Pasteurization Inactivates SARS-CoV-2–Spiked Breast Milk

Carina Conzelmann,[a]* Rüdiger Groß,[a]* Toni Luise Meister,[b]* Daniel Todt,[b,c] Adalbert Krawczyk,[d,e] Ulf Dittmer,[e] Steffen Stenger,[f] Jan Münch,[a] Eike Steinmann,[b] Janis A. Müller,[a] Stephanie Pfaender[b]

The current coronavirus disease 2019 pandemic, caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), raises unprecedented questions regarding virus transmission and risks for pregnant or breastfeeding women. We and others described that SARS-CoV-2 RNA is detectable in the breast milk of infected mothers.[1–3] In two cases in which the mother continued breastfeeding, the newborns also tested positive for SARS-CoV-2.[1,2] However, the origin of the infections of the newborns remained unclear and raised concerns of possible virus transmission via breast milk. The safety and feasibility of breastfeeding is of high importance because breast milk contains nutrients, hormones, and immunoprotective entities that are essential for the development, health, and protection of the neonate. So far, the World Health Organization recommends continued breastfeeding after maternal SARS-CoV-2 infection while taking measures to maintain strict hygiene.[4] We here explored the inactivation of SARS-CoV-2 spiked into human milk by Holder pasteurization to reduce the risk of a possible virus transmission while preserving the milk's beneficial properties.

METHODS

See the Supplemental Information for detailed methods.

Virus Strains

Viral isolates BetaCoV/France/IDF0372/2020 and BetaCoV/Netherlands/01 were obtained through the European Virus Archive Global and BetaCoV/Germany/Ulm/01/2020, BetaCoV/Germany/Ulm/02/2020, and BetaCoV/UKEssen from patient samples.

Milk Samples

Milk samples were obtained from 5 healthy human donors after ethical approval by the Ethics Commission of Hanover Medical School and the Ethics Committee of Ulm University. All mothers provided written informed consent for the collection of samples and subsequent analysis. Milk was collected freshly and stored at −80°C until further use as anonymized samples.

Tissue Culture Infectious Dose 50 End-point Titration

Tissue culture infectious dose 50 ($TCID_{50}$) was determined by infection of *Cercopithecus aethiops*–derived epithelial kidney (Vero E6) or human epithelial colorectal adenocarcinoma (Caco-2) cells. To this end, 20 000 cells were seeded per 96-well plate and, the next day, inoculated with serially diluted samples. For 3 to 6 days, cytopathic effect was monitored, and $TCID_{50}$ per milliliter was calculated according to the Spearman-Karber method.

RESULTS

To test if SARS-CoV-2 retains infectivity in human breast milk and to explore Holder pasteurization as a possible inactivation method, we spiked five different SARS-CoV-2 isolates from

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Dr Pfaender conceptualized and designed the study, organized samples, supervised the project, and drafted the manuscript; Dr Müller conceptualized and designed the study, performed experiments, analyzed data, supervised the project, and drafted the manuscript; Drs Münch and Steinmann conceptualized and designed the study and helped in writing; Mrs Conzelmann, Mr Groß, Mrs Meister, and Drs Todt, Krawczyk, and Dittmer performed experiments, analyzed the data, and critically reviewed the manuscript; Dr Stenger supervised biosafety level 3 work and helped in writing; and all authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

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Germany, France, and the Netherlands 1:10 into five individual breast milk samples or medium samples and incubated them for 30 minutes at room temperature or 63°C. Residual infectivity was determined as $TCID_{50}$ after titration on susceptible cells. All five tested SARS-CoV-2 isolates (BetaCoV/UKEssen, BetaCoV/Germany/Ulm/01/2020, BetaCoV/Germany/Ulm/02/2020, BetaCoV/France/IDF0372/2020, and BetaCoV/Netherlands/01) remained infectious in the milk samples that were incubated for 30 minutes at room temperature, with infectious titers of 0.09 to 1.2 $\times$ 10^5 $TCID_{50}$ per mL (Fig 1). Of note, in each milk sample and independent of the viral strain, we detected a 40.9% to 92.8% decrease of viral titers compared with the medium control. This might indicate an antiviral property of milk potentially due to free fatty acids.[5,6] Importantly, after pasteurization, no residual infectivity was detected in any of the samples (Fig 1). Thus, human breast milk potentially containing infectious SARS-CoV-2 can be efficiently inactivated by using standard Holder pasteurization.

DISCUSSION

Holder pasteurization (heating to 63°C for 30 minutes) is a standard procedure to inactivate viral and bacterial agents while at the same time preserving many beneficial and protective effects of human breast milk. Accumulating evidence reveals that SARS-CoV-2 RNA can be shed into the breast milk of infected mothers.[1–3] Although no transmission events via breastfeeding have been recorded and no infectious virus has been detected, the possibility needs to be considered, and measures providing safety and supporting continued feeding of breast milk need to be evaluated. Thus, we explored the efficiency of Holder pasteurization against SARS-CoV-2 in five milk samples and found that

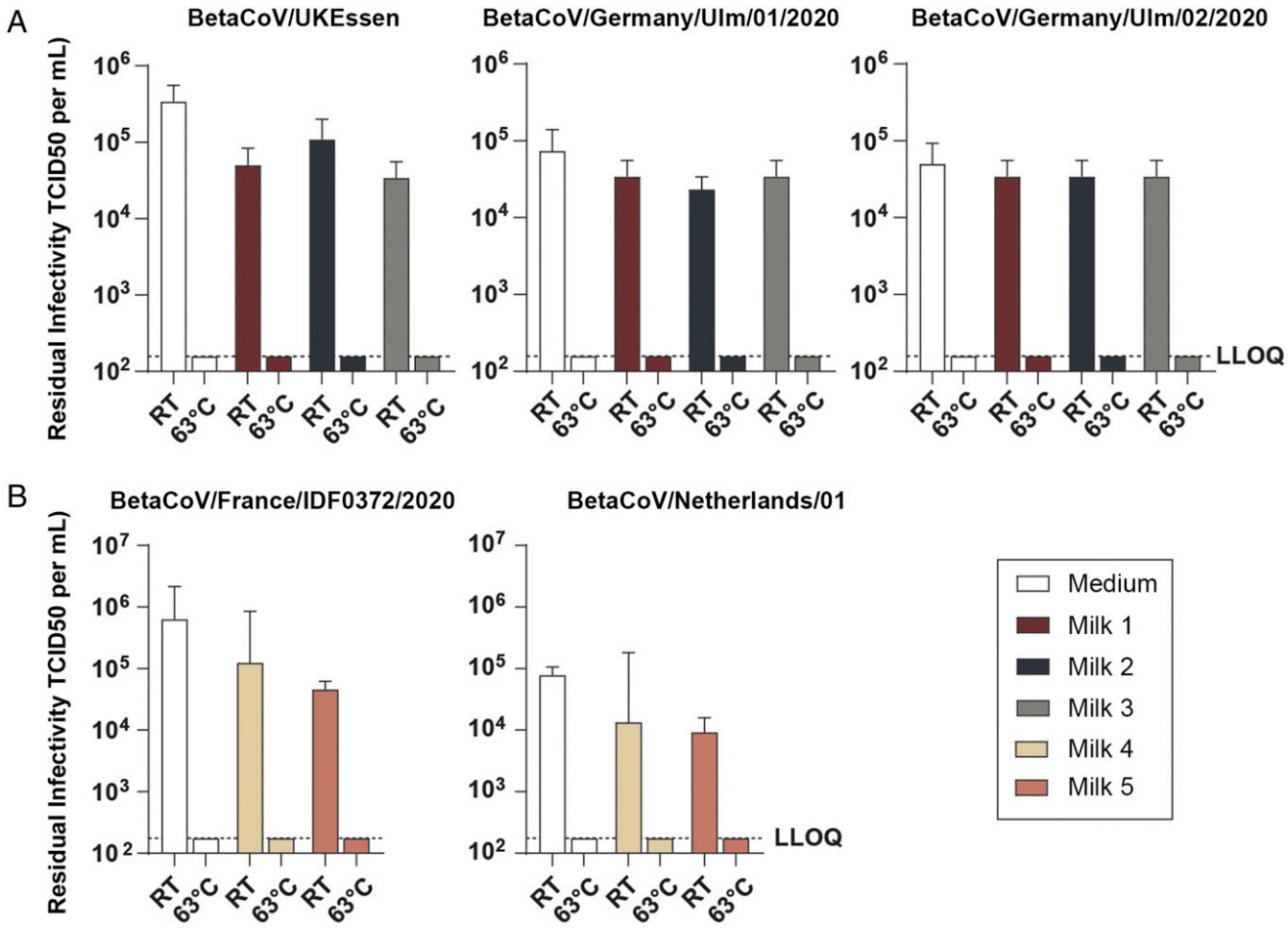

FIGURE 1

Holder pasteurization inactivates SARS-CoV-2 in human breast milk. A, SARS-CoV-2 isolates BetaCoV/UKEssen, BetaCoV/Germany/Ulm/01/2020, and BetaCoV/Germany/Ulm/02/2020 were spiked into a medium or individual breast milk samples from donors 1 to 3. B, Isolates BetaCoV/France/IDF0372/2020 and BetaCoV/Netherlands/01 were spiked into medium or milk samples from donors 4 and 5. Isolates were incubated for 30 minutes at room temperature (RT) or 63°C and titrated onto Vero E6 (A) or Caco-2 (B) cells to determine infectious titers. $TCID_{50}$ was calculated according to the Spearman-Karber method. Data indicate averages and SDs from two (B) or three (A) experiments. LLOQ, lower limit of quantitation (A, 158 $TCID_{50}$ per mL; B, 176 $TCID_{50}$ per mL).

infectivity of all five tested SARS-CoV-2 isolates is completely eliminated by this treatment. This confirms the findings of earlier studies in which Holder pasteurization of milk spiked with three SARS-CoV-2 isolates prevented viral infection of monkey-derived Vero E6 cells.[7–9] We verify the efficiency of Holder pasteurization after inclusion of additional viral strains encompassing the dominant pandemic variant D614G, which reveals increased fitness in cell culture (BetaCoV/Netherlands/01, BetaCoV/UKEssen),[10] as well as highly susceptible Caco-2 cells, which express the viral entry factor transmembrane serine protease 2. Thus, despite no infectious virus having been detected in milk so far, Holder pasteurization provides safety for the infant and in milk banks and reassurance for the mother, who might consider discontinuing breastfeeding and substituting for infant formula milk, which lacks many of human milk's important components.

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ABBREVIATIONS

Caco-2: human epithelial colorectal adenocarcinoma
SARS-CoV-2: severe acute respiratory syndrome coronavirus 2
$TCID_{50}$: tissue culture infectious dose 50
Vero E6: *Cercopithecus aethiops*–derived epithelial kidney

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REFERENCES

1. Groß R, Conzelmann C, Müller JA, et al. Detection of SARS-CoV-2 in human breastmilk. *Lancet*. 2020;395(10239):1757–1758
2. Tam PCK, Ly KM, Kernich ML, et al. Detectable severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in human breast milk of a mildly symptomatic patient with coronavirus disease 2019 (COVID-19) [published online ahead of print May 30, 2020]. *Clin Infect Dis*. doi:10.1093/cid/ciaa673
3. John Hopkins University. COVID-19, maternal and child health, nutrition. 2020. Available at: http://hopkinshumanitarianhealth.org/empower/advocacy/covid-19/covid-19-children-and-nutrition/. Accessed September 9, 2020
4. World Health Organization. Clinical management of COVID-19: interim guidance. 2020. Available at: https://www.who.int/publications/i/item/clinical-management-of-severe-acute-respiratory-infection-when-novel-coronavirus-(ncov)-infection-is-suspected. Accessed September 9, 2020
5. Pfaender S, Heyden J, Friesland M, et al. Inactivation of hepatitis C virus infectivity by human breast milk. *J Infect Dis*. 2013;208(12):1943–1952
6. Conzelmann C, Zou M, Groß R, et al. Storage-dependent generation of potent anti-ZIKV activity in human breast milk. *Viruses*. 2019;11(7):591
7. Chambers C, Krogstad P, Bertrand K, et al. Evaluation for SARS-CoV-2 in breast milk from 18 infected women. *JAMA*. 2020;324(13):1347–1348
8. Walker GJ, Clifford V, Bansal N, et al. SARS-CoV-2 in human milk is inactivated by Holder pasteurisation but not cold storage [published online ahead of print August 7, 2020]. *J Paediatr Child Health*. doi:10.1111/jpc.15065
9. Unger S, Christie-Holmes N, Guvenc F, et al. Holder pasteurization of donated human milk is effective in inactivating SARS-CoV-2. *CMAJ*. 2020;192(31):E871–E874
10. Korber B, Fischer WM, Gnanakaran S, et al.; Sheffield COVID-19 Genomics Group. Tracking changes in SARS-CoV-2 spike: evidence that D614G increases infectivity of the COVID-19 virus. *Cell*. 2020;182(4):812–827.e19

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