The Journal of Infectious Diseases

BRIEF REPORT

Decline of Antibodies to Major Viral and Bacterial Respiratory Pathogens During the COVID-19 Pandemic

Natalie Lorenz,[1,2] Alex James,[3] Tiaan Van Rooyen,[1] Aimee Paterson,[1] Ciara Ramiah,[1] Lauren H. Carlton,[1,2] Prachi Sharma,[1] Michael G. Baker,[2,4] Richard Charlewood,[1,5] Reuben McGregor,[1,2] and Nicole J. Moreland[1,2]

[1]Department of Molecular Medicine and Pathology, Faculty of Medical and Health Sciences, The University of Auckland, Auckland, New Zealand; [2]Maurice Wilkins Centre, The University of Auckland, Auckland, New Zealand; [3]Te Pūnaha Matatini and School of Mathematics and Statistics, University of Canterbury, Christchurch, New Zealand; [4]Department of Public Health, University of Otago, Wellington, New Zealand; and [5]New Zealand Blood Service, Auckland, New Zealand

Surges in infections caused by respiratory pathogens have been documented in multiple settings after relaxation of pandemic restrictions. Antibodies to major antigens from respiratory syncytial virus and group A *Streptococcus* waned significantly in a longitudinal adult cohort throughout the pandemic. This waning may have contributed to the pathogen-surges that followed.

Keywords. SARS-CoV-2; group A *Streptococcus*; respiratory syncytial virus; antibody; waning.

A reduction in the incidence of respiratory transmitted infections was reported in many settings concurrent with coronavirus disease 2019 (COVID-19) restrictions during the pandemic [1]. This was followed by upsurges in these infections after restrictions were lifted, with marked increases in hospitalizations due to influenza, respiratory syncytial virus (RSV), and group A *Streptococcus* (GAS) observed in multiple countries [2, 3]. Factors leading to this altered epidemiology may have been driven by both the pathogen and the host, and a role for "immunity gaps" has been postulated. For non-vaccine-preventable diseases, this refers to a decline in population-level immunity resulting from a lack of pathogen exposure [4, 5]. Indeed, there is now emerging evidence that RSV antibody levels in both children and adults decreased during the pandemic, and this may have contributed to increased susceptibility [3, 4].

Aotearoa New Zealand followed an elimination strategy during the COVID-19 pandemic with stringent border controls in place for 2 years, which was combined with intermittent lock-downs and other non-pharmaceutical interventions. Elimination of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in New Zealand was also associated with an absence of community transmission of RSV and influenza. These viral pathogens only returned briefly during a 3-month period of quarantine-free travel with Australia in 2021 until the country reopened in 2022 [6]. New Zealand has a high incidence of serious GAS disease including invasive infections and acute rheumatic fever, both of which decreased dramatically during the pandemic. First-episode acute rheumatic fever hospitalization rates fell to the lowest rate for decades in 2022 (1.6/100 000), before increasing to prepandemic levels in 2023 (3.5/100 000) [7, 8]. Postpandemic surges in invasive GAS in New Zealand mirrored those in other settings, albeit somewhat delayed, occurring in 2023 rather than late 2022 as in other countries [9, 10]. Less severe respiratory infections followed similar trends with both community crowdsourced data and community surveillance of flu-like symptoms (fever and cough) showing reductions while the New Zealand border was closed [6, 11].

Understanding the drivers of postpandemic surges to globally important pathogens is critical to inform how these unintended consequences can be better managed in future pandemic responses. Consequently, this study aimed to explore the biological basis of immunity gaps to RSV, GAS, and endemic human coronaviruses (HCoV) by quantifying pathogen-specific antibodies in a longitudinal cohort of New Zealand adults between 2020 and 2023. There were no vaccines available for these viral and bacterial pathogens throughout the pandemic, and while the first RSV vaccines have now been approved, vaccines for GAS and HCoV remain an unmet need. Temporal antibody responses to candidate vaccine antigens from these pathogens were measured in a multiplex bead-based assay. Given the distinct epidemiological effects of the New Zealand elimination strategy, which provided an extended 2-year period of low pathogen exposure, this setting provides a unique view of immunity dynamics.

METHODS

Study Cohort

Some 330 eligible New Zealand Blood Service blood donors who regularly donated in Auckland over the study timeframe

Received 09 September 2024; editorial decision 05 December 2024; accepted 12 December 2024; published online 14 December 2024

Correspondence: Nicole J. Moreland, PhD, Department of Molecular Medicine and Pathology, Faculty of Medical and Health Sciences, The University of Auckland, Private Bag 92019, Auckland, New Zealand (n.moreland@auckland.ac.nz); Reuben McGregor, PhD, Department of Molecular Medicine and Pathology, Faculty of Medical and Health Sciences, The University of Auckland, Private Bag 92019, Auckland, New Zealand (reuben.mcgregor@auckland.ac.nz).

The Journal of Infectious Diseases® 2025;231:e77–81

https://doi.org/10.1093/infdis/jiae611

(March 2020–March 2023) were screened for inclusion, and 150 were selected based on Auckland demographics (2018 census data, https://www.stats.govt.nz). Preferentially included were all donors that identified as Māori, Pacific, Asian, or other ethnicity, followed by all female donors. Remaining donors were randomly selected using weighted sampling to age match the census data. Final demographics of the cohort are shown in Supplementary Table 1. To identify annualized samples, donations were date sorted and those closest to the 1 March each year were selected (Supplementary Figure 1). This study was assessed by the Health and Disability Ethics Committee, and additional consent beyond that provided at donation was not required.

Multipathogen Immunoassays

Antibody responses were measured in a bead-based, multiplex immunoassay. Recombinant HCoV-HKU1 spike (S1) Protein, HCoV-NL63 spike (S1) protein, RSV glycoprotein G, and RSV pre-fusion F protein (pre-F) were obtained (SinoBiological) and coupled to MagPlex microspheres using xMAP Antibody Coupling Kit (Luminex) according to manufacturer's instructions. GAS antigens (streptolysin-O [SLO], deoxyribonuclease B [DNaseB], *Streptococcus pyogenes* nuclease A [SpnA] and streptococcal pyrogenic exotoxin A [SpeA]) and SARS-CoV-2 spike were coupled as previously described [9, 12, 13]. The final 9-plex had high specificity (>87% inhibition with homologous antigens and <8% with heterologous antigens) (Supplementary Figure 2). The multipathogen immunoassay was performed as previously described [12, 13]. In brief, serum was diluted 1:1000 in phosphate-buffered saline (PBS), 1% (w/v) immunoglobulin G (IgG)-free bovine serum albumin (BSA) and incubated with the 9-plex antigen bead solution (45 beads/μL/antigen) for 35 minutes. Beads were washed in PBS, 1% BSA (BioTek 50TS magnetic plate washer; Agilent) and incubated with phycoerythrin-labelled donkey anti-human IgG antibody (RRID:AB_2340519; Jackson ImmunoResearch Labs) diluted 1:120 in PBS, 1% BSA for 35 minutes. All incubation steps were carried out at room temperature with 800 rpm agitation and protected from light. Beads were washed and resuspended in xMAP Sheath Fluid Plus (Luminex) prior to data acquisition (Luminex200 instrument; Diasorin). Background values from wells without serum were subtracted from the sample readouts to calculate the net median fluorescence intensity (MFI).

Statistical Analysis

Data was graphed and analyzed in R (version 4.4.1), R-Studio (version 2024.04.1 + 748) and Matlab 2021b. *P* values of <.01 were considered significant. Antibody responses across the time period were modelled using a mixed effect model on the logged response for each antigen separately. Year was included as a linear fixed effect and donor was included as a random effect:

$$log(Response)\ Year + (1 \vee Donor)$$

A squared term for year was not supported by the data. As year is a temporal variable there may be some autocorrelation effects that were not modelled. However, the relatively long period (approximately 1 year) between each measurement would make any autocorrelation effects negligible.

RESULTS

To assess antibody levels in healthy adults before, during, and after pandemic border closures, donations from 150 donors nearest to 1 March every year were selected (Supplementary Table 1 and Supplementary Figure 1). Major border closures in New Zealand commenced on 19 March 2020 [6], thus samples from early March 2020 represent baseline. Antibodies specific for antigens from RSV (pre-F and RSV-G), GAS (SLO, DNaseB, SpnA, and SpeA) and endemic HCoV (HKU1 and NL63) were quantified using a multiplex bead-panel on at least 3 donations from each donor (n = 581 donations).

Two complementary analysis approaches were undertaken: paired analyses that included donors with annualized samples present in every year of the study (n = 131), and mixed effect modelling that included all donors (n = 150). While marked heterogeneity in antibody levels was observed between donors (Supplementary Figure 3), significant reductions in antibody levels were observed between 2020 and 2023 in both analyses (Table 1 and Supplementary Figure 4). A repeated measures ANOVA of paired data showed significant differences in mean antibody levels for all 9 antigens across the study timeframe (2020–2023) (Table 1). Follow-up *t* tests confirmed a significant drop in mean antibody levels between before border closure (March 2020) and after reopening (March 2023) for all antigens except for HCoV HKU1, and GAS SpeA (Figure 1*A*). Similarly, the mixed effect model showed antibodies for all antigens except HKU1 had a significant drop over the study period (Table 1 and Supplementary Figure 4).

The average percentage reduction ranged from 6% to 16% in the paired analysis and from 10% to 20% in the model, with DNaseB having the largest percentage drop in both analyses. While antibody levels reduced significantly for most antigens, the relative level and magnitude of change varied widely amongst those reaching significance (Table 1). The largest reduction was seen with SLO (1125 MFI units) followed by RSV pre-F (774 MFI units), with the smallest reduction observed for the HCoV NL63 (24 MFI units). In general, antigens with lower antibody levels at baseline showed a lower magnitude drop.

Antibodies targeting SARS-CoV-2 spike were also quantified via the multiplex assay. In contrast to endemic respiratory pathogens, these showed a dramatic, highly significant increase between 2021 and 2023 (Figure 1*A* and Supplementary Figure 4).

Table 1. Statistics for Changes in Antibodies Against 9 Antigens From 2020 to 2023

	Paired Analysis (n = 131)						Complete Analysis (n = 150)		
	Raw MFI			ANOVA	Paired *t* Test		Mixed Effects Model		
Antigen	Geometric Mean 2020, MFI	Geometric Mean 2023, MFI	MFI Change	*P* Value Significance	Raw MFI Change, %	*P* Value Significance	Model Predicted MFI Change, %	*P* Value Significance	Model Coefficient
SLO	10 257	9132	−1125	***	−11	**	−14.1	***	−.0219
RSV-F	7746	6972	−774	***	−10	*	−11.4	***	−.0175
DNaseB	2527	2116	−411	***	−16	***	−19.7	***	−.0318
SpnA	2028	1763	−266	***	−13	*	−16.8	***	−.0266
RSV-G	533	470	−63	***	−12	*	−14.2	***	−.0221
NL63	187	162	−24	***	−13	*	−17.4	***	−.0277
HKU1	180	168	−11	**	−6	ns	−11.4	ns	−.0175
SpeA	80	73	−7	**	−9	ns	−10.3	***	−.0158
SARS-CoV-2 spike	14	9050	9036	***	65 684	***	2393	***	1.1264

Ordered by magnitude of change by net MFI. ANOVA was a repeated measures ANOVA. Mixed effects model changes using predictions from mixed effect model. Model coefficient is for the year variable. *$P < .01$, **$P < .001$, **$P < .0001$.

Abbreviations: DNaseB, deoxyribonuclease B; MFI, median fluorescence intensity; ns, not significant; RSV, respiratory syncytial virus; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SLO, streptolysin-O; SpeA, streptococcal pyrogenic exotoxin A; SpnA, *Streptococcus pyogenes* nuclease A.

Finally, correlation analysis between antigens from the same pathogen (Figure 1*B* and 1*C*) showed significant positive relationships. There was a strong correlation between RSV antibodies against pre-F and G proteins ($r = 0.52$), and for GAS antibodies against SLO, DNaseB, and SpnA ($r \geq 0.45$), with relationships across years evident within donors. Interestingly, anti-SpeA antibodies only weakly correlated with other GAS antigens, suggesting these antibodies are somewhat distinct (Figure 1*C*).

DISCUSSION

By combining multiplex bead-based immunoassay technology with a longitudinal cohort analysis this study showed that antibodies to multiple antigens from respiratory pathogens decreased concurrent with pandemic restrictions. In contrast, SARS-CoV-2 antibodies increased exponentially over the same time period in line with mass vaccine rollout followed by widespread Omicron infection once borders reopened. The highest magnitude of waning was observed for SLO, a putative vaccine candidate for GAS [12], followed by pre-F, the antigenic basis of RSV vaccines [14], reinforcing the importance of these highly immunogenic antigens. Divergent from GAS and RSV, the lower relative level and magnitude change for HCoV antibodies may reflect the milder nature and reduced force of HCoV infections compared with more severe pathogens [15].

The relationships observed between antibodies from the same pathogen emphasize the patterns of antibody waning observed. While the relative level of anti-RSV pre-F antibodies was higher than for protein G, as expected [14], the responses were highly correlative. Similarly for GAS, correlations between antibodies for SLO, DNaseB, and SpnA suggest waning to multiple antigens is occurring, which is opposite to previously observed multi-antigen infection responses [12]. SpeA is a superantigen that is variably carried by different GAS strain types [9]. The weaker correlations and lower relative level of SpeA antibodies compared with the other 3 GAS antigens likely reflects this variable carriage. In keeping, a smaller proportion of the GAS *emm*1 lineage known to express high levels of SpeA ($M1_{UK}$) was observed in New Zealand prepandemic compared to other settings [2, 9].

Taken together, the overall reduction in antibodies to multiple RSV and GAS antigens supports the hypothesis that reduced pathogen circulation during the pandemic was associated with a decline in pathogen-specific antibodies in healthy adults. It suggests that the cycle of increased immunity from new infections, followed by waning antibody levels, was disrupted during the 2 years of border closure in New Zealand such that, on average, only waning has been observed. This is consistent with a recent modelling study, which found that alterations in infectious disease dynamics and pathogen exposure can impact population immunity [5].

A limitation of this study is that the cohort is restricted to healthy adults, and serological data on infants, children, and older people who experienced the highest burden of serious disease in postpandemic surges in New Zealand are lacking. However, as healthy adults are integral to transmission chains, it is possible that antibody waning in this group contributed to the increased infections observed following border reopening. Further studies to ascertain the time frame over which antibody levels return to prepandemic levels and the association between serological responses and the endemic pathogen equilibrium are warranted.

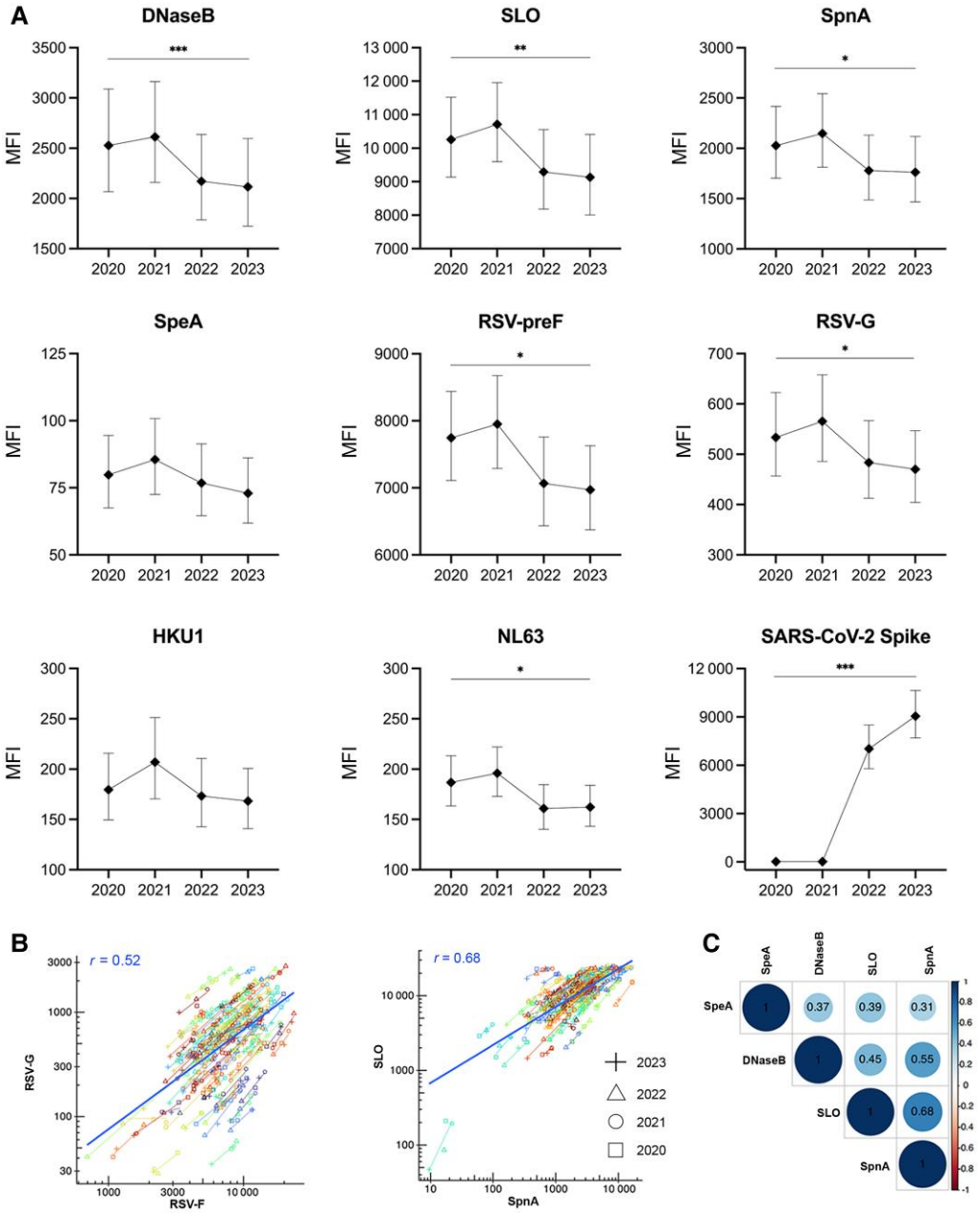

Figure 1. Multipathogen antibody analysis in a longitudinal cohort. *A*, Geometric mean and 95% confidence intervals annually across the 3-year study period; paired *t* test, n = 131. *B*, Dot plot correlating the 2 RSV antigens (pre-F and protein G) and 2 of the GAS antigens (SLO and DNaseB). Points colored by individual donors, with data from different years represented by symbols: 2020, square; 2021, circle; 2022, triangle; and 2023, cross. All lines are linear regression representations. Thick blue lines relates to linear regression of all data, with individual-colored lines relating to linear regression of each donor across the 4 years of the study. Blue text is the Spearman correlation coefficient, *r*. *C*, Spearman correlation matrix comparing 4 GAS antigens. Color, size, and text indicate Spearman correlation coefficient with positive correlations in blue. All correlations were significant $P < .01$, with analysis based on n = 131 donors included in the paired analysis. $^{*}P < .01$, $^{**}P < .001$, $^{***}P < .0001$. Abbreviations: DNaseB, deoxyribonuclease B; GAS, group A *Streptococcus*; MFI, median fluorescence intensity; RSV, respiratory syncytial virus; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SLO, streptolysin-O; SpeA, streptococcal pyrogenic exotoxin A; SpnA, *Streptococcus pyogenes* nuclease A.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online (http://jid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

Acknowledgments. We thank Professor Thomas Proft for the expression clone for SpeA; and Dr Alana Whitcombe for

previous purification of SLO, DNaseB, and SpnA antigens, both from the University of Auckland. We are also grateful to the team at New Zealand Blood Service's Auckland Donation Accreditation Laboratory for their work in retrieving the archived donor samples.

Financial support. This work was supported by the Maurice Wilkins Centre for Biodiscovery (grant number 3730361). T. V. R. was supported by the University of Auckland, Research Masters Scholarship.

Potential conflicts of interest. All authors: No reported conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. Bender RG, Sirota SB, Swetschinski LR, et al. Global, regional, and national incidence and mortality burden of non-COVID-19 lower respiratory infections and aetiologies, 1990–2021: a systematic analysis from the global burden of disease study 2021. Lancet Infect Dis **2024**; 24:974–1002.
2. Osowicki J, Nizet V. Malice in chains. J Infect Dis **2023**; 227:1117–8.
3. Jiang W, Xu L, Wang Y, Hao C. Exploring immunity debt: dynamic alterations in RSV antibody levels in children under 5 years during the COVID-19 pandemic. J Infect **2024**; 88:53–6.
4. den Hartog G, van Kasteren PB, Schepp RM, Teirlinck AC, van der Klis FRM, van Binnendijk RS. Decline of RSV-specific antibodies during the COVID-19 pandemic. Lancet Infect Dis **2023**; 23:23–5.
5. Munro APS, House T. Cycles of susceptibility: immunity debt explains altered infectious disease dynamics post-pandemic [published online ahead of print 11 October 2024]. Clin Infect Dis doi:10.1093/cid/ciae493.
6. Huang QS, Turner N, Wood T, et al. Impact of the COVID-19 related border restrictions on influenza and other common respiratory viral infections in New Zealand. Influ Other Respir Viruses **2024**; 18:e13247.
7. Health New Zealand. Reducing rheumatic fever, **2024**. https://www.tewhatuora.govt.nz/for-health-professionals/clinical-guidance/diseases-and-conditions/rheumatic-fever-guidance/reducing-rheumatic-fever/#first-episode-rheumatic-fever-hospitalisations-annual-rate-per-100000-new-zealand-2006-2023. Accessed 23 July 2024.
8. Bennett J, Zhang J, Leung W, et al. Prevalence of acute rheumatic fever and rheumatic heart disease among ethnic groups, New Zealand, 2000–2018. Emerg Infect Dis **2021**; 27:36–46.
9. Vesty A, Ren X, Sharma P, et al. The emergence and impact of the M1UK lineage on invasive group A *Streptococcus* disease in Aotearoa New Zealand. Open Forum Infect Dis **2024**; 11:ofae457.
10. Ammar S, Anglemyer A, Bennett J, et al. Post-pandemic increase in invasive group A strep infections in New Zealand. J Infect Public Heal **2024**; 17:102545.
11. FluTracking. https://info.flutracking.net. Accessed 23 July 2024.
12. Whitcombe AL, McGregor R, Bennett J, et al. Increased breadth of group A *Streptococcus* antibody responses in children with acute rheumatic fever compared to precursor pharyngitis and skin infections. J Infect Dis **2022**; 226: 167–76.
13. Whitcombe AL, McGregor R, Craigie A, et al. Comprehensive analysis of SARS-CoV-2 antibody dynamics in New Zealand. Clin Transl Immunol **2021**; 10:e1261.
14. Capella C, Chaiwatpongsakorn S, Gorrell E, et al. Prefusion F, Postfusion F, G antibodies, and disease severity in infants and young children with acute respiratory syncytial virus infection. J Infect Dis **2017**; 216:1398–406.
15. De Thoisy A, Woudenberg T, Pelleau S, et al. Seroepidemiology of the seasonal human coronaviruses NL63, 229E, OC43 and HKU1 in France. Open Forum Infect Dis **2023**; 10:ofad340.