Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

International Journal of Infectious Diseases 112 (2021) 165–172

Contents lists available at ScienceDirect

International Journal of Infectious Diseases

journal homepage: www.elsevier.com/locate/ijid

Differential pre-pandemic breast milk IgA reactivity against SARS-CoV-2 and circulating human coronaviruses in Ugandan and American mothers ☆

Thomas G. Egwang [a,*], Tonny Jimmy Owalla [a], Emmanuel Okurut [a], Gonzaga Apungia [a], Alisa Fox [b], Claire De Carlo [b], Rebecca L. Powell [b,**]

[a] Human Milk and Lactation Research Center, Med Biotech Laboratories, Kampala, Uganda
[b] Department of Medicine, Division of Infectious Diseases, Icahn School of Medicine at Mount Sinai, New York, USA

ARTICLE INFO

Article history:
Received 28 June 2021
Revised 9 September 2021
Accepted 15 September 2021

KEYWORDS:
Breast milk IgA
Cross-reactivity
Spike proteins
SARS-CoV-2
COVID-19
Human coronaviruses

ABSTRACT

Objective: Uganda has registered fewer coronavirus disease 2019 (COVID-19) cases and deaths per capita than Western countries. The lower numbers of cases and deaths might be due to pre-existing cross-immunity induced by circulating common cold human coronaviruses (HCoVs) before the COVID-19 pandemic. To investigate pre-existing mucosal antibodies against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the causative agent of COVID-19, a comparison was performed of IgA reactivity to SARS-CoV-2 and HCoVs in milk from mothers collected in 2018.

Methods: Ugandan and United States milk samples were run on an ELISA to measure specific IgA to SARS-CoV-2 and HCoVs NL63, OC43, HKU1, and 229E spike proteins. Pooled plasma from United States SARS-CoV-2-positive and negative cases were positive and negative controls, respectively.

Results: One Ugandan mother had high milk IgA reactivity against all HCoVs and SARS-CoV-2 spike proteins. Ugandan mothers had significantly higher IgA reactivity against the betacoronavirus HCoV-OC43 than United States mothers ($P = 0.018$). By contrast, United States mothers had significantly higher IgA reactivity against the alphacoronaviruses HCoV-229E and HCoV-NL63 than Ugandan mothers ($P < 0.0001$ and $P = 0.035$, respectively).

Conclusion: Some Ugandan mothers have pre-existing HCoV-induced IgA antibodies against SARS-CoV-2, which may be passed to infants via breastfeeding.

1. Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has spread worldwide, causing more than 177 100 000 coronavirus disease 2019 (COVID-19) cases by June 18, 2021 (World Health Organization, 2021). Uganda, like other African countries, has registered fewer COVID-19 cases and deaths per capita than non-African countries (World Health Organization, 2021). The lower numbers of cases and deaths in Africa by comparison with those in Western countries might be partly due to cross-immunity induced by circulating common cold human coronaviruses (HCoVs) (Doshi, 2020).

The HCoVs 229E, NL63, OC43, and HKU1 share high sequence similarity with SARS-CoV-2 proteins (Kaur et al., 2020), cause mild upper respiratory tract infections (common cold) (van der Hoek, 2007), and induce cross-reacting antibodies and T cells to SARS-CoV-2 in people never exposed to SARS-CoV-2 (Ng et al., 2020; Saletti et al., 2020). HCoV-induced antibodies with SARS-CoV-2 neutralizing activity targeting the S2 subunit of the SARS-CoV-2 spike protein were found to be present in pre-pandemic samples of 21/48 (44 %) children and 16/302 (5.3%) adults from the UK never exposed to SARS-CoV-2 (Ng et al., 2020). A Dutch study

☆ Author contributions: EO and GA in Uganda and AF and CD in the United States contributed equally. EO, GA, TJO, AF, and CD played a critical role in the acquisition of data. TGE acquired funding, designed, conceived and supervised the study in Uganda, analyzed and interpreted the data, and drafted and revised the manuscript for intellectual content. RLP acquired funding, designed, conceived and supervised the study in the United States, analyzed and interpreted the data, and drafted and revised the manuscript for intellectual content. All authors approved the publication.

* Corresponding authors. Thomas G. Egwang, Human Milk and Lactation Research Center, Med Biotech Laboratories, Kampala, Uganda.

** Rebecca L. Powell, Department of Medicine, Division of Infectious Diseases, Icahn School of Medicine at Mount Sinai, New York, USA.

E-mail addresses: tgegwang@gmail.com (T.G. Egwang), Rebecca.Powell@mssm.edu (R.L. Powell).

https://doi.org/10.1016/j.ijid.2021.09.039

similarly demonstrated pre-existing HCoV-specific T cells in young but not older adults (Saletti et al., 2020). Notably, the prevalence of HCoV-induced cross-reactive antibodies in pre-pandemic sub-Saharan African sera was 6–8-fold higher when compared with United States pre-pandemic sera (Tso et al., 2021). These findings support the premise that systemic cross-immunity induced by HCoVs partly explains the lower numbers of COVID-19 cases and deaths in younger individuals in general and in Africa as a geographical region. However, there is a paucity of studies on pre-existing cross-reactive mucosal immunity against SARS-CoV-2.

Mucosal immunity is predominantly effected by IgA and secretory IgA (IgA/sIgA) and IgM and secretory IgM (IgM/sIgM) at mucosal surfaces such as the gastrointestinal and reproductive tracts, lungs, tears, saliva, and breast milk (Brandtzaeg et al., 2010). There is increasing evidence of a robust mucosal immunity against SARS-CoV-2 (Russell et al., 2020). IgA antibody responses to SARS-CoV-2 have been reported in nasal fluids, tears, and saliva, while elevated IgA-secreting plasmablasts expressing the mucosal chemokine receptor CCR10 have been reported in peripheral blood of SARS-CoV-2-infected individuals (Isho et al., 2020; Cervia et al., 2021; Sterlin et al., 2021). CCR10 and its ligand CCL28 are uniquely involved in mucosal immunity (Xiong et al., 2012; Morteau et al., 2008).

Breast milk provides a unique window on mucosal immunity in mothers and provides protection against a legion of pathogens in infants (Le Doare et al., 2018). There is evidence that IgA antibodies are robustly induced by natural SARS-CoV-2 and HCoV infections and various COVID-19 vaccines. First, several studies have demonstrated IgA antibodies against SARS-CoV-2 in the breast milk of mothers with COVID-19 (Pace et al., 2021; Demers-Mathieu et al., 2021a; Fox et al., 2020a). The reported breast milk antibody isotypes include IgA/sIgA, IgM/sIgM, and IgG, with IgA being the predominant antibody (Pace et al., 2021; Demers-Mathieu et al., 2021a; Fox et al., 2020a; Zhu et al., 2021). Second, COVID-19 vaccination has been rolled out in several countries, and recently published reports have indicated that vaccination induces robust IgA and IgG antibody responses with neutralizing activity in breast milk, which may confer protection to breastfeeding infants (Collier et al., 2021; Perl et al., 2021; Shlomai et al., 2021; Gray et al., 2021). Finally, experimental infection with HCoV-229E was found to induce protective IgA antibodies, which reduced virus shedding in human volunteers (Callow, 1985).

The current limited access to COVID-19 vaccines in Africa has left millions of mothers and their infants vulnerable to SARS-CoV-2 infection and COVID-19-related illnesses. Protection for these mother–infant pairs may therefore depend in the interim on pre-existing cross-reactive mucosal immunity acquired by mothers before the pandemic. There is a dearth of information about pre-existing cross-reactive mucosal immunity induced by HCoVs. Two United States studies reported the presence of cross-reactive breast milk antibodies against HCoVs in United States pre-pandemic breast milk samples that recognize SARS-CoV-2 (Pace et al., 2021; Demers-Mathieu et al., 2021b). Pre-existing immunity in breastfeeding mothers might provide temporary protection against severe COVID-19 illness (Sagar et al., 2021) or reduce the duration of COVID-19 symptoms (Gouma et al., 2021) in mother–infant dyads with no immediate access to COVID-19 vaccines. This is particularly relevant to sub-Saharan African countries where COVID-19 vaccination rollout is expected to be slow due to limited vaccine supplies and vaccine hesitancy, which must be overcome (Wirsiy et al., 2021).

It appears that no study has investigated the presence of cross-reactive IgA antibodies against the spike (S) proteins of SARS-CoV-2 and HCoVs in pre-pandemic breast milk samples collected in Africa prior to 2019. This study was performed to test the hypothesis that breastfeeding rural Ugandan mothers who have never been exposed to SARS-CoV-2 have pre-existing cross-reactive IgA antibodies induced by endemic HCoVs that might be passed to infants via breast milk. Therefore IgA antibodies against the S proteins of SARS-CoV-2 and the four HCoVs 229E, NL63, OC43, and HKU1 were measured in pre-pandemic Ugandan milk samples collected in March 2018 before the emergence of the COVID-19 pandemic. IgA antibody responses in pre-pandemic Ugandan and United States breast milk samples were also compared to gauge differences in pre-existing cross-reactive mucosal immunity between the two study populations before the pandemic. The findings of this study support the hypothesis that breastfeeding Ugandan mothers who have never been exposed to SARS-CoV-2 may have pre-existing HCoV-induced IgA antibodies in milk that might be transferred to infants via breastfeeding.

2. Methods

2.1. Study samples

A cross-sectional study examining various breast milk parameters in a cohort of breastfeeding mother–infant dyads was undertaken in March 2018. The study was part of a larger malaria study that was reviewed and approved by the Research Ethics Committee of the Vector Control Division, Ministry of Health and the Uganda National Council for Science and Technology. The study was conducted at the Med Biotech Laboratories research clinic at St. Anne Health Center III in Usuk Subcounty, Katakwi District, in Northeastern Uganda. The study population comprised mothers with a mean age of 26 years (range 16–40 years) who were breastfeeding at the time of the study. Inclusion criteria were (1) willingness to donate breast milk; (2) age 18 years and above. The sole exclusion criterion was clinical suspicion or evidence of mastitis in one or both breasts. Details about these mothers have been published previously (Owalla et al., 2020). United States samples were collected in 2018–2019 in New York, NY from healthy women with a mean age of 34 years (range 21–44 years) as part of studies related to influenza and HIV under approved Ichan School of Medicine at Mount Sinai IRB protocols. All donors provided written informed consent.

2.2. Milk collection

For Ugandan samples, breast milk was collected manually with the assistance of a lactation nurse, as described previously (Slusher et al., 2012). Briefly, the skin around the areola and nipple was cleaned with an alcohol swab, the breast gently massaged, and 5-ml milk samples were manually expressed into 50-ml Falcon tubes, which were immediately plunged into dry ice. The milk samples were transported to the laboratory in Kampala where they were aliquoted and stored at −20 °C until shipped on dry ice to the United States. For United States samples, participants were asked to collect milk into a clean container using an electronic or manual pump at home. The milk was frozen in the participants' home freezers until samples were picked up by researchers and transferred on ice to the Mount Sinai Hospital, where they were stored at −80 °C until testing.

2.3. Enzyme-linked immunosorbent assay (ELISA)

The levels of SARS-CoV-2 and HCoV antibodies in human milk were examined using a modified ELISA that was recently developed and validated for use on blood serum/plasma (Amanat et al., 2020; Stadlbauer et al., 2020); this assay has been successfully adapted for use with human milk (Fox et al., 2020). The antigens were the spike protein of SARS-CoV-2 and the S1 domain of HCoV-HKU1, HCoV-OC43, HCoV-229E, and HCoV-NL63.

SARS-CoV-2 spike protein was prepared as described previously (Stadlbauer et al., 2020). HCoV antigens were purchased from Sino Biological Company; details of their preparations are given on the company website (https://www.sinobiological.com/recombinant-proteins/hcov-oc43-cov-spike-40607-v08h1; https://www.sinobiological.com/recombinant-proteins/hcov-hku1-cov-spike-40021-v08h; https://www.sinobiological.com/recombinant-proteins/hcov-nl63-cov-spike-40600-v08h; https://www.sinobiological.com/recombinant-proteins/hcov-229e-cov-spike-40601-v08h). The recombinant antigens were His-tagged and purified to homogeneity as confirmed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). The recombinant antigens were diluted in phosphate buffered saline (PBS), coated onto microtiter plates overnight at 4 °C at a concentration of 50 ng/well, and used within 24 h. Briefly, before antibody testing, milk samples were thawed, centrifuged at 800 *g* for 15 min at room temperature, fat was removed, and the supernatant was transferred to a new tube. Centrifugation was repeated twice to ensure the removal of all cells and fat. Skimmed acellular milk was aliquoted and frozen at −80 °C until testing. Both Ugandan and United States pre-pandemic milk samples were tested in duplicate, measuring IgA against the full trimeric spike protein of SARS-CoV-2, or the S1 domain of HCoV-HKU1, HCoV-OC43, HCoV-229E, or HCoV-NL63. Due to the limited availability of Ugandan samples, milk was used diluted 1 in 10 and titrated 2.5-fold in 1% bovine serum albumin (BSA)/PBS and added to the plate. Negative controls were wells without antigen, secondary antibody, or milk. Pooled milk or sera from SARS-CoV-2-positive or negative cases were also employed as positive and negative controls for assay-to-assay consistency. The plates were incubated at 4 °C overnight, washed in 0.1% Tween 20/PBS (PBS-T), and blocked in PBS/3% goat serum/0.5% milk powder/3.5% PBS-T for 1 h at room temperature. After 2 h incubation at room temperature, the plates were washed and incubated for 1 h at room temperature with horseradish peroxidase-conjugated goat anti-human-IgA (Rockland) diluted in 1% BSA/PBS. The plates were developed with 3,3′,5,5′-tetramethylbenzidine (TMB) reagent, followed by 2 N hydrochloric acid (HCl), and read at 450 nm on a BioTek PowerWave HT plate reader.

2.4. Data analysis

Endpoint dilution titers were determined from log-transformed titration curves using four-parameter non-linear regression and an optical density (OD) cutoff value of 1.0. Endpoint dilution positive cutoff values were determined as above. The Mann–Whitney *U*-test was used to determine whether the grouped Ugandan and United States pre-pandemic milk samples differed in terms of specific reactivity to a given antigen. Pearson correlation tests were performed to compare SARS-CoV-2 and HCoV reactivity. An outlier analysis was performed using Grubbs' test. All statistical tests were performed in GraphPad Prism and were two-tailed; the significance level was set at a *P*-value <0.05.

3. Results

3.1. Pre-pandemic milk IgA reactivity to SARS-CoV-2

Pre-pandemic Ugandan and United States milk samples ($n = 25$ per group) were assayed in duplicate by ELISA in order to compare the levels of IgA antibody reactivity to the spike protein (Figure 1). Pooled plasma from SARS-CoV-2-positive and negative donors were employed as positive and negative controls, respectively. Notably, one Ugandan sample exhibited very high reactivity to the SARS-CoV-2 spike, as evidenced from the titration curve ODs and endpoint titer (Figure 1A, F). In contrast, at the initial 1/10 dilution, grouped Ugandan and United States samples exhibited similar reactivity (Ugandan mean OD = 0.88, United States mean OD = 1.02; P = not significant (NS); Figure 1A and data not shown). OD values at each milk dilution tested were used to determine the endpoint titer. With the exception of the high-responder Ugandan sample, all other samples tested in both groups exhibited variable, low-level reactivity, with 16/25 samples in each group failing to reach the endpoint titer cutoff (OD = 1.0; Figure 1F). Ugandan samples exhibited a higher mean endpoint titer compared to that of United States samples (44.1 vs 12.7); however, this difference was not significant, as the outlier Ugandan high responder sample strongly influenced the higher mean (Figure 1F).

3.2. Pre-pandemic milk IgA reactivity to human alphacoronaviruses 229E and NL63

Milk samples were assayed against the HCoVs as described above. The Ugandan high-responder noted for SARS-CoV-2 was also highly reactive against both alphacoronaviruses (Figure 1). In contrast, Ugandan milk samples at 1/10 dilution as a group exhibited significantly lower reactivity compared to the United States samples against HCoV-229E (Ugandan mean OD = 0.87, United States mean OD = 1.95; $P < 0.0001$) and HCoV-NL63 (Ugandan mean OD = 1.27, United States mean OD = 1.62; $P = 0.0077$; Figure 1B, G and data not shown). Endpoint titers were significantly lower for the Ugandan samples compared to the United States samples against HCoV-229E (Ugandan mean endpoint titer = 87.2, United States mean endpoint titer = 238; $P = 0.0003$; Figure 1G). The statistical significance of the difference was increased if the Ugandan high-responder was removed from the dataset ($P < 0.0001$; Figure 1G, right panel). Against HCoV-NL63, endpoint titers were not significantly different if the Ugandan high responder was included in the data; however, if this outlier was removed, endpoint titers were also significantly lower for the Ugandan samples (Ugandan mean endpoint titer = 18.2, United States mean endpoint titer = 41.4; $P = 0.035$; Figure 1H).

3.3. Pre-pandemic milk IgA reactivity to human betacoronaviruses HKU1 and OC43

The Ugandan high-responder noted for SARS-CoV-2 was also highly reactive against both betacoronaviruses (Figure 1). Against the HKU1 spike, IgA reactivity was highly similar at 1/10 dilution between the Ugandan and United States sample groups (Ugandan mean OD = 2.16, United States mean OD = 2.26; P = NS; Figure 1D and data not shown). Endpoint titers were also not significantly different, although the Ugandan mean was increased due to the robust response of the Ugandan high responder (Ugandan mean endpoint titer = 162; United States mean endpoint titer = 107; P = NS; Figure 1I). Against HCoV-OC43, initially it was evident that a subset of Ugandan samples exhibited increased reactivity, although comparing the two groups on the whole did not yield significant differences (data not shown); therefore, 30 additional Ugandan and 27 additional United States samples were analyzed to determine whether the observed trend of higher reactivity might become significant. It was found for milk at 1/10 dilution that Ugandan samples exhibited significantly higher IgA reactivity against HCoV-OC43 (Ugandan mean OD = 1.76, United States mean OD = 1.37; $P = 0.0132$; Figure 1E and data not shown). Endpoint titers were also significantly higher for the Ugandan samples (Ugandan mean endpoint titer = 82, United States mean endpoint titer = 28.3; $P = 0.0182$; Figure 1J). The higher reactivity of the Ugandan samples remained significant even with the removal of the high-responder from the dataset ($P = 0.0258$; Figure1J).

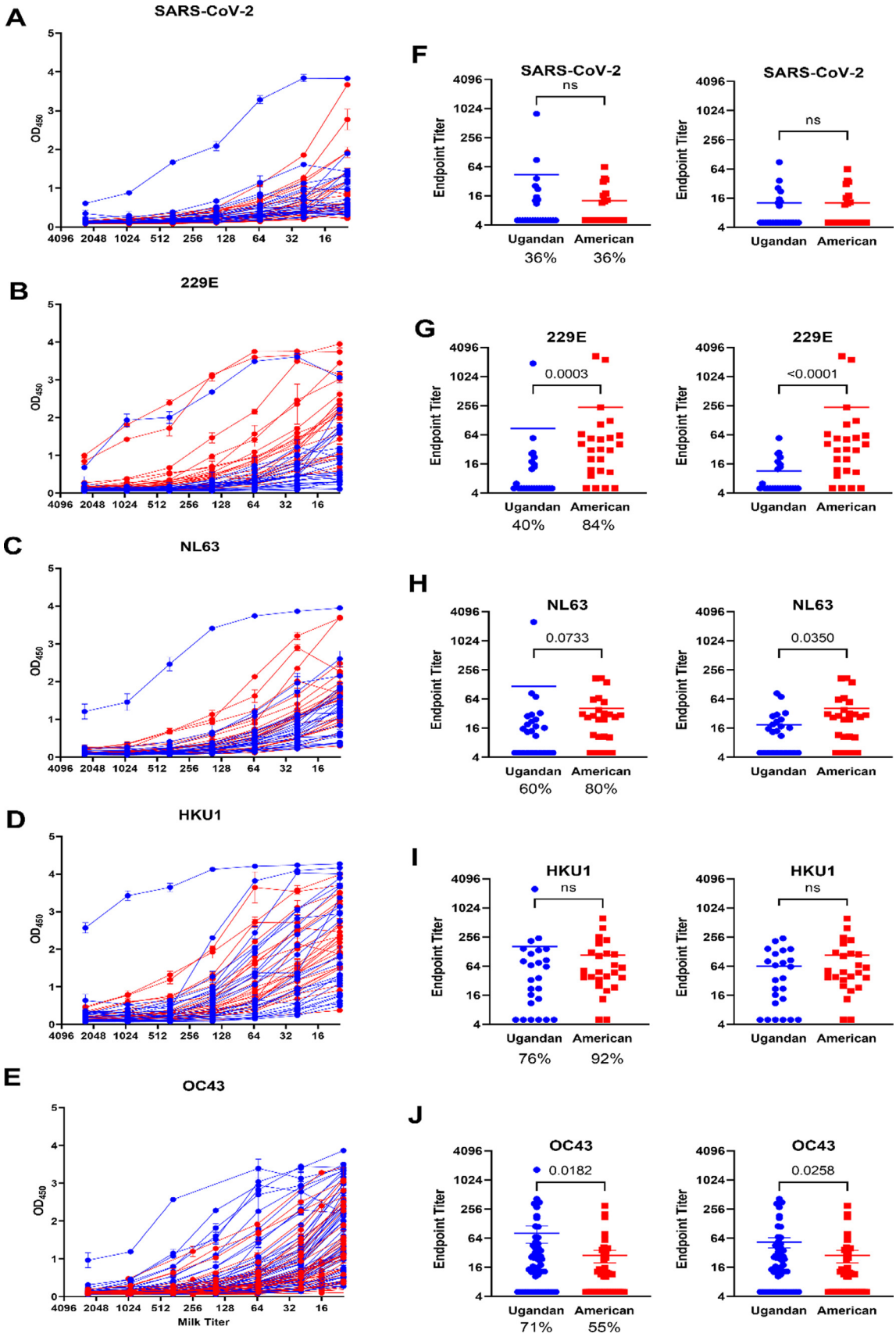

Figure 1. IgA reactivity in pre-pandemic milk obtained from Ugandan and United States donors against HCoVs and SARS-CoV-2. Milk was assayed by ELISA starting at 1/10 dilution. The full titrations against each spike protein are shown in the first panel. Titration curves were used to determine endpoint titers using an OD cutoff of 1.0. Endpoint titers for each antigen tested and the proportion of mothers with specific IgA antibodies are shown in the second panel. In the third panel, the Ugandan high responder was removed from the dataset in order to compare differences without this outlier. Assays were performed in duplicate. Median values of the scatter charts are indicated. Groups were compared by two-tailed Mann–Whitney test; *P*-values are shown.

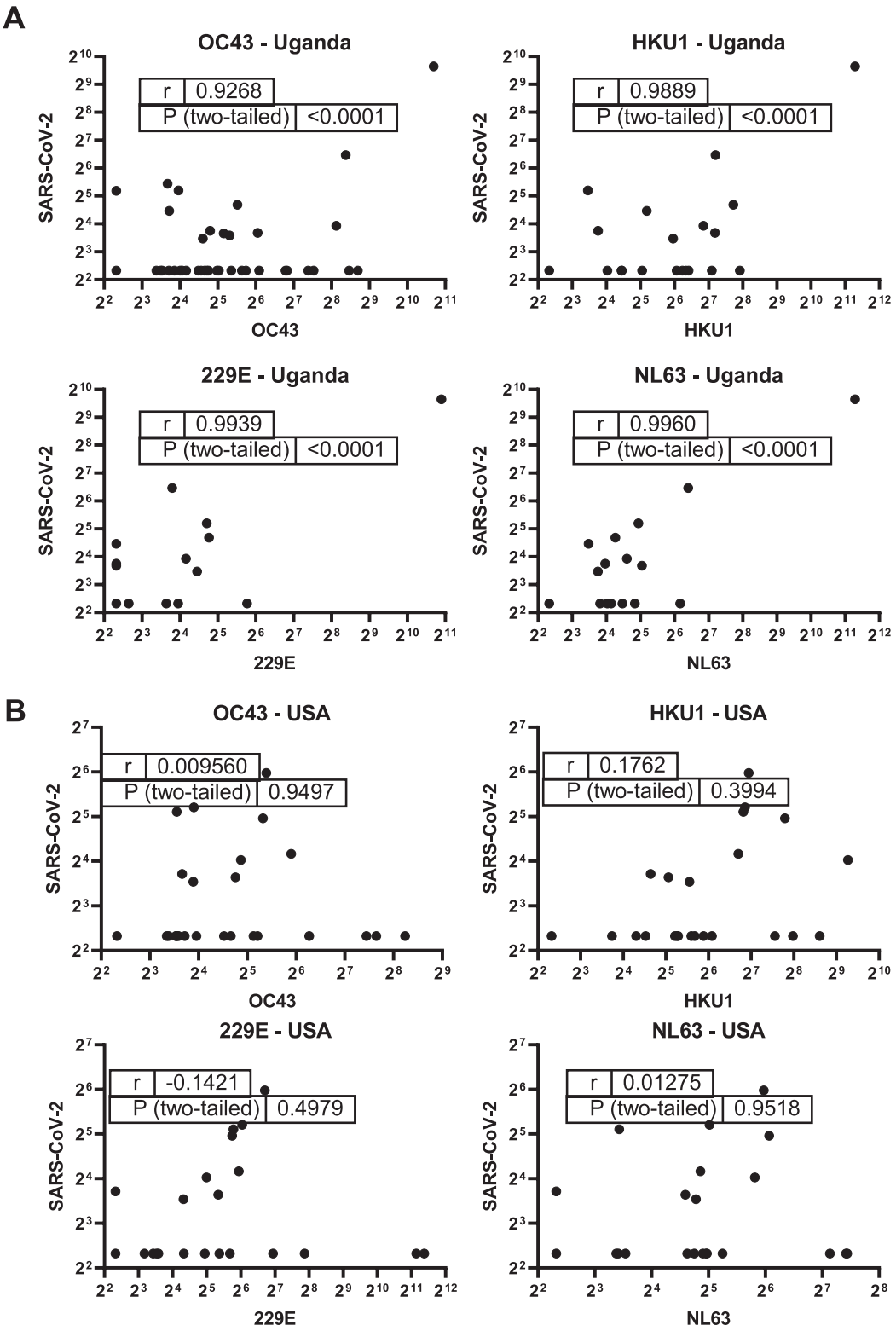

Figure 2. Correlation between HCoV and SARS-CoV-2 milk IgA reactivity. (A) Ugandan sample correlations. (B) United States sample correlations. Endpoint titers were used for these analyses in two-tailed Pearson correlation tests. *P*-values are shown.

3.4. Correlation of HCoV and SARS-CoV-2 IgA reactivity

Correlation analyses were performed to determine the relationship between pre-pandemic HCoV and SARS-CoV-2 IgA reactivity for each group of milk samples. For the Ugandan milk samples, a significant positive correlation was found for reactivity against each HCoV compared to SARS-CoV-2 (Figure 2A; $P < 0.0001$). However, if the Ugandan high responder sample was removed from the dataset, these correlations were no longer evident (data not shown). No correlation between HCoV and SARS-CoV-2 IgA reactivity was found for the United States samples (Figure 2B).

4. Discussion

African countries have registered fewer COVID-19 cases and deaths per capita than the United States and other Western countries (WHO, 2021). The lower numbers of cases and deaths might be partly due to cross-immunity induced by circulating common cold HCoVs, which share high sequence similarity with SARS-CoV-2 proteins (Kaur et al., 2020) and induce cross-reacting antibodies and T cells to SARS-CoV-2 in people never exposed to SARS-CoV-2 (Nga et al., 2020; Saletti et al., 2020). There is a paucity of studies on pre-pandemic breast milk antibodies against SARS-CoV-2 and HCoVs in African mothers. The rationale for this research focus on breast milk is because it is reflective of the common mucosal immune system and therefore relevant in terms of mucosal antibodies against COVID-19 in both adults and infants. This study is novel in reporting that pre-pandemic breast milk samples obtained from rural Ugandan mothers in 2018 contain IgA antibodies that are reactive to the spike proteins of all four HCoVs tested with low-to-moderate cross-reactivity to SARS-CoV-2. In comparing these data to those of pre-pandemic United States samples, it was evident that there is a considerable unique IgA reactivity profile in Ugandan and United States mothers for most of the HCoVs tested that is likely geographically based.

Ugandan mothers had significantly higher levels of milk IgA antibodies against HCoV-OC43 by comparison with United States mothers. There was also a higher prevalence of milk IgA antibodies against HCoV-OC43 in Ugandan mothers by comparison with United States mothers (71% vs 55%). By contrast, United States mothers had significantly higher levels and prevalence of milk IgA antibodies against the alphacoronaviruses HCoV-NL63 and HCoV-229E.

Younger individuals have a higher prevalence of pre-existing humoral and T cell immunity-induced by HCoVs (Ng et al., 2020; Saletti et al., 2020). However, it is unlikely that the observed differences in IgA reactivity in the present study populations were due to the age difference between Ugandan mothers (mean age 26 years) and United States mothers (mean age 34 years).

The differential breast milk IgA reactivity against HCoVs in Ugandan and United States mothers could be due to differences in the distribution of alphacoronaviruses and betacoronaviruses in the two study populations. The study data indicate that there was a higher prevalence of HCoV-OC43 in the Ugandan study population. A recent hospital-based study of Ugandan patients with influenza-like illness demonstrated a high seroprevalence of HCoVs, but used a nucleoprotein-based ELISA that could not distinguish between different species of HCoVs (Mulabbi et al., 2021). Molecular surveillance and seroprevalence studies in Kenya and Ghana, respectively, have demonstrated the predominance of HCoV-OC43 among HCoVs (Sipulwa et al., 2016; Owusu et al., 2021). These previous studies and the present study underscore the potential public health significance of certain HCoVs, especially OC43, in Africa in the context of pre-existing cross-reactive IgA against SARS-CoV-2. No previous studies have investigated the relationship between HCoV-OC43-induced antibodies and clinical outcomes of COVID-19 patients in Africa.

Over one third of Ugandan breastfeeding mothers exhibited pre-pandemic milk IgA reactivity against SARS-CoV-2 and over 70% exhibited pre-pandemic milk IgA reactivity against HCoV-OC43. Pre-existing HCoV-OC43-induced cross-reactive mucosal antibodies might provide moderate protection against COVID-19 in rural Ugandans including mothers and infants. This premise is supported by several published studies. First, recent endemic HCoV infections have been associated with a reduced severity of COVID-19 and risk of admission to the intensive care unit (Sagar et al., 2021) and a reduced duration of COVID-19 symptoms (Gouma 2021 et al., 2021). Second, previous viral symptoms have been shown to correlate with the level and duration of cross-reactive antibodies to S1 and S2 subunits of SARS-CoV-2 and HCoV-OC43 in breast milk (Demers-Matthieu et al., 2021b). Third, higher antibody levels against HCoV-OC43 S protein have been observed in individuals with pre-pandemic SARS-CoV-2 reactive antibodies than in those without (Anderson et al., 2021). Additionally, HCoV-OC43 immunity may impact the cross-reactive response in ways not measured in the present study, including an enhanced memory B cell population and/or reactive T cells. It also remains to be established how Ugandan milk IgA reactivity against HCoV-OC43 relates to mucosal immunity in other mucosal compartments such as the lungs, which are most affected by severe COVID-19 disease.

There is also a growing body of evidence that cross-reactive antibodies induced by betacoronaviruses may be associated with severe and fatal COVID-19 illness. First, SARS-CoV-2 infection boosts antibodies against betacoronaviruses (Anderson et al., 2021; Nguyen-Contant et al., 2020; Shrock et al., 2020), probably due to the original antigenic sin. Original antigenic sin occurs when a prior immune response compromises de novo responses to a closely related pathogen, variant, or serotype and has been well-characterized in influenza virus and dengue infections (Francis, 1960; Mongvolsapaya et al., 2003; Linderman et al., 2014). Second, SARS-CoV-2 back-boosting of antibodies to shared epitopes in the S2 subunits of SARS-CoV-2 and betacoronaviruses is accompanied by weaker and delayed de novo antibody responses to SARS-CoV-2 in severe and fatal COVID-19 cases (Westerhuis et al. 2020; McNaughton et al., 2021). In the present study, United States pre-pandemic samples exhibited significantly more milk IgA reactivity to HCoV-229E and HCoV-NL63 compared to Ugandan samples. It is therefore possible that this enhanced antibody response to alphacoronaviruses is accompanied by weaker and delayed antibodies to SARS-CoV-2 in United States mothers. It is also plausible that the strong antibody responses seen in Ugandan mothers against HCoV-OC43 may skew antibody responses against SARS-CoV-2. Suboptimal antibody responses can result in antibody-dependent enhancement of infection and antibody-mediated immune enhancement with poor outcomes (Iwasaki and Yang, 2020). Prospective studies in mothers with COVID-19 illness are required to establish whether increased HCoV-OC43-induced IgA responses are associated with poor clinical outcomes.

One Ugandan mother had high milk IgA reactivity against the spike proteins of SARS-CoV-2 and all four HCoVs. The high reactivity to SARS-CoV-2 was not seen in milk samples from other mothers from the same village. It is not clear whether the robust cross-reactive IgA response in this mother could have been due to a previous unique exposure to a zoonotic coronavirus circulating in this region that is highly related to SARS-CoV-2. Indeed, molecular surveillance in East Africa has identified bat coronaviruses that are relatives of the HCoVs Middle East respiratory syndrome coronavirus (MERS-CoV), NL63, and 229E (Anthony et al., 2017; Tao et al., 2017). A significant correlation was present between breast milk IgA reactivity to SARS-CoV-2 and all four HCoVs only when the Ugandan high responder mother was included. The correlation was not significant when this outlier was excluded. There is insufficient remaining milk sample from the outlier mother to undertake further studies to confirm immunological cross-reactivity with SARS-CoV-2 through competition ELISA. Therefore, we are undertaking further investigations to establish whether there are more Ugandan breastfeeding mothers with such potent milk cross-reactive IgA against SARS-CoV-2 for further studies.

This study has several limitations. First, only a small number of pre-pandemic breast milk samples were analyzed for IgA reactivity. It is therefore not possible to conclude whether the general population of Ugandan breastfeeding mothers has similar cross-reactive IgA antibodies. Second, due to the limited amounts of milk sam-

ples, it was not possible to undertake SARS-CoV-2 neutralization studies to determine the functional relevance of the highly reactive IgA antibodies in some pre-pandemic Ugandan milk samples. However, we have previously reported that breast milk IgA titers correlate with virus neutralization activity in United States mothers (Fox et al., 2021b). Furthermore, for the same reasons, competition ELISA with SARS-CoV-2 spike protein was not performed using Ugandan samples. There are plans to identify Ugandan pre-pandemic milk samples with high IgA reactivity and undertake neutralization and competition experiments to establish their functional relevance to protection and target specificity. Third, the cross-sectional design of the study precluded the generation of hypotheses regarding cause–effect relationships. The design was inevitable because samples were collected at a single time point before the COVID-19 pandemic. Fourth, the study only looked at IgA reactivity and did not investigate other antibody isotypes that may be relevant to humoral mucosal cross-immunity against COVID-19. Finally, the study did not correlate breast milk and serum antibody reactivity to establish the relationship between systemic and mucosal cross-reactive antibodies to SARS-CoV-2 and HCoVs in the study region. Some of these limitations will be addressed in a future study with a larger number of Ugandan pre-pandemic breast milk samples.

In conclusion, these preliminary findings support the premise that HCoV-induced mucosal antibody profiles vary geographically, and a proportion of rural Ugandan breastfeeding mothers who have not been exposed to SARS-CoV-2 have pre-existing HCoV-induced cross-reactive IgA antibodies. It is not clear whether these cross-reactive IgA antibodies protect against or increase the risk of COVID-19 illness in rural mothers and their infants.

Acknowledgements

We thank the mothers of Abwokodia Parish, Usuk Subcounty in Uganda, and NYC who provided milk samples and made this study possible.

Declarations

Data availability: The data underlying this article will be shared on reasonable request to the corresponding authors. Data suppression rules apply to ensure the anonymity of the study participants.

Funding: This work was supported by Grand Challenges Canada and Global Innovation Fund grant 0817-05 (to T.G.E.) and by the Icahn School of Medicine at Mount Sinai (to R.P.). The funders played no role in the study design, data collection and analysis, manuscript preparation, or decision to publish.

Conflict of interest: All authors declare that they have no financial or non-financial competing interests.

References

Amanat F, Stadlbauer D, Strohmeier S, Nguyen THO, Chromikova V, McMahon M, et al. A serological assay to detect SARS-CoV-2 seroconversion in humans. Nat Med 2020;26:1033–6.

Anderson EM, Goodwin EC, Verma A, Arevalo CP, Bolton MJ, Weirick ME. Seasonal human coronavirus antibodies are boosted upon SARS-CoV-2 infection but not associated with protection. Cell 2021;184 1858-64.e10. doi:10.1016/j.cell.2021.02.010.

Anthony SJ, Gilardi K, Menachery VD, Goldstein T, Ssebide B, Mbabazi R, et al. Further Evidence for Bats as the Evolutionary Source of Middle East Respiratory Syndrome Coronavirus. mBio 2017;8 e00373-17. doi:10.1128/mBio.00373-17.

Brandtzaeg P. The mucosal immune system and its integration with the mammary glands. J Pediatr 2010;156(2 Suppl):S8–15. doi:10.1016/j.jpeds.2009.11.014.

Callow KA. Effect of specific humoral immunity and some non-specific factors on resistance of volunteers to respiratory coronavirus infection. J Hyg (Lond) 1985;95:173–89. doi:10.1017/s0022172400062410.

Cervia C, Nilsson J, Zurbuchen Y, Valaperti A, Schreiner J, Wolfensberger A. Systemic and mucosal antibody responses specific to SARS-CoV-2 during mild versus severe COVID-19. J Allergy Clin Immunol 2021;147 545-557.e9. doi:10.1016/j.jaci.2020.10.040.

Collier AY, McMahan K, Yu J, Tostanoski LH, Aguayo R, Ansel J. Immunogenicity of COVID-19 mRNA Vaccines in Pregnant and Lactating Women. JAMA 2021. doi:10.1001/jama.2021.7563.

Demers-Mathieu V, DaPra C, Mathijssen GB, Medo E. Previous viral symptoms and individual mothers influenced the leveled duration of human milk antibodies cross-reactive to S1 and S2 subunits from SARS-CoV-2, HCoV-229E, and HCoV-OC43. J Perinatol 2021a;41:952–60. doi:10.1038/s41372-021-01001-0.

Demers-Mathieu V, Do DM, Mathijssen GB, Sela DA, Seppo A, Järvinen KM, et al. Difference in levels of SARS-CoV-2 S1 and S2 subunits- and nucleocapsid protein-reactive SIgM/IgM, IgG and SIgA/IgA antibodies in human milk. J Perinatol 2021b;41:850–9. doi:10.1038/s41372-020-00805-w.

Doshi P. Covid-19: Do many people have pre-existing immunity? BMJ 2020;370:m3563. doi:10.1136/bmj.m3563.

Fox A, Marino J, Amanat F, Krammer F, Hahn-Holbrook J, Zolla-Pazner S, et al. Robust and Specific Secretory IgA Against SARS-CoV-2 Detected in Human Milk. iScience 2020;23. doi:10.1016/j.isci.2020a.101735.

Fox A, Marino J, Amanat F, Oguntuyo K, Hahn-Holbrook J, Lee B, et al. The spike-specific IgA in milk commonly-elicited after SARS-Cov-2 infection is concurrent with a robust secretory antibody response, exhibits neutralization potency strongly correlated with IgA binding, and is highly durable over time. medRxiv preprint 2021b. doi:10.1101/2021.03.16.21253731.

Francis TJ. On the Doctrine of Original Antigenic Sin. Proc Am Philos Soc 1960;104(6):572–8. Available from: http://www.jstor.org/stable/985534.

Gouma S, Weirick ME, Bolton MJ, Arevalo CP, Goodwin EC, Anderson EM, et al. Health care worker seromonitoring reveals complex relationships between common coronavirus antibodies and COVID-19 symptom duration. JCI Insight 2021;6(16). doi:10.1172/jci.insight.150449.

Gray KJ, Bordt EA, Atyeo C, Deriso E, Akinwunmi B, Young N. COVID-19 vaccine response in pregnant and lactating women: a cohort study. Am J Obstet Gynecol 2021 S0002-9378:00187-3. doi:10.1016/j.ajog.2021.03.023.

Isho B, Abe KT, Zuo M, Jamal AJ, Rathod B, Wang JH. Persistence of serum and saliva antibody responses to SARS-CoV-2 spike antigens in COVID-19 patients. Sci Immunol 2020;5:eabe5511. doi:10.1126/sciimmunol.abe5511.

Iwasaki A, Yang Y. The potential danger of suboptimal antibody responses in COVID-19. Nat Rev Immunol 2020;20:334–9.

Kaur N, Singh R, Dar Z, Bijarnia RK, Dhingra N, Kaur T. Genetic comparison among various coronavirus strains for the identification of potential vaccine targets of SARS-CoV2. Infect Genet Evol 2020. doi:10.1016/j.meegid.2020.104490.

Le Doare K, Holder B, Bassett A, Pannaraj PS. Mother's Milk: A Purposeful Contribution to the Development of the Infant Microbiota and Immunity. Front Immunol 2018;9:361. doi:10.3389/fimmu.2018.00361.

Linderman SL, Chambers BS, Zost SJ, Parkhouse K, Li Y, Herrmann C, et al. Potential antigenic explanation for atypical H1N1 infections among middle-aged adults during the 2013-2014 influenza season. Proc Natl Acad Sci U S A 2014;111(44):15798–803. doi:10.1073/pnas.1409171111.

McNaughton AL, Paton RS, Edmans M, Youngs J, Wellens J, Phalora P, et al. Fatal COVID-19 outcomes are associated with an antibody response targeting epitopes shared with endemic coronaviruses. medRxiv 2021 05.04.21256571. doi:10.1101/2021.05.04.21256571.

Mongkolsapaya J, Dejnirattisai W, Xu XN, Vasanawathana S, Tangthawornchaikul N, Chairunsri A, et al. Original antigenic sin and apoptosis in the pathogenesis of dengue hemorrhagic fever. Nat Med 2003;9(7):921–7. doi:10.1038/nm887.

Morteau O, Gerard C, Lu B, Ghiran S, Rits M, Fujiwara Y. An indispensable role for the chemokine receptor CCR10 in IgA antibody-secreting cell accumulation. J Immunol 2008;181:6309–15.

Mulabbi EN, Tweyongyere R, Wabwire-Mangen F, et al. Seroprevalence of human coronaviruses among patients visiting hospital-based sentinel sites in Uganda. BMC Infect Dis 2021;21(1):585. doi:10.1186/s12879-021-06258-6.

Ng KW, Faulkner N, Cornish GH, Rosa A, Harvey R, Hussain S, et al. Preexisting and de novo humoral immunity to SARS-CoV-2 in humans. Science 2020;370:1339–43. doi:10.1126/science.abe1107.

Nguyen-Contant P, Embong AK, Kanagaiah P, Chaves FA, Yang H, Branche AR, et al. S Protein-Reactive IgG and Memory B Cell Production after Human SARS-CoV-2 Infection Includes Broad Reactivity to the S2 Subunit. mBio 2020;11 11 e01991-20. doi:10.1128/mBio.01991-20.

Owalla TJ, Okurut E, Apungia G, Ojakol B, Lema J, Murphy SC, et al. Using the ultrasensitive Alere *Plasmodium falciparum* Malaria Ag HRP-2™ Rapid Diagnostic Test in the field and clinic in Northeastern Uganda. Am J Trop Med Hyg 2020;103:778–84. doi:10.4269/ajtmh.19-0653.

Owusu M, Sylverken AA, El-Duah P, Acheampong G, Mutocheluh MY. Adu-Sarkodie Y. Sero-epidemiology of human coronaviruses in three rural communities in Ghana. Pan African Medical Journal 2021;38:244. doi:10.11604/pamj.2021.38.244.26110.

Pace RM, Williams JE, Järvinen KM, Belfort MB, Pace CDW, Lackey KA. Characterization of SARS-CoV-2 RNA, Antibodies, and Neutralizing Capacity in Milk Produced by Women with COVID-19. mBio 2021;12 e03192-20. doi:10.1128/mBio.03192-20.

Perl SH, Uzan-Yulzari A, Klainer H, Asiskovich L, Youngster M, Rinott E, et al. SARS-CoV-2-Specific Antibodies in Breast Milk After COVID-19 Vaccination of Breastfeeding Women. JAMA 2021. doi:10.1001/jama.2021.5782.

Russell MW, Moldoveanu Z, Ogra PL, Mestecky J. Mucosal immunity in COVID-19: a neglected but critical aspect of SARS-CoV-2 infection. Front Immunol 2020;11. doi:10.3389/fimmu.2020.611337.

Sagar M, Reifler K, Rossi M, Miller NS, Sinha P, White LF. Recent endemic coronavirus infection is associated with less-severe COVID-19. J Clin Invest. 2021;131. doi:10.1172/JCI143380.

Saletti G, Gerlach T, Jansen JM, Molle A, Elbahesh H, Ludlow M, et al. Older adults lack SARS CoV-2 cross-reactive T lymphocytes directed to human coronaviruses OC43 and NL63. Sci Rep 2020;10:21447. doi:10.1038/s41598-020-78506-9.

Shlomai NO, Kasirer Y, Strauss T, Smolkin T, Marom R, Shinwell ES. Neonatal SARS-CoV-2 Infections in Breastfeeding Mothers. Pediatrics 2021;147. doi:10.1542/peds.2020-010918.

Shrock E, Fujimura E, Kula T, Timms RT, Lee IH, Leng Y, et al. Viral epitope profiling of COVID-19 patients reveals cross-reactivity and correlates of severity. Science 2020;370:eabd4250. doi:10.1126/science.abd4250.

Sipulwa LA, Ongus JR, Coldren RL, Bulimo WD. Molecular characterization of human coronaviruses and their circulation dynamics in Kenya, 2009-2012. Virol J 2016;13:18. doi:10.1186/s12985-016-0474-x.

Slusher TM, Slusher IL, Keating EM, Curtis BA, Smith EA, Orodriyo E. Comparison of maternal milk (breastmilk) expression methods in an African nursery. Breastfeed Med 2012;7:107–11. doi:10.1089/bfm.2011.0008.

Stadlbauer D, Amanat F, Chromikova V, Jiang K, Strohmeier S, Arunkumar GA. SARS-CoV-2 Seroconversion in Humans: A Detailed Protocol for a Serological Assay, Antigen Production, and Test Setup. Curr Protoc Microbiol 2020;57:e100. doi:10.1002/cpmc.100.

Sterlin D, Mathian A, Miyara M, Mohr A, Anna F, Claër L. IgA dominates the early neutralizing antibody response to SARS-CoV-2. Sci Transl Med 2021;13:eabd2223. doi:10.1126/scitranslmed.abd2223.

Tao Y, Shi M, Chommanard C, Queen K, Zhang J, Markotter W, et al. Surveillance of Bat Coronaviruses in Kenya Identifies Relatives of Human Coronaviruses NL63 and 229E and Their Recombination History. J Virol 2017;91 e01953-16. doi:10.1128/JVI.01953-16.

Tso FY, Lidenge SJ, Peña PB, Clegg AA, Ngowi JR, Mwaiselage J. High prevalence of pre-existing serological cross-reactivity against severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) in sub-Saharan Africa. Int J Infect Dis 2021;102:577–83. doi:10.1016/j.ijid.2020.10.104.

van der Hoek L. Human coronaviruses: what do they cause? Antivir Ther 2007;12:651–8.

Wirsiy FS, Nkfusai CN, Ako-Arrey DE, Dongmo EK, Manjong FT, Cumber SN. Acceptability of COVID-19 Vaccine in Africa. Int J MCH AIDS 2021;10:134–8. doi:10.21106/ijma.482.

Westerhuis BM, Aguilar-Bretones M, Raadsen MP, de Bruin E, Okba NMA, Haagmans BL, et al. Severe COVID-19 patients display a back boost of seasonal coronavirus-specific antibodies. medRxiv 2020 10.10.20210070. doi:10.1101/2020.10.10.20210070.

World Health Organization Coronavirus Disease (COVID-19) Dashboard. Accessed 18 June 2021. https://covid19.who.int/?gclid=EAIaIQobChMI6OvIh-D06QIVVYfVCh1y9AotEAAYASAAEgJemfD_BwE.

Xiong N, Fu Y, Hu S, Xia M, Yang J. CCR10 and its ligands in regulation of epithelial immunity and diseases. Protein Cell 2012;3:571–80.

Zhu F, Zozaya C, Zhou Q, De Castro C, Shah PS. SARS-CoV-2 genome and antibodies in breastmilk: a systematic review and meta-analysis. Arch Dis Child Fetal Neonatal Ed 2021:F1–8. doi:10.1136/archdischild-2020-321074.