

Antibodies Against SARS-CoV-2 in Human Milk: Milk Conversion Rates in the Netherlands

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Abstract

Background: It has been demonstrated that human milk from mothers who have been infected with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) contains antibodies against the virus, which could play an important role in protecting the recipient infant against coronavirus disease 2019 (COVID-19). Seroconversion is measured frequently around the world, but the milk conversion rate is unknown.

Research Aims: To determine (1) the prevalence and (2) the dynamics of immunoglobulin A (IgA) antibodies against SARS-CoV-2 in human milk amongst lactating mothers in the Netherlands.

Methods: In this large prospective cohort study, lactating mothers ($N = 2312$) were included between October 12, 2020 and February 24, 2021. Enzyme-linked immunosorbent assay was used to determine levels of IgA antibodies in human milk and immunoglobulin G (IgG) antibodies in serum against the ectodomain of the SARS-CoV-2 spike protein.

Results: A total of 691 (30.6%) participants had SARS-CoV-2 specific antibodies in human milk and/or serum. Of these participants, 524 (23.1%) had IgA antibodies against SARS-CoV-2 in human milk, and 356 (15.7%) had IgG antibodies against SARS-CoV-2 in serum. A total of 199 (8.8%) participants had antibodies in both human milk and serum. SARS-CoV-2 specific IgA antibodies in human milk remain present at least 10 months after a polymerase chain reaction confirmed infection.

Conclusion: The prevalence of IgA antibodies against SARS-CoV-2 in human milk was 23.1% in our cohort. This high prevalence of antibodies in human milk might lead to passive immunity in many breastfed infants and may serve as protection against COVID-19.

Keywords

breastfeeding, breastmilk, coronavirus, COVID-19, immunoglobulins, lactation secretory IgA, spike protein

Background

The coronavirus disease 2019 (COVID-19) pandemic emerged in December 2019 (Li et al., 2020). It rapidly became a public health emergency of international concern due to its high dispersion rate influencing public health, society, and the economy across the world (Guner et al., 2020). Therefore, the World Health Organization declared the outbreak a pandemic on March 11, 2020.

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is part of the *Coronaviridae* family and causes COVID-19. Transmission between humans occurs mainly through droplets caused by sneezing or coughing or inhalation of aerosols, which are subsequently picked up by other people (Guner

et al., 2020). The disease has a wide range of clinical presentations, from asymptomatic infection or only experiencing cold-like symptoms to severe pneumonia, hospital admission, or even death (Guner et al., 2020).

In general, people with an impaired immune function, like the elderly, experience the most severe symptoms of COVID-19. Newborn infants also have an impaired immune function due to the lack of a fully developed immune system; therefore, they

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may be seriously affected by SARS-CoV-2, although usually mildly (Adeyinka et al., 2021; Barrero-Castillero et al., 2020; De Rose et al., 2020; Kim et al., 2020; Raschetti et al., 2020; Shah & Saugstad, 2020). Human milk is considered to be the main source of passive and active immunity for newborns and infants, as it contains a large array of bioactive factors, including antibodies, oligosaccharides, nucleic acids, and cytokines, that help enrich an infant's immune system (Mosca & Gianni, 2017). During the first 6 months of life, breastfed infants have a 2.2-fold lower mortality risk due to fewer infections than non-breastfed infants (Sankar et al., 2015). This highlights the protective effects of human milk against infectious diseases.

The most abundant antibody in human milk is immunoglobulin A (IgA), comprising approximately 90% of the total immunoglobulins (Hurley & Theil, 2011; Palmeira & Carneiro-Sampaio, 2016). IgA in human milk is mostly polymeric and bound to a secretory component (Goldman et al., 2011). In general, IgA antibodies in human milk confer protection for the infant by eliminating invading pathogens through their inhibiting effect to bind to host-receptors of intestinal epithelial cells or entrapping microorganisms within the mucus (Palmeira & Carneiro-Sampaio, 2016). Specific IgA antibodies have been identified in human milk of previously infected mothers against various viruses, including SARS-CoV, human immunodeficiency virus (HIV) and respiratory syncytial virus (Palmeira & Carneiro-Sampaio, 2016; Robertson et al., 2004).

Much data are available about the prevalence of seroconversion throughout the world, mainly measured by blood banks collecting samples from their regular donors. Seroconversion rates range from 0.02%–53.40%, depending on the prevalence of SARS-CoV-2 infection in specific regions (Ioannidis, 2021). In the Netherlands, seroconversion in donors from our national blood bank (Sanquin) reached between 10%–15% at the end of 2020 (Zaaijer et al., 2021). Lactating mothers comprise a specific group whose behavior might be more self-protecting compared to the population that donates blood due to pregnancy or care for a newborn child.

There is limited evidence regarding the presence of antibodies against SARS-CoV-2 in human milk. However, it has been demonstrated that human milk from mothers who had previously been infected contains SARS-CoV-2 specific antibodies and that these antibodies were capable of neutralizing the virus in small sample sizes (Fox et al., 2020; Keulen et al., 2020*; Pace et al., 2021). Since lactating mothers with COVID-19 rarely infect their infants, a protective role of human milk in the context of this

Key Messages

- Human milk of previously infected mothers contains antibodies against the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which could play an important role in protecting the recipient infant against coronavirus disease 2019.
- From October 2020–March 2021, the prevalence of SARS-CoV-2 specific immunoglobulin A antibodies in human milk was 23.1% in the Netherlands.
- Human milk antibodies remain present at least 10 months after confirmed coronavirus disease 2019.

infectious disease has been suggested (Dumitriu et al., 2021). Breastfeeding has been documented as safe under certain conditions, including measures to reduce the risk of transmission, for example, wearing a face mask and washing hands. To date, replication competent SARS-CoV-2 has not been isolated from human milk and transmission of the virus to the infant through human milk has not been reported (Auriti et al., 2020; Chambers et al., 2020; Pace et al., 2021; Salvatori et al., 2021). Despite the discovery of viral particles in human milk, breastfeeding was not associated with SARS-CoV-2 infection, suggesting that viral transmission through human milk, if any, is very rare (Auriti et al., 2020).

Antibodies against SARS-CoV-2 have been demonstrated in human milk in small sample sizes; larger studies regarding IgA against SARS-CoV-2 in human milk are lacking. As human milk antibodies can play an important role in child immune function and, subsequently, child health, our study aims were to determine (1) the prevalence and (2) the dynamics of IgA against SARS-CoV-2 in human milk amongst lactating mothers in the Netherlands.

Methods

Research Design

The COVID MILK—POWER MILK study is a prospective cohort study. To determine the prevalence of IgA antibodies against SARS-CoV-2 in the human milk of lactating mothers in the Netherlands, a large number of samples were collected. The study was approved by the Ethics Committee of the Amsterdam

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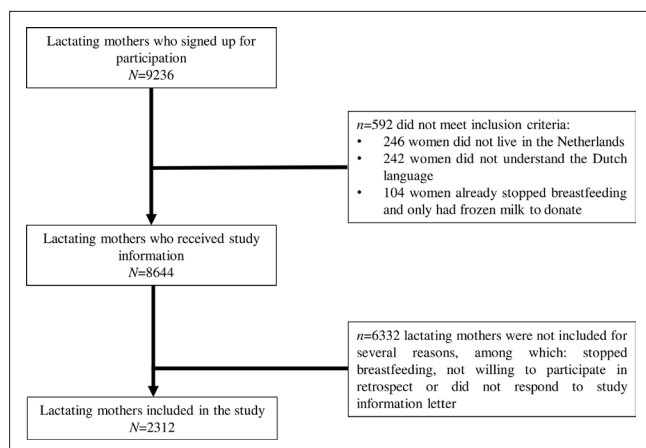


Figure 1. PRISMA of the Study Population.

University Medical Centre on September 28, 2020 (NL74752.029.20).

Setting and Relevant Context

In the Netherlands, approximately 170,000 infants are born annually (Centraal Bureau voor de Statistiek [Statistics Netherlands], 2020). Breastfeeding, feeding human milk to the infant, is highly recommended after birth; approximately 70% of mothers start breastfeeding. After 6 months, 20% of these mothers are still breastfeeding their child (Engelse & Dommelen, 2020). During the study period, there was a lockdown in the Netherlands to minimize the third wave of SARS-CoV-2 infections.

Sample

Lactating mothers were recruited through the media (e.g., social media, television, newspapers, and radio). All lactating mothers were eligible to participate. There were no exclusion criteria. In total, 9239 lactating mothers were interested in participation. After screening, 8644 lactating mothers were eligible to participate in the study and received the study information letter. At the analysis stage, a total of 2312 participants had been included in the study (Figure 1). This sample size is assumed to be adequate to answer the research question. At that time, seroconversion rates were 10%–15% (Zaaijer et al., 2021).

Measurement

To obtain information about participant characteristics, a questionnaire in Dutch was sent to the participants (see supplemental Material S1). Before analysis, the collected human milk and serum samples were stored at the Amsterdam University Medical Center at -80°C . To assess the SARS-CoV-2 specific IgA antibodies in human milk and immunoglobulin G (IgG) antibodies in serum, an enzyme-linked

immunosorbent assay (ELISA) with the SARS-CoV-2 spike protein was used. Soluble perfusion-stabilized spike protein of SARS-CoV-2 was generated and immobilized overnight on a 96-well plate (Greiner) using 0.1M NaHCO_3 followed by a 1-hr blocking step with 1% casein Phosphate Buffered Saline (PBS; Thermo Scientific). The human milk samples were diluted at 1:10, and the serum samples 1:50 in 1% casein PBS (Thermo Scientific) and incubated on the spike protein coated 96-well plates, for 2 hr to allow binding to the target protein. Finally, a 1:3000 diluted horseradish peroxidase (HRP)-labeled goat anti-human IgG (Jackson, Immunoresearch) in 1% casein PBS was used to detect specific IgG antibodies in the serum samples. In contrast, a 1:5000 diluted HRP-labeled goat anti-human IgA (Biolegend) in 1% casein PBS was used for the human milk samples. After 1 hr incubation, 3,3',5,5'-Tetramethylbenzidine (TMB) was used for the read-out at 450 nm. For the determination of the cut-off value, a relative operating characteristic curve analysis was performed for both milk and serum samples using pre-pandemic negative samples and polymerase chain reaction (PCR) proven positive samples. The milk samples were considered positive at an optical density (OD) 450 nm cut-off value of 0.502, and a cut-off value of 0.452 for the serum samples. With these cut-off values, the sensitivity was 67.9% (95% CI [61.0%,74.1%]) for IgA antibodies in human milk with a specificity of 99.0% (95% CI [94.7%,100.0%]) and for serum IgG antibodies the sensitivity was 95.9 (95% CI [92.9%–97.6%]) with a specificity of 99.1 (95% CI [94.9%,100%]).

Data Collection

Data collection took place between October 12, 2020 and February 24, 2021 and written informed consent was obtained prior to sample collection from all participants. Participants were requested to collect their milk from the first feeding moment in the morning of their study appointment. They were instructed to empty one breast in the morning before feeding their child. After mixing the milk, 10–30 ml was donated in a sterile container (SteriFeed[®]) that was provided by the researchers and subsequently the collected human milk was stored in the refrigerator at $2-8^{\circ}\text{C}$. To assess the prevalence of serum IgG antibodies against SARS-CoV-2, a phlebotomist performed a venipuncture and collected a 5 ml vial of blood during the study appointment. Participant characteristics were obtained by a questionnaire. All scientific information collected as part of this study was treated confidentially and a unique code was assigned to each study participant to ensure anonymity, and stored safely at the study side.

Data Analysis

Statistical analyses were performed by using IBM SPSS (Version 26). Characteristics were described in descriptive

Table 1. Participants' Characteristics (N = 2312).

Characteristics	Total	Positive for SARS-CoV-2 IgA antibodies	Negative for SARS-CoV-2 IgA antibodies
Mother			
Age mother years - median (IQR)	33 (30,36)	33 (31,36)	33 (30,36)
Body Mass Index - median (IQR)	23.3 (21.3,26.0)	23.2 (21.2,25.9)	23.4 (21.3,26.1)
Chronic diseases - n (%)	248 (11.3)	58 (11.5)	190 (11.3)
Autoimmune disease - n (%)	66 (2.9)	8 (1.5)	58 (3.3)
Smoking - n (%)	42 (1.9)	11 (2.2)	30 (1.8)
Infant			
Age infant weeks - median (IQR)	34 (24,50)	40 (27,63)	32 (23,47)
Gestational age at birth weeks - median (IQR)	40 (39,40)	40 (39,40)	40 (39,40)
COVID-19			
Positive PCR test - n (%)	165 (7.3)	98 (19.2)	61 (3.6)
Weeks post positive PCR - median (IQR)	8 (3,15)	9 (4,17)	7 (3,14)

Note. IgA = immunoglobulin A, PCR = polymerase chain reaction, IQR = interquartile range, SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2, COVID-19 = coronavirus disease 2019.

statistics including frequencies or median with interquartile ranges (IQR), depending on the distribution. Graphpad Prism 8.2.1. for Windows was used to display the prevalence and the dynamics of IgA antibodies in human milk and IgG antibodies in serum over time. To test the difference in prevalence of SARS-CoV-2 specific antibodies over the different months, a Chi-square test was performed.

Results

Characteristics of the Sample

Lactating participants provided 2276 human milk samples and 2267 serum samples and 2294 participants completed the questionnaire. The participant characteristics are depicted in Table 1. Of the participants, 165 (7.3%) had a previous PCR confirmed SARS-CoV-2 infection, on average 8 weeks before sample collection. Of those participants, 159 reported symptoms, of which a runny nose ($n = 121$), fatigue ($n = 121$), headache ($n = 110$), and loss of smell ($n = 110$), were the most reported. Fever was reported 87 times. None of the participants received a SARS-CoV-2 vaccine prior to participation.

Aim 1: The Prevalence

Of the participants, 691 (30.6%) had SARS-CoV-2 specific antibodies in human milk and/or serum, of whom 524 (23.1%) had them only in human milk, and 356 (15.7%) only in serum. A total of 199 (8.8%) participants had SARS-CoV-2 specific antibodies in human milk and serum (Figure 2). Figure 3 demonstrates the prevalence of SARS-CoV-2 specific antibodies in human milk and serum over time during the study period. The prevalence of human milk

SARS-CoV-2 specific IgA antibodies increased during the study period ($p < .001$), whereas the prevalence of serum SARS-CoV-2 specific IgG antibodies showed a more variable pattern with increases and decreases ($p = .011$). Of the participants with SARS-CoV-2 specific IgA antibodies in human milk, 427 (81%) had had COVID-19 related symptoms.

Aim 2: The Dynamics

Figure 4 demonstrates the dynamics of SARS-CoV-2 specific IgA antibodies in human milk of those participants who

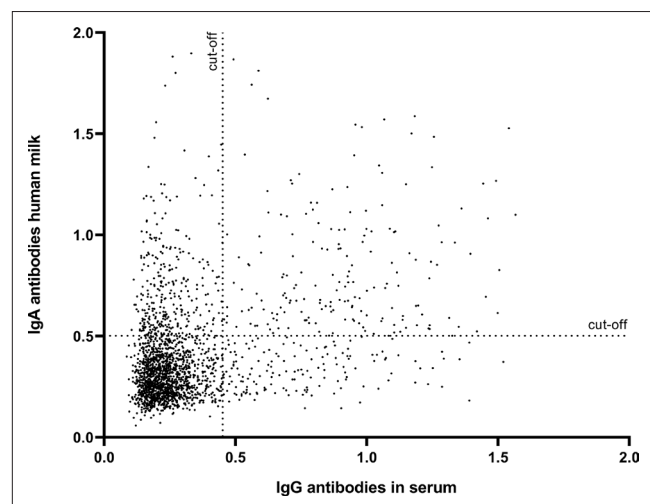


Figure 2. SARS-CoV-2 Specific Antibody Levels in Human Milk Versus Serum.

Note. IgA = immunoglobulin A; IgG = immunoglobulin G; SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2.

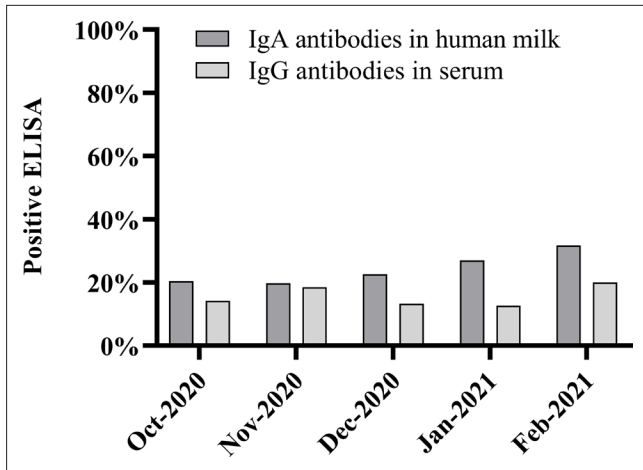


Figure 3. Prevalence of SARS-CoV-2 Specific Antibodies Over the Study Period.
 Note. IgA = immunoglobulin A; IgG = immunoglobulin G; ELISA = Enzyme-linked immunosorbent assay.

had recovered from PCR-confirmed COVID-19. We demonstrated that these antibodies remain stable over time. SARS-CoV-2 specific IgA antibodies in human milk remain present up to at least 10 months after infection. Out of the 165 participants who had had a previously proven SARS-CoV-2 infection by PCR, 98 (59%) participants showed SARS-CoV-2 specific IgA antibodies in their milk. In 61 (37%) of the 165 PCR confirmed SARS-CoV-2 infected participants, IgA antibodies were not detected in their milk, which is consistent with the lower sensitivity of the ELISA for milk samples.

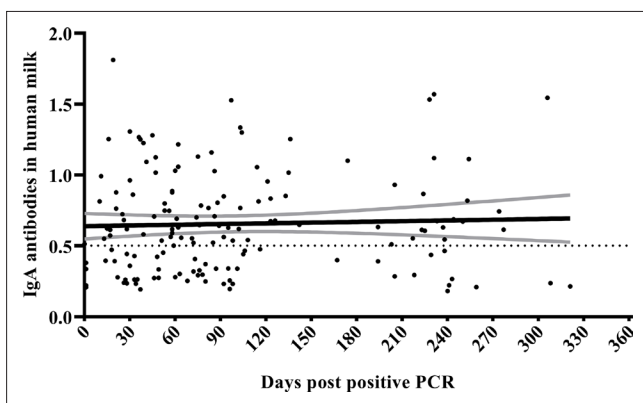


Figure 4. Dynamics of SARS-CoV-2 Specific sIgA in Human Milk in Lactating Mothers Who Recovered From PCR-Confirmed COVID-19.
 Note. Curved lines represent 95% CI. IgA = immunoglobulin A; PCR = polymerase chain reaction; SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2; COVID-19 = coronavirus disease 2019.

Discussion

The prevalence of IgA antibodies against SARS-CoV-2 in human milk was 23.1% and, in serum, the prevalence of IgG antibodies against SARS-CoV-2 was 15.7%. The Blood Bank of the Netherlands (Sanquin) reported a seroprevalence of SARS-CoV-2 in its blood donors of 2.7% 1 month after the start of the pandemic (Slot et al., 2020). From October 2020 until February 2021, 10%–15% of the blood donors showed antibodies (Zaaijer et al., 2021). Presumably, the difference in prevalence with our study cohort is due to an overrepresentation of the actual prevalence, as lactating mothers with previous symptoms of COVID-19 might have been more likely to participate in this study.

Whereas some participants who had tested positive showed antibodies in both human milk and serum, in most of them, SARS-CoV-2 specific antibodies were found only in human milk or serum. Previously, researchers studying SARS-CoV-2 infected patients, showed that serum and mucosal IgA antibodies had been detected earlier in the immune reaction than serum IgG antibodies (Sterlin et al., 2021), suggesting that the systemic IgG antibody response might be slightly slower compared to the mucosal IgA antibody response. After a rapid early IgA antibody response, a decline in IgA antibody serum levels was observed earlier than a decline in serum IgG antibodies. Therefore, IgA antibodies seem to play an especially important role during early SARS-CoV-2 infection (Sterlin et al., 2021).

Much of our sample had not been tested for SARS-CoV-2, even though most participants experienced several episodes of mild symptoms that could be attributed to COVID-19 or other infectious diseases. Consequently, we were not able to determine the time between SARS-CoV-2 infection and time of sampling within this study for many of the participants with detectible SARS-CoV-2 antibodies. It might well be that participants who only showed IgA antibodies in human milk were more likely to have been included more immediately after their SARS-CoV-2 infection, whereas participants only showing IgG antibodies in serum were more likely to be included further from the moment of infection. Moreover, the severity of symptoms might have influenced the duration of stay of antibodies in human milk (Demers-Mathieu et al., 2021).

A higher average signal was observed during the analysis of human milk samples compared to serum samples, which might be explained by non-specific reactivity. This was also the case for pre-pandemic controls. This could be due to the viscousness of human milk samples and the limited dilution possibilities because of lower antibody concentrations compared to serum. The high background might lead to an overestimation of the actual prevalence due to false-positive samples. To minimize false-positive samples, a cut-off specificity level of 99% was used.

One of the strengths of this study was the large sample size, which increased the possibility of obtaining an accurate

representation of the prevalence of SARS-CoV-2 specific antibodies in the Netherlands. Another strength was the standardized way of milk sampling. Since hindmilk contains more fat than foremilk, and antibodies are more prone to bind to fat (Schroten et al., 1999), it is important to collect human milk samples in a standardized way. Therefore, our participants received clear instructions on timing and human milk collection methods, to avoid variation in antibody levels due to sampling issues.

Limitations

Our study might be limited by a selection bias. Lactating mothers signed themselves up to participate, which might lead to a misrepresentation of the actual prevalence of antibodies in lactating mothers, as mothers with symptoms may have signed up more frequently than mothers without symptoms. Furthermore, in the first months after the outbreak of SARS-CoV-2, the testing capacity in the Netherlands was limited, restricting the determination of the dynamics of SARS-CoV-2 specific antibodies in confirmed cases over time. In the future, researchers should incorporate not only the presence of SARS-CoV-2 specific antibodies in human milk, but also the maturation, affinity, and functionality of these antibodies.

Conclusions

The prevalence of IgA antibodies against SARS-CoV-2 in human milk was 23.1% between October 2020 and February 2021. After a polymerase chain reaction confirmed infection, SARS-CoV-2 specific IgA antibodies was stable over time and remained present up to at least 10 months in human milk. The high prevalence of antibodies in human milk might lead to passive immunity in breastfed infants and may serve as protection against COVID-19.

*Editor's Note

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**Editor's Note

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authors have reviewed all references and take responsibility for their quality.

Authors' Note

Marit J. van Gils and Britt J. van Keulen contributed equally to this study.

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Disclosures and conflicts of interest

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Supplemental Material

Supplemental material for this article is available online.

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