themselves of SARS-CoV-2 antibody testing at any of a large number of pediatricians' offices since the beginning of the COVID-19 pandemic. The data thus collected reveal a rapid and marked increase in seropositivity among children and adolescents since the appearance of the Omicron variant in Germany at the end of 2021. By summer 2022 the monthly seroprevalences had risen to 73.5% in the cohort as a whole, 66.6% in the group of 1- to 4-year-olds, and 84.4% in the 5- to 10-year-olds. Data from the Fr1da studies in northern Germany and in Saxony confirm these findings—albeit with smaller numbers of tests—and show that the seroprevalence is already very high in other regions of Germany too, not just in Bavaria. Of note, this increase has occurred despite regular testing in schools and day-care centers and mandatory mask wearing in schools—protective measures that in Bavaria, for example, were in force until May 2022. In the cohort analyzed, particularly in the fifth wave of infection, school-age children showed a higher seroprevalence than preschool children. A potential explanation for this is that school-age children have more interpersonal contacts, e.g., in the course of their leisure activities, than do younger children, only some of whom, for example, attend day-care centers. It remains to be seen how the case numbers develop; under the current

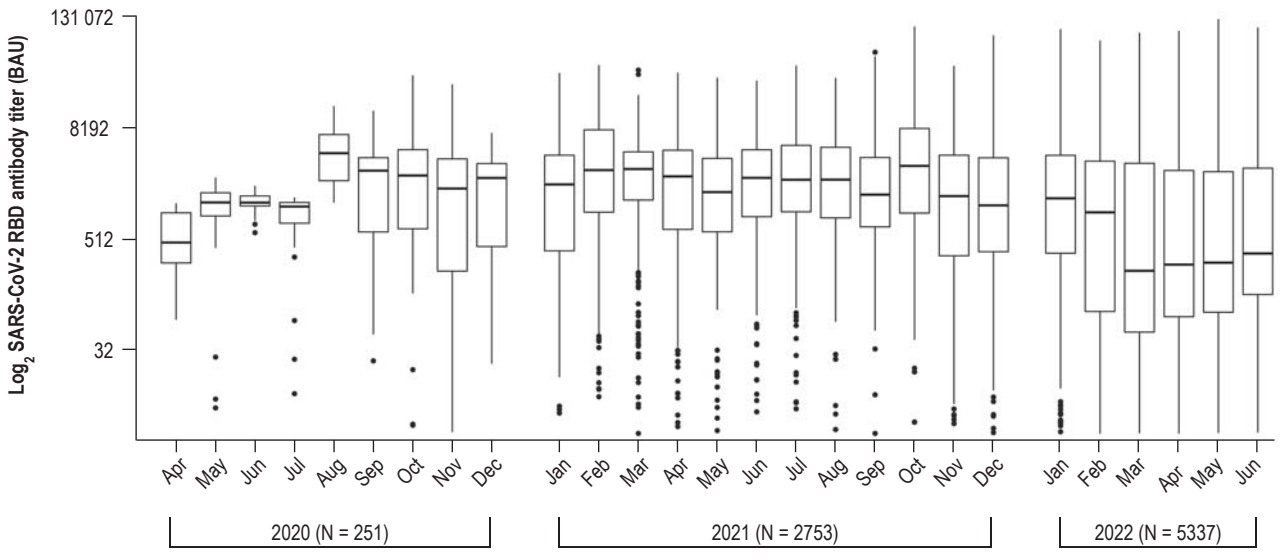
TABLE

SARS-CoV-2 seroprevalence in the Fr1da study in Bavaria, stratified into age groups 1–4 years and 5–10 years, during the five waves of infection in Germany

Period [*1]	Wave	Number of months in period	Total number of children tested	Number of children seropositive	Number of children vaccinated without infection or with much earlier infection	Seroprevalence, % [*2] median (minimum–maximum)
Age group 1–4 years						
Mar–Apr 2020	1	2	1938	6	–	0.3 (0.1–0.5)
Sep 2020–Jan 2021	2	5	7202	165	–	2.1 (0.6–5.4) [*3]
Feb–May 2021	3	4	6242	452	–	7.6 (5.4–8.7) [*3]
Aug–Dec 2021	4	5	5626	684	–	12.1 (8.6–16.2) [*3]
Jan–May 2022	5	5	6071	2654	143	44.6 (19.1–63.0) [*4]
Age group 5–10 years						
Mar–Apr 2020	1	2	612	3	–	0.4 (0.0–0.8)
Sep 2020–Jan 2021	2	5	3694	131	–	3.5 (0.7–8.1) [*3]
Feb–May 2021	3	4	4236	441	–	10.2 (8.4–13.1) [*3]
Aug–Dec 2021	4	5	3474	500	–	13.5 (7.3–24.1) [*3]
Jan–May 2022	5	5	3418	2136	404	63.0 (34.9–80.9) [*4]

[*1] The duration of each wave of infection in was defined on the basis of data from the Robert Koch Institute. The fifth wave ended in May 2022 (13).
[*2] Comparison of seroprevalence between the two age groups in each wave of infection ([*3] $p < 0.001$; [*4] $p < 0.01$)
SARS-CoV-2, Severe acute respiratory syndrome coronavirus 2

FIGURE 2



Distribution of the SARS-CoV-2 RBD immunoglobulin-G antibody titers of the positive samples (> 25 BAU) in the Fr1da study in Bavaria from April 2020 to June 2022. Box plots (median and IQR) with lines for measurements within 1.5 IQR and dots for values exceeding 1.5 IQR. N is the number of RBD-positive children and adolescents (age 1–17 years) in each separate period (see cluster analysis in *eFigure 2*).
BAU, Binding antibody units; IQR, interquartile range; RBD, receptor binding domain; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2

circumstances, however, e.g., low vaccination acceptance on the part of children and adolescents, dominance of the Omicron variant, and suspension of measures protecting against infection, further growth in the number of seropositive children and adolescents is initially likely.

With regard to RBD antibody titers, the range of values measured became wider from November 2021 onwards, a trend that accelerated in February 2022. A clear reduction in median titers followed in March 2022, but the dispersal remained high. Accordingly, from March 2022 onwards more children and

adolescents had low antibody titers; strikingly, however, the range of titers is broad. A possible explanation is that the antibody assay was developed using the viral protein constructs of wild-type SARS-CoV-2, which react less intensively with the antibodies directed against the Omicron variant present in the children tested. Another reason could be the lower antibody response to infection with the currently dominant Omicron variant (15).

Participation in the Fr1da study and testing for SARS-CoV-2 antibodies are voluntary, so selection bias cannot be ruled out; this can be the case in virtually all investigations of this kind. Under- or over-representation of the true monthly seroprevalence figures is therefore possible. Nevertheless, the strengths of our research are the relatively large size of the sample, the long observation period, and the wide geographic distribution of the participating pediatricians, providing substantial coverage of the Bavarian population.

The analysis of SARS-CoV-2-antibody-positive children and adolescents presented here shows a steep increase in seroprevalence since November 2021. By June 2022 more than two thirds of the children in the cohort as a whole, over 60% of the preschool children, and over 80% of children of school age were seropositive. These high case numbers, also discernable in other regions of Germany, should be considered in future health policy decision making.

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Conflict of interest statement
Dr. Ewald is honorary chairperson of the Bavaria state branch of the Professional Association of Pediatricians (*Berufsverband der Kinder- und Jugendärzte*).

The remaining authors declare that no conflict of interest exists.

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► **Supplementary material**

eMethods, eFigures:
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Supplementary material to:

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eMETHODS

Method

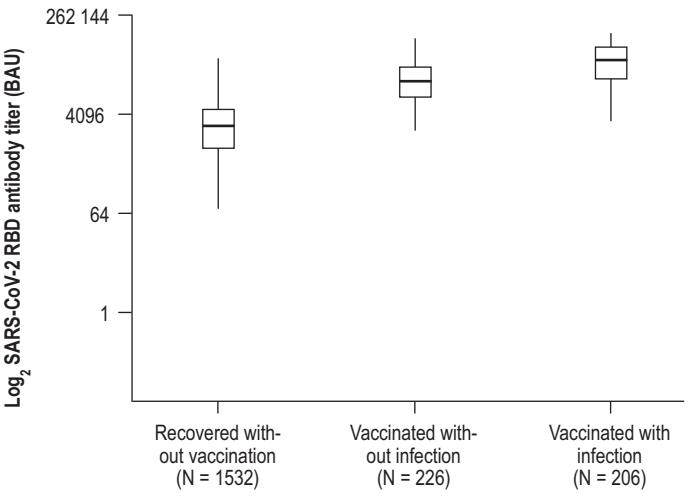
SARS-CoV-2 antibody quantification

Antibodies were determined in capillary serum using a highly specific screening method with sensitivity of > 95% and specificity of 100% that has been described in detail elsewhere (7). In the first phase, a nano-luciferase was used to quantify antibodies to the receptor binding domain (RBD) of the SARS-CoV-2 spike protein in a luciferase immunoprecipitation system (LIPS). The relative light levels measured by this means were converted to arbitrary units (AU) using a calibration curve from serum of a PCR-positive sample (Munich Diabetes Bioresource, Munich, Germany) diluted in PCR-negative serum of 0.1–100 AU. In accordance with international standards, the AU measurements were converted to binding antibody units (BAU). The internal cut-off for RBD positivity was set at > 1 AE (> 5 BAU). In the second phase, the samples from text processing were investigated for antibodies to the SARS-CoV-2 nucleocapsid protein (NP) by means of the LIPS method. The cut-off for positive NP samples was set at > 13 AE. The nano-luciferase virus protein constructs used, based on the sequence of the Wuhan/SARS-CoV-2 wild-type virus variant, were developed and kindly made available by Dr. Vito Lampasona (San Raffaele Hospital, Milan, Italy).

The same method was applied in the Fr1da study in Saxony, although there a specially developed nano-luciferase virus protein construct was used (Dr. Ezio Bonifacio, Technical University of Dresden). The samples from the Fr1da study in northern Germany were analyzed at the Institute for Diabetes Research laboratory in Munich using the method described above.

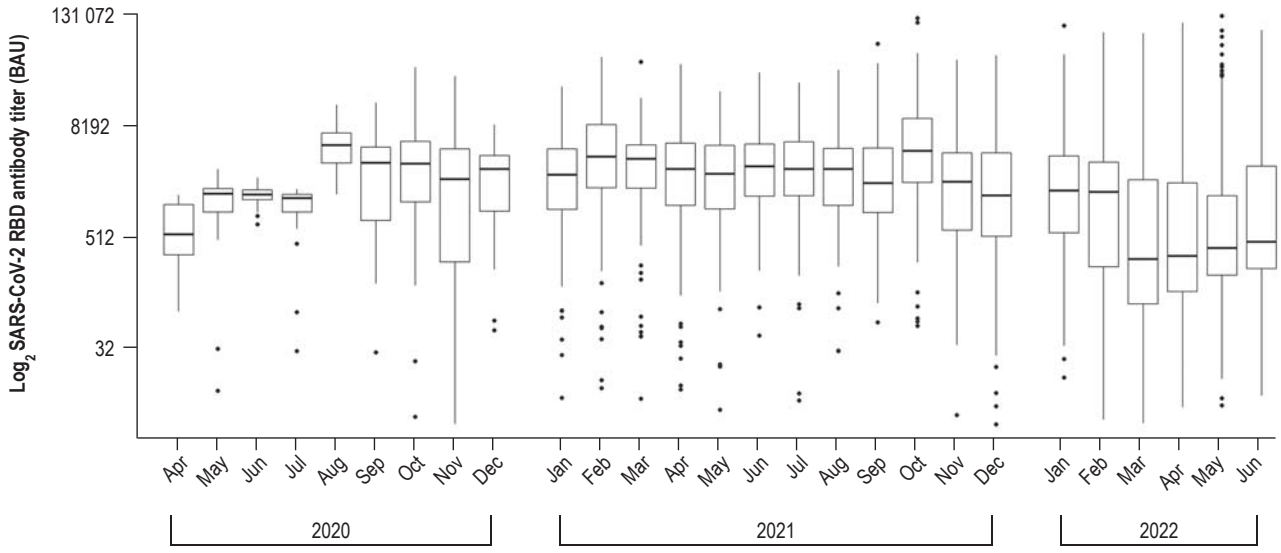
A double-positive antibody response to both RBD and NP was interpreted as “recovered from infection with/exposure to SARS-CoV-2” or “recovered and vaccinated”; a positive antibody response to RBD (> 5 AU or > 25 BAU) and a negative antibody response to NP (< 13 AU) was interpreted as “vaccinated against SARS-CoV-2 without prior infection” or “infection/exposure long ago”. Children with antibody titers of < 1 AU against RBD or titers of 1–5 AU against RBD and < 13 AU against NP were classed as “antibody-negative”.

eFIGURE 1



SARS-CoV-2 RBD antibody titer in recovered and vaccinated children and adolescents of the Fr1da study in Bavaria. The figure shows the RBD antibody titers (> 25 BAU) of recovered persons without vaccination (all RBD-positive and NP-positive cases between January 2020 and July 2021) and of vaccinated persons with or without prior infection (confirmed vaccination, RBD positive and NP positive or negative). Box plots (median and IQR) with lines for measurements within 1.5 IQR. All group differences are significant (Wilcoxon test, $p < 0.001$).
BAU, Binding antibody units; IQR, interquartile range;
NP, nucleocapsid protein; RBD, receptor binding domain;
SARS-CoV-2, severe acute respiratory syndrome coronavirus 2

eFIGURE 2



Distribution of the SARS-CoV-2 RBD immunoglobulin-G antibody titers in the positive samples (> 25 BAU) per pediatrician's office in the Fr1da study in Bavaria from April 2020 to June 2022. The median antibody titers from each pediatrician's office are expressed as box plots (median and IQR) with lines for measurements within 1.5 IQR and dots for values exceeding 1.5 IQR.

BAU, Binding antibody units; IQR, interquartile range; RBD, receptor binding domain; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2