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BRIEF REPORT

Examination of Common Coronavirus Antibodies in SARS-CoV-2-Infected and Uninfected Participants in a Household Transmission Investigation

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We compared paired serum specimens from household contacts of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) cases with detectable SARS-CoV-2 seroconversion with contacts who remained seronegative. No protection from SARS-CoV-2 infection was associated with human coronavirus antibodies; however, an increase in common betacoronavirus antibodies was associated with seroconversion to SARS-CoV-2 in mild to moderately ill cases.

Keywords. common coronaviruses; COVID-19; human coronaviruses; immunology; SARS-CoV-2.

While the understanding of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection continues to evolve, knowledge of the immune response to SARS-CoV-2 is currently limited. Four other more common human coronaviruses (HCoVs), HCoV-NL63 and HCoV-229E (alphacoronaviruses) and HCoV-OC43 and HCoV-HKU1 (betacoronaviruses), generally cause mild symptoms [1]. Most individuals have been infected by these 4 common HCoVs by the age of 5 years [2–7]. The spike (S) proteins of coronaviruses have some homology and are the targets of neutralizing antibodies [8, 9]. Antibodies to the common human coronaviruses could therefore affect the outcome of SARS-CoV-2 exposure and infections; however, the role for cross-reactive antibodies is still under investigation. This investigation aims to address the impact of common coronavirus antibodies on SARS-CoV-2 infection by comparing common HCoV antibody levels at the time of SARS-CoV-2 exposure and after infection and development of SARS-CoV-2 antibodies using serum specimens from individuals with known exposures.

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METHODS

We used serum specimens from an investigation of household transmission of SARS-CoV-2 conducted during 2020 prior to SARS-CoV-2 vaccine introduction [10], which recruited reverse transcriptase polymerase chain reaction (RT-PCR)–confirmed SARS-CoV-2 cases and their household contacts. All household contacts were interviewed to obtain demographics and medical histories. Blood (ie, serum) and nasopharyngeal (NP) swabs were collected from investigation participants at the initial household visit (enrollment) and 14 days later (follow-up). Nasopharyngeal (NP) swabs and blood draws were collected from household contacts of confirmed SARS-CoV-2 cases at an initial enrollment visit and ~14 days later. If household contacts developed symptoms during the 14-day follow-up period, an additional NP swab was collected at an interim visit. NP swabs were tested for SARS-CoV-2 using the Centers for Disease Control and Prevention (CDC) 2019 Novel Coronavirus Real Time RT-PCR assay, and blood was processed by the local public health agency laboratory [11]. Any contacts positive at enrollment for SARS-CoV-2 antibodies by enzyme-linked immunosorbent assay (ELISA) were excluded from this analysis.

We tested serum specimens from enrollment and day 14 using both ELISA and multiplexed immunoglobulin G (IgG) immunoassay. SARS-CoV-2 spike ELISAs were performed using the method described by Freeman et al. [12]. A 10-spot, highly sensitive V-PLEX COVID-19 Serology Kit (Meso Scale Discovery, Rockville, MD, USA) was used to quantitatively measure antibodies to antigens related to SARS-CoV-2, SARS-CoV-1, and circulating common coronaviruses HCoV-OC43 spike, HCoV-HKU1 spike, HCoV-229E spike, and HCoV-NL63 spike, plus a negative control (bovine serum albumin [BSA]). Briefly, antibodies in the samples bind to the antigens on the spots, and anti-IgG antibodies conjugated with Meso Scale Discovery (MSD) SULFO-TAG are used for detection. Serum samples were added at 1:500, 1:5000, and 1:50 000 dilutions and incubated at room temperature with shaking (~700 rpm) for 2 hours. SULFO-TAG-labeled anti-IgG detection antibody was added, and electrochemiluminescence signal was read using the MSD instrument. Results were reported as assigned arbitrary units (AU)/mL interpolated from a standard curve.

We used linear regression models to test for significant differences in log 10 transformed common coronavirus levels at enrollment by age, sex, race/ethnicity, underlying medical conditions, and smoking status. We compared common coronavirus levels at enrollment between SARS-CoV-2 seroconverters and those who remained seronegative using a Mann-Whitney *U* test and

differences in common coronavirus levels between days 0 and 14 in these participant groups using a Wilcoxon matched-pairs signed rank test. Data were analyzed using Discovery Workbench, Microsoft Excel, GraphPad Prism 9, and R, version 3.6.3.

This activity was reviewed by the CDC and was conducted consistent with applicable federal laws and CDC policies (see, eg, 45 C.F.R. part 46, 21 C.F.R. part 56; 42 U.S.C. §241(d); 5 U.S.C. §552a; 44 U.S.C. §3501 et seq.).

RESULTS

Sixty-two households were enrolled, including 198 exposed household contacts. In total, 158 contacts who provided serum specimens were seronegative for SARS-CoV-2 antibodies at enrollment by SARS-CoV-2 spike ELISA. Of these 158, 141 provided a follow-up blood specimen at day 14, and 29 were confirmed rRT-PCR positive for SARS-CoV-2 by the follow-up visit(s).

Younger age was associated with lower common HCoV levels at enrollment (Supplementary Table 1). Children in the 0–4-year-old age group had lower antibody levels at enrollment ($P \leq .0001$ for all HCoVs) for the 4 common HCoVs (Figure 1). HCoV-229E levels were also significantly lower among the 5–17-year-old age group than the 18–49-year-old reference group, but no difference was seen among these age groups for the other 3 HCoVs (Figure 1D; Supplementary Table 1), as noted previously [13]. An association was also found between presence of an underlying medical condition and HCoV-229E antibody levels (Supplementary Table 1).

Of 138 contacts age >5 years who provided paired serum specimens and were seronegative for SARS-CoV-2 antibodies at enrollment, 26 developed anti-SARS-CoV-2 antibodies during the investigation (we excluded 3 children <5 years old with paired specimens due to the small size of this age group). Most individuals who seroconverted to SARS-CoV-2 had mild illness; the most commonly reported symptoms were headache, nasal congestion, rhinorrhea, and fatigue, and only 6 patients reported shortness of breath. No SARS-CoV-2-seropositive household contacts were asymptomatic, and none were hospitalized. We did not find a

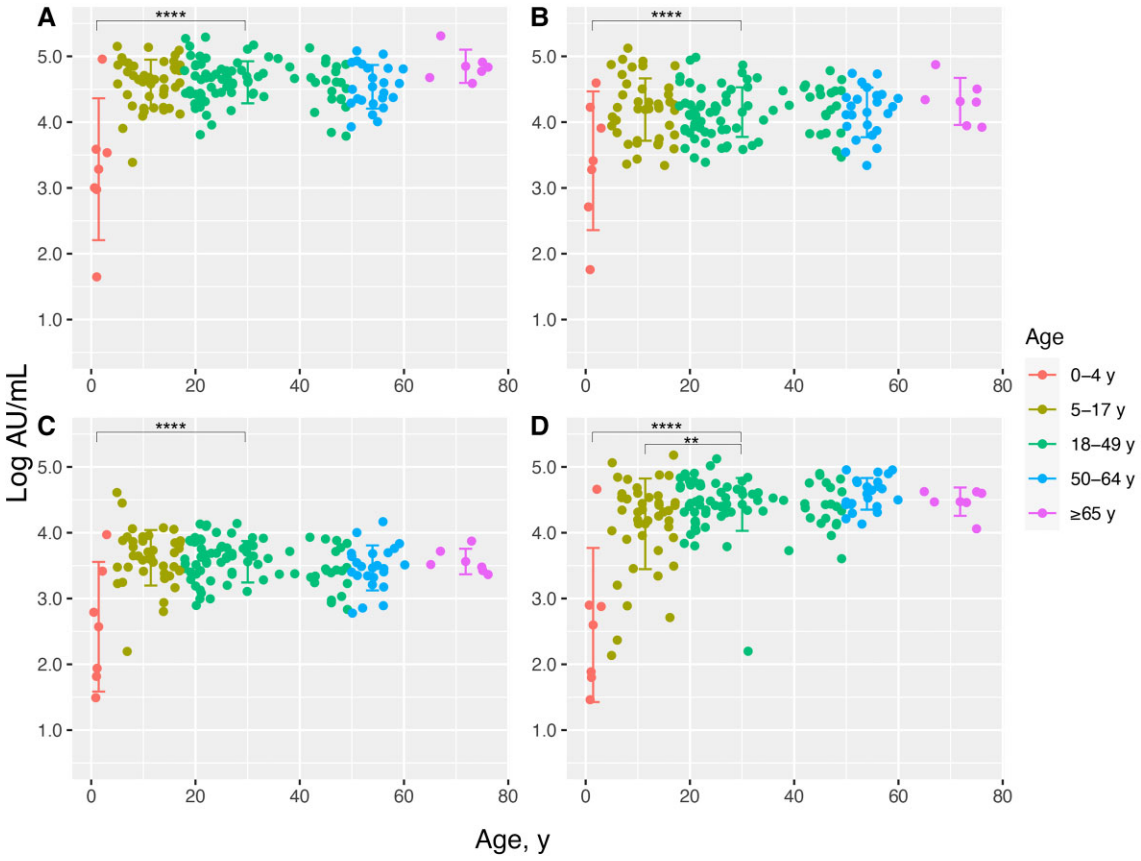

Figure 1. Human coronavirus calculated antibody levels (as measured by MSD) by age (n = 158). Log-transformed antibody levels (in MSD AU/mL) at enrollment in household contacts seronegative for SARS-CoV-2 at enrollment visit against (A) HCoV-OC43, (B) HCoV-HKU1, (C) HCoV-NL63, and (D) HCoV-229E interpolated by a standard curve. Colored lines, categorized by age group, represent the mean and SD. Tested for significance using a linear regression model. ****$P \leq .0001$; ***$P \leq .001$; **$P \leq .01$; *$P \leq .05$. Abbreviations: AU, arbitrary units; HCoV, human coronavirus; MSD, Meso Scale Discovery; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

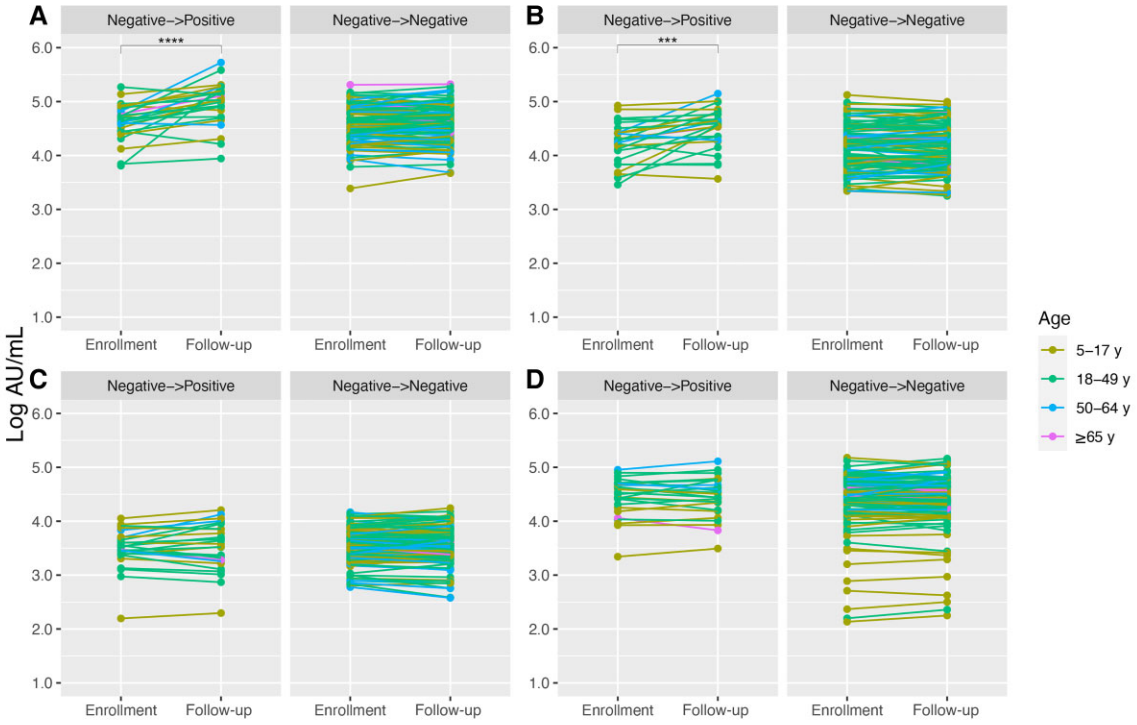

Figure 2. Differences in calculated HCoV antibody levels at enrollment vs follow-up (as measured by MSD) in seroconverters (n = 26) and seronegative (n = 112) household contacts. Log-transformed antibody levels (in MSD AU/mL) in household contacts seronegative for SARS-CoV-2 at enrollment visit against (A) HCoV-OC43, (B) HCoV-HKU1, (C) HCoV-NL63, and (D) HCoV-229E interpolated by a standard curve. Left panels indicate contacts who seroconverted during the 2-week investigation period, and right panels represent contacts who remained seronegative at investigation follow-up. Colored lines connect individual contacts' serum antibody levels at enrollment and follow-up visits. Significance tested using Wilcoxon matched-pairs signed rank test. ****$P \leq .0001$; ***$P \leq .001$; **$P \leq .01$; *$P \leq .05$. Abbreviations: AU, arbitrary units; HCoV, human coronavirus; MSD, Meso Scale Discovery; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

significant difference in HCoV antibody levels at enrollment between household contacts who became SARS-CoV-2 seropositive during the investigation and those who remained seronegative by the second sample collection (Supplementary Figure 1).

Antibody levels to the betacoronaviruses HCoV-OC43 and HCoV-HKU1 were significantly higher at follow-up compared with enrollment in contacts who developed anti-SARS-CoV-2 antibodies during the investigation. No difference was seen in HCoV-OC43 and HCoV-HKU1 antibodies at follow-up compared with enrollment in those who did not develop SARS-CoV-2 antibodies during the investigation, and there was no significant difference in levels of antibody to alphacoronaviruses HCoV-229E and HCoV-NL63 between the 2 time points in either group (Figure 2A and B).

DISCUSSION

We observed that common HCoV serum antibodies are universally present in older children and adults, but present at lower levels in children <5 years old, potentially due to fewer previous HCoV infections. This is consistent with expected exposure timelines for the common HCoVs and suggests that antibody levels may increase with each HCoV exposure up until adulthood [2–7, 14]. An association was also found between presence of an underlying medical condition and HCoV-229E antibody levels. Increased anti-HCoV-229E antibody levels have been previously linked to immunocompromising conditions [4], but due to insufficient power we were unable to investigate an association between HCoV antibodies and specific medical conditions.

High levels of serum HCoV antibodies could theoretically affect the outcome of SARS-CoV-2 infection by providing effective cross-immunity. However, in our investigation, levels of HCoV serum antibodies at enrollment did not differ between the participants who did and did not become SARS-CoV-2 seropositive. This suggests that HCoV antibodies did not provide sterilizing immunity, consistent with other studies [15]. Protective neutralizing antibodies would need to compete with spike–ACE2 interaction, which is high affinity [16]. It has been demonstrated that endemic coronavirus infections and infection with SARS-CoV-2 result in antibody responses with relativity low affinity/avidity, and therefore the lack of cross-protection is not surprising [17, 18].

We did observe boosting of betacoronavirus serum antibodies in older children and adults who developed SARS-CoV-2 antibodies over the course of the investigation. These data are consistent with other studies that have observed boosting of human coronavirus antibodies during seroconversion to SARS-CoV-2 in individuals with severe illness [15]. In this investigation, all infections were mild, indicating that the phenomenon of back boosting is not specific to severe illness. HCoV-HKU1 and HCoV-OC43 are more closely related to SARS-CoV-2 as betacoronaviruses, with 24.8% and 26.4% amino acid identity in the spike as compared with 21.0% and 22.0% of HCoV-229E and HCoV-NL63, respectively. Using a blocks substitution matrix 45 (BLOSUM45) to compare amino acid similarity in the spike protein, these percentages increase to 54.1% and 55.0% for HCoV-HKU1 and HCoV-OC43, and increase to 50.0% and 52.0% for HCoV-229E and HCoV-NL63, when compared with the SARS-CoV-2 spike. It is logical, then, that SARS-CoV-2 infection might lead to a boost in the more similar viral antibodies that are cross-reactive with these antigens' spike proteins. In contrast, this boost was not observed for alphacoronaviruses, HCoV-229E and HCoV-NL63, and is potentially explained by their decreased relatedness (Figure 2C and D) [19, 20]. Notably, OC43 and HKU1 use 9-O-acetylated sialic acid as cellular receptors, whereas SARS-CoV-2 use ACE2. It is, therefore, not surprising that antibodies against the other beta coronaviruses would not be cross protective. The back-boosting of antibodies is suggestive of common epitopes in more conserved areas of spike that are not involved in receptor binding.

This investigation is subject to several limitations. We may have missed detection of seroconversion in household contacts who did not seroconvert during the investigation period. Additionally, it might be possible to determine whether the boosts in HCoV-OC43 and HCoV-HKU1 antibodies were the product of newly formed B-cell populations targeting the conserved epitopes on betacoronavirus spikes or perhaps an anamnestic response from pre-existing HCoV-OC43- and HCoV-HKU1-specfic B cell populations in this investigation. If the boosts were from new B cells, the avidity of these antibodies would be very low, which could be examined in follow-up investigations. Additionally, we did not have enough serum specimens from children <5 years old to be able to compare back boosting in this age group.

We initiated this analysis to examine the hypothesis that pre-existing HCoV antibodies could affect the outcome of household SARS-CoV-2 exposure. In this group of household contacts of SARS-CoV-2 cases, our data does not indicate any correlation between HCoV antibody titers and whether an individual developed antibodies to SARS-CoV-2. However, our data does demonstrate a rise in common beta HCoV antibodies after SARS-CoV-2 infections in mildly ill persons, consistent with other studies demonstrating this HCoV antibody rise in hospitalized SARS-CoV-2 cases [15, 21–23]. Back-boosting of non- or weakly neutralizing antibodies could lead to reduced viral clearance in older adults, enhance neutralizing activity, or not affect viral clearance. Ultimately, the degree to which this back-boosting response may interfere with or augment effective neutralization activity and virus clearance should be investigated further.

Supplementary Data

Supplementary materials are available at Open Forum Infectious Diseases online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

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Author contributions. M.M.S., N.J.T., and M.E.K. drafted the manuscript. M.M.S., B.F., L.M., and S.L. collected the experimental data. V.T.C. and H.L.K. led the field epidemiologic investigation that provided the participant epidemiologic data, RT-PCR lab results, and serum specimens. M.M.S. and M.E.K. conducted the statistical analyses. M.M.S., M.E.K., B.F., L.M., and N.J.T. analyzed and interpreted the data. M.M.S., M.E.K., and N.J.T. designed the serologic investigation. N.J.T., M.E.K., and H.L.K. conceived the investigation.

Patient consent. Participants aged >18 years provided written consent; participants aged 7–18 years provided assent with written parental consent; participants aged <7 years had written parental consent provided.

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