Antibodies to seasonal coronaviruses rarely cross react with SARS-CoV2: findings from an African birth cohort

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Conflicts of Interest and Source of Funding:
This work was supported by the UK-Medical Research Council Global Effort on Covid (GECO) award (GEC1111), the Wellcome Trust Centre for Infectious Diseases Research in Africa (CIDRI), the Bill & Melinda Gates Foundation, USA (grant number OPP1017641, OPP1017579) and the National Institutes of Health H3 Africa (grant numbers U54HG009824, U01AI110466]. HZ is supported by the South African Medical Research Council. MPN is supported by an Australian National Health and Medical Research Council Investigator Grant (APP1174455).

The authors have no conflicts of interest to declare.

Keywords: seasonal coronavirus, cross protection, antibodies, child, COVID-19

Abbreviated title: Antibodies to sHCoV rarely cross protect against SARS-CoV2

Running title: Antibodies and cross protection coronavirus

Abstract

Antibodies to seasonal human-coronaviruses (sHCoV) may cross-protect against SARS-CoV2. We investigated antibody responses in biobanked serum obtained before the pandemic from infants with PCR-confirmed sHCoV. Amongst 141 samples with antibodies to sHCoV, 4(2.8%) were positive for SARS-CoV2-S1 and 8(5.7%) for SARS CoV2-S2. Antibodies to sHCoV rarely cross-react with SARS-CoV2 antigens and are unlikely to account for mild pediatric illness.

Background

Children have been largely spared in the COVID-19 pandemic, developing predominantly asymptomatic or mild disease.[1] Globally, children constitute around 8% of infections, less than 2% of hospitalisations and less than 1% of all COVID-19 associated mortality in high and low-middle income countries (LMICs).[2] In South Africa, 9% of infections and <0.1% of COVID deaths occur in children or adolescents, who comprise more than 30% of the population.[3] Although pneumonia remains a major cause of mortality and morbidity in children in LMICs, risk factors for severe pneumonia such as malnutrition, HIV or prematurity have also not emerged as risk factors for COVID-19.[4]

A key knowledge gap is why paediatric disease is relatively mild. One hypothesis is that cross-protection to SARS-CoV2 may occur from immunity to one of the four seasonal coronaviruses (sHCoVs; 229E, NL63, OC43 and HKU1), which are common and circulate seasonally worldwide.[5-9] Recently, individuals, including children, unexposed to SARS-CoV-2, were reported to have antibodies to the S2 subunit of SARS-CoV2 spike (S) protein from presumed prior sHCoV infection.[7] Shared sequence conservation between sHCoVs and SARS-CoV2, raises the possibility that immunity against sHCoV may cross-protect against SARS-CoV2.

We recently reported the epidemiology of sHCoV infection in infants preceding the COVID-19 pandemic in an African birth cohort, the Drakenstein Child Health study (DCHS).[10] By leveraging this unique dataset and matching biobank of samples, we investigated cross-reactivity of antibodies induced by PCR-confirmed prior sHCoV infection against SARS-CoV2.

Methods

We investigated serological responses to sHCoVs and to SARS-CoV-2 spike (S) antigen in biobanked samples collected prior to the pandemic. Samples were collected from infants with PCR-confirmed sHCoV and age-matched controls without documented sHCoV. Infants enrolled in the DCHS, a birth cohort study in a low-income community, followed infants from birth at 6, 10, 14 weeks and 6, 9 and 12 months, during which serum was collected and biobanked.[11] Intensive follow-up was done, in a subset who chose to participate, comprising fortnightly nasopharyngeal sample collection through the first year of life. Active surveillance for pneumonia, using WHO case definitions, was done. At each pneumonia episode, a nasopharyngeal swab and a serum sample was taken; convalescent serum was also obtained 4 to 6 weeks after pneumonia.

Nasopharyngeal swabs from the time of pneumonia and 2 weekly up to 90 days prior to pneumonia were tested with qPCR to detect sHCoV -229E, -NL63, -OC43, and -HKU1, as previously described.[12] Swabs from age-matched control children without pneumonia in the cohort, were also tested over the equivalent period.

The study was approved by the Human Research Ethics Committee, Faculty of Health Sciences University of Cape Town. Mothers provided written informed consent.

Microbiological testing

Nasopharyngeal swabs preserved in PrimeStore nucleic acid preservation medium (Longhorn Vaccines and Diagnostics, San Antonio, TX, USA), transported on ice and frozen at –80°C for batch testing. Swabs underwent mechanical lysis on a Tissuelyzer LT (Qiagen, Hilden, Germany) followed by total nucleic acid extraction (QIAsymphony Virus/Bacteria Mini Kit,

Qiagen, Hilden, Germany). Quantitative, multiplex, real-time PCR (qPCR) with FTDResp33 (Fast-Track Diagnostics, Esch-sur-Alzet, Luxembourg) identified potential respiratory pathogens including sHCoV (-NL63, -229E, -OC43, -HKU1). Standard curves were derived using standards supplied by the manufacturer.

Antibody measurements

Biobanked serum samples matched to sHCoV-tested nasopharyngeal samples collected at the time of pneumonia were tested for antibodies. In addition, matched convalescent samples taken 4-6 weeks after a pneumonia episode were also tested, when available. Serum was aliquoted, and frozen until batch shipping to the WHO International Reference laboratory for Pneumococcal Serology at University College London where samples were tested for IgG to each of the 4 sHCoV. Samples were also analysed in a multiplexed assay of IgG to SARS-CoV2 of S1and S2 and trimeric spike antigen (MSD® SARS-Coronavirus Plate 1, Rockville, MD) as described, as spike provides the greatest sensitivity and specificity for SARS-CoV-2.[13]

Analysis

Data were analysed using STATA 14.1 (STATA Corporation, College Station, TX USA) and GraphPad Prism version 9.0.2 (GraphPad, San Diego, CA). Data were summarized as frequencies (percent) if categorical and median (interquartile range (IQR)) if continuous. Wilcoxon rank-sum test (Mann-Whitney U test), Kruskal-Wallis test and Chi-square or Fisher's exact were used for crude comparisons, as appropriate. The antibody titres for sHCoV, CoV2-S and CoV2-S2 were reported as geometric means (95% CI).

Results

We identified 42 pneumonia cases positive for sHCoV from whom serum was available at the time of episode with 33 matched convalescent serum samples at 4-6 weeks after pneumonia, all collected pre-COVID. These were matched to 39 pneumonia cases negative for sHCoV, but with other identified organisms. We also included identified 16 samples from children who were asymptomatic but had sHCoV detected (with matched serum available), and matched these to 21 samples from asymptomatic children without sHCoV. In total, there were 151 biobanked serum samples available from 114 children [median age 6 (3.1-7.3) months]. Four children had more than one episode of pneumonia; the median (IQR) time between pneumonia episodes was 141 (96-186) days, so each episode was included as an independent episode. Children with sHCoV-associated pneumonia were younger than those with asymptomatic sHCoV infection (median age 4.6 vs 6 months, p=0.010) (see Table, Supplemental Digital Content 1). OC43 was the commonest sHCoV, occurring in 29 (24.6%), followed by NL63 (14, 11.9%), HKU1 (12, 10.2%) and 229E (4, 3.4%).

Geometric mean (95% CI) IgG antibody titres for each sHCoV were higher in those who were PCR positive (at the same time point) for the corresponding sHCoV compared to those who were negative (Table 1). GMTs were similar in sHCoV pneumonia cases compared to asymptomatic sHCoV-positive controls [24.61 (14.40-42.06) vs 33.49 (14.78-75.90) for OC43, p=0.402; 62.84 (34.43-114.67) vs 42.19 (17.29-102.99) for NL63, p=0.396; 25.64 (14.87-44.21) vs 26.77 (9.52-75.26), p=0.972 for HKU1; 18.44 (11.32-30.03) vs 8.80 (5.20-14.88) for 229E, p=0.098] (Figure, Supplemental Digital Content 2). Amongst children with sHCoV-associated pneumonia, there was an increase in GMTs in matched pneumonia and convalescent sera [31.88 (10.76-94.42) vs 113.95 (37.67-344.74) for OC43; p=0.098; 60.50 (13.02-281.18) vs 194.57 (89.16-424.60) for NL63, p=0.252; 13.70 (4.13-45.48) vs 90.71

(29.36-280.27), p=0.024 for HKU1; 61.35 (10.18-369.74) vs 267.87 (10.43-6876.75) for 229E, p=0.248] (Figure, Supplemental Digital Content 3).

Antibodies were specific to each sHCoV, with no cross reactivity across each of the 4 sHCoVs (Table 1). There was no clear pattern of cross reactivity for SARS-CoV2-S1 or S2, by presence of any sHCoV (Table 1). Amongst 141 samples above the lower limit of detection for antibodies to a sHCoV, only 4 (2.84%) were positive for SARS-CoV2-S1 while 8 (5.7%) were weakly positive for SARS CoV2-S2 (3 of which were also positive to SARS-CoV2-S1).

Discussion

This study, using samples collected preceding the COVID-19 pandemic, found that antibody responses to documented sHCoV infection or disease are robust and specific for each sHCoV in infants in an African birth cohort. While antibody levels did not differ between infants who had symptomatic compared to asymptomatic infection, titres increased in convalescence, following pneumonia. However, little cross reactivity against SARS-CoV2, occurred, indicating that antibodies to sHCoV are unlikely to cross-protect against COVID-19. The data on lack of cross-reactivity between different sHCoV also support our previous finding that infection with different sHCoV occurs within short intervals of each other.[10]

Several explanations have been proposed for lower rates of infection and mild disease from SARS-CoV2 globally in children. These include testing practices with lower case ascertainment due to asymptomatic or mild disease,[1] lower expression of angiotensin-converting-enzyme-2 viral receptor in pediatric compared to adult airway epithelial cells,[14]

more robust innate immune responses in children[8] or induction of trained immunity following BCG immunization or infection,[15] that protects against SARS-CoV2 disease. Immunity to sHCoV with seasonal circulation, has also been hypothesised as a mechanism for protection.[5-7]

In this study, IgG antibodies to sHCoVs rarely cross-reacted with SARS-CoV2-S including the S1 and S2 components. Our findings differ from those recently published in which IgG antibodies binding to the S2 component of SARS-CoV2 were detected in some individuals prior to the pandemic, using a flow cytometry assay.[7] Differences in methodology, populations sampled or interpretation of findings may explain such differences. Only some individuals were reported to have cross reactivity on flow cytometry (for example only 5 of 34 subjects with confirmed sHCoV infection), compared to our findings of 8 of 114 children with cross reactivity. Cross reactivity was rare in healthy donor cohorts (occurring only in 16/302; 5.3%) but the highest prevalence of cross reactivity occurred in donors 6 -16 yrs. A strength of our study is that infants had PCR-confirmed sHCoV infection prior to the pandemic, and cross reactivity was assessed both at the time of disease and 4 to 6 weeks after when titres increased. It is possible that cross reactivity may occur following several infections, and therefore occur later in childhood. Further, pre-existing cross-reactive cellular T cell immune responses to SARS-CoV2, presumably due to prior infection with sHCoV, have been demonstrated in some studies, and may provide a different mechanism for protection against SARS-CoV2.[6, 16, 17]

A limitation of this study is that serological responses to sHCoV were investigated only during the first year of life; however, this age group has the highest incidence of childhood pneumonia and respiratory infections, as previously shown.[12] Another limitation is that T-cell

responses were not evaluated. Strengths are strong surveillance for pneumonia,[18] PCR confirmation of sHCoV episodes, matching antibody measurements including convalescent sera, and the inclusion of a matched control group in a LMIC population-based cohort.

In summary, while sHCoV infections were common and associated with robust antibody responses in infants, minimal cross reactivity against SARS-CoV2 spike antigen was detected. Antibodies to sHCoV are unlikely to provide substantial cross protection against COVID-19, but other mechanisms such as cross-reactive cellular immune responses may be important in ameliorating disease in children.

Funding

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Acknowledgements

We thank the children and families participating in the DCHS. We acknowledge the study staff, and the clinical and administrative staff of the Western Cape Government Health Department for their support of the study.

List of Supplemental Digital Content

Supplemental Digital Content 1. Table describes the characteristics of episodes by sHCoV.

Supplemental Digital Content 2. Figure illustrates antibody IgG titres from PCR-positive sHCoV pneumonia cases and controls.

Supplemental Digital Content 3. Figure illustrates antibody IgG titres at time of PCR-positive sHCoV pneumonia and in matched convalescent serum.

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Table 1. Antibody titres in children by PCR-positive sHCoV and cross reactivity to SARS-CoV-S (S1, S2)

	229E PCR			OC43 PCR			HKU1 PCR			NL63 PCR		
	Positive n=4	Negative n=114	P	Positive n=29	Negative n=89	P	Positive n=12	Negative n=106	P	Positive n=14	Negative n=104	P
229E IgG	**61.35 (10.18-369.74)**	**14.40 (11.09-18.71)**	**0.026**	14.68 (8.11-26.58)	15.28 (11.42-20.44)	0.645	8.91 (5.94-13.36)	16.06 (12.09-21.34)	0.277	15.29 (5.93-39.40)	15.11 (11.51-19.83)	0.724
OC43 IgG	13.57 (1.98-93.05)	24.21 (17.92-32.71)	0.550	**56.24 (29.90-105.76)**	**17.93 (13.08-24.57)**	**0.001**	14.60 (5.69-37.48)	25.08 (18.36-3427)	0.233	14.69 (5.77-37.40)	25.33 (18.54-34.60)	0.181
HKU1 IgG	13.32 (1.64-108.38)	23.39 (17.16-31.89)	0.549	30.77 (15.17-62.41)	20.86 (14.93-29.13)	0.331	**44.25 (12.42-157.63)**	**21.31 (15.63-29.04)**	**0.208**	17.11 (6.60-44.35)	23.87 (1728-32.99)	0.395
NL63 IgG	176.67 (24.55-1271.48)	54.66 (38.78-77.05)	0.173	33.11 (15.95-68.76)	67.85 (46.53-98.94)	0.054	55.00 (21.07-143.57)	57.10 (39.74-82.03)	0.936	**106.22 (34.25-329.37)**	**52.29 (36.72-74.48)**	**0.167**

SARS-CoV2-S1 IgG	0.54 (0.54-0.54)	0.56 (0.54-0.58)	0.744	0.59 (0.52-0.67)	0.55 (0.54-0.56)	0.084	0.62 (0.47-0.81)	0.55 (0.54-0.57)	0.170	0.54 (0.54-0.54)	0.56 (0.54-0.58)	0.522
SARS-CoV2-S2 IgG	8.16 (1.72-38.73)	11.04 (8.86-13.75)	0.583	12.41 (7.24-21.26)	10.48 (8.33-13.19)	0.667	21.85 (6.73-70.98)	10.10 (8.24-12.38)	0.078	8.44 (5.37-13.28)	11.31 (8.93-14.33)	0.489

Footnote: results are geometric means (95% CI); bolded values show comparison of antibody levels for specific sHCoV by PCR positivity for that sHCoV

Abbreviations: sHCoV= seasonal human coronaviruses; SARS-CoV2-S = SARS-CoV2-spike; PCR = polymerase chain reaction

Supplementary Table 1. Characteristics of episodes by sHCoV

	All	Pneumonia sHCoV PCR-positive	Pneumonia sHCoV PCR-negative	Asymptomatic sHCoV PCR-positive	Asymptomatic sHCoV PCR-negative	P-value
N (%)	118	42 (35.6)	39 (33.0)	16 (13.6)	21 (17.8)	
Median (IQR) age, months	6.0 (3.1-7.4)	4.6 (2.8-7.3)	4.6 (2.8-7.3)	6.1 (6.0-11.3)	6.0 (6.0-11.3)	0.010
Male	68 (57.6)	26 (61.6)	28 (71.8)	6 (37.5)	8 (38.1)	0.024
<37 wks. gestation	12 (10.2)	8 (19.1)	1 (2.6)	0 (0.00	3 (14.3)	0.040
HIV exposed	28 (23.7)	11 (26.2)	10 (25.6)	3 (18.8)	4 (19.1)	0.875
OC43	29 (24.6)	21 (50.0)	0 (0.0)	8 (50.0)	0 (0.0)	<0.001
229E	4 (3.4)	4 (9.5)	0 (0.0)	0 (0.0)	0 (0.0)	0.058
HKU1	12 (10.2)	8 (19.1)	0 (0.0)	4 (25.0)	0 (0.0)	0.003
HL63	14 (11.9)	10 (23.8)	0 (0.0)	4 (25.0)	0 (0.0)	0.001

Abbreviations: sHCoV= seasonal human coronaviruses; PCR = polymerase chain reaction; IQR = interquartile range

All values are n (%) unless otherwise indicated

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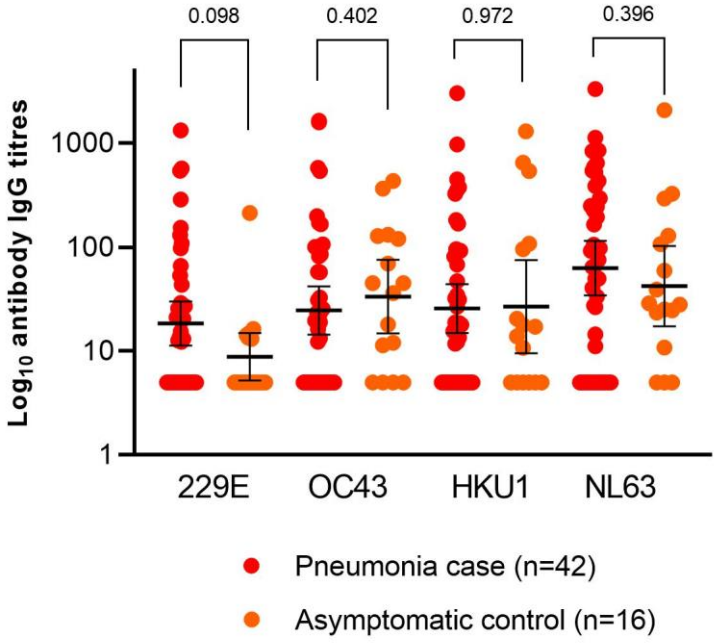
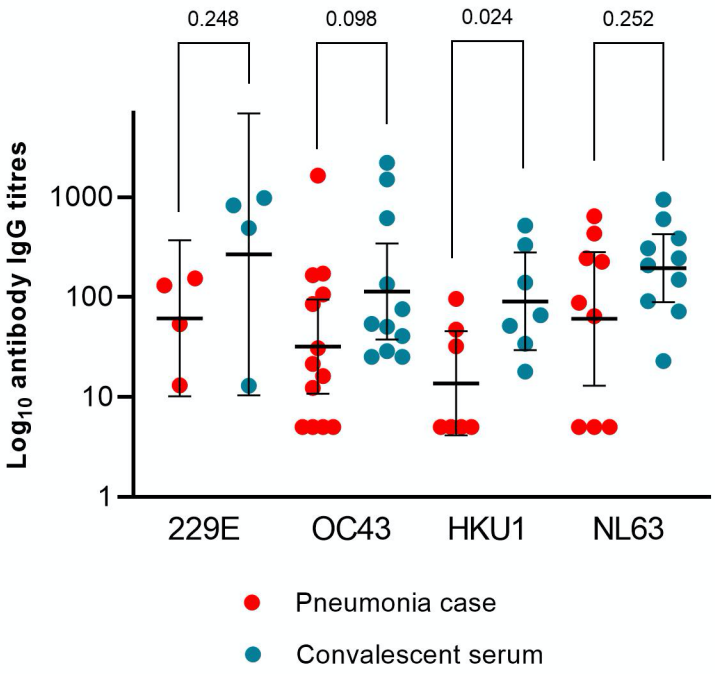


Supplementary Figure 2. Antibody IgG titres in PCR-positive sHCoV pneumonia cases and asymptomatic controls



Supplementary Figure 3. Antibody IgG titres at the time of PCR-positive sHCoV pneumonia (n=33) and in matched convalescent serum

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Background

Children have been largely spared in the COVID-19 pandemic, developing predominantly asymptomatic or mild disease.[1] Globally, children constitute around 8% of infections, less than 2% of hospitalisations and less than 1% of all COVID-19 associated mortality in high and low-middle income countries (LMICs).[2] In South Africa, 9% of infections and <0.1% of COVID deaths occur in children or adolescents, who comprise more than 30% of the population.[3] Although pneumonia remains a major cause of mortality and morbidity in children in LMICs, risk factors for severe pneumonia such as malnutrition, HIV or prematurity have also not emerged as risk factors for COVID-19.[4]

A key knowledge gap is why paediatric disease is relatively mild. One hypothesis is that cross-protection to SARS-CoV2 may occur from immunity to one of the four seasonal coronaviruses (sHCoVs; 229E, NL63, OC43 and HKU1), which are common and circulate seasonally worldwide.[5-9] Recently, individuals, including children, unexposed to SARS-CoV-2, were reported to have antibodies to the S2 subunit of SARS-CoV2 spike (S) protein from presumed prior sHCoV infection.[7] Shared sequence conservation between sHCoVs and SARS-CoV2, raises the possibility that immunity against sHCoV may cross-protect against SARS-CoV2.

We recently reported the epidemiology of sHCoV infection in infants preceding the COVID-19 pandemic in an African birth cohort, the Drakenstein Child Health study (DCHS).[10] By leveraging this unique dataset and matching biobank of samples, we investigated cross-reactivity of antibodies induced by PCR-confirmed prior sHCoV infection against SARS-CoV2.

Methods

We investigated serological responses to sHCoVs and to SARS-CoV-2 spike (S) antigen in biobanked samples collected prior to the pandemic. Samples were collected from infants with PCR-confirmed sHCoV and age-matched controls without documented sHCoV. Infants enrolled in the DCHS, a birth cohort study in a low-income community, followed infants from birth at 6, 10, 14 weeks and 6, 9 and 12 months, during which serum was collected and biobanked.[11] Intensive follow-up was done, in a subset who chose to participate, comprising fortnightly nasopharyngeal sample collection through the first year of life. Active surveillance for pneumonia, using WHO case definitions, was done. At each pneumonia episode, a nasopharyngeal swab and a serum sample was taken; convalescent serum was also obtained 4 to 6 weeks after pneumonia.

Nasopharyngeal swabs from the time of pneumonia and 2 weekly up to 90 days prior to pneumonia were tested with qPCR to detect sHCoV -229E, -NL63, -OC43, and -HKU1, as previously described.[12] Swabs from age-matched control children without pneumonia in the cohort, were also tested over the equivalent period.

The study was approved by the Human Research Ethics Committee, Faculty of Health Sciences University of Cape Town. Mothers provided written informed consent.

Microbiological testing

Nasopharyngeal swabs preserved in PrimeStore nucleic acid preservation medium (Longhorn Vaccines and Diagnostics, San Antonio, TX, USA), transported on ice and frozen at –80°C for batch testing. Swabs underwent mechanical lysis on a Tissuelyzer LT (Qiagen, Hilden, Germany) followed by total nucleic acid extraction (QIAsymphony Virus/Bacteria Mini Kit,

Qiagen, Hilden, Germany). Quantitative, multiplex, real-time PCR (qPCR) with FTDResp33 (Fast-Track Diagnostics, Esch-sur-Alzet, Luxembourg) identified potential respiratory pathogens including sHCoV (-NL63, -229E, -OC43, -HKU1). Standard curves were derived using standards supplied by the manufacturer.

Antibody measurements

Biobanked serum samples matched to sHCoV-tested nasopharyngeal samples collected at the time of pneumonia were tested for antibodies. In addition, matched convalescent samples taken 4-6 weeks after a pneumonia episode were also tested, when available. Serum was aliquoted, and frozen until batch shipping to the WHO International Reference laboratory for Pneumococcal Serology at University College London where samples were tested for IgG to each of the 4 sHCoV. Samples were also analysed in a multiplexed assay of IgG to SARS-CoV2 of S1and S2 and trimeric spike antigen (MSD® SARS-Coronavirus Plate 1, Rockville, MD) as described, as spike provides the greatest sensitivity and specificity for SARS-CoV-2.[13]

Analysis

Data were analysed using STATA 14.1 (STATA Corporation, College Station, TX USA) and GraphPad Prism version 9.0.2 (GraphPad, San Diego, CA). Data were summarized as frequencies (percent) if categorical and median (interquartile range (IQR)) if continuous. Wilcoxon rank-sum test (Mann-Whitney U test), Kruskal-Wallis test and Chi-square or Fisher's exact were used for crude comparisons, as appropriate. The antibody titres for sHCoV, CoV2-S and CoV2-S2 were reported as geometric means (95% CI).

Results

We identified 42 pneumonia cases positive for sHCoV from whom serum was available at the time of episode with 33 matched convalescent serum samples at 4-6 weeks after pneumonia, all collected pre-COVID. These were matched to 39 pneumonia cases negative for sHCoV, but with other identified organisms. We also included identified 16 samples from children who were asymptomatic but had sHCoV detected (with matched serum available), and matched these to 21 samples from asymptomatic children without sHCoV. In total, there were 151 biobanked serum samples available from 114 children [median age 6 (3.1-7.3) months]. Four children had more than one episode of pneumonia; the median (IQR) time between pneumonia episodes was 141 (96-186) days, so each episode was included as an independent episode. Children with sHCoV-associated pneumonia were younger than those with asymptomatic sHCoV infection (median age 4.6 vs 6 months, p=0.010) (see Table, Supplemental Digital Content 1). OC43 was the commonest sHCoV, occurring in 29 (24.6%), followed by NL63 (14, 11.9%), HKU1 (12, 10.2%) and 229E (4, 3.4%).

Geometric mean (95% CI) IgG antibody titres for each sHCoV were higher in those who were PCR positive (at the same time point) for the corresponding sHCoV compared to those who were negative (Table 1). GMTs were similar in sHCoV pneumonia cases compared to asymptomatic sHCoV-positive controls [24.61 (14.40-42.06) vs 33.49 (14.78-75.90) for OC43, p=0.402; 62.84 (34.43-114.67) vs 42.19 (17.29-102.99) for NL63, p=0.396; 25.64 (14.87-44.21) vs 26.77 (9.52-75.26), p=0.972 for HKU1; 18.44 (11.32-30.03) vs 8.80 (5.20-14.88) for 229E, p=0.098] (Figure, Supplemental Digital Content 2). Amongst children with sHCoV-associated pneumonia, there was an increase in GMTs in matched pneumonia and convalescent sera [31.88 (10.76-94.42) vs 113.95 (37.67-344.74) for OC43; p=0.098; 60.50 (13.02-281.18) vs 194.57 (89.16-424.60) for NL63, p=0.252; 13.70 (4.13-45.48) vs 90.71

(29.36-280.27), p=0.024 for HKU1; 61.35 (10.18-369.74) vs 267.87 (10.43-6876.75) for 229E, p=0.248] (Figure, Supplemental Digital Content 3).

Antibodies were specific to each sHCoV, with no cross reactivity across each of the 4 sHCoVs (Table 1). There was no clear pattern of cross reactivity for SARS-CoV2-S1 or S2, by presence of any sHCoV (Table 1). Amongst 141 samples above the lower limit of detection for antibodies to a sHCoV, only 4 (2.84%) were positive for SARS-CoV2-S1 while 8 (5.7%) were weakly positive for SARS CoV2-S2 (3 of which were also positive to SARS-CoV2-S1).

Discussion

This study, using samples collected preceding the COVID-19 pandemic, found that antibody responses to documented sHCoV infection or disease are robust and specific for each sHCoV in infants in an African birth cohort. While antibody levels did not differ between infants who had symptomatic compared to asymptomatic infection, titres increased in convalescence, following pneumonia. However, little cross reactivity against SARS-CoV2, occurred, indicating that antibodies to sHCoV are unlikely to cross-protect against COVID-19. The data on lack of cross-reactivity between different sHCoV also support our previous finding that infection with different sHCoV occurs within short intervals of each other.[10]

Several explanations have been proposed for lower rates of infection and mild disease from SARS-CoV2 globally in children. These include testing practices with lower case ascertainment due to asymptomatic or mild disease,[1] lower expression of angiotensin-converting-enzyme-2 viral receptor in pediatric compared to adult airway epithelial cells,[14]

more robust innate immune responses in children[8] or induction of trained immunity following BCG immunization or infection,[15] that protects against SARS-CoV2 disease. Immunity to ~~circulating~~ sHCoV with seasonal ~~hich~~ circulatione ~~seasonally~~, has also been hypothesised as a mechanism for protection.[5-7]

In this study, IgG antibodies to sHCoVs rarely cross-reacted with SARS-CoV2-S including the S1 and S2 components. Our findings differ from those recently published in which IgG antibodies binding to the S2 component of SARS-CoV2 were detected in some individuals prior to the pandemic, using a flow cytometry assay.[7] Differences in methodology, populations sampled or interpretation of findings may explain such differences. Only some individuals were reported to have cross reactivity on flow cytometry (for example only 5 of 34 subjects with confirmed sHCoV infection), compared to our findings of 8 of 114 children with cross reactivity. Cross reactivity was rare in healthy donor cohorts (occurring only in 16/302; 5.3%) but the highest prevalence of cross reactivity occurred in donors 6 -16 yrs. A strength of our study is that infants had PCR-confirmed sHCoV infection prior to the pandemic, and cross reactivity was assessed both at the time of disease and 4 to 6 weeks after when titres increased. It is possible that cross reactivity may occur following several infections, and therefore occur later in childhood. Further, pre-existing cross-reactive cellular T cell immune responses to SARS-CoV2, presumably due to prior infection with sHCoV, have been demonstrated in some studies, and may provide a different mechanism for protection against SARS-CoV2.[6, 16, 17]

A limitation of this study is that serological responses to sHCoV were investigated only during the first year of life; however, this age group has the highest incidence of childhood pneumonia and respiratory infections, as previously shown.[12] Another limitation is that T-cell

responses were not evaluated. Strengths are strong surveillance for pneumonia,[18] PCR confirmation of sHCoV episodes, matching antibody measurements including convalescent sera, and the inclusion of a matched control group in a LMIC population-based cohort.

In summary, while sHCoV infections were common and associated with robust antibody responses in infants, minimal cross reactivity against SARS-CoV2 spike antigen was detected. Antibodies to sHCoV are unlikely to provide substantial cross protection against COVID-19, but other mechanisms such as cross-reactive cellular immune responses may be important in ameliorating disease in children.

Funding

This work was supported by the UK-Medical Research Council Global Effort on Covid (GECO) award (GEC1111), the Wellcome Trust Centre for Infectious Diseases Research in Africa (CIDRI), the Bill & Melinda Gates Foundation, USA (grant number OPP1017641, OPP1017579) and the National Institutes of Health H3 Africa (grant numbers U54HG009824, U01AI110466]. HZ is supported by the South African Medical Research Council. MPN is supported by an Australian National Health and Medical Research Council Investigator Grant (APP1174455).

Acknowledgements

We thank the children and families participating in the DCHS. We acknowledge the study staff, and the clinical and administrative staff of the Western Cape Government Health Department for their support of the study.

List of Supplemental Digital Content

Supplemental Digital Content 1. Table describes the characteristics of episodes by sHCoV.

Supplemental Digital Content 2. Figure illustrates antibody IgG titres from PCR-positive sHCoV pneumonia cases and controls.

Supplemental Digital Content 3. Figure illustrates antibody IgG titres at time of PCR-positive sHCoV pneumonia and in matched convalescent serum.

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