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 assessment of household mitigation measures; nasopharyngeal, saliva and stool PCR testing; along with mucosal and systemic SARS-CoV-2 specific antibodies, to comprehensively characterise 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 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 non-respiratory samples 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 characterised by lower respiratory Ct-values than low transmission families (Median 22.62 vs 32.91; IQR 17.06 to 28.67 vs 30.37 to 34.24). High transmission families were associated with enhanced plasma antibody responses to multiple SARS-CoV-2 antigens and the presence of neutralising 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** Utilising respiratory and non-respiratory PCR testing, along with 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.

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Running Title

SARS-CoV-2 household infection and immunity

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Conflict of interest

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

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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 assessment of household mitigation measures; nasopharyngeal, saliva and stool PCR testing; along with mucosal and systemic SARS-CoV-2 specific antibodies, to comprehensively characterise 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 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 non-respiratory samples 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 characterised by lower respiratory Ct-values than low transmission families (Median 22.62 vs 32.91; IQR 17.06 to 28.67 vs 30.37 to 34.24). High transmission families were associated with enhanced plasma antibody responses to multiple SARS-CoV-2 antigens and the presence of neutralising 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

Utilising respiratory and non-respiratory PCR testing, along with 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.

Key words

Immunology; COVID-19; children; novel coronavirus; SARS-CoV-2, household transmission

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, 3, 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 which 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 [6 4].

Transmission dynamics vary within households for reasons that are still not well understood. Clustering of infection in the household can occur, where transmission is characterised 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 detection of transmission routes.

Understanding the host immune responses to SARS-CoV-2 in controlling the infection are important in determining susceptibility. The immune responses to SARS-CoV-2 differ with age; children are less likely to experience 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 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, 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.

METHODS

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.[26] [27]. The Australian FFX study was led by the Doherty Institute, The University of Melbourne[28]. Families were invited to join either, or both studies at 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.

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 [29]. Serial samples of saliva, NPS, and stool were self-collected by all family members, every week for one 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) (Supplementary Table 1). The household secondary attack rate was calculated as the total number of secondary cases over the total number of household contacts.

Viral Identification

NPS were processed by the lab and their extraction was processed using the automated MagNA Pure system (Roche, Basel, Switzerland). The majority of samples were tested with the LightMix® Modular SARS and Wuhan CoV E-gene kit (targeting the E-gene; TIB Molbiol, Berlin, Germany) 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 by Roche LightCycler 480 Instrument II viral panel[30].

Viral RNA was manually extracted from 140 µL of NPS, saliva and 140 µL of 20% (w/v) faecal suspension [31] and then eluted in 60 µL sterile, molecular grade water (Life Technologies, Australia), using the QIAamp

viral RNA kit (QIAGEN, Hilden, Germany) 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[32]. 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 7 households. Briefly, viral RNA from saliva or stool (extracted as described above) was amplified using the ARTIC version 3 primers and published protocols and subjected to Illumina sequencing as previously described[33]. Following quality trimming, reads were aligned to the reference genome (Wuhan Hu-1; GenBank MN908947.3) and consensus sequences generated utilising Geneious Prime. Samples were classified into the recognised SARS-CoV-2 lineages using Pangolin[34].

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 degC 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 1h incubation with biotinylated anti-mouse IgG detection antibody, 1:1,000; 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. Colour 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 was defined as two standard deviations above the maximum titre from the corresponding negative controls.

Serological Immunity

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, China) 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- (1:10,000) horseradish peroxidase (HRP) conjugated secondary antibody 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, United Kingdom). Results were reported in International Units/mL. The cut-off for seropositivity was 8.36 IU/mL based on pre-pandemic samples, while seronegative samples were given half of the seropositive cut-off value.

Coronavirus antibody multiplex assay (blood and saliva)

A novel coronavirus multiplex bead array was designed as previously described[19] 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[35], 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[36] 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 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 pre-pandemic control data.

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 utilised using MATLAB version 9.6 (including machine learning and statistical toolbox) (The MathWorks, Inc., Natick, MA) and Eigenvector PLS toolbox (Eigenvector, Manson, WA). Heatmaps were generated using Morpheus (https://software.broadinstitute.org/morpheus). Prism GraphPad version 9.0.2 (GraphPad Software, San Diego, CA) were used to illustrate final figures and to conduct any univariate analysis. For univariate analysis p value of 0.05 was set as the level for statistical significance, unless otherwise stated.

Data Normalization

For all multivariate analysis, 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 was then log transformed to ensure that the majority of features were normally distributed, by using the following equation $\log_{10}$ (x+1). Values were subsequently normalized by mean centering and variance scaling by calculating respective z -scores.

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[37]. Cross validation was performed iteratively (repeated 1,000 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.

ElasticNet and PLS-R

To identify the key contributing antibody signatures from the data set, elasticNet regression was utilised as previously described[19, 38] The Elastic Net hyperparameter was set to have equal weights between L1norm and L2norm i.e. α=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).

Neutralising Antibodies

Microneutralization assay

SARS-CoV-2 isolate CoV/Australia/VIC01/2020[39] 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; viral cytopathic effect was read on day 5. The neutralizing antibody titer was calculated using the Reed/Muench method[40].

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 generalising estimating equations (assuming an exchangeable correlation structure and distribution of dependent variable as binomial) controlling for the number of contacts within a household (Figure 2 A). The correlation between immune parameters were assessed using tetrachoric correlation as the immune parameters are dichotomous (Figure 2 B).

RESULTS

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 to 4.0). Overall, 47% (43/92) of participants were female, and 43% (40/92) children with a median age of 3.9 years (IQR 1.9 to 7.6). SARS-CoV-2 was detected in 54% (50/92) of participants (25 children, 25 adults) on NPS (Supplementary Figure 1). 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 8 households also tested positive by PCR for SARS-CoV-2 in stool, with fifteen out of 92 participants testing positive in saliva (Supplementary Figure 1). 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.

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, 20). High transmission families were positive for most respiratory and non-respiratory samples (Supplementary Figure 1). High transmission families were also largely characterised by lower respiratory Ct-values than low transmission families (Supplementary Figure 2A; High vs low transmission families; Median 22.62 vs 32.91; IQR 17.06 to 28.67 vs 30.37 to 34.24; $p = 0.007$). 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 neutralising antibodies as determined by microneutralization assay (denoted by the asterisk on Supplementary Figure 1). Furthermore, neutralising antibodies were only detected in participants from high transmission families.

Factors associated with transmission

The probability of transmission to household members, detected by NPS, increased if the index case was an adult compared to a child (62% vs 12%, odds ratio (OR) 12.4, 95% confidence interval (95 Cl) 1.8 to 84.8, p = 0.10), or had a Ct-value below 32 compared to [?]32 (54% vs 21%, OR 4.4, 95 CI 1.1 to 17.1, p = 0.034) (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 to 3.4, p = 0.329). Households who employed any non-pharmaceutical intervention measure were less likely to have household transmission compared to those without (31% vs 46%, OR 0.5, 95 CI 0.1 to 2.0, p = 0.358), specifically, household separation (27% vs 45%, OR 0.46, 95 CI 0.1 to 2.2, p = 0.330) and mask wearing (16% vs 43%, OR 0.2, 95 CI 0.0 to 1.6, p = 0.149) were associated with lower secondary transmission (Figure 2A). *Correlation between immune parameters*

SARS-CoV-2 virus detection in saliva positively correlated with evidence of symptoms (r = 1, p = 0.002) (Figure 2B). Detection of SARS-CoV-2 in saliva also correlated with SARS-CoV-2 specific plasma IgG (r = 1.0, p <0.001) and IgM/IgA (r = 1.0, p<0.001) (Figure 2B). Similarly, detection of SARS-CoV-2 in stool positively correlated with SARS-CoV-2-specific plasma IgG (r = 1.0, p <0.001) and IgM/IgA (r = 1.0, p = 0.0015), and showed concordance with detection of SARS-CoV-2 in saliva (r = 0.83, p<0.001) (Figure 2B). In SARS-CoV-2 positive children, 72% (13/18) produced Spike 1-specific salivary antibodies but had no detected serum antibodies (Supplementary Figure 1).

Differences in humoral responses between children and adults

Multiplex systems analysis of antibody responses in saliva found distinct SARS-CoV-2 specific antibody responses in infected children compared to adults during the acute phase of infection (Figure 3A). Three SARS-CoV-2 antibody features (IgA1 to Spike 1, IgA1 to nucleocapsid protein (NP), IgA2 to NP, Figure 3B) were identified by dimensionality reducing analysis (least absolute shrinkage and selection operator (LASSO)) as significantly elevated responses in adult saliva compared to children (Supplementary Figure 3, p =0.0023, p <0.001, p <0.001 respectively), thus suggesting that adults and children generate distinct mucosal antibody responses during the acute phase of infection.

Given we observed different antibody profiles in the saliva between adults and children, multiplex systems analysis was also conducted on acute children and adult plasma samples. This analysis identified three elevated antibody features that were unique to adult plasma (C1q Trimer S C1q (Marker of antibody-mediated complement activation) to Trimer S, IgG4 to RBD, IgA2 to NP, Supplementary Figure 4).

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, Supplementary Figure 1). Therefore, the secondary attack rate when respiratory (NPS) and non-respiratory 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.

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 detection of widespread infection with higher secondary attack rates when a comprehensive set of biological specimens were analysed compared with NPS alone. Enhanced plasma antibody levels were observed in individuals from high transmission families compared to 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.

Whilst new VOC, such as the Delta and Omicron variants, are associated with higher reproductive numbers compared with the ancestral strain[16], our data suggests 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 rate[17]. With 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 amongst adults and elderly in comparison to children due to pre-existing memory[18, 19, 20]. Our study suggests that this cross-reactive antibody priming occurs for both mucosal and systemic antibody responses.

Like other studies, we identified prolonged faecal shedding beyond respiratory sample detection [21, 22, 23]. Fifteen out of 22 patients in an Italian paediatric 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[24]. Stool specimens in this study remained positive when NPS were negative, with a median duration of 14 days (range 10-15) from 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[25].

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 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[16] is unclear. Comparative analyses between our data and VOC in the future will be important.

CONCLUSION

Utilising 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 detection of SARS-CoV-2 in saliva and stool, and an acute and robust blood and saliva response which is only detected following comprehensive assessment of biological samples. Denser sampling methods provide a more comprehensive assessment of infection and highlights 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 emerge.

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Stephen J. Kent provided pre-pandemic healthy saliva samples which were used to create a positive threshold for saliva studies.

Impact Statement

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

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