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Virology and immune dynamics reveal high household transmission of ancestral SARS-CoV-2 strain

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Abstract

Background: Household studies are crucial for understanding the transmission of SARS-CoV-2 infection, which may be underestimated from PCR testing of respiratory samples alone. We aim to combine the assessment of household mitigation measures; nasopharyngeal, saliva, and stool PCR testing; along with mucosal and systemic SARS-CoV-2–specific antibodies, to comprehensively characterize SARS-CoV-2 infection and transmission in households.

Methods: Between March and September 2020, we obtained samples from 92 participants in 26 households in Melbourne, Australia, in a 4-week period following the onset of infection with ancestral SARS-CoV-2 variants.

Results: The secondary attack rate was 36% (24/66) when using nasopharyngeal swab (NPS) PCR positivity alone. However, when respiratory and nonrespiratory samples

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were combined with antibody responses in blood and saliva, the secondary attack rate was 76% (50/66). SARS-CoV-2 viral load of the index case and household isolation measures were key factors that determine secondary transmission. In 27% (7/26) of households, all family members tested positive by NPS for SARS-CoV-2 and were characterized by lower respiratory Ct values than low transmission families (Median 22.62 vs. 32.91; IQR 17.06–28.67 vs. 30.37–34.24). High transmission families were associated with enhanced plasma antibody responses to multiple SARS-CoV-2 antigens and the presence of neutralizing antibodies. Three distinguishing saliva SARS-CoV-2 antibody features were identified according to age (IgA1 to Spike 1, IgA1 to nucleocapsid protein (NP)), suggesting that adults and children generate distinct mucosal antibody responses during the acute phase of infection.

Conclusion: Utilizing respiratory and nonrespiratory PCR testing, along with the measurement of SARS-CoV-2–specific local and systemic antibodies, provides a more accurate assessment of infection within households and highlights some of the immunological differences in response between children and adults.

KEYWORDS
children, COVID-19, immunology, novel coronavirus, SARS-CoV-2, household transmission

1 | INTRODUCTION

The COVID-19 pandemic has uniformly identified households as the highest risk setting for SARS-CoV-2 transmission,[1] even when community transmission is reduced.[2–4] Occupants of a household face higher risk through sharing a closed space, being in close contact without personal protective equipment, and potential crowding.[2,5] Numerous household transmission studies have identified factors that contribute to higher secondary attack rates, including a symptomatic index case, spouses compared with other household members, and that adults are more likely to transmit than children.[4,6]

Transmission dynamics vary within households for reasons that are still not well understood. Clustering of infection in the household can occur, where transmission is characterized by higher secondary transmission rates, whilst in other households, there may be no transmission.[4] SARS-CoV-2 is transmitted primarily by exposure to respiratory fluids when individuals cough or breathe, through contact and droplet or airborne transmission.[7,8] Individuals who are symptomatic often have higher nasopharyngeal viral RNA concentrations early in the course of symptomatic infection.[9] In addition to respiratory fluid, SARS-CoV-2 has been detected in other biological samples, such as saliva, stool, and urine.[10,11] Prolonged excretion has been shown to occur following negative respiratory viral testing.[12] These factors may account for higher transmission in household settings and testing from multiple sample types may improve sensitivity in the detection of transmission routes.

Understanding the host immune responses to SARS-CoV-2 in controlling the infection is important in determining susceptibility. The immune responses to SARS-CoV-2 differ with age; children are less likely to experience the severe disease as compared to adults, and both children and adults can mount an immune response to SARS-CoV-2 without virological confirmation of infection.[13,14] Immune differences and endothelial/clotting function are proposed hypotheses for the age-related severity of COVID-19.[15] Emerging variants of concern (VOC) may induce different immune responses and cause varying severity of the disease.

Most transmission studies have relied on SARS-CoV-2 PCR testing of nasopharyngeal swabs (NPS) and symptoms in contacts to describe secondary infection and clinical attack rates.[4] However, the timing of NPS, host viral load, and swab collection quality may miss the pervasive nature of the infection and underestimate transmission routes. Higher density analyses of multiple biological specimens at numerous timepoints, together with the antibody-mediated immune response following COVID-19, may provide a more comprehensive profile of SARS-CoV-2 transmission. In this study, we describe the extent of SARS-CoV-2 infection and host immune responses behind transmission dynamics with ancestral SARS-CoV-2 in households.

Key Message

When respiratory and nonrespiratory samples were combined with antibody responses in blood and saliva, a much higher secondary attack rate of SARS-CoV-2 in households was identified. Lower viral load and mitigation measures reduced transmission. Saliva and serum antibody analyses show differences in immune responses between adults and children.

2 | METHODS

2.1 | Study design

This study was aligned with the Australian FFX study, which ran concurrent to this project and is aligned to the WHO First Few X Protocol.[16,17] The Australian FFX study was led by the Doherty Institute, The University of Melbourne.[18] Families were invited to join either, or both studies at the time of first contact, for more intensive biosampling and follow-up. This study was approved by the Royal Children's Hospital Research and Ethics Committee (#63666 and #63101).

Suspected SARS-CoV-2 cases and close contacts were tested by PCR of nasopharyngeal swabs (NPS) at The RCH from March 2020 to September 2020. These dates correspond to the first two epidemiological peaks of SARS-CoV-2 in Melbourne, Australia. Confirmed cases and their household members were recruited if all household members consented to participate.

2.2 | Clinical data and sample collection

Daily symptoms and household isolation measures (e.g., mask use, household separation) were recorded in a standardized diary and disease severity was classified according to WHO criteria.[19] Serial samples of saliva, NPS, and stool were self-collected by all family members, every week for 1 month following the date of the first positive swab of the index case. Blood samples were collected approximately at baseline and 28 days after onset of infection. Data were compiled for each participant, for SARS-CoV-2 (positive/negative), case (index/secondary), symptoms (symptomatic/asymptomatic), stool (positive/negative), saliva (positive/negative), salivary antibodies (positive/negative) and serology (positive/negative) (Table S1). The household secondary attack rate was calculated as the total number of secondary cases over the total number of household contacts.

2.3 | Viral identification

NPS were processed by the lab and their extraction was processed using the automated MagNA Pure system (Roche). The majority of samples were tested with the LightMix® Modular SARS and Wuhan CoV E-gene kit (targeting the E-gene; TIB Molbiol) for the SARS-CoV-2 PCR. Some were tested using the AusDiagnostics Respiratory Pathogens 16-well assay (Mascot, Australia), on the AusDiagnostics High-Plex 24 system (the SARS-CoV-2 target of this assay is the ORF-1 gene). Respiratory panel testing was done by Roche LightCycler 480 Instrument II viral panel.[20]

Viral RNA was manually extracted from 140 µl of NPS, saliva, and 140 µl of 20% (w/v) fecal suspension[21] and then eluted in 60 µl sterile, molecular-grade water (Life Technologies), using the QIAamp viral RNA kit (QIAGEN, Hilden) according to the manufacturer's instructions. The Centers for Disease Control and Prevention (CDC) developed a real-time reverse transcription PCR panel targeting nucleocapsid protein genes, N1 and N2.[22] CDC's validated platform was selected for saliva and stool analyses. SARS-CoV-2 standard (Exact Diagnostic, USA) was used as the standard curve in each assay to determine viral load.

Whole-genome sequencing was conducted on a subset of 12 participants from seven households. Briefly, viral RNA from saliva or stool (extracted as described above) was amplified using the ARTIC version three primers and published protocols and subjected to Illumina sequencing as previously described.[23] Following quality trimming, reads were aligned to the reference genome (Wuhan Hu-1; GenBank MN908947.3) and consensus sequences generated utilizing Geneious Prime. Samples were classified into the recognized SARS-CoV-2 lineages using Pangolin.[24]

2.4 | Salivary antibodies

Parents self-collected saliva in a 50 ml conical Falcon tube. Children were provided a SalivaBio swab and Salimetrcs swab-storage tube. Children produced between 0.1–1 ml of saliva from the swab, and parents provided on average 2 ml. After centrifugation, saliva samples were aliquoted and stored at −80 °C until analysis. Immuno MaxiSorp 96-well ELISA plates (Thermo Fisher Scientific, USA) were coated overnight at 4 °C with 2 µg/ml recombinant SARS-CoV-2/2019-nCoV S1 protein (Sino Biologicals) diluted in PBS. Wells were blocked with 10% skim milk in PBST (PBS + 0.1% Tween 20) at room temperature for 1 h. Two-fold serial dilutions of saliva samples in PBST were transferred to the ELISA plates (in duplicate) and incubated at room temperature for 1 h. Saliva from an asymptomatic individual confirmed negative for SARS-CoV-2 by clinical testing was used as a negative control. Saliva from a convalescent individual recently infected with SARS-CoV-2 was used as a positive control and pre-COVID saliva samples were used as negative controls. Antibody binding was detected with anti-human secretory IgA (sIgA, 1:10,000; Merck; followed by 1 h incubation with biotinylated anti-mouse IgG detection antibody, 1:1000; Southern Biotech) and biotinylated IgG (1:10,000; Assay Matrix) for 1 h at room temperature, then Streptavidin-HRP (1:5000; Life Technologies) in PBST for 45 min at room temperature. The color was developed with TMB solution (Sigma-Aldrich) and H_2O_2 with the reaction stopped using 2 M H_2SO_4. Absorbance at 450 nm was read on a microplate reader and used to calculate end point titres of samples. Cut-off values for each antibody class were defined as two standard deviations above the maximum titer from the corresponding negative controls.

2.5 | Serological immunity

2.5.1 | Plasma S1 and RBD ELISA

The ELISA method used to measure IgG, IgM, and IgA levels to SARS-COV-2 S1 and RBD protein was based on the Mount Sinai

Laboratory method previously described. Briefly, 96-well high-binding plates were coated with receptor-binding domain (RBD) or S1 (Sino Biological) antigen diluted in PBS at 2 μg/ml. Serum samples were first screened with RBD antigen, and potential seropositive samples were then confirmed with S1 antigen. Goat anti-human IgG-horseradish peroxidase (HRP) conjugated secondary antibody (1:10,000) was used, and the plates were developed using 3.3′, 5.5′-tetramethylbenzidine substrate solution. Seropositive samples were titrated and calculated based on a World Health Organization (WHO) SARS-CoV-2 pooled serum standard (National Institute of Biological Standards and Controls). Results were reported in International Units/mL. The cut-off for seropositivity was 8.36 IU/ml based on prepandemic samples, while seronegative samples were given half of the seropositive cut-off value.

2.5.2 | Coronavirus antibody multiplex assay (blood and saliva)

A novel coronavirus multiplex bead array was designed as previously described[25,26] consisting of SARS-CoV-2 spike 1 (Sino Biological), spike 2 (ACRO Biosystems), spike trimer (kind gift from Adam Wheatley), RBD (BEI Resources) and nucleoprotein (ACRO Biosystems). Tetanus toxoid (Sigma-Aldrich), influenza hemagglutinin (H1Cal2009; Sino Biological), and SIV gp120 (Sino Biological) were also included in the assay as positive and negative controls, respectively. Antigens were covalently coupled to magnetic carboxylated beads (Bio Rad) using a two-step carbodiimide reaction and blocked with 0.1% BSA, before being resuspended and stored in PBS 0.05% sodium azide till use.

The antigen-coupled beads were combined to form a coronavirus multiplex bead cocktail to investigate serological signatures from plasma and saliva samples. Briefly, 20 μl of working bead mixture (1000 beads per bead region) and 20 μl of diluted plasma (final dilution 1:200) or 20 μl of diluted saliva (final dilution 1:50) were added per well in 384 well plates and incubated overnight at 4°C on a shaker. Fourteen different Fc detectors were used to assess coronavirus-specific antibodies as previously described[26] including phycoerythrin (PE)-conjugated mouse anti-human pan-IgG, IgG1-4, and IgA1-2 (Southern Biotech; 1.3 μg/ml, 25 μl/well). IgM (biotinylated mouse anti-human IgM (mab MT22; Mabtech; 1.3 μg/ml, 25 μl/well), C1q protein (MP Biomedicals) and FcγR dimers (higher affinity polymorphisms FcγRIIa-H131, lower affinity polymorphisms FcγRIIa-R131, FcγRIIb, higher affinity polymorphisms FcγRIIIa-V158, lower affinity polymorphisms FcγRIIIa-F158; 1.3 μg/ml, 25 μl/well; kind gifts from Bruce Wines and Mark Hogarth[27] were first added to the beads, washed, and followed by the addition of PE-conjugated streptavidin (1.3 μg/ml, 25 μlμ/well). Assays were read on a Flexmap 3D with x-PONENT 4.2 software and performed in duplicate. Antibody levels are reported as median fluorescent intensity (MFI) of the PE signal associated with each bead. Pre-SARS-CoV-2 pandemic samples were used as controls in a multiplex assay. SARS-CoV-2 plasma antibodies (positive/negative) and salivary antibodies (positive/negative) cut-off thresholds were determined by calculating the average plus two standard deviations of respective prepandemic control data.

2.6 | Systems serology analysis

To holistically examine the spectrum of antibody signatures obtained via the above-mentioned SARS-CoV-2 multiplex array, multivariate analysis techniques were utilized using MATLAB version 9.6 (including machine learning and statistical toolbox) (The MathWorks, Inc.) and Eigenvector PLS toolbox (Eigenvector). Heatmaps were generated using Morpheus (https://software.broadinstitute.org/morpheus). Prism GraphPad version 9.0.2 (GraphPad Software) was used to illustrate final figures and to conduct any univariate analysis. For univariate analysis p value of .05 was set as the level for statistical significance, unless otherwise stated.

2.7 | Data normalization

For all multivariate analyses, positive antigens were removed (Tetanus and H1Cal2009). If any antibody feature included negative values, right-shifting was performed (by adding the minimum value for each respective feature back to all samples). Data were then log-transformed to ensure that the majority of features were normally distributed, by using the equation $\log_{10}(x+1)$. Values were subsequently normalized by mean centering and variance scaling by calculating respective z-scores.

2.8 | LASSO and PCA

To determine the minimal number of antibody features that distinguish between different groups, a least absolute shrinkage and selection operator (LASSO) feature reduction method was employed as previously described.[28] Cross-validation was performed iteratively (repeated 1000 times; 10-fold cross-validation) to identify the optimal value of the regularized parameters. Unsupervised principal component analysis (PCA) was then performed on LASSO-selected antibody features (which resolves multiple variables into principal components that describe the variance within the data set). The contribution of each variable in describing the variance within each principal component is represented on loading plots.

2.9 | ElasticNet and PLS-R

To identify the key contributing antibody signatures from the data set, elasticNet regression was utilized as previously described.[25,29]

The Elastic Net hyperparameter was set to have equal weights between L1norm and L2norm, i.e., $\alpha = 0.5$. Model performance was evaluated iteratively (1000 iterations, 4-fold cross-validation). Partial least squares regression (PLS-R) was performed on ElasticNet-selected antibody features to visualize the relationship between antibody signatures with continuous variables, i.e., Ct (cycle threshold) PCR values (determined via Roche LightCycler 480 Instrument II).

2.10 | Neutralizing antibodies

2.10.1 | Microneutralization assay

SARS-CoV-2 isolate CoV/Australia/VIC01/2020[30] passaged in Vero cells was stored at −80°C. Serial two-fold dilutions of heat-inactivated plasma were incubated with 100 TCID50 of SARS-CoV-2 for 1 h and residual virus infectivity was assessed in quadruplicate wells of Vero cells; the viral cytopathic effect was read on day 5. The neutralizing antibody titer was calculated using the Reed/Muench method.[31]

2.11 | Statistical analysis for factors associated with transmission and correlation between immune parameters

Associations between household members being positive to SARS-CoV-2 detected by NPS with demographic characteristics, clinical parameters, and preventive measures were assessed using generalizing estimating equations (assuming an exchangeable correlation structure and distribution of dependent variable as binomial) controlling for the number of contacts within a household. The correlation between immune parameters was assessed using tetrachoric correlation as the immune parameters are dichotomous.

3 | RESULTS

3.1 | Demographics and transmission dynamics

We included 92 participants from 26 households, recruited between 1st March and 30th September 2020. The median family size was 3.5 (inter-quartile range [IQR] 3.0–4.0). Overall, 47% (43/92) of participants were female, and 43% (40/92) were children with a median age of 3.9 years (IQR 1.9–7.6). SARS-CoV-2 was detected in 54% (50/92) of participants (25 children, 25 adults) on NPS (Figure S1). There were 26 index cases and 24 secondary cases, hence the secondary attack rate using NPS results alone was 36% (24/66). Genetic analysis was available from 15 samples, which reflected the circulating community original Wuhan strain or "Alpha" variants of SARS-CoV-2 at the time of recruitment, all of lineage D.2 except one which was B.1.338.

Twelve participants from eight households also tested positive by PCR for SARS-CoV-2 in stool, with 15 out of 92 participants testing positive in saliva (Figure S1). Those who tested positive in saliva or stool were also positive on NPS PCR for SARS-CoV-2. NPS positivity rate decreased over time with 13% (6/46) positive 28 days following onset of infection. SARS-CoV-2 in stool was detected for the longest period of all virological samples, with 42% (5/12) positive at day 28.

3.2 | Higher transmission vs lower transmission families

In 27% (7/26) of households, all family members tested positive by NPS for SARS-CoV-2, which we have termed high transmission families (families 3, 4, 6, 11, 12, 13 and 20). High transmission families were positive for most respiratory and nonrespiratory samples (Figure S1). High transmission families were also largely characterized by lower respiratory Ct values than low transmission families (Figure S2A; High vs low transmission families; Median 22.62 vs. 32.91; IQR 17.06–28.67 vs. 30.37–34.24; $p = .007$). Feature selection analysis identified antibody signatures associated with lower Ct values (Figure 1A,B). A heatmap including only these selected antibody features illustrates that individuals from high transmission households (green) generally had higher plasma antibody responses to SARS-CoV-2 antigens (indicated by red heat signatures) and clustered separately from the low transmission households, which had largely lower SARS-CoV-2 plasma antibody levels (indicated by blue signatures). As before, this pattern of clustering between high and low transmission families largely coincided with their differences in measured Ct values (low to high; white to purple) (Figure 1C). Interestingly, all family members from 6/7 high transmission families demonstrated evidence of neutralizing antibodies as determined by microneutralization assay (denoted by the asterisk on Figure S1). Furthermore, neutralizing antibodies were only detected in participants from high transmission families.

3.3 | Factors associated with transmission

The probability of transmission to household members, detected by NPS, increased if the index case was an adult compared with a child (62% vs. 12%, odds ratio (OR) 12.4, 95% confidence interval (95 CI) 1.8–84.8, $p = .10$), or had a Ct-value below 32 compared with ≥32 (54% vs. 21%, OR 4.4, 95 CI 1.1–17.1, $p = .034$) (Figure 2A). The probability of transmission among household members increased by 10% in cases who were symptomatic, relative to asymptomatic cases (34% vs. 44%, OR 1.5, 95 CI 0.7–3.4, $p = .329$). Households who employed any nonpharmaceutical intervention measure were less likely to have household transmission compared to those without (31% vs. 46%, OR 0.5, 95 CI 0.1–2.0, $p = .358$), specifically, household separation (27% vs. 45%, OR 0.46, 95 CI 0.1–2.2, $p = .330$) and

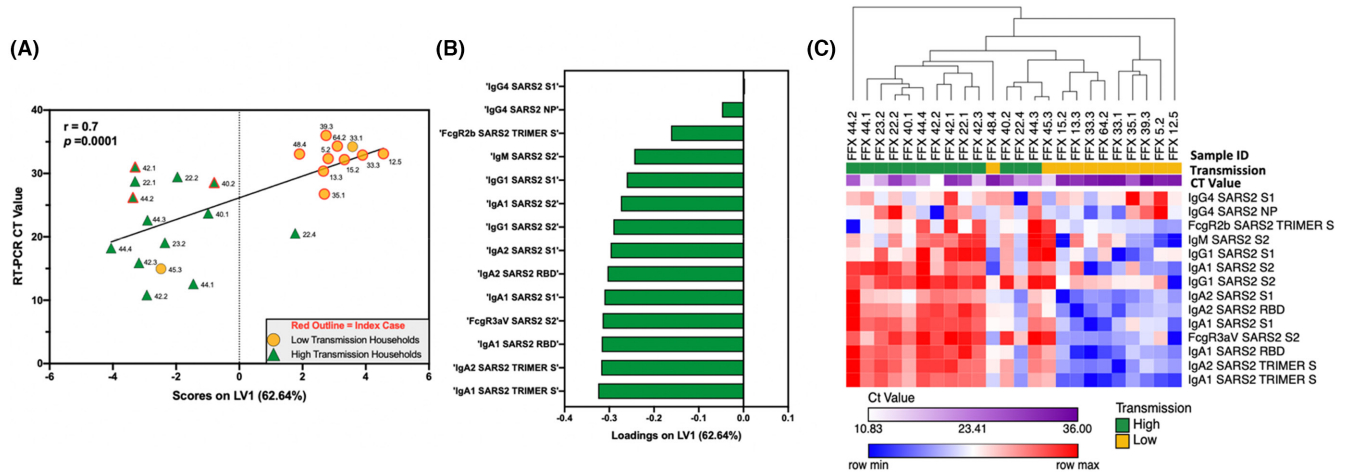

FIGURE 1 SARS-CoV-2 antibody responses correlate with viral load and distinguish high versus low transmission COVID households. Feature selection (elasticNet) identified SARS-CoV-2 antibody signatures measured via multiplex from acute plasma (within 14 days of symptom onset/positive swab) of household members stratified into two groups: high transmission families (defined by majority of household contacts becoming RT-PCR positive for SARS-CoV-2) (green; $n = 13$) versus low transmission families (defined by an absence-to-minimal RT-PCR positive cases among household contacts) (yellow; $n = 11$). PLS-R analysis demonstrated R^2 calibration = 0.48 and R^2 cross-validation = 0.14, and the (A) latent variable 1 (LV1) scores correlated (spearman) against RT-PCR Ct (cycle threshold) values, with (B) loadings depicting the contribution of each antibody signature. Index cases are outlined in red. 4/7 of the high transmission index cases are not shown due to the absence of Ct values. Variance is captured on each axis in parenthesis. (C) Hierarchical clustering was performed on elasticNet-selected antibody features represented in the heatmap from low (blue) to high (red). t values are spread over a purple spectrum (low Ct: light purple; high Ct: dark purple)

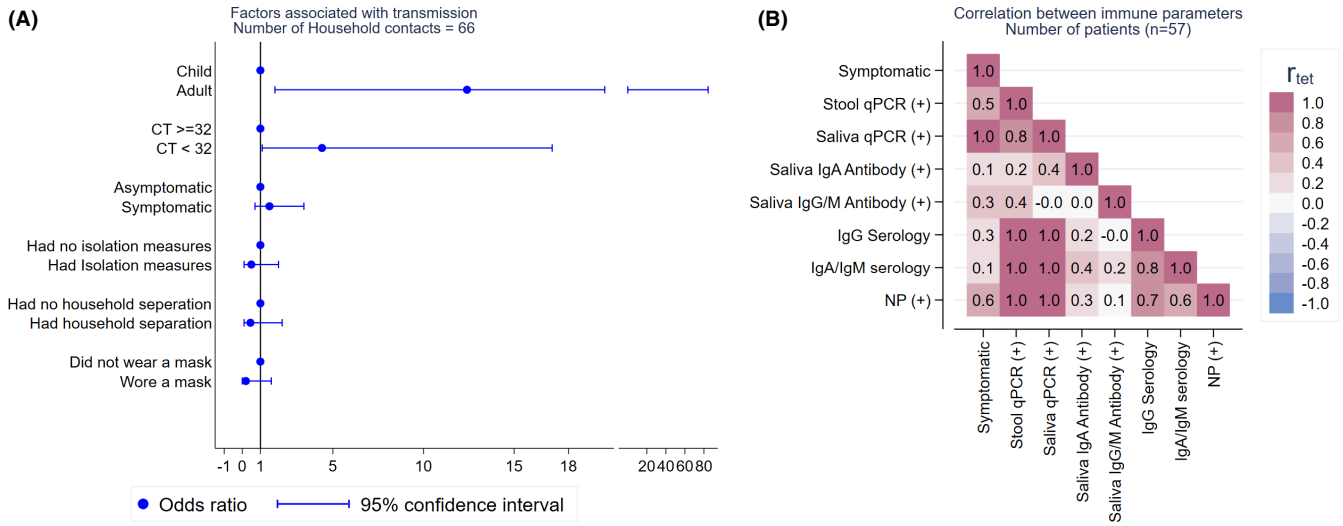

FIGURE 2 SARS-CoV-2 transmission dynamics and immunological characteristics in a household cohort study. Associations between household members being positive to SARS-CoV2 detected by nasopharyngeal swab with demographic characteristics, clinical parameters, and preventive measures were assessed using generalizing estimating equations (assuming an exchangeable correlation structure and distribution of dependent variable as binomial) controlling for the number of contacts within a household (A). The correlation between immune parameters were assessed using tetrachoric correlation as the immune parameters are dichotomous (B)

mask wearing (16% vs. 43%, OR 0.2, 95 CI 0.0–1.6, $p = .149$) were associated with lower secondary transmission (Figure 1A).

3.4 | Correlation between immune parameters

SARS-CoV-2 virus detection in saliva positively correlated with evidence of symptoms ($r = 1$, $p = .002$) (Figure 2B). Detection of SARS-CoV-2 in saliva also correlated with SARS-CoV-2 specific plasma IgG ($r = 1.0, p < .001$) and IgM/IgA ($r = 1.0, p < .001$) (Figure 2B). Similarly, detection of SARS-CoV-2 in stool positively correlated with SARS-CoV-2-specific plasma IgG ($r = 1.0$, $p < .001$) and IgM/IgA ($r = 1.0$, $p = .0015$), and showed concordance with detection of SARS-CoV-2 in saliva ($r = 0.83$, $p < .001$) (Figure 2B). In SARS-CoV-2 positive children, 72% (13/18) produced Spike 1-specific salivary antibodies but had no detected serum antibodies (Figure S1A). Of the

13 children with salivary but no serum antibodies, only 5 (38%) were positive for SARS-CoV-2, in contrast to 5 out of 5 (100%) of children with both a salivary and serum antibody responses. Moreover, of the 18 children demonstrating salivary antibodies against SARS-CoV2, only one was from a high transmission family.

3.5 | Differences in humoral responses between children and adults

Multiplex systems analysis of antibody responses in saliva found infected children and adults displayed distinct SARS-CoV-2 specific antibody responses during the acute phase of infection (Figure 3A). Three SARS-CoV-2 antibody features (IgA1 to spike subunit-1 (S1), IgA1 to nucleocapsid protein (NP), IgA2 to NP) (Figure 3B), identified by dimensionality reducing analysis (least absolute shrinkage and selection operator (LASSO)), were strongly associated with the adult salivary antibody response compared with children, with 80.67% of separation occurring across the first principal component (PC1) (Figure 3A,B). Indeed, salivary IgA responses to several SARS-CoV-2 antigens, including S1, NP, RBD, were significantly elevated in adults compared with children across IgA1 (S1: $p = .002$, NP: $p < .0001$, RBD: $p = .006$) and IgA2 subclasses (S1: $p = .003$, NP: $p < .0001$) (Figure S3), suggesting that adults and children generate distinct mucosal antibody responses during the acute phase of infection. Interestingly, plasma IgA responses to NP were significantly higher for adults compared to that for children (IgA1: $p = .01$; IgA2: $p = .005$) (Figure S4); however, no differences in plasma IgA responses to spike antigens (S1, S2, RBD, trimer) were observed, suggesting the potential for elevated levels of pre-existing cross-reactive immunity in adults at mucosal vs systemic sites that is boosted following infection.

3.6 | Secondary attack rate when including comprehensive virological and antibody assessment

Evidence of SARS-CoV-2 exposure was observed in saliva and plasma antibody responses in 62% of household contacts who tested negative by NPS (26/42: 7/42 serology, 24/42 saliva antibodies, Figure S1). Therefore, the secondary attack rate when respiratory (NPS) and nonrespiratory measures were included (saliva PCR, stool PCR, plasma antibodies, saliva antibodies) was 76% (50/66). There was no onward transmission from participants who tested negative by NPS, even if they were PCR positive in other biological samples.

4 | DISCUSSION

This study provides a detailed virological and immunological profile of families exposed to SARS-CoV-2 "Alpha" variants in 2020–2021 in a low COVID-19 incidence country, Australia. A key finding was the detection of widespread infection with higher secondary attack rates when a comprehensive set of biological specimens were analyzed compared with NPS alone. Enhanced plasma antibody levels were observed in individuals from high transmission families compared with low transmission families. We show that SARS-CoV-2 specific salivary antibodies were detected in a high proportion of participants and that antibody features in blood and saliva differ between children and adults. Moreover, salivary antibody responses to S1 protein were frequently present in the absence of detectable serological responses. Such salivary responses, especially in children, were associated with reduced infectivity and were predominantly present in families of low transmission, a finding supportive of a previous case study we have examined. These associations suggest that salivary antibodies might protect against the establishment of

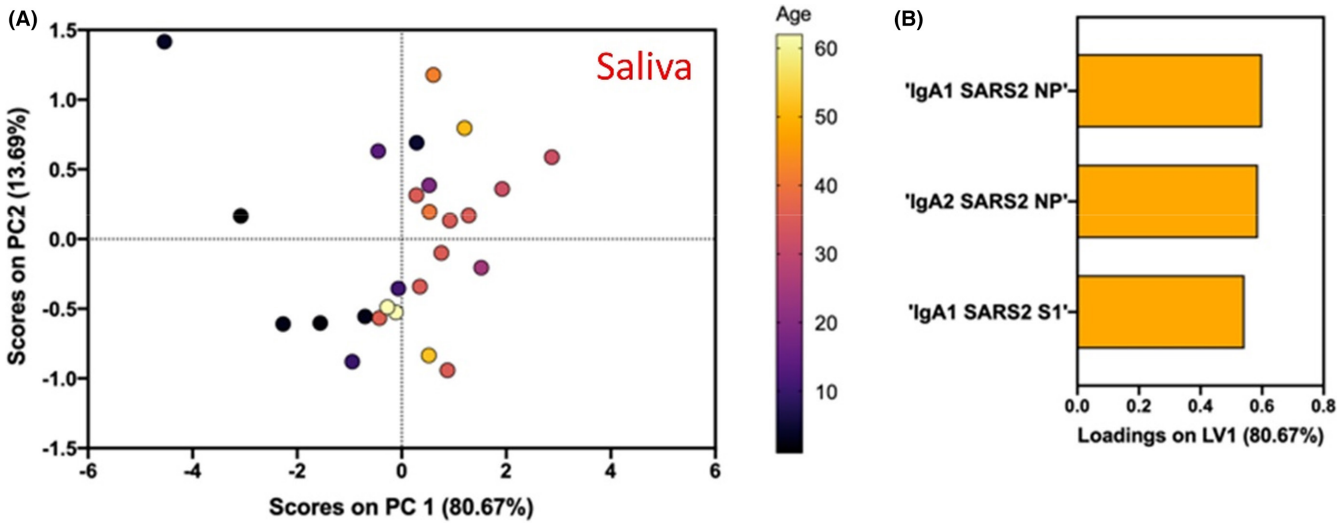

FIGURE 3 Higher IgA responses in saliva to SARS-CoV-2 antigens during acute phase of infection in adults but not in children. (A) PCA scores plot of acute saliva samples (within 14 days of symptom onset/positive swab) from adults (above 19 years of age; $n = 17$) and children (18 years of age or below; $n = 9$) using LASSO feature selected antibody signatures measured via multiplex. Circles are colored over spectrum indicating age (years) range (younger, black/purple; older, yellow). (B) Loadings plots. Variance is captured on each axis in parenthesis

SARS-CoV2 infection, thereby preventing the downstream generation of serum antibody response. Consistent with this, the LASSO analyses indicated that adults mounted a greater salivary antibody response to children. A longer and more inflammatory infection in adults would be expected to induce a larger immune response, as indicated by the higher antibody levels, than if the infection were either more readily controlled, or did not become established, as seemed to be the case in some children.

Our description of the salivary and humoral immune response to SARS-CoV-2 adds to our understanding of the differences in immunity between children and adults. Children typically experience mild disease compared with adults and exhibit distinct innate[13] and adaptive[32] immune pathways that have been proposed to account for these differences. Studies have shown that airway epithelial and immune cells in children are primed for virus sensing, resulting in stronger innate antiviral responses compared with adults.[33] This increased local response may restrict SARS-CoV-2 spread in children, resulting in the more limited systemic immune cell activation observed in children when compared to adults.[34]

Whilst new VOC, such as the Delta and Omicron variants, are associated with higher reproductive numbers compared with the ancestral strain,[35] our data suggest that high levels of household transmission of the ancestral virus are detected when extensive virology and immune assessments are collected. This highlights that dense sampling protocols are more likely to identify infected household members and could be used as a more accurate assessment of secondary attack rates.[36] With the emergence of new VOC, determining the true extent of infection will be important in comparing virulence and transmission dynamics associated with each variant.

In this study, IgA responses in saliva, especially to the SARS-CoV-2 NP antigen, were identified during the acute phase of infection in adults but not in children. Interestingly, elevated antibody responses to NP were observed in both adult saliva and plasma. Previous studies have demonstrated that SARS-CoV-2 NP is highly cross-reactive with NP from other human coronaviruses, thus cross-reactive antibodies are more rapidly induced upon SARS-CoV-2 exposure within the blood, especially among adults and elderly in comparison to children due to pre-existing memory.[25,37,38] Our study suggests that this cross-reactive antibody priming occurs for both mucosal and systemic antibody responses. Furthermore, greater levels of IgA were detected in SARS-CoV-2 spike in adult saliva compared with children, though this disparity was less pronounced within the plasma, supporting cross-reactive class-switched antibody responses are boosted following infection, likely from prior exposure to human coronaviruses.[39] Because seasonal coronaviruses typically infect the upper respiratory tract, one explanation for the disparity between plasma and saliva is that cross-reactive immunity at the level of the mucosa may be more readily boosted during the early stages (within 14 days) of infection.

Like other studies, we identified prolonged fecal shedding beyond respiratory sample detection.[40–42] Fifteen out of 22 patients in an Italian pediatric cohort had RNA detected in stool at diagnosis, independently from gastrointestinal symptoms. Similarly, prolonged SARS-CoV-2 positivity was detected in a study by Xu et al., 8 out of 10 children persistently tested positive on rectal swabs even after nasopharyngeal testing was negative.[43] Stool specimens in this study remained positive when NPS was negative, with a median duration of 14 days (range 10–15) from the onset of symptoms compared with 8 days (range 2–17) for NPS, providing an opportunity for diagnosing SARS-CoV-2 beyond the period of acute infection.[44]

This study has some limitations. Transmission to household contacts was assumed to have occurred within the household, and not due to infections acquired outside the household. This assumption was made due to quarantine rules restricting movement from identification of the first positive case; however, a family may have had a shared external exposure. This study includes lineages D.2 and B.1.338, and the applicability of our findings following the emergence of the Delta and Omicron strains with higher transmission rates[35] is unclear. Comparative analyses between our data and VOC in the future will be important.

5 | CONCLUSION

Utilizing multiple virological and immunological specimens, it is possible to show evidence of infection much greater than those detected from SARS-CoV-2 NPS alone. High transmission in families is associated with the detection of SARS-CoV-2 in saliva and stool, and an acute and robust blood and saliva response, which is only detected following a comprehensive assessment of biological samples. Denser sampling methods provide a more comprehensive assessment of infection and highlight some of the immunological differences in response between children and adults. This profile of infection within households provides a basis for comparison in future studies as VOC emerges.

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CONFLICT OF INTEREST

All authors have indicated they have no conflicts to declare relevant to this study.

PEER REVIEW

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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