



# OPEN Neutralizing antibodies to SARS-CoV-2 variants of concern: a pediatric surveillance study

Douglas D. Fraser<sup>1,2,3,4✉</sup>, Devika Singh<sup>1</sup>, Enis Cela<sup>2,4</sup>, Maitray A. Patel<sup>5</sup>, Maysaa Assaf<sup>1</sup>, Maritza Quintero<sup>1</sup>, Michael Knauer<sup>6</sup>, Michael R. Miller<sup>1,4</sup>, Melina Bellini<sup>7</sup>, Angela Li<sup>7</sup>, Paul Hahn<sup>7</sup>, Marta Hrek<sup>7</sup> & Jorge Cruz-Aguado<sup>7</sup>

Knowledge regarding the pediatric immune response to SARS-CoV-2 infection and/or vaccination remains limited, particularly for the variants of concern (VOC). Our objective was to evaluate the neutralizing antibody response against SARS-CoV-2 VOC in the naturally infected and/or vaccinated pediatric population. Participants aged 5–12 years who presented to either an outpatient clinic or emergency room were eligible for participation in this study. Participants were divided into four groups based on infection and vaccination status. Plasma was tested using immunoassays targeting anti-SARS-CoV-2 IgG, spike protein, and nucleocapsid. A total of 619 participants met study inclusion. Natural infection was identified in 189/619 children (31%), 284/619 were vaccinated (46%) and 69/619 were both naturally infected and vaccinated (11%). Participants that were vaccinated had received one ( $n=169/619$ ; 27%) or two ( $n=115/619$ ; 19%) vaccine doses. The median time between the 1st and 2nd vaccine doses was 56 days, interquartile range 50–56. A general upward trend in antibody positivity was observed across all VOC as the study proceeded over a 5-month period. Omicron antibody responses were lower than those of other VOC, both in relation to the percentage of positive cases and over time. Neither asthma nor diabetes altered antibody responses, but antibody titres were reduced for a variety of VOC in those children receiving immunotherapy or with leukopenia. This study demonstrated decreased neutralizing antibody responses against the Omicron variant, regardless of past infection or vaccination status. These findings emphasize the need for continued neutralizing antibody surveillance.

SARS-CoV-2 ignited a global health crisis that spawned multiple waves of infection and necessitated the development of diagnostic tools, treatments, and vaccines. The emergence of variants of concern (VOC) undermines efforts to contain the virus when antibodies generated by prior infection or vaccination cannot neutralize emerging VOC. Antibodies resulting from SARS-CoV-2 infections exhibit varying durations, without a definitive threshold defining a protective correlate against reinfection<sup>1,2</sup>. Previous research primarily focused on evaluating testing approaches and the antibody responses in individuals during the acute infection phase and the immediate aftermath of infection<sup>3–5</sup>. Data from surveillance studies have demonstrated the persistence of nucleocapsid antibodies in adults from 6 to 12 months following infection<sup>6–10</sup>. However, limited data, especially concerning antibodies targeting VOC, were available for children<sup>11–17</sup>. Despite children's infrequent moderate or severe illness, they are susceptible to frequent infections<sup>18</sup>.

The COVID-19 vaccination was approved in Canada for children aged 5–11 years on November 19, 2021. While it had been previously shown that all vaccinated children had antibodies against the SARS-CoV-2 spike protein, compared to only half of unvaccinated children, the unique immune response in this pediatric cohort remains unclear<sup>19</sup>. Essential questions persist about the extent of immunity development against common variants, such as Alpha, Beta, Gamma, and Delta; the duration of such immune responses and whether these children will acquire immunity against newer circulating variants and emerging strains, such as Omicron. Variants holding mutations in the receptor binding domain (RBD) should be a main focus, given that this region is targeted by ~90% of the neutralizing antibody activity<sup>20</sup>. Health surveillance and early intervention strategies rely on antibody cross-reactivity to reduce future outbreaks. The emergence of SARS-CoV-2 VOC has raised

<sup>1</sup>Pediatrics, Western University, London, ON, Canada. <sup>2</sup>Physiology and Pharmacology, Western University, London, ON, Canada. <sup>3</sup>Clinical Neurological Sciences, Western University, London, ON, Canada. <sup>4</sup>Children's Health Research Institute, London, ON, Canada. <sup>5</sup>Epidemiology and Biostatistics, Western University, London, ON, Canada. <sup>6</sup>Pathology and Laboratory Medicine, Western University, London, ON, Canada. <sup>7</sup>Diagnostics Biochem Canada Inc, London, ON, Canada. ✉email: douglas.fraser@lhsc.on.ca

concerns about the effectiveness of neutralizing antibodies induced by SARS-CoV-2 vaccines<sup>21</sup>. In children, there were substantially lower neutralizing antibody titres against Omicron compared to the original, wild-type (WT) virus or Beta variant<sup>22</sup>. The emergence of vaccinations targeting new SARS-CoV-2 VOC demands that we understand pediatric antibody responses to natural infections and vaccinations.

The primary objective of this study was to measure the prevalence and quantity of neutralizing antibodies against SARS-CoV-2 VOC in plasma samples collected from children aged 5–12 years. Additional objectives included assessing the neutralizing antibody responses to SARS-CoV-2 VOC based on virus exposure, vaccination history, and primary medical service. To address the latter, participants were divided into four groups based on SARS-CoV-2 test results, the presence of antibodies to the nucleocapsid protein (NCP), and vaccination status.

## Methods

### Study participants

This study was conducted at the Children's Hospital, London Health Sciences Centre (London, Ontario, Canada) over a five-month period, from December 2021 to May 2022, following the vaccine rollout for children aged 5–11 years on November 19, 2021 (Pfizer-BioNTech COVID-19 10 mcg). Pediatric blood samples collected for clinical purposes and subsequently designated for discard by the hospital laboratory were screened. Samples obtained from children aged 5–12 years who had presented either to the emergency room or outpatient clinics were selected for the study. The blood had been collected using lithium heparin Plasma Separator Tubes. Plasma was isolated by centrifugation (3000 g, 7 min, 16 °C) and aliquoted using a Roche Cobas 8100 automated preanalytical instrument. All selected samples were frozen at –80 °C until further use, avoiding freeze/thaw cycles. Demographic (age and sex) and clinical data (reason for medical service, comorbidities, possible immunosuppression and white blood cell counts) were collected from the electronic medical records (PowerChart, Cerner Corporation). Leukopenia, neutropenia, and lymphopenia were defined as cell counts falling below the normal range established by the hospital laboratory standards. SARS-CoV-2 testing results, both polymerase chain reaction (PCR) and serological, and vaccination history were collected from provincial health administrative data repositories. These datasets were linked using encoded identifiers and analyzed at the Institute for Clinical Evaluative Sciences (ICES).

Based on ICES data, participants were sorted into the following groups: (1) no COVID-19 vaccination and no past identified SARS-CoV-2 infection, (2) no COVID-19 vaccination and a possible previous COVID-19 infection as determined by a past positive PCR or serological test, (3) previously vaccinated against COVID-19 and no past identified SARS-CoV-2 infection, and (4) previously vaccinated against COVID-19 and a possible previous COVID-19 infection as determined by a past positive PCR or serological test. Groups 3 and 4 were each further subdivided into two groups, 1–2V based on the number of vaccinations received (Fig. 1).

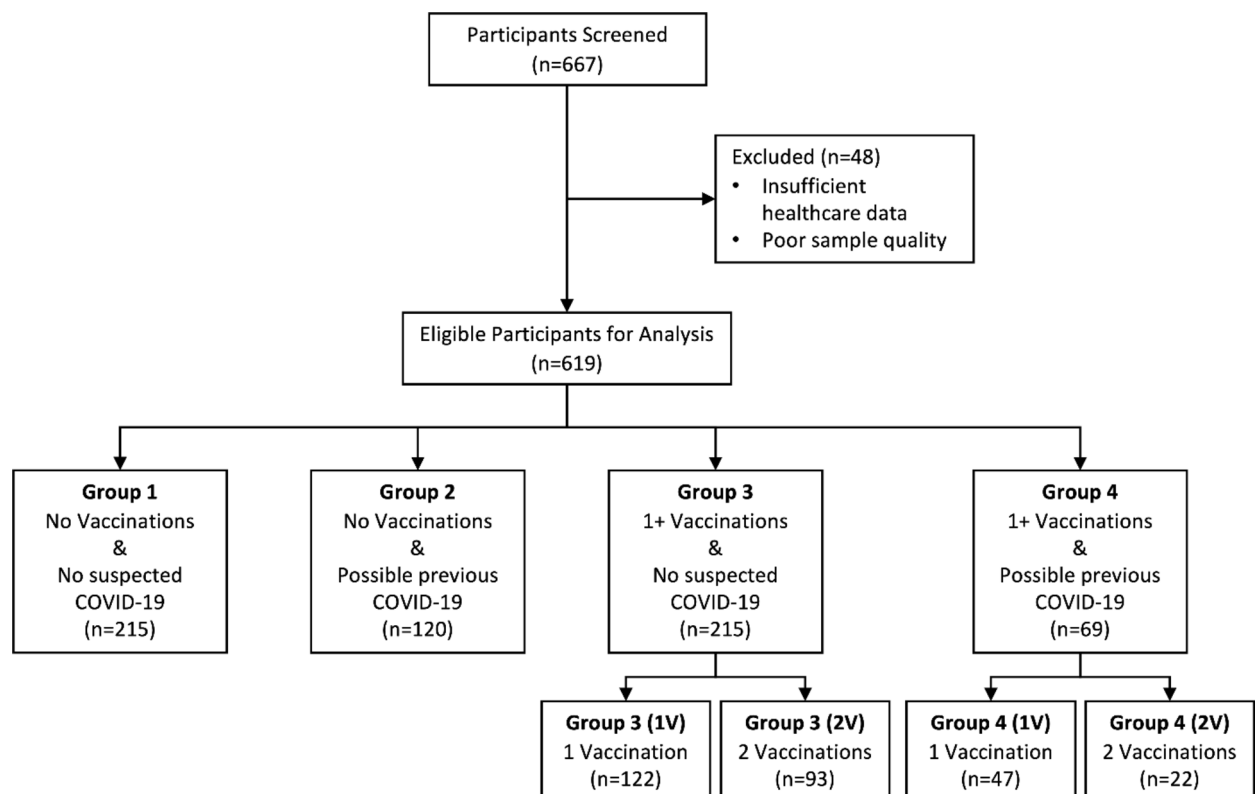
### Immunoassays

Anti-SARS-CoV-2 Immunoglobulin G (IgG) antibodies were detected with enzyme-linked immunosorbent assays (ELISAs). Antibodies against the RBD of the viral spike protein were detected using an ELISA kit produced by Diagnostics Biochem Canada (DBC) (DBC anti-SARS-CoV-2 ELISA DBC-IGG-19; London, Ontario, Canada). The DBC ELISA was designed against antigens formulated by recombinant proteins (aa 319–541) created from the wild type (WT) RBD sequence or representing the RBDs of prevalent VOC. Antigens were produced in mammalian protein expression systems available at the time of study commencement (Hek- and Cho-expressed proteins produce equivalent *in vitro* biological activity)<sup>23</sup> and RBD sequences were modified to incorporate the mutations characteristic of each VOC (Table 1). We previously showed that DBC ELISA kits detect neutralizing antibodies similarly to Genscript cPass SARS-CoV-2 Neutralization Antibody Kits<sup>24</sup>. Antibodies against the nucleocapsid protein (NCP) were detected with an ELISA kit manufactured by Euroimmun (anti-SARS-CoV-2 NCP ELISA(IgG), EI 2606–9601-2 G; Lübeck, Germany).

Immunoassays were performed per manufacturer instructions. The ratio of the optical density (OD) of the sample and the cut-off (CO), [Ratio = OD of sample/CO], was used to determine a binary 'positive' or 'negative' antibody result. For anti-spike protein assays the CO, [CO = (Mean of 3 Negative Control results) x factor 1.5], was used to interpret a Positive (Ratio ≥ 1.2), Negative (Ratio ≤ 1.0), or Borderline (Ratio between 1.0 and 1.2) result. In the anti-NCP assay, the ratio between the OD of the sample and the CO [CO = Negative Control] was interpreted as a Positive (Ratio ≥ 1.1), Negative (Ratio < 0.8), or Borderline (Ratio between 0.8 and 1.1) result. These ratios have limited utility when comparing medians across assays that rely on different antigens because of potential bias in antibody binding of the negative control. Hence, comparisons between variants were based on ODs. We have previously published additional details on this interpretation strategy<sup>24</sup>.

### Statistical analysis

Both prevalence (antibody positivity) and antibody quantity (OD) were used to compare groups. Prevalence was compared to categorical variables using a chi-square test (or Fisher's exact test when applicable) unless otherwise specified. Comparisons of antibody quantity were made using the Kruskal-Wallis test, followed by a Bonferroni corrected Many-To-One Dunn Test with WT Cho or WT Hek as controls (calculated using  $R = 4.3.1$ ,  $\text{PMCMRplus} = 1.9.7$ ). To address multiple comparisons, a Bonferroni correction was applied, and adjusted  $P$  values were calculated.  $P$  values at the 5% significance level were used to establish differences between variables. This analysis was calculated using Python = 3.10.0, SciPy = 1.11.2, and NumPy = 1.26.0.



**Fig. 1.** Participant Inclusion and Groups. A schematic illustrates the participant enrollment and division into 4 groups based on COVID-19 diagnosis and vaccination status. The classification “Possible previous COVID-19” refers to a positive result for the nucleocapsid antibody test or a positive PCR on a nasopharyngeal swab. The classification “No suspected COVID-19” refers to a negative nucleocapsid antibody test result with or without a negative PCR on a nasopharyngeal swab.

VOC name	Alpha	Beta	Gamma	Delta	Omicron
<i>Expression system</i>	Cho	Cho	Cho	Cho	Hek
<i>Lineage (PANGO)</i>	B.1.1.7	B.1.351/501Y.V2	P.1/B.1.1.28.1	B.1.617.2	B.1.1.529
<i>Mutations</i>	N501Y	K417N, E484K N501Y	K417T, E484K, N501Y	L452R, T478K	G339D, S371L, S373P, S375F, K417N, N440K, G446S, S477N, T478K, E484A, Q493R, G496S, Q498R, N501Y, Y505H

**Table 1.** Summary of the antigens used in this study. Note: All target spike protein. PANGO: Phylogenetic Assignment of Named Global Outbreak Lineages.

## Results

### Measurement of neutralizing antibodies

To establish the CO value for each VOC type, the antibody OD of each group and all participants were compared against WT (Supplemental Table 1). The difference from WT with a 95% confidence interval (CI) was determined by the shift in OD for each VOC within each group (Supplemental Table 2). Both Hek- and Cho-expressed WT proteins were similar (Fig. 2A; Supplemental Fig. 1).

## Study population

A total of 667 children were screened for inclusion in this study; 48 were excluded due to insufficient health care data or poor sample quality (Fig. 1). Subject age, sex, reason for medical service, comorbidities, possible immunosuppression, previous SARS-CoV-2 PCR and/or serological testing, and SARS-CoV-2 vaccination history are listed in Table 2. SARS-CoV-2 infection history is presented in Table 3. Of the 619 eligible participants (median age in years = 7, IQR 6–9), 344 (56%) were male. Groups 1 and 3 each had 215 participants eligible for analysis, Group 2 had 120 participants and Group 4 had 69 participants. Group 1 consisted of participants with absent or negative SARS-CoV-2 testing, absent NCP antibodies, and without a vaccination. SARS-CoV-2 swab tests and/or NCP antibody detection identified 189 participants that were considered positive for a previous SARS-CoV-2 infection (31%; Groups 2 and 4). A total of 284 participants were vaccinated (46%; Groups 3 and 4); 169 (27%) had received one vaccine dose and 115 (19%) had received two vaccine doses. The median time between 1st and 2nd vaccine doses was 56 days, IQR 50–56. None of the participants had received a third vaccination at the time of study.

## Prevalence (antibody positivity) of SARS-CoV-2 antibodies

A general upward trend in the prevalence of SARS-CoV-2 antibodies was observed over time, indicating an increase in antibody positivity for all VOCs as the study progressed over 5 months (Fig. 2A). Omicron and NCP (natural infection) antibody positivity were lower in comparison to the percentage of positive cases observed for the WT strain and other VOCs. The percentage of participants with antibody positivity, broken down by both VOC and Group, is shown in Fig. 2B and Supplemental Table 3. Groups 1 (No Vaccination, No Suspected COVID-19), 2 (No Vaccination, Possible Previous COVID-19), and 3 (1V; No Suspected COVID-19, 1 Vaccination), and 4 (1V; Possible Previous COVID-19, 1 Vaccination) all exhibited lower levels of antibody positivity for the Omicron compared to other VOCs. The associations between antibody positivity and participant Groups are illustrated in Fig. 2C. The non-vaccinated participant Groups 1 and 2, regardless of previous COVID-19 status, exhibited lower antibody positivity compared to vaccinated participant Groups 3 and 4.

## Quantity (OD) of SARS-CoV-2 antibodies

The quantity of antibodies to the Omicron variant was lower than that observed for the WT strain and all other VOCs when compared within participant groups (Fig. 3A and B; Supplemental Tables 1 and 2). The antibody quantity to the Omicron variant remained lower regardless of whether participants received one or two vaccinations. Regarding the other VOCs, the Beta antibody quantity was lower for participants in Groups 3(1V) and 3(2V), suggesting that previous COVID-19 plays a key role in optimal antibody production. The Gamma antibody quantity was also lower in participants from Groups 1 and 3(2V), further indicating that prior COVID-19 may contribute to enhanced antibody quantity.

## Prevalence (antibody positivity) and antibody quantity between medical services

Beta ( $P < 0.046$ ) and Omicron ( $P < 0.044$ ) antibody responses were significantly lower (Supplemental Table 4), which corresponded to the proportion of samples testing positive for antibodies in the Haematology/Oncology and Surgery services, respectively (Supplemental Table 5).

## Prevalence (antibody positivity) and chronic medical conditions and immunosuppression

Antibody positivity responses were analyzed in participants with two chronic conditions: asthma (Supplemental Table 6) and diabetes (Supplemental Table 7). In both conditions, antibody positivity responses to the WT strain and all VOCs were comparable to those observed in participants without chronic conditions. In contrast, participants undergoing active immunotherapy exhibited lower antibody positivity rates across all VOCs compared to those without immunosuppression ( $P < 0.05$ ; Table 4). Specifically, antibody positivity for various WT and VOCs was reduced in participants with leukopenia, lymphopenia, and/or neutropenia ( $P < 0.05$ ; Table 4). Steroid treatment did not significantly affect neutralizing antibody responses.

## Discussion

With the ongoing presence of SARS-CoV-2, understanding the dynamics of neutralizing antibody responses in children is of paramount importance, particularly considering emerging variants and the unique challenges posed by this age group. This study measured neutralizing antibodies against prevalent SARS-CoV-2 VOC in plasma samples from children aged 5–12 years, with the objective of elucidating antibody responses within distinct cohorts and against emerging variants. Our data suggest that a significant percentage of children do not demonstrate neutralizing antibodies against the Omicron variant.

A major advantage of this study was the use of ICES data, which accurately linked SARS-CoV-2 infections and vaccinations with all participants. According to our data, 31% of children had a SARS-CoV-2 infection and 46% were vaccinated. Children who were both naturally infected and vaccinated constituted 11% of the population. Of those vaccinated, 27% of children received one vaccination and 19% received two doses. Over a 5-month period, the study observed a general upward trend in neutralizing antibody responses across all VOC. Omicron antibody responses were lower than those of WT and other VOC, both in relation to the percentage of positive cases and over time. While two chronic conditions, asthma and diabetes, did not alter antibody responses, active immunotherapy and leukopenia resulted in blunted responses to some VOC. Taken together, these findings emphasize the need for continued surveillance and vaccine optimization.

Multiple pediatric studies have measured plasma antibodies to the spike and NCP antibodies<sup>8–10</sup>, but they did not examine the neutralizing ability of circulating antibodies to specific VOCs. High throughput mapping of antibody sequence to antigen specificity has been used in a pediatric population, but this study relied on patient

self-reporting for infection and vaccination data, and they examined only a limited number of pooled samples<sup>25</sup>. Our study examined a large cohort of children with accurate SARS-CoV-2 testing and vaccination data, together with individual antibody studies to elucidate responses with higher fidelity.

Vaccination plays a crucial role in generating a neutralizing immune response against SARS-CoV-2<sup>26</sup>. The safety and effectiveness of the Pfizer-BioNTech COVID-19 vaccination in children aged 5–11 years have been demonstrated, with no serious adverse events reported<sup>27</sup>. Children with SARS-CoV-2 infection (symptomatic or asymptomatic) developed neutralizing antibodies lasting longer than adults<sup>28</sup>. However, these antibodies gradually diminished in concentration over time, along with decreases in the levels of broad IgG antibodies. Notably, the levels of neutralizing antibodies post-infection were lower than those observed after vaccination. Among children aged 5–11 years, the mRNA vaccine demonstrated moderate effectiveness in protecting against infections caused by Omicron<sup>29</sup>. However, these vaccines were likely to be highly protective against severe COVID-19 cases requiring hospitalization. Pediatric and adult SARS-CoV-2 patients have also demonstrated alignment in protective neutralizing antibody responses<sup>30</sup>. There was an increase in symptomatic COVID-19 infections among non-hospitalized children over the past two years, particularly with the emergence of VOC<sup>31</sup>. Neutralizing antibodies in children remained strong for up to 16 months after infection, with no differences based on gender or symptoms. Children under 5 years old showed a quicker rise in antibody levels during infection and less decline over time compared to older individuals, suggesting a lower risk of SARS-CoV-2 reinfection and milder clinical disease in this age group<sup>11</sup>. However, it has also been suggested that children exhibit comparable antibody levels in response to SARS-CoV-2, irrespective of their age<sup>12</sup>. These findings emphasize the need for further research to better understand pediatric responses to emerging variants.

Numerous studies have reported elevated rates of symptomatic infection as a result of Omicron's heightened transmissibility and decreased vaccine efficacy<sup>32,33</sup>. Omicron, while more transmissible and capable of evading some vaccine-induced immunity, still allows first-generation vaccines to be effective against severe disease and death. Recent studies have shown that COVID-19 vaccines can still exhibit neutralizing potency against Omicron<sup>34</sup>. Immunized individuals have also demonstrated the ability to generate neutralizing antibodies against variants<sup>35</sup>. However, over time, vaccine effectiveness against Omicron decreases<sup>36</sup>. In comparison, infection with the Omicron BA.2.38 variant induced the production of antibodies that responded not only to Omicron BA.2.38 but also to other Omicron variants (Omicron BA.1-BA.5)<sup>37</sup>. Both children and adults infected with the ancestral strain of SARS-CoV-2 developed robust serological and T-cell memory responses persisting for at least 12 months<sup>38</sup>, suggesting an enduring immunological memory and partial cross-reactivity against the Omicron BA.1 variant.

The majority of people develop anti-SARS-CoV-2 antibodies through vaccination or infection<sup>39</sup>. However, less than half of people exhibited neutralizing activity against the currently circulating Omicron BA.5 subvariant. Notably, hybrid immunity obtained through booster vaccination and prior infection provided the highest level of neutralization capacity, even against Omicron. Neutralizing activity against Delta and Omicron variants was significantly lower compared to the WT strain, and did not improve even after a third vaccine dose<sup>40</sup>. In contrast, infection with SARS-CoV-2 provided higher levels of neutralization against these variants. These findings collectively emphasize the potential challenges of mounting an effective immune response against the Omicron variant.

Recent research has shed light on various aspects of pediatric immune responses to SARS-CoV-2. For instance, vaccination of children with inflammatory bowel disease was both immunogenic and safe<sup>41</sup>. However, the study also raised concerns about vaccine efficacy against Omicron<sup>42</sup>. This aligns with our findings of variations in antibody positivity between groups, emphasizing the importance of vaccination to bolster immunity in vulnerable populations. Additionally, when children with SARS-CoV-2 infection and neuroinflammatory diseases received CD20 monoclonal antibodies compared to other treatments, SARS-CoV-2 antibodies decreased<sup>43</sup>. The serologic responses to SARS-CoV-2 vaccination in children with a history of multisystem inflammatory syndrome revealed significant reductions in neutralizing titres to Omicron<sup>44</sup>.

This study adds to the growing body of research on the immune response in children to SARS-CoV-2 infection and vaccination. Together with previous research, our findings underscore the importance of continued surveillance, vaccination strategies, and tailored medical interventions to address the evolving landscape of SARS-CoV-2 variants in children. These insights are critical for public health decision-making and safeguarding children's well-being in the face of this global health challenge. Specifically, our study questions the efficacy of current vaccines against Omicron in pediatric populations; booster shots and variant-specific vaccines may become essential tools in protecting children from this highly transmissible variant.

While this study offers valuable insights into the immune response of children aged 5–12 years to SARS-CoV-2 variants of concern (VOCs), we acknowledge several limitations. First, our focus on known variants up to a specific point in time limits our ability to assess responses to newer emerging variants that may have arisen after the study period. Second, the reliance on plasma samples collected from specific pediatric populations in a single geographical location may restrict the generalizability of our findings to other regions or demographic groups. Third, the design of our surveillance study did not allow for the evaluation of the immune response timeline following acute infection, reporting primarily prevalence rates. Additionally, we utilized two different antigen expression systems based on availability; however, both Hek- and Cho-expressed proteins demonstrated equivalent *in vitro* biological activity, and both WT Hek and WT Cho proteins exhibited similar antibody profiles. Fourth, while we focused on measuring neutralizing antibodies—an essential component of immunity—other aspects of the immune response, such as cellular immunity, were not explored. Finally, while this study provides important data on antibody prevalence, it does not directly assess clinical outcomes such as cross-reactivity or immune protection against VOCs. Despite these limitations, our findings have important implications for public health interventions and surveillance strategies, highlighting the need for additional vaccination doses in immunocompromised populations.

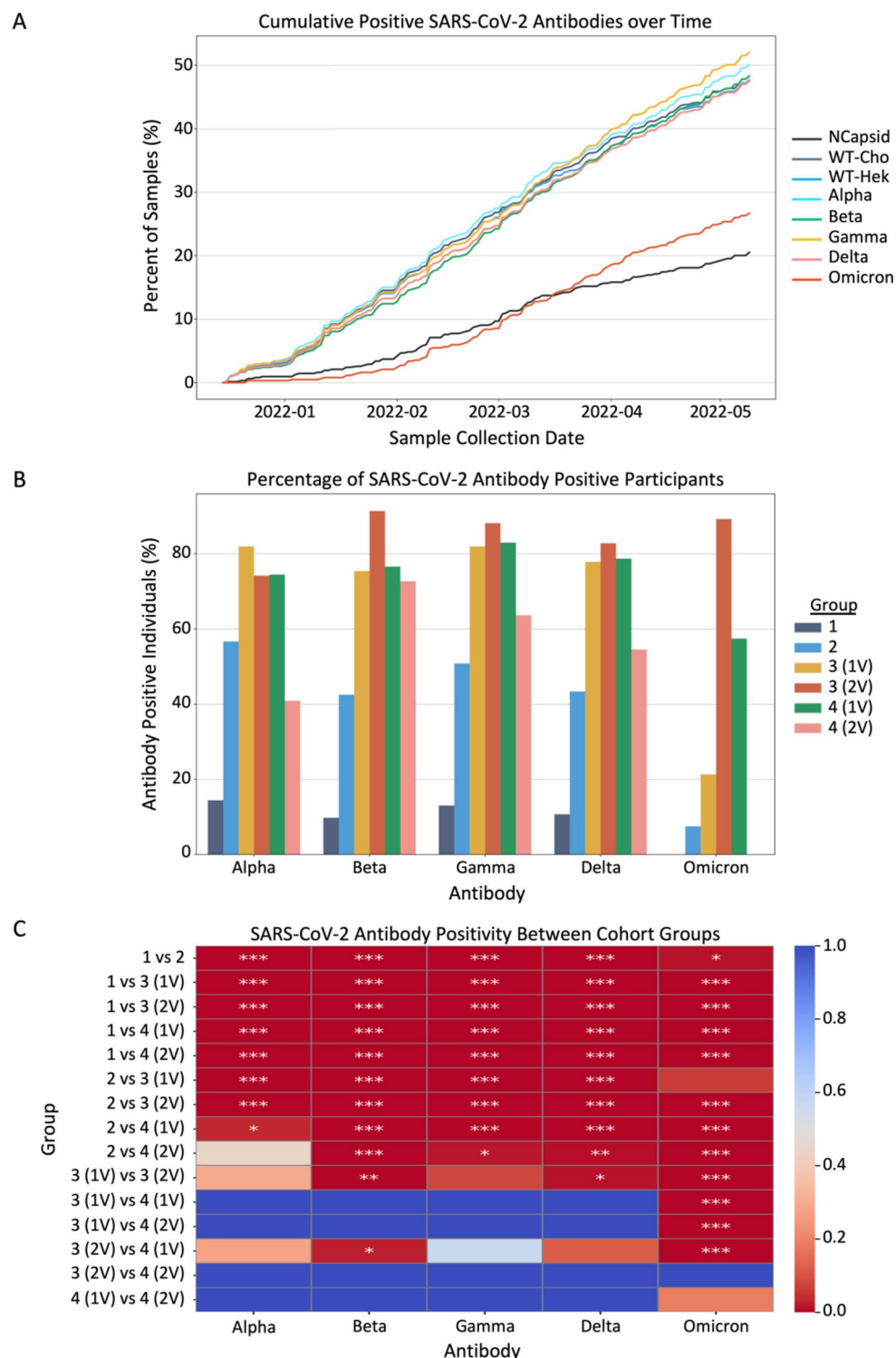


Variable	N = 619
Male sex, n (%)	344 (56)
Age (yrs), median (IQR)	7 (6–9)
<b>Age group, n (%)</b>	
5 years old	112 (18)
6 years old	97 (16)
7 years old	108 (17)
8 years old	97 (16)
9 years old	90 (15)
10 years old	105 (17)
11 years old	≤ 10 (≤ 1)
12 years old	≤ 5 (≤ 1)
<b>Medical service, n (%)</b>	
Emergency	232 (37)
Specialty service	174 (28)
Haematology/Oncology	108 (17)
Medicine	87 (14)
Surgery	18 (3)
<b>Comorbidities, n (%)</b>	
Asthma	117 (19)
Diabetes	13 (2)
<b>Possible Immuno-compromised, n (%)</b>	
Leukopenia <sup>1</sup>	86 (14)
Neutropenia <sup>1</sup>	35 (6)
Lymphopenia <sup>1</sup>	165 (28)
Active Immunotherapy	81 (13)
Steroid Medication	38 (6)
<b>COVID-19 vaccine doses before sample collection, n (%)</b>	
0 doses	335 (54)
1 dose	169 (27)
2 doses	115 (19)
<b>Days between 1st and 2nd dose, median (IQR)</b>	56 (50–56)

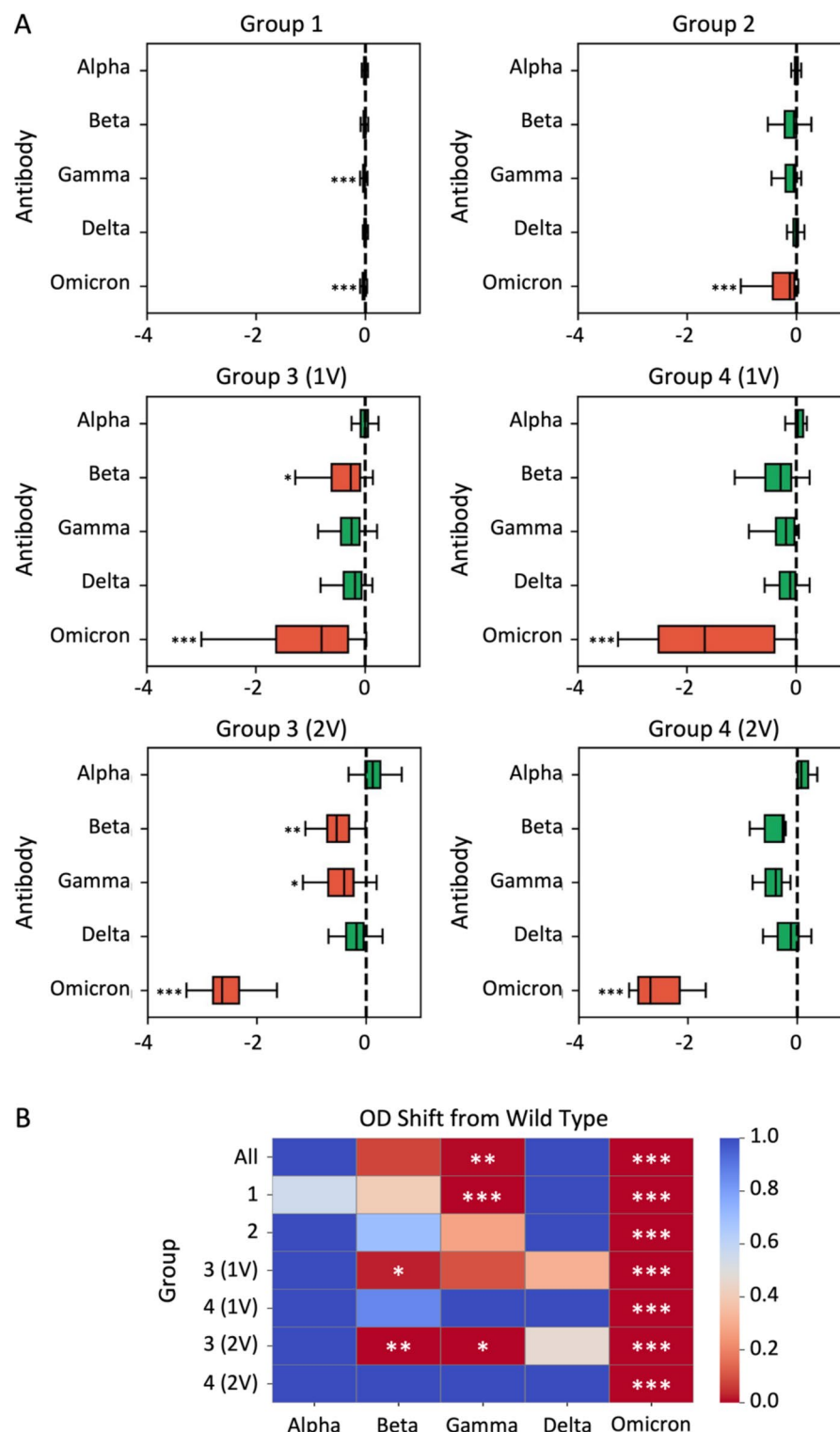
**Table 2.** Participant demographic and clinical data. Note: ≤ used to maintain subject privacy. <sup>1</sup>Variable % calculated excluding those with missing data. Leukopenia, neutropenia, and lymphopenia were defined as cell counts falling below the normal range established by the hospital laboratory standards.

Variable	No vaccines	1 Vaccine	2 Vaccines	P Value
Total Participants from Cohort, n (%)	335 (54.1)	169 (27.3) ***	115 (18.6) *** †††	<0.001
<b>Swab Test, n (%)</b>				<b>0.040</b>
Positive	49 (14.6)	26 (15.4)	8 (7.0) *	
Negative	188 (56.1)	119 (70.4)	83 (72.2)	
<b>Nucleocapsid Antibody, n (%)</b>				<b>0.006</b>
Positive	86 (25.7)	26 (15.4) *	17 (14.8) *	
Negative	249 (74.3)	143 (84.6)	98 (85.2)	
<b>Swab Test or Nucleocapsid, n (%)</b>				<b>0.002</b>
Positive	120 (35.8)	47 (27.8)	22 (19.1) **	
Negative	215 (64.2)	122 (72.2)	93 (80.9)	

**Table 3.** SARS-CoV-2 infection summary. Note: P Value represents the Chi-square test of independence comparing the difference in proportions between the No Vaccines, 1 Vaccine, and 2 Vaccine groups. Post-hoc pairwise Chi-square Bonferroni adjusted P Values are shown as <0.05 = \* or †, <0.01 = \*\* or ††, <0.001 = \*\*\* or †††. Comparisons to the No Vaccine group are marked with \*, while comparison between the 1 Vaccine and 2 Vaccine group are marked with †.



**Fig. 2. Antibody Prevalence in the Participant Cohort.** (A) A timeline depicting the collection date for all samples and the cumulative percentage of children with positive antibody testing. The cumulative percentage is calculated as the proportion of positive samples to date relative to all samples collected ( $n = 619$ ). The percentage of samples with positive antibodies across the sample collection period is similar for all variants except for Omicron and Nucleocapsid (NCapsid). (B) A bar plot demonstrates the percentage of antibody positive participants within their respective group, reflecting prevalence rates. The proportion of antibody positive participants with Omicron for Group 1 and Group 4 (2V) were concealed to maintain subject privacy. A visual representation of Supp. Table 3. (C) Heatmap showing pairwise comparisons of antibody positivity rates between cohort groups (Bonferroni-adjusted Chi-square test). P Value  $< 0.05 = *$ ,  $< 0.01 = **$ ,  $< 0.001 = ***$ .



**Fig. 3. Antibody Quantity (Optical Density Measurements) between Groups.** (A) A shift diagram comparing the antibody concentrations for the variants of concern as compared to the wild-type measurement. Alpha, Beta, Gamma, and Delta were compared to WT Cho measurements, while Omicron was compared to WT Hek (a comparison of WT Cho to WT Hek showed no significant differences). The bars represent the minimum and maximum differences, and red highlights the significantly different antibodies (Bonferroni adjusted  $P < 0.05$ ). Four groups are illustrated: Group 1 (No Vaccination, No suspected COVID-19 infection); Group 2 (No vaccination, Possible previous COVID-19 infection); Group 3 (1 vaccination (1V) or 2 vaccinations (2V), and No suspected COVID-19 infection); and Group 4 (1 vaccination (1V) or 2 vaccinations (2V), and Possible previous COVID-19 infection). (B) A heatmap depicting the variant antibody optical density measurement shift from their corresponding WT by subject groups.  $P$  Value  $< 0.05 = *$ ,  $< 0.01 = **$ ,  $< 0.001 = ***$ .



Antibody	Active immunotherapy	Steroid medication	Leukopenia	Lymphopenia	Neutropenia
WT (Cho cells)	0.063	0.530	0.147	<b>0.001</b>	0.925
WT (Hek cells)	<b>0.043</b>	0.737	0.195	<b>0.004</b>	0.435
Alpha	0.167	1.000	0.249	<b>0.018</b>	0.171
Beta	<b>0.006</b>	0.225	<b>0.011</b>	<b>0.000</b>	0.111
Gamma	<b>0.013</b>	0.230	0.071	0.054	0.064
Delta	<b>0.006</b>	0.151	<b>0.024</b>	<b>&lt;0.001</b>	0.280
Omicron	<b>0.001</b>	0.174	0.066	0.791	<b>0.020</b>
NCapsid	0.152	1.000	0.363	1.000	0.680

**Table 4.** Antibody positivity (prevalence) comparison by immunocompromised clinical variables. Note: Fisher Exact Test, followed by Bonferroni correction per clinical variable.

## Conclusion

In conclusion, this study highlights the reduced neutralizing antibody responses to the Omicron variant in pediatric participants, regardless of prior infection or vaccination status. While overall antibody positivity increased over the 5-month study period, responses to Omicron remained significantly lower compared to WT and other VOCs. The quantity of antibodies to Beta and Gamma were also lower in participants without previous COVID-19. Factors such as asthma and diabetes did not appear to significantly affect antibody responses; however, children undergoing immunotherapy or those with leukopenia exhibited notably lower antibody positivity. These findings underscore the importance of ongoing surveillance to assess neutralizing antibody responses, particularly in vulnerable populations, and inform future vaccination strategies. Additionally, the lower responses to Omicron suggest that alternative or enhanced strategies may be needed to improve immunity against this variant in the pediatric population. Future prospective studies with long-term follow-up are essential to evaluate the clinical implications regarding disease prevalence and severity.

## Data availability

The dataset from this study is held securely in coded form at Institute for Clinical Evaluative Sciences (ICES). While legal data sharing agreements between ICES and data providers (e.g., healthcare organizations and government) prohibit ICES from making the dataset publicly available, access may be granted to those who meet pre-specified criteria for confidential access, available at [www.ices.on.ca/DAS](http://www.ices.on.ca/DAS) (email: [das@ices.on.ca](mailto:das@ices.on.ca)). The full dataset creation plan and underlying analytic code are available from the authors upon request (DDE, corresponding author), understanding that the computer programs may rely upon coding templates or macros that are unique to ICES and are therefore either inaccessible or may require modification.

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## Author contributions

DDF and JC-A conceptualized and designed the study. DDF, DS, EC, MAP, MA, MK, MRM, MB, AL, PH, MH, and JC-A acquired, analyzed and interpreted data. DDF, DS, EC, MQ, and JC-A drafted the initial manuscript. All authors critically reviewed and revised the manuscript.

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## Declarations

### Competing interests

The authors declare no competing interests.

### Conflict of interest

MB, AL, PH, MH, and JC-A are employed by Diagnostics Biochem Canada Inc. (London, Ontario, Canada). The other authors have no conflicts of interest to disclose.

### Ethics approvals

This study was approved by the Western University, Health Sciences Research Ethics Board (HSREB Project ID: 120315), and by the Institute for Clinical Evaluative Sciences. All research was performed in accordance with relevant guidelines/regulations. Research involving human research participants must have been performed in accordance with the Declaration of Helsinki.

### Consent to participate

Informed consent was waived by the Western University, Health Sciences Research Ethics Board (HSREB Project ID: 120315) as the research used left-over blood samples, which otherwise would have been discarded, and it was deemed safe and low risk (not obtaining consent would not cause harm p to participants).

### Additional information

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**Correspondence** and requests for materials should be addressed to D.D.F.

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