

1 **Holder Pasteurization Inactivates SARS-CoV-2 in Human Breast Milk**

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21 **Abstract**

22 SARS-CoV-2 RNA has been detected in the human breast milk of infected mothers, raising
23 concerns regarding the safety of breastfeeding upon infection. We here show that holder
24 pasteurization inactivates SARS-CoV-2 and provides an alternative and safe option for
25 infected mothers to continue feeding breast milk to their infants.

26 **Introduction**

27 The current coronavirus pandemic, caused by the severe acute respiratory syndrome
28 coronavirus 2 (SARS-CoV-2), raises unprecedented questions regarding virus transmission
29 and risks for pregnant or breastfeeding women. We and others recently described that SARS-
30 CoV-2 is detectable in breast milk of infected mothers ¹⁻⁵ and found viral RNA in single or
31 multiple breast milk samples of mothers suffering from coronavirus disease 2019 (COVID-
32 19). In two cases where the mother continued breastfeeding, the newborns were also tested
33 positive for SARS-CoV-2 and one infant developed severe respiratory disease ^{1,5}. However,
34 the origin of the infections of the newborns remained unclear and raises concerns of possible
35 virus transmission via breast milk.

36 The safety and feasibility of breastfeeding is of high importance as breast milk contains
37 nutrients, hormones, and immunoprotective entities that are essential for the development,
38 health, and protection of the neonate from infections ⁶. So far, the World Health Organization
39 (WHO) recommends to continue breastfeeding upon SARS-CoV-2 infection of the mother
40 while taking measures of strict hygiene and wearing masks to protect the child from droplets
41 or aerosols ⁷. The Centers for Disease Control and Prevention (CDC) states that the decision
42 for breastfeeding lays with the mother, family and health care providers ⁸. Generally, stopping
43 breastfeeding is not advised, however, given the recent detection of SARS-CoV-2 in breast
44 milk samples, a possible transmission of the virus via breastfeeding cannot be ruled out.
45 Therefore, measures to provide safety of infants are urgently required. Thus, we here explored
46 the inactivation of SARS-CoV-2 in human milk by holder pasteurization (heating to 63°C for
47 30 minutes) to reduce the risk of a possible virus transmission while preserving many of
48 milk's beneficial properties.

49

50 **Results**

51 To test if SARS-CoV-2 retains infectivity in human breast milk and to explore holder
52 pasteurization as a possible inactivation method, we spiked five different SARS-CoV-2
53 isolates from Germany, France and the Netherlands into five individual breast milk samples,
54 and incubated them for 30 minutes at room temperature or 63°C. Samples were then titrated
55 on cells and residual infectivity determined as tissue culture infectious dose 50 (TCID₅₀). All
56 five tested SARS-CoV-2 isolates (UKEssen, Ulm/01, Ulm/02, France/IDF0372,
57 Netherlands/01) remained infectious in the milk samples that were incubated for 30 minutes at
58 room temperature, with infectious titers of 0.09 to 1.2×10^5 TCID₅₀/mL (**Figure 1A, B**). Of
59 note, in each milk sample, we detected a 40.9 – 92.8% decrease of viral titers compared to the
60 medium control. This was independent of the viral strain, suggesting that this partial
61 inactivation of virus is an intrinsic property of human milk, as previously described for many
62 enveloped viruses including hepatitis C and Zika virus^{9–11}. Importantly, upon pasteurization,
63 no residual infectivity was detected in any of the samples (**Figure 1A, B**). Thus, human breast
64 milk containing infectious SARS-CoV-2 can be efficiently inactivated using standard holder
65 pasteurization.

66

67 **Discussion**

68 Breastfeeding provides many health benefits and is therefore recommended as the optimal
69 feeding option for infants. Currently, the WHO recommends that SARS-CoV-2 infected
70 mothers continue breastfeeding while taking measures of strict hygiene to protect the child
71 from droplets or aerosols⁷. As initial reports failed to detect the presence of viral RNA in
72 milk samples of infected mothers^{12,13}, the risk of transmission via breastfeeding was deemed
73 to be very low. Recent reports, however, demonstrate that SARS-CoV-2 RNA can be detected
74 in breast milk samples after virus infection, associated with at least one newborn with severe
75 respiratory disease^{1–5}. These findings raise the concern of safety during breastfeeding and a

76 possible alternative or additional way of virus transmission to the infant. Holder
77 pasteurization (heating to 63°C for 30 minutes) has been used for a long time to inactivate
78 viral and bacterial agents, while at the same time preserving the many beneficial and
79 protective effects of human breast milk ⁶. Here, we evaluated holder pasteurization as an easy
80 and inexpensive methods to inactivate infectious SARS-CoV-2 in breast milk. Our data show
81 that independent of the tested SARS-CoV-2 isolates or the breast milk sample, viral
82 infectivity is completely eliminated by this treatment. Thus, holder pasteurization provides
83 safety for the infant and reassurance for the mother, who might consider discontinuing
84 breastfeeding and substituting for infant formula milk that lacks many of the human milk's
85 important components. In conclusion, we here provide a safe and feasible option, when in
86 doubt, to continue feeding of the infant with breast milk upon symptomatic SARS-CoV-2
87 infection of the mother.

88

89 **Methods**

90 **Cell culture.** Vero E6 (*Cercopithecus aethiops* derived epithelial kidney) cells were grown in
91 Dulbecco's modified Eagle's medium (DMEM, Gibco) which was supplemented with 2.5%
92 heat-inactivated fetal calf serum (FCS), 100 units/mL penicillin, 100 µg/mL streptomycin, 2
93 mM L-glutamine, 1 mM sodium pyruvate, and 1x non-essential amino acids (DMEM
94 complete). Caco-2 (human epithelial colorectal adenocarcinoma) cells were grown in DMEM
95 complete but with supplementation of 10% FCS. All cells were grown at 37°C in a 5% CO₂
96 humidified incubator.

97 **Virus strains and virus propagation.** Viral isolate BetaCoV/France/IDF0372/2020 and
98 BetaCoV/Netherlands/01 were obtained through the European Virus Archive global. The viral
99 isolates BetaCoV/Germany/Ulm/01/2020, BetaCoV/Germany/Ulm/02/2020 and UKEssen
100 were obtained from patient samples. Virus was propagated by inoculation of 70% confluent
101 Vero E6 cells in 75 cm² cell culture flasks with 100 µl SARS-CoV-2 isolates in 3.5 ml serum-

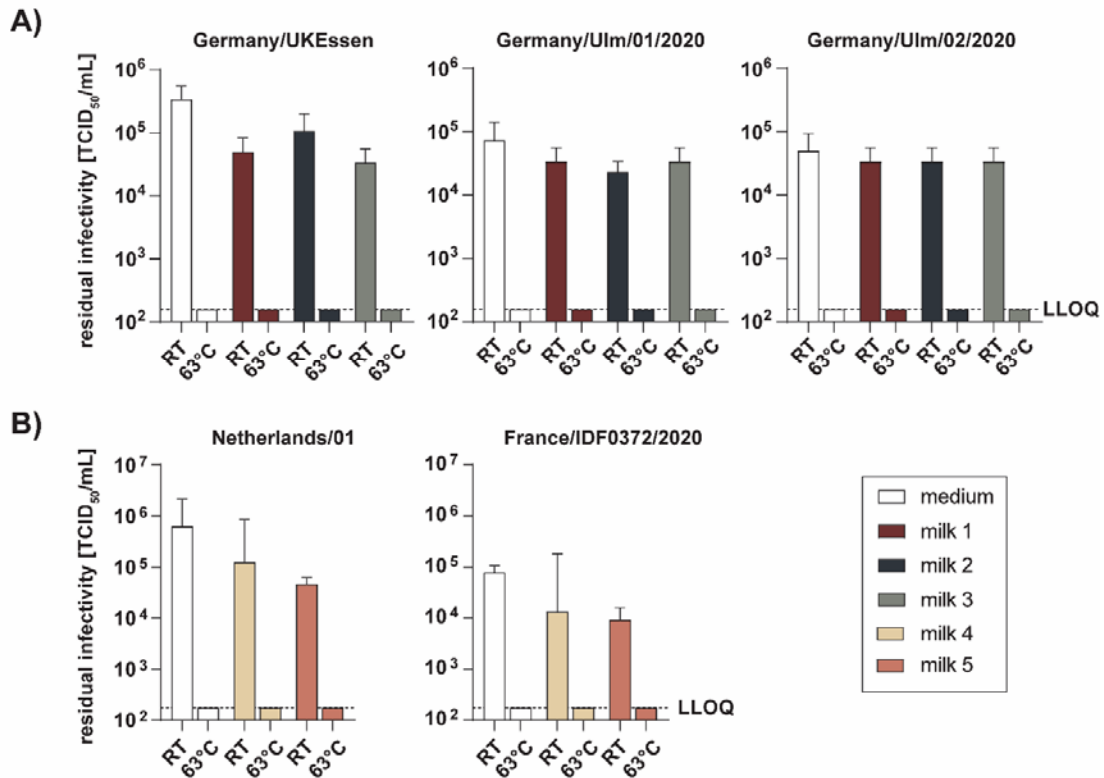
102 free medium containing 1 $\mu\text{g/mL}$ trypsin. Cells were incubated for 2 h at 37°C, before adding
103 20 ml medium containing 15 mM HEPES. Supernatant was harvested at day 3 post
104 inoculation when a strong cytopathic effect (CPE) was visible. Supernatants were centrifuged
105 for 5 min at 1,000 \times g to remove cellular debris, and then aliquoted and stored at -80°C as
106 virus stocks. Infectious virus titer was determined as plaque forming units or TCID₅₀/mL.

107 **Milk samples.** Milk samples were obtained from five healthy human donors after ethical
108 approval by the ethics commission of Hanover Medical School, Hanover, Germany and the
109 ethics committee of Ulm University. All mothers provided written informed consent for the
110 collection of samples and subsequent analysis. Milk was collected freshly and stored at -80°C
111 until further use as anonymized samples.

112 **TCID₅₀ endpoint titration.** To determine the tissue culture infectious dose 50 (TCID₅₀),
113 virus stocks or samples were serially diluted and used to inoculate Vero E6 or Caco-2 cells.
114 To this end, 20,000 cells were seeded per well in 96 flat bottom well plates and incubated
115 over night. Cells were infected with SARS-CoV-2 in serial dilutions and incubated for 3-6
116 days and monitored for CPE. TCID₅₀/mL was calculated according to Spearman-Kärber.

117

Figure 1



118

119

120 **Figure legends:**

121 **Figure 1. Holder Pasteurization inactivates SARS-CoV-2 in human breast milk.** SARS-
122 CoV-2 isolates UKEssen, Ulm/01, or Ulm/02 were spiked into medium or individual breast
123 milk samples from donors 1-3 (A), or isolates France/IDF0372 and Netherlands/01 into milk
124 samples from donors 4-5 (B), incubated for 30 minutes at room temperature (RT) or 63°C and
125 titrated onto Vero E6 (A) or Caco-2 (B) cells to determine infectious titers. Tissue culture
126 infectious dose 50 (TCID₅₀) was calculated according to Spearman-Kärber. Data indicate
127 averages and standard deviation from two (B) or three (A) experiments. LLOQ lower limit of
128 quantitation (A, 158 TCID₅₀/mL; B, 176 TCID₅₀/mL).

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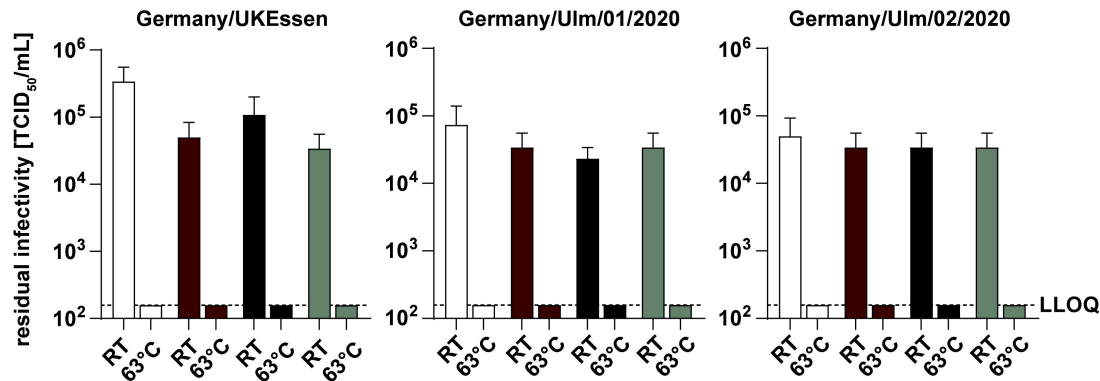
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Figure 1**A)****B)**