

# Seroprevalence and silent infection rate during SARS-CoV-2 pandemic among children and adolescents in Western Pomerania: a multicenter, cross-sectional study—the COVIDKID study

Marcus Vollmer<sup>1,\*</sup>, Daniela Kuthning<sup>2,\*</sup>, Jana Gramenz<sup>2</sup>, Arevik Scholz<sup>2</sup>, Robin Michael<sup>2</sup>, Nico Wittmann<sup>2,3</sup>, Udo Gesser<sup>4</sup>, Christian Niesyutto<sup>5</sup>, Antje Vogler<sup>6</sup>, Vanda Tuxhorn<sup>7</sup>, Ute Lenschow<sup>8</sup>, Anja Lange<sup>2</sup>, Kristina Linnemann<sup>9</sup>, Holger Lode<sup>10</sup>, Lars Kaderali<sup>1</sup> and Almut Meyer-Bahlburg<sup>2,3</sup>

<sup>1</sup> Institute of Bioinformatics, University Medicine Greifswald, Greifswald, Germany

<sup>2</sup> Pediatric Rheumatology, Department Pediatric and Adolescent Medicine, University Medicine Greifswald, Greifswald, Germany

<sup>3</sup> German Center for Child and Adolescent Health (DZKJ). Partner site Greifswald/Rostock, Greifswald, Germany

<sup>4</sup> Children's Hospital, Sana Hospital Rügen, Bergen, Germany

<sup>5</sup> Department of Pediatrics, AMEOS Hospital Anklam, Anklam, Germany

<sup>6</sup> Department of Pediatrics, Asklepios Clinic Pasewalk, Pasewalk, Germany

<sup>7</sup> Department of Pediatrics, Kreiskrankenhaus Demmin, Demmin, Germany

<sup>8</sup> Department of Pediatrics, Helios HanseKlinikum Stralsund, Stralsund, Germany

<sup>9</sup> Pediatrics Outpatient Clinic, Greifswald, Germany

<sup>10</sup> Pediatric Hematology and Oncology, Department Pediatric and Adolescent Medicine, University Medicine Greifswald, Greifswald, Germany

\* These authors contributed equally to this work.

## ABSTRACT

**Background.** Limited data on SARS-CoV-2 seroprevalence in rural areas of northern Germany necessitate comprehensive cohort studies. We aimed to evaluate the seroprevalence, silent infection (SI) rates and risk factors for infections among children and adolescents in Western Pomerania from December 2020 to August 2022.

**Methods.** In this cross-sectional study, serum or plasma samples from children and adolescents (6 months to 17 years) were collected during routine blood draw. SARS-CoV-2 specific antibodies (S1 and nucleocapsid) and their neutralizing capacity were analyzed using commercially available enzyme-linked immunosorbent and neutralization assays. Socio-demographic data and information about SARS-CoV-2 infection or vaccination were obtained. Multivariable logistic regression was used to identify independent risk factors for SARS-CoV-2 infections and SI.

**Results.** A total of 1,131 blood samples were included into the study. Overall, SARS-CoV-2 seroprevalence was 25.1%, strongly influenced by the pandemic course, predominant virus variants, age and approval of vaccination. SI rate was 5.4% (95%-CI [3.7%–6.8%]) among unvaccinated and undiagnosed children over the entire study period with highest rates among adolescents. Main risk factor despite the time at risk for silent infections was an infected household member (Odds ratio = 9.88, 95%-CI [4.23–22.9],  $p < 0.001$ ). Factors associated with overall infections (known and silent)

Submitted 18 April 2024  
Accepted 1 October 2024  
Published 11 November 2024

Corresponding author  
Almut Meyer-Bahlburg,  
almut.meyer-bahlburg@med.uni-greifswald.de

Academic editor  
Joël Mossong

Additional Information and  
Declarations can be found on  
page 16

DOI 10.7717/peerj.18384

© Copyright  
2024 Vollmer et al.

Distributed under  
Creative Commons CC-BY 4.0

OPEN ACCESS

also include the infection of a household member (Odds ratio = 17.8, 95%-CI [10.7–29.6],  $p < 0.001$ ).

**Conclusions.** We believe that the introduction of governmental measures and systematic test strategies in schools strongly impacted on the SI rate, as we suspect that asymptomatic cases have already been identified, resulting in surprisingly low SI identified in our study.

**Subjects** Epidemiology, Infectious Diseases, Pediatrics, Public Health, COVID-19

**Keywords** SARS-CoV-2, Covid-19, Serology, Pediatric, Silent infection, Pandemic, IgG-S1, IgA-S1, NCP-IgG, Seroprevalence

## INTRODUCTION

Since the first description of severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2) in December 2019 ([Zhu et al., 2020](#)) and its subsequent global expansion resulting in a pandemic ([World Health Organization, 2024](#)), the new coronavirus causing respiratory coronavirus disease 2019 (COVID-19) was responsible for 32,145,157 cases in Germany and 582,669 cases in the federal state of Mecklenburg-Western Pomerania (Mecklenburg-Vorpommern, MV) by the end of August 2022 ([Robert Koch Institute, 2022](#)). The pandemic was characterized by emergence of several variants, each defined by altered levels of infectivity, symptom presentation and immune escape ([Whitaker et al., 2022](#); [Sumner et al., 2023](#)).

To fight the pandemic, several preventive measures were implemented. Together with the introduction of vaccination against SARS-CoV-2 for adults by the end of 2020 and subsequently for children from May 2021 ([European Medicines Agency, 2024](#)) they impacted the course of the pandemic. In contrast to adults, children and adolescents are often only mildly affected by acute SARS-CoV-2 infection, with only mild or no symptoms, resulting in significant under-ascertainment rate. This underlines the importance of seroprevalence studies.

In addition, infection rates in densely populated urban regions may differ from those in sparsely populated rural areas, with several studies demonstrating different results ([Kleyhans et al., 2021](#); [Cantu et al., 2024](#); [Anzalone et al., 2023](#); [Uschner et al., 2022](#); [Czerwiński et al., 2023](#); [Bignami-Van Assche et al., 2024](#)). Seroprevalence among children for urban regions of Germany is available ([Hippich et al., 2021](#); [Kirsten et al., 2022](#); [Leone et al., 2022](#); [Sorg et al., 2022](#); [Wachter et al., 2022](#); [Engels et al., 2023](#)), but there is less information for more rural areas like MV. Western Pomerania is covered by three districts, which belong to the 6% least populated districts in Germany (each with 60/70/47 inhabitants per km<sup>2</sup>) ([Statistisches Bundesamt, 2023](#)).

In our present study, we aimed to analyze temporal changes of seroprevalence for SARS-CoV-2 in Western Pomerania, estimate the rate of silent infections (SI) and identify associated risk factors. With this, our study sheds light on the dynamic changes in seroprevalence and rate of SI in children and adolescents during SARS-CoV-2 pandemic,

most importantly with the emergence of Omicron, as many studies concentrated on earlier phases of the pandemic.

## MATERIALS & METHODS

### Cohort selection

For this multicenter, cross-sectional study, participants (6 months to 17 years) with permanent residence in Western Pomerania were recruited in six participating pediatric hospitals and two outpatient practices. The indication for participation in the study was routine blood sampling at the participating clinics. Our study itself was not the reason for the blood sampling, but rather general indications, such as the monitoring of laboratory parameters in chronically ill children, the planned blood sampling for further diagnostics in children with certain pathological symptoms (e.g., arthralgia) or for further diagnostics in acutely ill children and adolescents. Participation included written informed consent, collection of serum or plasma during routine blood draw and completion of a questionnaire. Three patients with known primary immunodeficiencies or under immunoglobulin replacement therapy were excluded from the study. Repeated participation after six months was allowed as exposure to the virus, life circumstances, and serology may have changed in the interim. The children were categorized in three distinct age groups: children under 5 years of age, between 5 and 11 years old (school children), and between 12 and 17 years old (adolescents).

The study was registered in the German Clinical Trials Register on March 9, 2021 (Trial ID: DRKS00024635).

### Outcomes

SARS-CoV-2 specific antibodies in serum or plasma were analyzed by enzyme-linked immunosorbent assays (ELISA), using commercially available kits (EUROIMMUN AG, Lübeck, Germany) against the S1 domain (IgG-S1 and IgA-S1) and the nucleocapsid antigen (NCP-IgG) according to the manufacturer's instructions. Samples were considered positive at a ratio  $\geq 1.1$ , as recommended by the manufacturer. (Borderline) positive sera and samples from SARS-CoV-2 infected participants were tested for total neutralizing antibodies (NAbs) with SARS-CoV-2 Surrogate Virus Neutralization Test cPass (GenScript Biotech, Piscataway, NJ, USA). Data from this semi-quantitative test are presented as percentage of inhibition with  $\geq 30\%$  inhibition capacity classified as positive.

Seropositivity was asserted when one of the following conditions was true: (1) positive IgG-S1; (2) borderline IgG-S1 and at least one additional positive test; (3) IgG-S1 negative, but at least 2 other positive tests. For the assessment of risk factors for both overall infection (OI) and SI, all vaccinated participants and those with incomplete information about their vaccination or infection history were excluded. SI was defined as seropositivity in participants without a history of SARS-CoV-2 infection or vaccination reported by questionnaire. OI was defined as either samples tested seropositive or participants declared an infection anamnestically.

## Statistical analysis

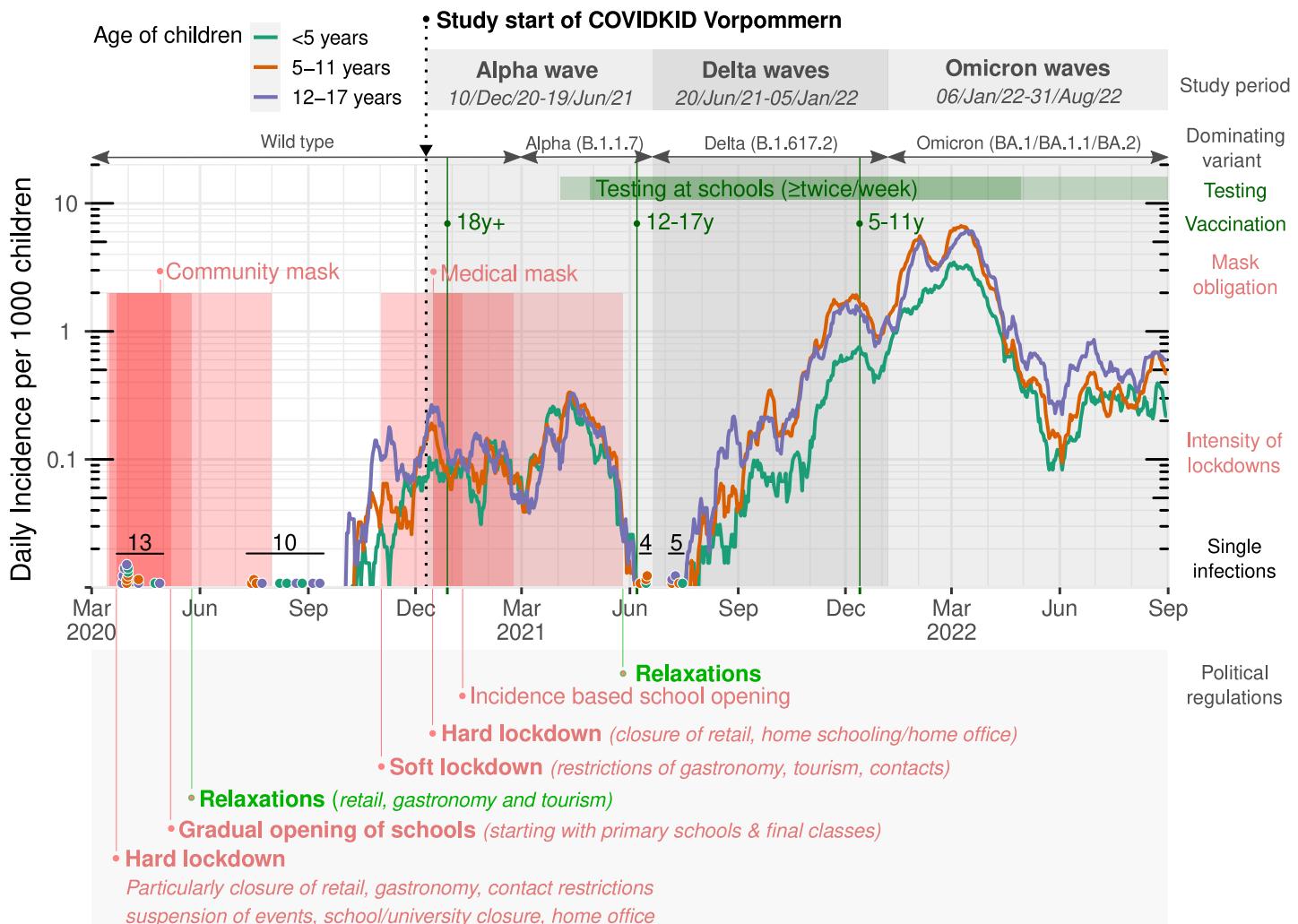
The analysis of the study includes the use of external data (see [Methods S1](#)). Official register data was used to check the representativeness of age, sex, and vaccination rate of our children's sample. Centered 7-day averages of daily case numbers in the three districts were used to compute the incidence of daily infections per 1,000 children in the respective age groups. Turning dates of the new dominating variants were interpolated from freely available data from the CoMV-Gen study center, which reported weekly fractions of challenging variants (interpolated fraction >50% for respective variant) determined from variant-polymerase chain reaction (PCR) or whole genome sequencing ([Kohler et al., 2023](#); [CoMV-Gen, 2023a](#); [CoMV-Gen, 2023b](#); [CoMV-Gen, 2023c](#); [CoMV-Gen, 2023d](#)).

Three study periods were defined based on dominating variant: Alpha, Delta and Omicron waves. Time course analysis was done using locally weighted scatterplot smoothing (LOESS). SI were compared among the age groups for each study period by Fisher's exact test. To evaluate associating factors, multivariable logistic regression were performed for OI and SI. Since this requires complete data, we created 10 imputed data frames using random forest imputation ([Shah et al., 2014](#)). We applied four-fold cross-validated elastic net with a fair mix of L1- and L2-regularization ( $\alpha = 0.5$ ) onto the imputed data frames to reduce model size for better generalizability ([Tay, Narasimhan & Hastie, 2023](#)). The extracted variables at a specific lambda value (one standard error from optimal lambda) entered the final model formula. In the following, we trained the regression coefficients on each imputed data frame and pooled the estimations to increase reliability. As blood were sampled at different dates and stages of the pandemic, the time exposed to an infectious environment and the severity of infectiousness was taken into account when modeling OI and SI. We therefore have cumulated the incidence numbers reported since the start of the pandemic till blood sampling date of the respective age groups and districts. The infection risk variable was defined as the resulting cumulative numbers normalized by the number of children in each age group of the three districts to allow a better comparison of the estimated odds ratios (OR). We performed Type-III likelihood ratio tests and computed OR with 95% confidence intervals from the regression models. Multicollinearity was checked using variance inflation factors (VIF) with default cutoffs:  $VIF > 5$  for concern,  $VIF > 10$  for serious collinearity ([James et al., 2021](#)).

All statistical tests were conducted two-sided with R version 4.4.1 ([R Core Team, 2024](#)). The significance level was set a priori at 5%.

## Ethics approval

This study was performed in line with the principles of the Declaration of Helsinki. Ethics approval was obtained from the Ethics Committee of University Medicine Greifswald (BB188/20 and BB188/20a).



**Figure 1** Infection rates in children and adolescents in the study region of Western Pomerania, Germany. Regional incidences per 1,000 children for each age group printed as 7-day average, with single infections shown as dots. Dominating variants were calculated from variant PCR (change points were defined at 50% domination). Study periods of the COVIDKID study are defined at these change points. Start of vaccination campaigns for adults and children are indicated by green bars, mandatory wearing of masks is indicated in red. Red areas showing lockdown measures depending on restriction intensity and relaxations labeled at major events.

Full-size DOI: 10.7717/peerj.18384/fig-1

## RESULTS

### Course of virus variants, political decisions, vaccination and SARS-CoV-2 infection in the study region

The course of the pandemic was evaluated for the study area as defined in the methods section. Normalized incidences of SARS-CoV-2 infection, approval of vaccination starts in different age groups, mask obligations and preventive measures implemented by the federal state government of MV are summarized in Fig. 1. The amount of available data in the particular variables can be read from column N of Table 1.

**Table 1** Characteristics of the study population stratified by age.

Variable	N	Included children in Western Pomerania, Germany		
		Children under 5	School children	Adolescents
		Age <5 years N = 277	Age 5–11 years N = 400	Age 12–17 years N = 454
Sex, n (%)				
Male		144 (52.0)	215 (53.8)	203 (44.7)
Female		133 (48.0)	185 (46.2)	251 (55.3)
Sampling at, n (%)	1,131			
Alpha wave (10/Dec/2020–19/Jun/2021)		87 (31.4)	167 (41.8)	214 (47.1)
Delta waves (20/Jun/2021–05/Jan/2022)		103 (37.2)	104 (26.0)	103 (22.7)
Omicron waves (06/Jan/2022–31/Aug/2022)		87 (31.4)	129 (32.2)	137 (30.2)
Sample taken after approval of vaccination, n/N (%)	1,131	0/277 (0.0%)	139/400 (34.8%)	254/454 (55.9%)
Self-disclosure about vaccination and infection, n (%)	1,102			
Vaccinated and infected		1 (0.4)	6 (1.5)	25 (5.6)
Vaccinated only		1 (0.4)	15 (3.8)	86 (19.4)
Infected only		44 (16.5)	77 (19.6)	41 (9.3)
Neither vaccinated nor infected		220 (82.7)	295 (75.1)	291 (65.7)
IgG-S1, Median (IQR)	1,131	0.13 (0.08, 0.49)	0.14 (0.09, 0.48)	0.17 (0.10, 3.93)
IgA-S1, Median (IQR)	1,130	0.12 (0.07, 0.34)	0.10 (0.06, 0.28)	0.09 (0.06, 0.23)
IgG-NCP, Median (IQR)	1,129	0.20 (0.13, 0.38)	0.23 (0.16, 0.43)	0.34 (0.19, 1.54)
NAbs (%), Median (IQR)	376 <sup>a</sup>	21 (6, 87)	35 (7, 91)	94 (32, 97)
Seropositive, n/N (%)	1,131	53/277 (19.1%)	82/400 (20.5%)	149/454 (32.8%)
Overall infection in unvaccinated children, n (%)	968			
Not infected		202 (76.5)	283 (76.1)	280 (84.3)
Infected (Self-disclosed or SI)		62 (23.5)	89 (23.9)	52 (15.7)
Day care facility, n (%)	1,042			
Not in a day care facility		36 (14.2)	7 (1.9)	40 (9.6)
Nursery/Nanny		114 (44.9)	1 (0.3)	0 (0.0)
Kindergarten		101 (39.8)	71 (19.1)	1 (0.2)
School with after-school care		1 (0.4)	71 (19.1)	3 (0.7)
School without after-school care		2 (0.8)	222 (59.7)	372 (89.4)
Medication intake at sampling, n/N (%)	1,079	165/258 (64.0%)	168/380 (44.2%)	231/441 (52.4%)
Chronic diseases, n (%)	1,077			
No chronic diseases		219 (84.2)	268 (70.3)	283 (64.9)
Chronic diseases except respiratory		29 (11.2)	79 (20.7)	127 (29.1)
Chronic diseases including respiratory		12 (4.6)	34 (8.9)	26 (6.0)
Smoking parent, n/N (%)	1,091	117/262 (44.7%)	164/388 (42.3%)	202/441 (45.8%)
Child is smoking, n/N (%)	1,095	3/263 (1.1)	9/389 (2.3)	47/443 (10.6)
Self-disclosure about infection, n (%)	1,081			
Neither parents nor child		186 (71.8)	285 (74.0)	348 (79.6)
Only a parent		33 (12.7)	20 (5.2)	27 (6.2)
Only child		5 (1.9)	11 (2.9)	18 (4.1)
Parent and child		35 (13.5)	69 (17.9)	44 (10.1)

(continued on next page)

Table 1 (continued)

Variable	N	Included children in Western Pomerania, Germany		
		Children under 5	School children	Adolescents
		Age <5 years N = 277	Age 5–11 years N = 400	Age 12–17 years N = 454
Household member works in the healthcare system with patient contact (including nursing home, nursing service and others), n/N (%)	1,080	84/262 (32.1)	123/379 (32.5)	135/439 (30.8)
Highest level of education of the household, n (%)	860			
no high school diploma (yet)		6 (2.6)	11 (3.7)	26 (7.8)
Secondary school (“Hauptschule”)		13 (5.7)	21 (7.0)	22 (6.6)
Junior high school (“Mittlere Reife”)		88 (38.8)	119 (39.5)	143 (43.1)
University entrance qualification (“Hochschulreife”)		48 (21.1)	63 (20.9)	77 (23.2)
Academic degree (“Hochschulabschluss”)		72 (31.7)	87 (28.9)	64 (19.3)
Number of household members, n (%)	1,063			
1 or 2		10 (3.9)	23 (6.1)	42 (9.8)
3		75 (29.1)	97 (25.7)	122 (28.5)
4		120 (46.5)	163 (43.2)	149 (34.8)
5		36 (14.0)	67 (17.8)	59 (13.8)
6 or more		17 (6.6)	27 (7.2)	56 (13.1)
Household composition, n/N (%)				
Small child (<5 years) <sup>b</sup>	748	61/177 (34.5%)	68/271 (25.1%)	39/300 (13.0%)
Senior (≥60 years) <sup>b</sup>	748	86/177 (48.6%)	122/271 (45.0%)	118/300 (39.3%)
Animals	1,029	123/248 (49.6%)	221/359 (61.6%)	254/422 (60.2%)
Traveled beyond the district during the past 6 months, n/N (%)	1,096	112/263 (42.6%)	183/389 (47.0%)	185/444 (41.7%)
Burdens of the respondent parent considered to be the most serious, n/N (%)	886			
Occupational insecurity		53/229 (23.1%)	64/311 (20.6%)	66/346 (19.1%)
Fear of their own illness		71/229 (31.0%)	71/311 (22.8%)	79/346 (22.8%)
Fear of illness in close relatives		106/229 (46.3%)	130/311 (41.8%)	151/346 (43.6%)

## Notes.

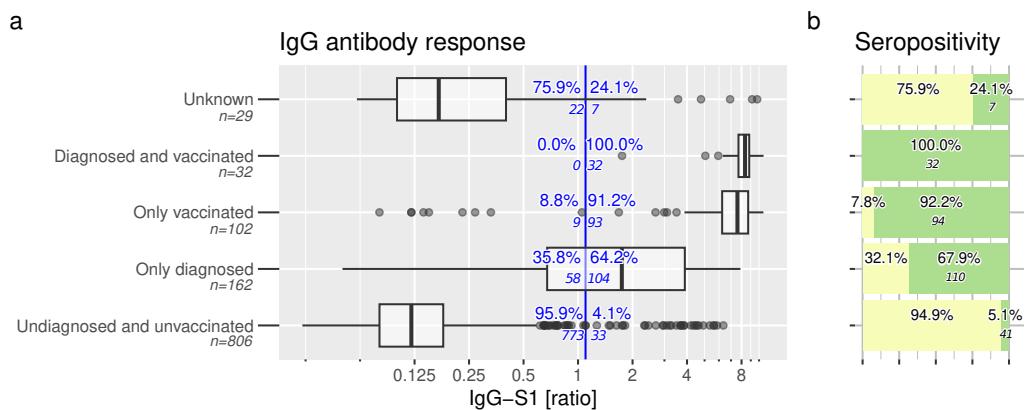
<sup>a</sup>Only positive or borderline positive sera as well as samples from patients reporting a history of SARS-CoV-2 infection were tested for total neutralizing antibodies (Nabs).

<sup>b</sup>Belongs to the household or is one of the very close contacts of the household community.

## Characteristics of study population in Western Pomerania

In total, 1,131 samples from 1,093 children and adolescents were included. Median age at sample collection was 10 years (quartile range 5 to 14 years). Sex distribution across the three age groups was slightly, but significantly different (female: 48.0%, 46.2% and 55.3%,  $p = 0.02$ ). Overall, the pediatric population of the study region is well represented, as the observed participant numbers follow an ideal representative age distribution and vaccination rates estimated from the study sample follows official numbers from RKI monitoring (see Figs. S1–S3). 1-year old children might be overrepresented in the the study sample (95 in the sample, 57 expected).

As the recruitment was hospital-based and involved recruitment in several special out-patient clinics, 28.5% of participants have at least one pre-existing condition. Of note, 17.9% of patients reported at least one known SARS-CoV-2 infection, 12.0% of participants



**Figure 2** Anti-SARS-CoV-2 IgG response and seropositivity. Anti-SARS-CoV-2-antibodies are evident in children and adolescents. (A) Anti-S1 IgG response and (B) seropositivity of all study participants at time of study inclusion, contingent upon self-disclosed SARS-CoV-2 vaccination and infection status. Vertical blue line indicates manufacturer's recommended cut-off (ratio IgG-S1  $\geq 1.1$ ). Seropositivity (green boxes in B) was inferred based on defined rules. Samples with missing information about infection and vaccination status were categorized as "unknown".

Full-size DOI: [10.7717/peerj.18384/fig-2](https://doi.org/10.7717/peerj.18384/fig-2)

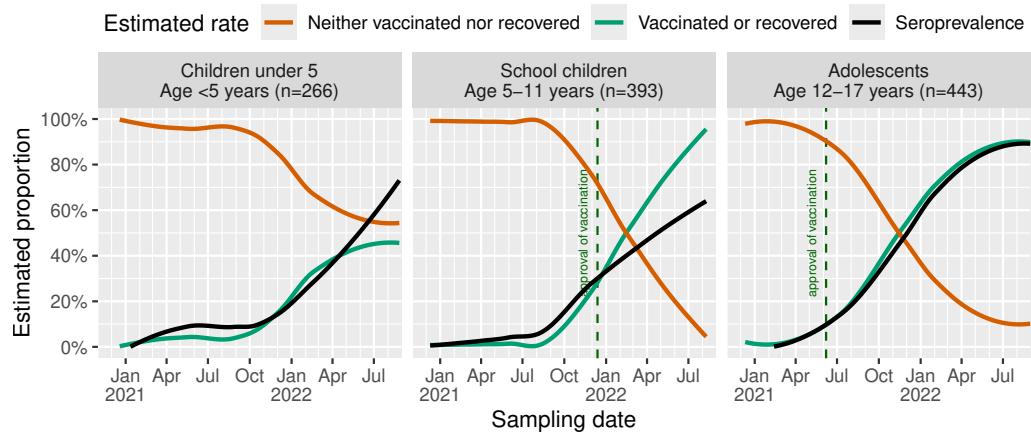
disclosed at least one vaccination. 21.1% of all children and adolescents reported to had contact to at least one SARS-CoV-2 infected household member. Participants characteristics and serological test results stratified by age are summarized in [Table 1](#).

### Estimation of seroprevalence among children and adolescents during the pandemic in Pomerania

[Figure 2A](#) shows the primary outcome of IgG-S1 antibodies. The highest rate of IgG-S1-positivity was achieved in vaccinated participants with or without prior SARS-CoV-2 infection ( $32/32 = 100\%$  and  $93/102 = 91.2\%$  respectively). In addition, 64.2% (104/162) of unvaccinated but infected participants were positive for IgG-S1. In the group of SARS-CoV-2 undiagnosed and unvaccinated participants, 4.1% were positive for IgG-S1 (33/806).

Overall seropositivity rate among these groups is shown in [Fig. 2B](#) and differs slightly from IgG-S1 positivity rates. Detailed information of tested participants is displayed in [Table S1](#) and [Figs. S4–S5](#).

For all age groups, seroprevalence increases in late 2021 ([Fig. 3](#)). In adolescents, a rapid increase of "vaccinated or recovered" participants was found with the estimated seroprevalence reaching comparable rates of approximately 90% at the end of the study. In school children, we see a strong deviation between both rates with approximately 90% "vaccinated or recovered" but an estimated seroprevalence of only about 60%. In children under 5, seroprevalence rates resemble those of school children despite slightly higher rates until mid-2021 and a lower rate of anamnestically recovered children. Of note, estimated seroprevalence and the rate of "vaccinated or recovered" children under 5 are diverging, especially during spring/summer 2021 and from May 2022 with emerging Omicron. At the end of the study, nearly 70% of this group were seropositive. In summary, at the end of



**Figure 3** Estimated rates of recovered or SARS-CoV-2-vaccinated children and seroprevalence. Seroprevalences across age groups were estimated using LOESS. Dashed lines shows the official vaccination start in the study region depending on the respective age group.

[Full-size](#) DOI: [10.7717/peerj.18384/fig-3](https://doi.org/10.7717/peerj.18384/fig-3)

**Table 2** Silent infection rates among undiagnosed and unvaccinated children and adolescents in Western Pomerania, stratified based on the time of study enrollment.

Study period	Children under 5 Age <5 years	School children Age 5–11 years	Adolescents Age 12–17 years	p-value <sup>a</sup>
Alpha wave (10/Dec/20–19/Jun/21), n/N (%)	3/79 (3.8%)	4/163 (2.5%)	2/204 (1.0%)	0.23
Delta waves (20/Jun/21–05/Jan/22), n/N (%)	4/89 (4.5%)	7/93 (7.5%)	2/62 (3.2%)	0.55
Omicron waves (06/Jan/22–31/Aug/22), n/N (%)	11/52 (21.2%)	1/39 (2.6%)	7/25 (28.0%)	0.006

#### Notes.

Notation: Number of silent infections/Total number of naive children or adolescents (silent infection rate in %).

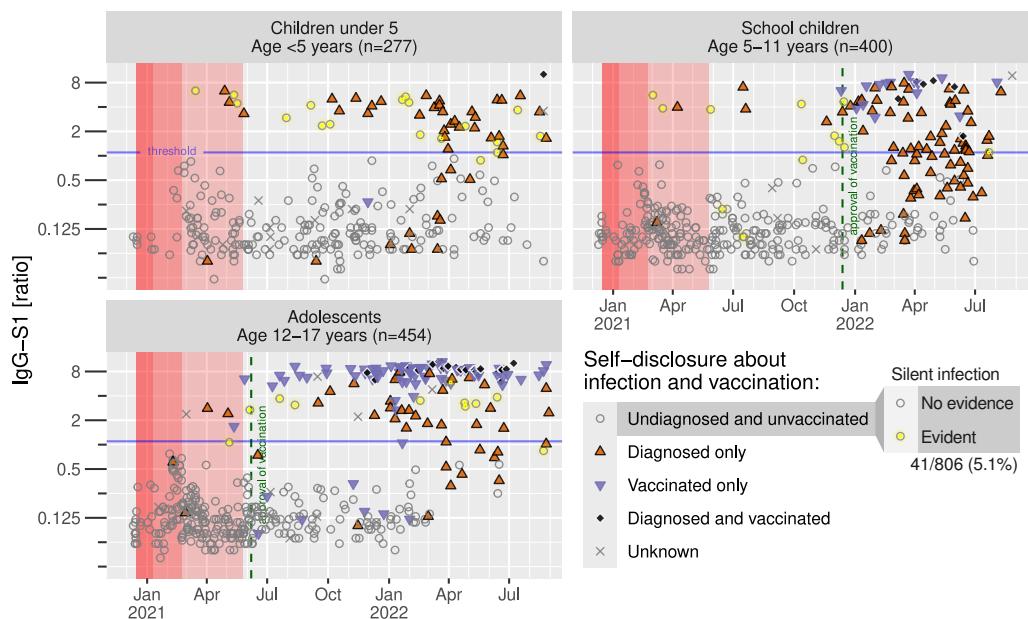
<sup>a</sup>Fisher's exact test.

August 2022, children and adolescents showed seroprevalence of 60% to 90%. During the first wave, we noticed slightly higher rates among children under 5. For subsequent Delta and Omicron waves, it reverses to the opposite with increasing rates with age.

#### Estimation of silent infection rate

In the presumptive negative samples, 41 undetected infections were confirmed, which corresponds to an SI rate of 5.4% (41/806, 95% Clopper–Pearson confidence interval 3.7% to 6.8%, see Fig. 2B). The distribution of these identified infections across age groups and major variants are summarized in Table 2. While the SI rates during Alpha dominance ranged between 1.0% to 3.8%, with no significant association with age, we found an increase in SI rate in all age groups, with significantly higher rates during Omicron dominance in children under 5 (11/52 = 21.2%) and in adolescents (7/25 = 28.0%) compared to school children (1/39 = 2.6%, Fisher's exact test  $p = 0.006$ ). However, the absolute number of undiagnosed and unvaccinated adolescents remarkably drops during Omicron domination.

Next, we analyzed IgG-S1 seroprevalence within the three groups in respect to date of study inclusion. Under strong restrictions (Fig. 4, marked in red shading), only 10 infections were diagnosed (upper red triangles), particularly from March 2021 on, and 7 SI were



**Figure 4** Anti-SARS-CoV-2-antibodies in time course. Individual anti-S1 antibodies of recruited participants related to their anamnestic infection and vaccination status. Dashed lines indicating vaccination approvals in respective age groups, red areas indicating infection control measures in Western Pomerania, depending on harshness of restrictions.

Full-size DOI: 10.7717/peerj.18384/fig-4

confirmed across all age groups until June 2021. The number of vaccinated participants (lower blue-magenta triangles) rapidly increased from June 2021 in adolescents and November 2021 in school children. Bulk infections were identified from November 2021 in all age groups. It is noteworthy that most school children were aware of their infection after the start of the vaccination campaign in January 2022 (72 of 138 reported an infection). Only three silent infections were identified among the remaining 46 undiagnosed and unvaccinated children.

### Evaluation of potential risk factors for OI and SI

Table 3 shows the results of the multivariable logistic regression for OI and SI. OI was significantly associated with infection risk (OR = 1.00, 95%-CI [0.96–1.05] [children under 5], 1.02, 95%-CI [0.98–1.05] [school children], 1.05, 95%-CI [1.01–1.09] [adolescents] for each percent increase in the case numbers in the respective age group). Moreover, the dominating variant at the time of study enrollment is significantly associated with OI, with significantly higher OR during Delta and Omicron waves compared to the Alpha wave (OR = 2.52, 95%-CI [1.33–4.77],  $p = 0.004$  and OR = 6.57, 95%-CI [2.41–17.9],  $p < 0.001$ , respectively). Our results demonstrate the strongest association for OI and SI when a household member was diagnosed with SARS-CoV-2 infection (OR = 17.8, 95%-CI [10.7–29.6],  $p < 0.001$  and OR = 9.88, 95%-CI [4.23–22.9],  $p < 0.001$ , respectively). All other socio-demographic characteristics considered did not make it into the regression equation.

**Table 3** Results of logistic regressions for associations with overall and silent infections.

Characteristic	Overall infections (OI) among unvaccinated (n = 968)		Silent infections (SI) among unvaccinated and uninfected (n = 806)	
	OR (95% CI) <sup>a</sup>	p-value	OR (95% CI) <sup>a</sup>	p-value
Infection risk <sup>b</sup> in interaction with age [OR per 1% increase of infected]				
Children under 5 (age: <5 years)	1.00 (0.96 to 1.05)	0.88	1.05 (1.01 to 1.10)	0.016
School children (age: 5-11 years)	1.02 (0.98 to 1.05)	0.35	0.95 (0.91 to 1.0)	0.045
Adolescents (age: 12-17 years)	1.05 (1.01 to 1.09)	0.011	1.03 (0.98 to 1.08)	0.216
Dominating variant				
During Alpha wave (10/12/2020-19/06/2021)	1.00 (Reference)			
During Delta waves (20/06/2021-05/01/2022)	2.52 (1.33 to 4.77)	0.004		
During Omicron waves (06/01/2022-31/08/2022)	6.57 (2.41 to 17.9)	<0.001		
At least one household member was infected	17.8 (10.7 to 29.6)	<0.001	9.88 (4.23 to 22.9)	<0.001

**Notes.**<sup>a</sup>OR, multivariable adjusted Odds Ratio, CI, Confidence Interval of the OR.<sup>b</sup>Infection risk is increasing with the time at risk and defined by the cumulative cases reported to LAGuS of each age group of the three study districts. Cumulative case numbers were divided by the total population of children in the respective age group of all three districts to allow meaningful interpretation of OR.

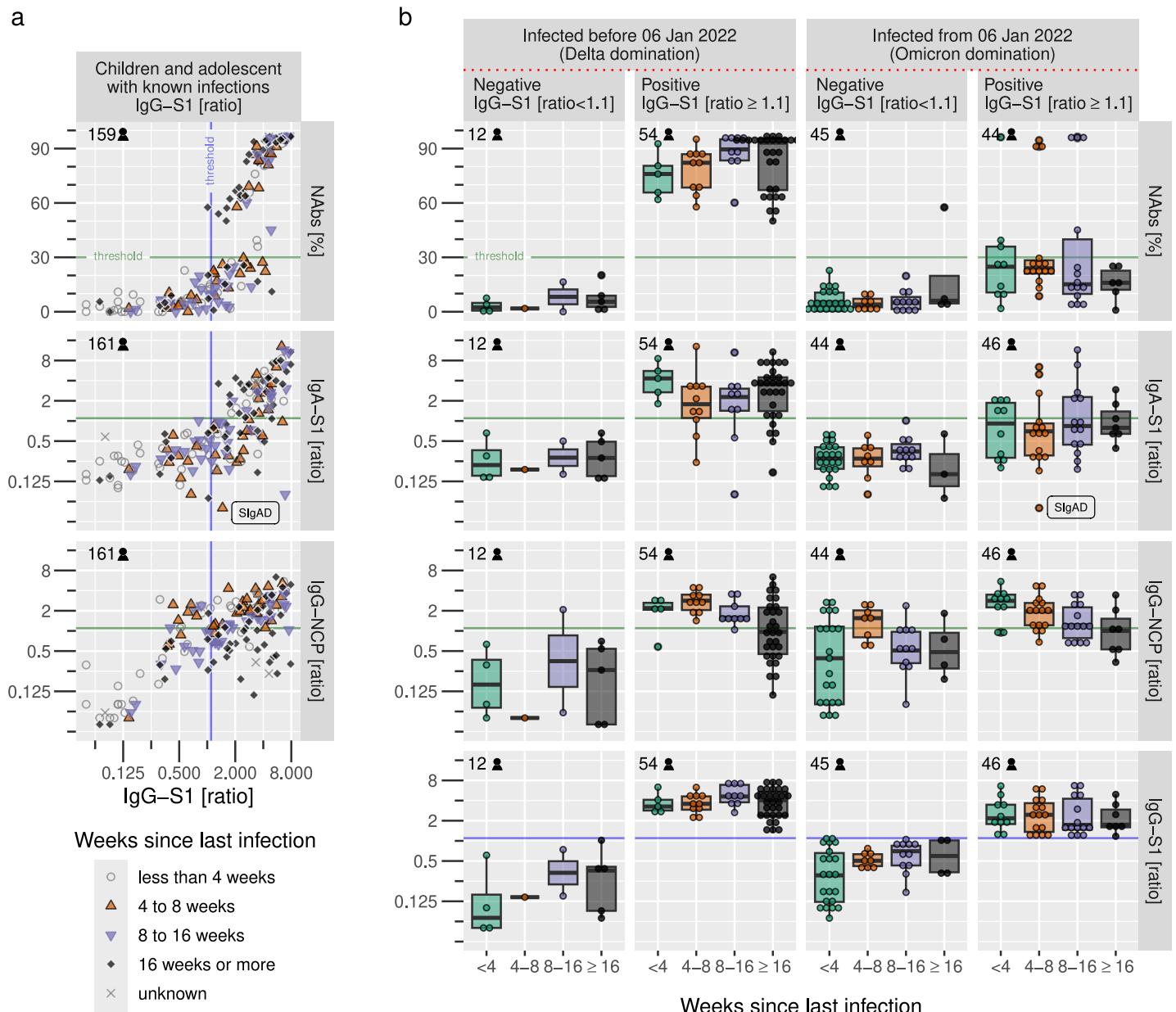
## Power of performed serological tests

We noticed an increasing number of samples found to be positive for IgG-S1 but negative for all other serological tests, most notably neutralizing antibodies testing by cPass (Fig. 5A). When comparing IgG-S1 positive and negative samples from diagnosed participants before and after 6 January 2022, our data demonstrate a significant loss of power for NAbS and IgA-S1 with emerging Omicron variants, as only 10 of 44 IgG-S1-positive samples were also NAb-positive (see top-right of Fig. 5B) and only 15 of 46 IgG-S1-positive samples were also IgA-S1-positive. IgG-S1 and IgG-NCP suffered from decreased antibody ratios but were still able to detect antibodies during the Omicron wave to a similar extent as during previous waves. In summary, IgG-S1 performed best to identify positive samples after confirmed infection, and IgG-NCP is particularly useful to detect antibodies irrespective of SARS-CoV-2-variants and vaccination just a few weeks after infection.

## DISCUSSION

### Principal findings

In our study we aimed to investigate the seroprevalence of SARS-CoV-2 specific antibodies in children and adolescents in Western Pomerania, part of a rural federal state in northeast of Germany from mid-December 2020 to August 2022. At the beginning of the pandemic, immunity against SARS-CoV-2 and SI rates were extremely low in all age groups of our study cohort. Seroprevalence increased during the Delta and Omicron waves with vaccine approval and relaxation of hygiene measures. Household contacts with infected individuals posed highest risk for SI. The SI rate was remarkably low at the start of our study, ranging



**Figure 5** Sensitivity of serological tests before and after emerging Omicron variants. (A) Correlation of IgG-S1 ratio with other serological tests in relation to the time since self-reported infection in children and adolescents, (B) Sensitivity of serological tests in combination with the IgG-S1 ratio and the dominating variant at the time of infection. Horizontal and vertical lines indicate the manufacturer's cut-offs for the respective tests.

Full-size DOI: 10.7717/peerj.18384/fig-5

from 1.0% to 3.8%, and increased particularly with the rise of Omicron variants in an age-dependent manner from 2.6% to 28.0%. However, this 28% SI rate in adolescents during Omicron should not be overestimated because the majority of all adolescents had already been vaccinated or knowingly infected. There were only 25 supposedly immunologically naïve adolescents in which we were able to detect an undetected infection in 7 subjects.

## Interpretation

Initial seroprevalence rates were very low in all age groups under strict restrictions, with first infections detected in participants recruited in March 2021, and remained low under strict contact restrictions resulting in only few infections. However, seropositivity strongly increased with both the approval of vaccinations and the relaxation of contact restrictions during emerging Delta and especially Omicron waves. At the end of the study in August 2022, adolescents showed a high estimated seroprevalence of 90% whereas children under 12 years of age had a seroprevalence rate of only about 60% to 70%, suggesting a higher immunity in adolescents than in younger children. This is in line with comparable studies across Europe ([Engels et al., 2023](#); [Boey et al., 2022](#); [Ratcliffe et al., 2023](#); [Sapronova et al., 2023](#)). Interestingly, our data from the first study period until June 2021 demonstrated a higher seroprevalence among very young children under 5 similar to the results from other German studies ([Leone et al., 2022](#); [Sorg et al., 2022](#)).

The slight overrepresentation of 1-year-old children is most likely due to the fact that children of this age are hospitalised more frequently than older children and that we recruited the majority of patients in hospital. However, we do not believe this affects the overall result of the study because we stratified our analyses by age group.

In school-aged children, our data suggested the lowest immunity with insufficient rates of antibody production or detection. In contrast, seroprevalence was significantly higher than anamnestically reported infection rates in children under 5, indicating a relatively high rate of SI in this group. Other seroprevalence studies from Germany showed similar results for children in different age groups, with low seroprevalences in low-incidence settings until late 2020 and increasing rates during 2021 ([Leone et al., 2022](#); [Wachter et al., 2022](#); [Engels et al., 2022](#); [Jank et al., 2023](#)). Only limited seroprevalence data are available for north-eastern Germany with the exception of the “Fr1da” study who is reporting a seroprevalence of 76.7% in children between August 2021 and June 2022 ([Ott et al., 2022](#)).

Our study aimed to provide additional information on seroprevalences in children of different age groups in a rural region. Several studies suggest differences in infection rates between urban and rural areas, with contrasting results, but only few studies have analysed the paediatric population: In a study from South Africa seroprevalence was lowest in children under 5 years of age in rural communities and highest in adults in urban areas ([Kleyhans et al., 2022](#)). In contrast, younger children in rural areas were more likely to be hospitalized for SARS-CoV-2 infections in a study from the US ([Cantu et al., 2024](#)). In Poland, higher seroprevalence rates were observed in rural compared to urban areas during early stages of the pandemic but this study did not specifically addressed the pediatric population ([Czerwiński et al., 2023](#)). A German/Italian study showed that the risk of dying from COVID-19 was lower for people living in rural areas, but only if hospitalisation was not required. In contrast, for those who were hospitalised in rural areas, the risk of dying was higher than in urban areas ([Bignami-Van Assche et al., 2024](#)). Overall, however, the seroprevalence rates in our study of children and adolescents from a rural area do not appear to differ significantly from others from urban regions in Germany. However, there is no direct comparison therefore the conclusion is somewhat limited.

In infectious disease epidemiology, silent infection refers to the unnoticed development of immune protection following an asymptomatic or clinically inapparent course of an infection. Under normal circumstances, this is a desirable effect, as a high rate of silent infection means that a large proportion of the population is immune to the pathogen in question. During the pandemic, however, the population was overwhelmingly SARS-CoV-2 naïve. To prevent too many people falling ill at the same time, which would have led to an overload of the healthcare system, SARS-CoV-2 infected people were to remain in quarantine. To avoid mass infections, it was therefore necessary and sensible to identify asymptotically infected people. The risk for SI in our study was highest in adolescents but almost completely disappeared among school children beginning from December 2021. The revised testing strategy in schools may have led to early and rapid detection of infections in children. The testing strategy included regular testing in schools for students and employees at least twice a week, and was mandatory between 28 April 2021 and 29 April 2022. As a result, SARS-CoV-2 positive children were kept in quarantine to prevent transmission of the virus. In kindergarten, starting in April 2021, only symptomatic children were tested, while many day care facilities remained closed, which could explain the higher SI rate due to undetecting in this age group. Therefore, the testing strategy implemented in schools seems to have been successful in avoiding SI. The main risk factor for children and adolescents being silently infected was having an infected household member, followed by the time at risk and the dominating variant.

Consistent with other studies, our data could not demonstrate a protective association with a lower risk of infection or increased seroprevalence when health care workers are in the household community ([Ratcliffe et al., 2023](#); [Almeida Carvalho et al., 2022](#); [Steensels et al., 2020](#)). Other socio-demographic characteristics may also be associated with SI, but were considered to be of minor importance in the elastic net regression. Increased risk for children in lower educated households, as reported in previous studies, was not evident in our cohort ([Brinkmann et al., 2022](#)).

The risk of OI was higher among adolescents during Delta and Omicron waves and with at least one infected household member. Similar results were found in a pediatric cohort from the US ([Smith et al., 2023](#)).

We believe that the introduction of government policies and systematic testing strategies in schools and many public and health-related institutions has had a strong impact on the rate of SI in children, as we suspect that asymptomatic cases have already been identified, resulting in the surprisingly low rate of SI identified in our study.

During our analysis, we recognized that the serological results are subject to a change point as NAbs and IgA-S1 show a remarkable decrease in IgG-S1 positive samples after 6 January 2022. This decline may be attributed, at least in part, to the immune escape mechanisms of various SARS-CoV-2 variants.

## Limitations

There are also some limitations to our study. We had to rely on questionnaire-based reports of diagnosis and vaccination. Identified SI cannot be attributed to causing variants and time of infection. Furthermore, we found reduced sensitivity of the cPass test for neutralization

capacity, also described in other publications, leading to the need of variant-specific testing as performed elsewhere ([Springer et al., 2022](#); [Kim et al., 2022](#)). A minor concern is cross-reactivity with other beta-coronaviruses which is still being debated ([Tammelin, Salminen & Blazevic, 2021](#); [Wong et al., 2023](#)). As the recruitment was voluntary and hospital-based, a selection bias cannot be ruled out. In particular, the number of included blood samples are not uniformly distributed across the study period and there were more inclusions in spring 2021 and less inclusions in summer 2022, leading to a biased SI rate. However, a study from the US and a worldwide meta-analysis demonstrated similar seroprevalences for population-based, school-based and hospital-based studies ([Clarke et al., 2023](#); [Naeimi et al., 2023](#)). Furthermore, the study sample follows the pediatric vaccination rate of the region and the age distribution is also representative. Therefore, we assume that our data allows the identification of under-ascertainment rates from late 2020 until August 2022, covering different SARS-CoV-2 variants in this well-defined region. Confounding variables can be missed in the multivariable models that may bias the estimation of independent effects. Moreover, we included a disproportionate number of patients with chronic diseases, including mainly rheumatic diseases, type 1 diabetes mellitus and respiratory diseases. The vast majority of these patients participate in life without any impairment, and severe courses of COVID-19 were not more common in these pediatric patient groups ([Sengler et al., 2021](#); [Danne & Kordonouri, 2021](#); [Edqvist et al., 2023](#); [Dolby et al., 2023](#)). However, we cannot rule out the possibility that these children and adolescents were tested more frequently, which could have led to a reduced SI rate.

## CONCLUSIONS

The COVIDKID study showed a significant increase in seroprevalence in children and adolescents of all age groups, particularly with the approval and introduction of vaccination and the emergence of the Delta and Omicron waves. The highest risk factor for infection, known or silent, adjusted for time at risk and dominant variant is infection of a household member. In addition, we saw higher rates when the Delta and Omicron variants were dominant. In the study sample, the SI rate in undiagnosed and unvaccinated children and adolescents was relatively low at only 5.4%, which we believe is due to systematic testing strategies in schools.

## ACKNOWLEDGEMENTS

We thank all study participants and their families for participating in the COVIDKID study. We thank the teams of physicians and nurses in all participating clinics for their support in sample acquisition. Special thanks go to Roswitha Bruns, who died in August 2021, for supporting this study. We also thank the diagnostics department of Medical Microbiology (Head: Prof. Dr. Karsten Becker, Dr. Zimmermann, Kathrin Hollmann, Maria Schuparis) for sample analysis and the Institute of Clinical Chemistry (especially Dr. Winter) as well as IMD Greifswald for sample transport. For his expertise and support we thank Prof. Dr. Martin Groschup.

## ADDITIONAL INFORMATION AND DECLARATIONS

### Funding

This study was supported by University Medicine Greifswald, Germany and funded by the Ministry for Economics, Labour and Health Mecklenburg-Vorpommern (406-00000-2020/002-018). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

### Grant Disclosures

The following grant information was disclosed by the authors:

University Medicine Greifswald, Germany.

Ministry for Economics, Labour and Health Mecklenburg-Vorpommern:  
406-00000-2020/002-018.

### Competing Interests

Almut Meyer-Bahlburg received funding for the project by the Ministry for Economics, Labour and Health Mecklenburg-Vorpommern. Lars Kaderali was a member of the Corona Expert Council advising the Federal Government of Germany.

### Author Contributions

- Marcus Vollmer analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the article, and approved the final draft.
- Daniela Kuthning conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the article, and approved the final draft.
- Jana Gramenz performed the experiments, authored or reviewed drafts of the article, and approved the final draft.
- Arevik Scholz performed the experiments, authored or reviewed drafts of the article, and approved the final draft.
- Robin Michael performed the experiments, authored or reviewed drafts of the article, and approved the final draft.
- Nico Wittmann performed the experiments, authored or reviewed drafts of the article, and approved the final draft.
- Udo Gesser performed the experiments, authored or reviewed drafts of the article, and approved the final draft.
- Christian Niesytto performed the experiments, authored or reviewed drafts of the article, and approved the final draft.
- Antje Vogler performed the experiments, authored or reviewed drafts of the article, and approved the final draft.
- Vanda Tuxhorn performed the experiments, authored or reviewed drafts of the article, and approved the final draft.
- Ute Lenschow performed the experiments, authored or reviewed drafts of the article, and approved the final draft.

- Anja Lange performed the experiments, authored or reviewed drafts of the article, and approved the final draft.
- Kristina Linnemann performed the experiments, authored or reviewed drafts of the article, and approved the final draft.
- Holger Lode performed the experiments, authored or reviewed drafts of the article, and approved the final draft.
- Lars Kaderali analyzed the data, authored or reviewed drafts of the article, and approved the final draft.
- Almut Meyer-Bahlburg conceived and designed the experiments, performed the experiments, analyzed the data, authored or reviewed drafts of the article, and approved the final draft.

## Human Ethics

The following information was supplied relating to ethical approvals (i.e., approving body and any reference numbers):

This study was performed in line with the principles of the Declaration of Helsinki. Ethics approval was obtained from the Ethics Committee of University Medicine Greifswald (BB188/20 and BB188/20a).

## Data Availability

The following information was supplied regarding data availability:

The code for data processing and statistical analysis are available in the [Supplemental Files](#).

## Supplemental Information

Supplemental information for this article can be found online at <http://dx.doi.org/10.7717/peerj.18384#supplemental-information>.

## REFERENCES

Almeida Carvalho JM, Camargo CN, De Souza Luna LK, Rabha AC, Conte DD, Mariano RF, De Oliveira Junior FI, Barbosa GR, Moreira LVL, Chaves APC, Perosa AH, Bellei N. 2022. Household transmission of COVID-19 according to index case: children, parents, and healthcare workers. *Brazilian Journal of Microbiology* 53:1345–1348 DOI [10.1007/s42770-022-00762-w](https://doi.org/10.1007/s42770-022-00762-w).

Anzalone AJ, Sun J, Vinson AJ, Beasley WH, Hillegass WB, Murray K, Hendricks BM, Haendel M, Geary CR, Bailey KL, Hanson CK, Miele L, Horswell R, McMurry JA, Porterfield JZ, Vest MT, Bunnell HT, Harper JR, Price BS, Santangelo SL, Rosen CJ, McClay JC, Hodder SL. 2023. Community risks for SARS-CoV-2 infection among fully vaccinated US adults by rurality: a retrospective cohort study from the National COVID Cohort Collaborative. *PLOS ONE* 18:e0279968 DOI [10.1371/journal.pone.0279968](https://doi.org/10.1371/journal.pone.0279968).

Bignami-Van Assche S, Ferraccioli F, Riccetti N, Gomez-Ramirez J, Ghio D, Stilianakis NI. 2024. Urban-rural disparities in COVID-19 hospitalisations and mortality: a

population-based study on national surveillance data from Germany and Italy. *PLOS ONE* 19:e0301325 DOI 10.1371/journal.pone.0301325.

**Boey L, Roelants M, Merckx J, Hens N, Desombere I, Duysburgh E, Vandermeulen C. 2022.** Age-dependent seroprevalence of SARS-CoV-2 antibodies in school-aged children from areas with low and high community transmission. *European Journal of Pediatrics* 181:571–578 DOI 10.1007/s00431-021-04222-9.

**Brinkmann F, Diebner HH, Matenar C, Schlegtendal A, Eitner L, Timmesfeld N, Maier C, Lücke T. 2022.** Seroconversion rate and socio-economic and ethnic risk factors for SARS-CoV-2 infection in children in a population-based cohort, Germany, 2020 to 2021. *Eurosurveillance* 27:2101028 DOI 10.2807/1560-7917.ES.2022.27.37.2101028.

**Cantu RM, Sanders SC, Turner GA, Snowden JN, Ingold A, Hartzell S, House S, Frederick D, Chalwadi UK, Siegel ER, Kennedy JL. 2024.** Younger and rural children are more likely to be hospitalized for SARS-CoV-2 infections. *PLOS ONE*. DOI 10.1371/journal.pone.0308221.

**Clarke KEN, Kim Y, Jones J, Lee A, Deng Y, Nycz E, Iachan R, Gundlapalli AV, MacNeil A, Hall A. 2023.** Pediatric infection-induced SARS-CoV-2 seroprevalence increases and seroprevalence by type of clinical care—September 2021 to February 2022. *The Journal of Infectious Diseases* 227:364–370 DOI 10.1093/infdis/jiac423.

**CoMV-Gen. 2023a.** SARS-CoV-2 in Mecklenburg-Vorpommern: Genetische Analyse und Nachverfolgung -Berichtswoche: 7. KW. Available at [http://www.comv-gen.de/fileadmin/user\\_upload/Berichte/2022\\_04\\_07\\_Bericht\\_KW13.pdf](http://www.comv-gen.de/fileadmin/user_upload/Berichte/2022_04_07_Bericht_KW13.pdf) (accessed on 22 December 2023).

**CoMV-Gen. 2023b.** SARS-CoV-2 in Mecklenburg-Vorpommern: Genetische Analyse und Nachverfolgung -Berichtswoche: 13. KW. Available at [http://www.comv-gen.de/fileadmin/user\\_upload/Berichte/2022\\_04\\_07\\_Bericht\\_KW13.pdf](http://www.comv-gen.de/fileadmin/user_upload/Berichte/2022_04_07_Bericht_KW13.pdf) (accessed on 22 December 2023).

**CoMV-Gen. 2023c.** SARS-CoV-2 in Mecklenburg-Vorpommern: Genetische Analyse und Nachverfolgung —Berichtswoche: 21. KW. Available at [http://www.comv-gen.de/fileadmin/user\\_upload/Berichte/2021\\_06\\_04\\_Bericht\\_KW21.pdf](http://www.comv-gen.de/fileadmin/user_upload/Berichte/2021_06_04_Bericht_KW21.pdf) (accessed on 22 December 2023).

**CoMV-Gen. 2023d.** SARS-CoV-2 in Mecklenburg-Vorpommern: Genetische Analyse und Nachverfolgung —Berichtswoche: 34. KW. Available at [http://www.comv-gen.de/fileadmin/user\\_upload/Berichte/2021\\_09\\_03\\_Bericht\\_KW34.pdf](http://www.comv-gen.de/fileadmin/user_upload/Berichte/2021_09_03_Bericht_KW34.pdf) (accessed on 22 December 2023).

**Czerwiński M, Stępień M, Juszczysz G, Sadkowska-Todys M, Zieliński A, Rutkowski J, Rosińska M. 2023.** Reversed urban–rural gradient in COVID-19 seroprevalence and related factors in a nationally representative survey, Poland, 29 March to 14 May 2021. *Eurosurveillance* 28:2200745 DOI 10.2807/1560-7917.ES.2023.28.35.2200745.

**Danne T, Kordonouri O. 2021.** COVID-19 (coronavirus disease 2019) und Typ-1-Diabetes: Kinder: Risiko Ketoazidose. *Der Diabetologe* 17:647–656 DOI 10.1007/s11428-021-00776-2.

**Dolby T, Nafilyan V, Morgan A, Kallis C, Sheikh A, Quint JK. 2023.** Relationship between asthma and severe COVID-19: a national cohort study. *Thorax* **78**:120–127 DOI [10.1136/thoraxjnl-2021-218629](https://doi.org/10.1136/thoraxjnl-2021-218629).

**Edqvist J, Lundberg C, Andreasson K, Björck L, Dikaio P, Ludvigsson J, Lind M, Adiels M, Rosengren A. 2023.** Severe COVID-19 infection in type 1 and type 2 diabetes during the first three waves in Sweden. *Diabetes Care* **46**:570–578 DOI [10.2337/dc22-1760](https://doi.org/10.2337/dc22-1760).

**Engels G, Hecker K, Forster J, Toepfner N, Hick E, Pietsch F, Heuschmann P, Berner R, Härtel C, Kurzai O, Petersmann A, Streng A, Liese J. 2022.** High seroprevalence of SARS-CoV-2 in preschool children in July 2022. *Deutsches Ärzteblatt International* **119**:771–772 DOI [10.3238/arztebl.m2022.0345](https://doi.org/10.3238/arztebl.m2022.0345).

**Engels G, Oechsle A-L, Schlegtental A, Maier C, Holzwarth S, Streng A, Lange B, Karch A, Petersmann A, Streeck H, Blaschke-Steinbrecher S, Härtel C, Schroten H, Von Kries R, Berner R, Liese J, Brinkmann F, Toepfner N, Forster J, Kurzai O, Pietsch F, Hick E, Hecker K, Lücke T, Hoffmann A, Schwarzbach M, Höppner J, Drinka D, Armann J, Blankenburg J, Falke U, Schneider J, Jäger V, Rücker V, Harries M, Hassenstein M, Dreier M, Von Holt I, Budde A, Kurosinski M-A, Bartz A, Brandhorst G, Brinkmann M, Budde K, Deckena M, Fenzlaff M, Hovardovska O, Kehl K, Kohls M, Krüger S, Meyer-Schlinkmann K, Ottensmeyer P, Reese J-P, Rosenkranz D, Rübsamen N, Schattschneider M, Schäfer C, Schlinkert S, Schulze-Wundling K, Störk S, Tiemann C, Völzke H, Winter T, Heuschmann P, Nauck M. 2023.** SARS-CoV-2 sero-immunity and quality of life in children and adolescents in relation to infections and vaccinations: the IMMUNEBRIDGE KIDS cross-sectional study, 2022. *Infection* **51**:1531–1539 DOI [10.1007/s15010-023-02052-5](https://doi.org/10.1007/s15010-023-02052-5).

**European Medicines Agency. 2024.** COVID-19 medicines. Available at <https://www.ema.europa.eu/en/human-regulatory-overview/public-health-threats/coronavirus-disease-covid-19/covid-19-medicines> (accessed on 26 August 2024).

**Hippich M, Holthaus L, Assfalg R, Zapardiel-Gonzalo J, Kapfelsperger H, Heigermoser M, Haupt F, Ewald DA, Welzhofer TC, Marcus BA, Heck S, Koelln A, Stock J, Voss F, Secchi M, Piemonti L, De la Rosa K, Protzer U, Boehmer M, Achenbach P, Lampasona V, Bonifacio E, Ziegler A-G. 2021.** A public health antibody screening indicates a 6-fold higher SARS-CoV-2 exposure rate than reported cases in children. *Med* **2**:149–163 DOI [10.1016/j.medj.2020.10.003](https://doi.org/10.1016/j.medj.2020.10.003).

**James G, Witten D, Hastie T, Tibshirani R. 2021.** *An introduction to statistical learning: with applications in R*. New York: Springer US. DOI [10.1007/978-1-0716-1418-1](https://doi.org/10.1007/978-1-0716-1418-1).

**Jank M, Oechsle A-L, Armann J, Behrends U, Berner R, Chao C-M, Diffloth N, Doenhardt M, Hansen G, Hufnagel M, Lander F, Liese JG, Muntau AC, Niehues T, Both UVon, Verjans E, Weil K, Von Kries R, Schroten H. 2023.** Comparing SARS-CoV-2 variants among children and adolescents in Germany: relative risk of COVID-19-related hospitalization, ICU admission and mortality. *Infection* **51**:1357–1367 DOI [10.1007/s15010-023-01996-y](https://doi.org/10.1007/s15010-023-01996-y).

**Kim W-J, Choi S-H, Park JY, Song JS, Chung J-W, Choi ST. 2022.** SARS-CoV-2 Omicron escapes mRNA vaccine booster-induced antibody neutralisation in patients

with autoimmune rheumatic diseases: an observational cohort study. *Annals of the Rheumatic Diseases* **81**:1585–1593 DOI [10.1136/ard-2022-222689](https://doi.org/10.1136/ard-2022-222689).

**Kirsten C, Kahre E, Blankenburg J, Schumm L, Haag L, Galow L, Unrath M, Czyborra P, Schneider J, Lück C, Dalpke AH, Berner R, Armann J.** 2022. Seroprevalence of SARS-CoV-2 in German secondary schools from 2020 to 2021: a longitudinal study. *Infection* **50**:1483–1490 DOI [10.1007/s15010-022-01824-9](https://doi.org/10.1007/s15010-022-01824-9).

**Kleynhans J, Tempia S, Wolter N, von Gottberg A, Bhiman JN, Buys A, Moyes J, McMorrow ML, Kahn K, Gómez-Olivé FX, Tollman S, Martinson NA, Wafawanaka F, Lebina L, Du Toit J, Jassat W, Neti M, Brauer M, Cohen C.** 2021. SARS-CoV-2 seroprevalence in a rural and urban household cohort during first and second waves of infections, South Africa, July 2020–March 2021. *Emerging Infectious Diseases* **27**:3020–3029 DOI [10.3201/eid2712.211465](https://doi.org/10.3201/eid2712.211465).

**Kleynhans J, Tempia S, Wolter N, Von Gottberg A, Bhiman JN, Buys A, Moyes J, McMorrow ML, Kahn K, Gómez-Olivé FX, Tollman S, Martinson NA, Wafawanaka F, Lebina L, Du Toit JD, Jassat W, Neti M, Brauer M, Cohen C.** 2022. SARS-CoV-2 seroprevalence after third wave of infections, South Africa. *Emerging Infectious Diseases* **28**:1055–1058 DOI [10.3201/eid2805.220278](https://doi.org/10.3201/eid2805.220278).

**Kohler C, King J, Stacker L, Goller KV, Moritz J, Pohlmann A, Nath N, Tzvetkova A, Rieck M, Paraskevopoulou S, Beslic D, Hölzer M, Fuchs S, Ziemann J, Kaderali L, Beer M, Hübner N-O, Becker K.** 2023. Neighbourhood watch: genomic epidemiology of SARS-CoV-2 variants circulating in a German federal state, Mecklenburg-Western Pomerania, in 2020–2022. *Emerging Microbes & Infections* **12**:2245916 DOI [10.1080/22221751.2023.2245916](https://doi.org/10.1080/22221751.2023.2245916).

**Leone V, Meisinger C, Temizel S, Kling E, Gerstlauer M, Fröhwald MC, Burkhardt K.** 2022. Longitudinal change in SARS-CoV-2 seroprevalence in 3-to 16-year-old children: the Augsburg Plus study. *PLOS ONE* **17**:e0272874 DOI [10.1371/journal.pone.0272874](https://doi.org/10.1371/journal.pone.0272874).

**Naeimi R, Sepidarkish M, Mollalo A, Parsa H, Mahjour S, Safarpour F, Almukhtar M, Mechaal A, Chemaitelly H, Sartip B, Marhoommirzabak E, Ardekani A, Hotez PJ, Gasser RB, Rostami A.** 2023. SARS-CoV-2 seroprevalence in children worldwide: a systematic review and meta-analysis. *eClinicalMedicine* **56**:101786 DOI [10.1016/j.eclim.2022.101786](https://doi.org/10.1016/j.eclim.2022.101786).

**Ott R, Achenbach P, Ewald DA, Friedl N, Gemulla G, Hubmann M, Kordonouri O, Löff A, Marquardt E, Sifft P, Sporreiter M, Zapardiel-Gonzalo J, Ziegler A-G.** 2022. SARS-CoV-2 seroprevalence in preschool and school-age children: population screening findings from January 2020 to June 2022. *Deutsches Ärzteblatt International* **119**:765–770 DOI [10.3238/arztebl.m2022.0355](https://doi.org/10.3238/arztebl.m2022.0355).

**R Core Team.** 2024. R: a language and environment for statistical computing. Version 4.4.1. Vienna: R Foundation for Statistical Computing. Available at <https://www.r-project.org>.

**Ratcliffe H, Tiley KS, Andrews N, Amirthalingam G, Vichos I, Morey E, Douglas NL, Marinou S, Plested E, Aley P, Galiza EP, Faust SN, Hughes S, Murray CS, Roderick M, Shackley F, Oddie SJ, Lees T, Turner DPJ, Raman M, Owens S, Turner**

**P, Cockerill H, Bernal JL, Linley E, Borrow R, Brown K, Ramsay ME, Voysey M, Snape MD. 2023.** Community seroprevalence of SARS-CoV-2 in children and adolescents in England, 2019–2021. *Archives of Disease in Childhood* **108**:123–130 DOI [10.1136/archdischild-2022-324375](https://doi.org/10.1136/archdischild-2022-324375).

**Robert Koch Institute. 2022.** RKI - Coronavirus SARS-CoV-2 - Situation report - 31 August 2022. Available at [https://www.rki.de/DE/Content/InfAZ/N/Neuartiges\\_Coronavirus/Situationsberichte/Aug\\_2022/2022-08-31-en.pdf](https://www.rki.de/DE/Content/InfAZ/N/Neuartiges_Coronavirus/Situationsberichte/Aug_2022/2022-08-31-en.pdf) (accessed on 29 September 2022).

**Sapronova K, Kake R, Pavāre J, Grāvele D, Šēla I, Ērgle E, Isarova D, Grīnberga Z, Zavadska D. 2023.** SARS-CoV-2 seroprevalence among children in Latvia: a cross-sectional study. *Medicine* **102**:e32795 DOI [10.1097/MD.00000000000032795](https://doi.org/10.1097/MD.00000000000032795).

**Sengler C, Eulert S, Minden K, Niewerth M, Horneff G, Kuemmerle-Deschner J, Siemer C, Berendes R, Girschick H, Hühn R, Borte M, Hospach A, Emminger W, Armann J, Klein A, Kallinich T. 2021.** Clinical manifestations and outcome of SARS-CoV-2 infections in children and adolescents with rheumatic musculoskeletal diseases: data from the National Paediatric Rheumatology Database in Germany. *RMD Open* **7**:e001687 DOI [10.1136/rmdopen-2021-001687](https://doi.org/10.1136/rmdopen-2021-001687).

**Shah AD, Bartlett JW, Carpenter J, Nicholas O, Hemingway H. 2014.** Comparison of random forest and parametric imputation models for imputing missing data using MICE: a CALIBER study. *American Journal of Epidemiology* **179**:764–774 DOI [10.1093/aje/kwt312](https://doi.org/10.1093/aje/kwt312).

**Smith H, Mahon A, Moss A, Rao S. 2023.** SARS-CoV-2 infection in children evaluated in an ambulatory setting during Delta and Omicron time periods. *Journal of Medical Virology* **95**:e28318 DOI [10.1002/jmv.28318](https://doi.org/10.1002/jmv.28318).

**Sorg A-L, Bergfeld L, Jank M, Corman V, Semmler I, Goertz A, Beyerlein A, Verjans E, Wagner N, Von Bernuth H, Lander F, Weil K, Hufnagel M, Spiekerkoetter U, Chao C-M, Naehrlich L, Muntau AC, Schulze-Sturm U, Hansen G, Wetzke M, Jung A-M, Niehues T, Fricke-Otto S, Von Both U, Huebner J, Behrends U, Liese JG, Schwerk C, Drosten C, Von Kries R, Schroten H. 2022.** Cross-sectional seroprevalence surveys of SARS-CoV-2 antibodies in children in Germany, June 2020 to May 2021. *Nature Communications* **13**:3128 DOI [10.1038/s41467-022-30482-6](https://doi.org/10.1038/s41467-022-30482-6).

**Springer DN, Perkmann T, Jani CM, Mucher P, Prüger K, Marculescu R, Reuberger E, Camp JV, Graninger M, Borsodi C, Deutsch J, Lammel O, Aberle SW, Puchhammer-Stöckl E, Haslacher H, Höltl E, Aberle JH, Stiasny K, Weseslindtner L. 2022.** Reduced sensitivity of commercial spike-specific antibody assays after primary infection with the SARS-CoV-2 Omicron variant. *Microbiology Spectrum* **10**:e02129-22 DOI [10.1128/spectrum.02129-22](https://doi.org/10.1128/spectrum.02129-22).

**Statistisches Bundesamt (Destatis). 2023.** Kreisfreie Städte und Landkreise nach Fläche, Bevölkerung und Bevölkerungsdichte am 31.12.2022. Available at <https://www.destatis.de/DE/Themen/Laender-Regionen/Regionales/Gemeindeverzeichnis/Administrativ/04-kreise.html> (accessed on 26 August 2024).

**Steensels D, Oris E, Coninx L, Nuyens D, Delforge M-L, Vermeersch P, Heylen L. 2020.**  
Hospital-wide SARS-CoV-2 antibody screening in 3056 staff in a tertiary center in Belgium. *JAMA* **324**:195–197 DOI [10.1001/jama.2020.11160](https://doi.org/10.1001/jama.2020.11160).

**Sumner MW, Xie J, Zemek R, Winston K, Freire G, Burstein B, Kam A, Emsley J, Gravel J, Porter R, Sabhaney V, Mater A, Salvadori MI, Berthelot S, Beer D, Poonai N, Moffatt A, Wright B, Freedman SB. 2023.** Comparison of symptoms associated with SARS-CoV-2 variants among children in Canada. *JAMA Network Open* **6**:e232328 DOI [10.1001/jamanetworkopen.2023.2328](https://doi.org/10.1001/jamanetworkopen.2023.2328).

**Tamminen K, Salminen M, Blazevic V. 2021.** Seroprevalence and SARS-CoV-2 cross-reactivity of endemic coronavirus OC43 and 229E antibodies in Finnish children and adults. *Clinical Immunology* **229**:108782 DOI [10.1016/j.clim.2021.108782](https://doi.org/10.1016/j.clim.2021.108782).

**Tay JK, Narasimhan B, Hastie T. 2023.** Elastic net regularization paths for all generalized linear models. *Journal of Statistical Software* **106**:1–31 DOI [10.18637/jss.v106.i01](https://doi.org/10.18637/jss.v106.i01).

**Uschner D, Bott M, Lagarde WH, Keating J, Tapp H, Berry AA, Seals AL, Munawar I, Schieffelin J, Yukich J, Santacatterina M, Gunaratne M, Fette LM, Burke B, Strylewicz G, Edelstein SL, Ahmed A, Miller K, Sanders JW, Herrington D, Weintraub WS, Runyon MS. 2022.** Breakthrough SARS-CoV-2 infections after vaccination in North Carolina. *Vaccines* **10**:1922 DOI [10.3390/vaccines10111922](https://doi.org/10.3390/vaccines10111922).

**Wachter F, Regensburger AP, Peter AS, Knieling F, Wagner AL, Simon D, Hoerning A, Woelfle J, Überla K, Neubert A, Rauh M. 2022.** Continuous monitoring of SARS-CoV-2 seroprevalence in children using residual blood samples from routine clinical chemistry. *Clinical Chemistry and Laboratory Medicine (CCLM)* **60**:941–951 DOI [10.1515/cclm-2022-0037](https://doi.org/10.1515/cclm-2022-0037).

**Whitaker M, Elliott J, Bodinier B, Barclay W, Ward H, Cooke G, Donnelly CA, Chadeau-Hyam M, Elliott P. 2022.** Variant-specific symptoms of COVID-19 in a study of 1,542,510 adults in England. *Nature Communications* **13**:6856 DOI [10.1038/s41467-022-34244-2](https://doi.org/10.1038/s41467-022-34244-2).

**Wong LSY, Loo EXL, Huang C-H, Yap GC, Tay MJY, Chua RXY, Kang AYH, Lu L, Lee BW, Shek LP-C, Zhang J, Chia WN, Wang L-F, Tham EH, Tambyah PA. 2023.** Early seasonal coronavirus seroconversion did not produce cross-protective SARS-CoV-2 antibodies. *Journal of Infection* **86**:e10–e12 DOI [10.1016/j.jinf.2022.08.032](https://doi.org/10.1016/j.jinf.2022.08.032).

**World Health Organization. 2024.** WHO Director-General's opening remarks at the media briefing on COVID-19-11 2020. Available at <https://www.who.int/director-general/speeches/detail/who-director-general-s-opening-remarks-at-the-media-briefing-on-covid-19---11-march-2020> (accessed on 26 August 2024).

**Zhu N, Zhang D, Wang W, Li X, Yang B, Song J, Zhao X, Huang B, Shi W, Lu R, Niu P, Zhan F, Ma X, Wang D, Xu W, Wu G, Gao GF, Tan W. 2020.** A novel coronavirus from patients with pneumonia in China, 2019. *New England Journal of Medicine* **382**:727–733 DOI [10.1056/NEJMoa2001017](https://doi.org/10.1056/NEJMoa2001017).