

## PEER REVIEW HISTORY

BMJ Paediatrics Open publishes all reviews undertaken for accepted manuscripts. Reviewers are asked to complete a checklist review form and are provided with free text boxes to elaborate on their assessment. These free text comments are reproduced below.

### ARTICLE DETAILS

<b>TITLE (PROVISIONAL)</b>	Excretion of SARS-CoV-2 in breast milk - a single centre observational study
<b>AUTHORS</b>	Prasad, Arun N, Yankappa Kumar, Pradeep Chaudhary, Bhavesh Kant Pati, Binod Kumar Anant, Monika

### VERSION 1 – REVIEW

<b>REVIEWER</b>	Reviewer name: Dr. Melisa Medina-Rivera Institution and Country: Cornell Univ, United Kingdom of Great Britain and Northern Ireland Competing interests: None
<b>REVIEW RETURNED</b>	30-Mar-2021

<b>GENERAL COMMENTS</b>	<p>Review for manuscript bmjpo-2021-001087: Excretion of SARS-CoV-2 in breastmilk of 50 infected mothers and outcome of neonates delivered and breastfed by them.</p> <p>I. Summary: This manuscript describes an observational study of 50 expectant mothers with positive SARS-CoV-2 infection, a known culprit of COVID-19. The authors took nasopharyngeal swaps from both mother and neonate alongside breastmilk samples to evaluate the presence of SARS-CoV-2 via RT-PCR.</p> <p>The narrative suggests that the main goals of this manuscript are to:</p> <ol style="list-style-type: none"><li>1. Detect for the excretion of SARS-CoV-2 in breastmilk</li><li>2. Assess the health of breastfed infants, whose mother was positive to SARS-CoV-2 during pregnancy</li></ol> <p>Corresponding to aim1, the authors collected breastmilk samples from mothers that tested positive to SARS-CoV-2 during pregnancy for their assessment via RT-PCR. Breastmilk samples were collected 7 days post-partum.</p> <p>On aim 2, two nasopharyngeal samples were collected from neonates at birth (24 and 48hr) and assessed via RT-PCR. If able to feed, the neonates were fed with expressed breastmilk. Maternal and neonate's symptoms were recorded during the hospital stay and follow-up by phone up to one month after hospital discharge.</p> <p>II. Paper Strengths and contributions to the field: The authors sought to investigate the possible excretion of SARS-CoV-2 within breastmilk samples from COVID-19 positive women and the health of infants up to 1 month follow-up. Such information is of vital importance to further define potential patterns of viral vertical transmission and to inform breastfeeding guidelines.</p> <p>III. Major Concerns:</p> <ul style="list-style-type: none"><li>• From the manuscript it is not clear the timeline of when COVID-19</li></ul>
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	<p>maternal symptom onset and diagnosis relative to the birth and breastmilk samples collection. This brings problems of interpretation of results and limitations with respect to viral detection within a timeline where RNA, if present in the breastmilk sample, can actually be detected via RT-PCR. Moreover, even if detected by RT-PCR, defining RNA presence within a breastmilk sample is not sufficient to define breastmilk samples as infective. Other tests such as viral culturing are still needed.</p> <ul style="list-style-type: none"> <li>• There is an incomplete description of expressed milk collection and breastfeeding practices.</li> <li>• Other potential routes of SARS-CoV-2 transmission were not evaluated or considered.</li> </ul> <p>IV. Additional comments following the structure of the paper</p> <p>Abstract:</p> <ul style="list-style-type: none"> <li>• Disclosing abbreviations: LSCS</li> <li>• Method of SARS-CoV-2 detection is missing in this section</li> </ul> <p>Introduction:</p> <ul style="list-style-type: none"> <li>• Appropriate</li> </ul> <p>Methods:</p> <ul style="list-style-type: none"> <li>• The description of sample collection is somewhat limited. Was the mother using recommended guidelines (as described in the introduction) to express her breastmilk? Were the breast pumps provided at the clinic? If so, cleaning between collections?</li> <li>• Include a better description of the analytical performance of RT-PCR assay employed. Was it commercially available or made at the clinic?</li> </ul> <p>Results:</p> <ul style="list-style-type: none"> <li>• First table with demographic information is missing. General characteristics of study participants are included in two tables; however, it would be more useful to include a summary table. The existing tables could stay as supplementary data.</li> <li>• I would suggest including a graphical representation that includes maternal symptom onset (if any) or suspected disease, and sample collection with respect to birth.</li> <li>• How many neonates were breastfed during or after the hospital stay? Where there any mix-fed (formula + breastmilk)? If so, how many for how long?</li> <li>• I found interesting that 2 out of the 50 infants that were positive for SARS-CoV-2 infection were receiving formula. Could the authors elaborate in the discussion of how these two children might have contracted COVID-19?</li> <li>• Please provide a better description of the follow-up interview/measurements. Also, during follow-up, was the child with the cold assessed for COVID-19?</li> <li>• If breastmilk samples are still available it would be interesting to also measure antibody levels within the samples.</li> </ul> <p>Discussion:</p> <ul style="list-style-type: none"> <li>• In the last update from the WHO (December 23, 2020) there were 67 published articles where breastmilk was assessed for the presence of SARS-CoV-2. These articles include a total of 413 breastmilk samples that were assessed via RT-PCR and 72 samples that were assessed for anti-SARS-CoV-2 antibodies via ELISA. The systematic analysis of this data found that only 13 out of the 423 breastmilk samples were positive, suggesting that vertical transmission of SARS-CoV-2 is not common. Noteworthy, a big limitation on interpreting this data was that many reports did not include information on breastfeeding practices, which is as important as measuring the presence or absence of the virus. Another limitation is the lack of measuring/reporting other possible routes of viral transmission. Pertaining to this manuscript, this reviewer agrees with the authors in that the excretion of the virus through</li> </ul>
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	<p>breastmilk could suggest potential vertical transmission. However, as the discussion is drafted it is misleading. RT-PCR data by itself is not sufficient evidence to support or discard the possibility of viral transmission through breastmilk.</p> <ul style="list-style-type: none"> <li>• Study limitations should be expanded.</li> </ul> <p>Other materials:</p> <ul style="list-style-type: none"> <li>• Figures are missing, not clearly labeled, or too small for viewing.</li> </ul> <p>V. Other minor issues:</p> <ul style="list-style-type: none"> <li>• Spelling SARS-Cov-2, breast milk or breastmilk</li> <li>• Spelling misuse of capitalizing words throughout the document</li> <li>• This reviewer suggests rephrasing the following sentence as it is misleading (symptoms are not COVID-19 related). "Overall, 8/51(16%) neonates were symptomatic, Birth asphyxia being most common symptom (4, 8%) followed by Neonatal hyperbilirubinemia (2, 4%), Abdominal distension due to Mesenteric cyst (1, 2%), and transient feed intolerance (1, 2%)."</li> </ul> <p>VI. Reviewer's conclusion:</p> <p>Based on the narrative and the goals that the author set out to accomplish, this manuscript successfully describes the 50 pregnant women positive pregnant women with SARS-CoV-2, assesses their breastmilk for the presence of RNA, and observes infant outcomes. This manuscript, however, could be improved by establishing a clear timeline between disease onset, and sample collection between mothers and infants. Also, the result section could be significantly improved by providing a better description of breastfeeding practices, re-sizing the figures/labels, and, if still available, performing additional analysis on the samples. Further elaborating in these areas might help not only to put this study into context with relevant literature but also include a broader picture of potential viral transmission routes.</p>
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<b>REVIEWER</b>	<p>Reviewer name: Dr. Harshil Bhatt Institution and Country: Goshen Hospital, United States Competing interests: None</p>
<b>REVIEW RETURNED</b>	28-Mar-2021

<b>GENERAL COMMENTS</b>	<ol style="list-style-type: none"> <li>1. Minor suggestion: The title might suggest that the study is about 50 mothers already positive with SARS-CoV-2 in the breastmilk. You can look into changing the title accordingly. "Excretion of SARS-CoV-2 in breastmilk....." vs "Study for evaluating excretion....." or something similar.</li> <li>2. Abstract – unexplained acronym in abstract – LSCS, consider writing the full name instead.</li> <li>3. 6/21, line 26- "We also assessed the health condition of babies at ≥ 1 month of their age.." The duration of this follow-up is actually missing from the abstract. You could mention in the abstract that follow-up was done at more than one month of age.</li> <li>4. Minor – SARS-CoV-2 is a correct word/format to use, SARS-Cov-2 is not.</li> <li>5. Minor - RT-PCR word should be described when using it for the first time in the manuscript.</li> <li>6. I believe readers will appreciate if in the abstract/methods it is mentioned that the newborns were also screened by RT-PCR, and also twice (within 24 hours and another after 24 hours).</li> <li>7. Neonates were fed with EBM from the mothers. (7/21, line 31) It means the neonates did not have any direct contact with their mothers (for breastfeeding). Mention if it is true.</li> <li>8. 7/21, line 40 – Were mothers advised to continue expressed breast milk feeding only, or were they told to do contact breastfeeding after discharge?</li> </ol>
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	<p>9. Figures were not numbered at the end of the file, making it difficult to identify them according to the numbers mentioned in the text.</p> <p>10. Discussion – Well-written with the examination of other studies and comparing the study with other authors. But still, some of the points mentioned in the discussion are more suitable for the results section and in fact are already mentioned in the results. Hence, the discussion could be more streamlined to include only the inference of the findings and authors' interpretation but rather not the findings/results themselves with data/numbers (example – line 46 onwards).</p> <p>11. "Findings of our study supports advocating the policy of breastfeeding by SARS-CoV-2 positive mothers, with due precaution....." Please specify what due precautions you are referring to – is it the safety measures, or using expressed breastmilk only, etc... Are you advocating for maternal-child contact for breastfeeding or what would be your advice on that based on your research? Kindly specify.</p> <p>Thank you for presenting this comprehensive study.</p> <p>Regards.</p>
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<b>REVIEWER</b>	<p>Reviewer name: Dr. aakash pandita</p> <p>Institution and Country: Sanjay Gandhi Post Graduate Institute of Medical Sciences, India</p> <p>Competing interests: None</p>
<b>REVIEW RETURNED</b>	05-Apr-2021

<b>GENERAL COMMENTS</b>	<p>A very good study from India but requires few clarifications</p> <ol style="list-style-type: none"> <li>1. Testing breast milk sample once is not sufficient that to over a wide range of 7 days as we know that excretion in breast milk is intermittent</li> <li>2. Was it a prospective or a retrospective study ?</li> <li>3. How many covid positive moms were delivered during study period which is not mentioned?</li> <li>4. Did you admit all neonates born to covid positive mothers?</li> <li>5. Bed strength of NICU?</li> <li>6. Birth asphyxia being most common symptom (4, 8%) followed by Neonatal hyperbilirubinemia (2, 4%), Abdominal distension due to Mesenteric cyst (1, 2%).....these cannot be symptoms of neonatal covid</li> <li>7. Were all the babies exclusive breast fed till discharge ?</li> <li>8. How many mothers were symptomatic and sick?</li> <li>9. What happened to feeding of such mothers?</li> <li>10. No clinical characteristic of neonates are provided</li> <li>11. The manuscript needs a thorough revision for language and syntax errors</li> </ol>
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<b>REVIEWER</b>	<p>Reviewer name: Dr. Stephanie Pfaender</p> <p>Institution and Country: Department of Molecular and Medical Virology, United Kingdom of Great Britain and Northern Ireland</p> <p>Competing interests: None</p>
<b>REVIEW RETURNED</b>	23-Mar-2021

<b>GENERAL COMMENTS</b>	<p>In this study the authors analyzed the presence of SARS-CoV-2 RNA in the breast milk of infected mothers to evaluate the potential risk of breastfeeding for the transmission of SARS-CoV-2. From their study cohort (n=50), no evidence for the presence of SARS-CoV-2 RNA in the breast milk was detected. In general, this study supports the current opinion that breastfeeding does not increase the risk for the infant to become infected with possible benefits clearly</p>
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	<p>outweighing potential risks. It thereby contributes to an increased understanding about possible transmission routes and supports current recommendations. However, there are several concerns. One of the major limitations concerns the timepoint of milk sampling. It is not clear if the mothers were tested positive at the time point of milk sampling. The authors state that inclusion criteria involve diagnosed SARS-CoV-2 infection within 10 preceding days of delivery and collection of milk samples within 7 days of delivery, at which time point the infection could have been resolved. Please clarify and if necessary, re-phrase. Furthermore, there are no details given regarding the material and methods (RNA extraction, RT-PCR). As there are several technical issues associated with RNA extraction from milk samples, including decreased RNA recovery in the presence of milk, specific controls should be included to avoid false negative reports. In addition, the viral loads of the infected mothers should be added in the table as this could potentially influence viral shedding.</p> <p>Minor points: The title does not support the conclusion of the study and should be re-phrased. Language editing throughout the manuscript would be required.</p>
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<b>REVIEWER</b>	<p>Reviewer name: Dr. Richa Mukhra Institution and Country: Department of Anthropology (UGC Centre of Advanced Study), Panjab University, India Competing interests: None</p>
<b>REVIEW RETURNED</b>	25-Mar-2021

<b>GENERAL COMMENTS</b>	<p>This article is well done; the research is well designed and the questions underneath it is well formulated. It is true, that only few studies have focused on Excretion of SARS-CoV-2 in breast milk, therefore the results of present manuscript could contribute in the knowledge. Such studies should be promoted and researchers should be encouraged to work of such arenas of SARS-CoV-2 so that awareness among the individuals, especially among the infected expectant mothers should rise so that the neonates should not be deprived of the nourishment from the breastmilk. I would recommend the article for publication, but not in the present form. There are few issues that the authors need to reconsider and carefully revise before publication.</p> <ol style="list-style-type: none"> <li>1. The introduction part of the manuscript must be more elaborated with the existing knowledge. The guidelines must be elaborated in brief to get an overview of the present scenario.</li> <li>2. In case of twins, any difference was found in the transmission of virus?</li> <li>3. what was the criteria of the telephonic follow up after discharge?</li> <li>4. The manuscript states that after the follow up of 37 respondents it was found that the neonates were found to be asymptomatic. What if they were COVID positive but asymptomatic in nature? Was there any criteria to cover that part of the examination?</li> <li>5. the references were found to be dated from the month of August 2020. I would recommend to update the references with new references. Consider in-text and end-references thoroughly.</li> <li>6. I also suggest passing the manuscript from Plagiarism check software.</li> </ol>
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<b>REVIEWER</b>	<p>Reviewer name: Dr. Jim Thornton Institution and Country: University of Nottingham, United Kingdom of Great Britain and Northern Ireland Competing interests: None</p>
<b>REVIEW RETURNED</b>	18-Mar-2021

<b>GENERAL COMMENTS</b>	<p>Important topic. It has already been shown that SARS-CoV-2 is not (or rarely) transmitted in breast milk</p>
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	<p>e.g. Chambers C, Krogstad P, Bertrand K, et al. Evaluation for SARS-CoV-2 in Breast Milk From 18 Infected Women. JAMA. Published online August 19, 2020. doi:10.1001/jama.2020.15580</p> <p>Bertino E, Moro GE, De Renzi G, et al. Detection of SARS-CoV-2 in Milk From COVID-19 Positive Mothers and Follow-Up of Their Infants. Front Pediatr. 2020;8:597699. Published 2020 Oct 27. doi:10.3389/fped.2020.597699</p> <p>Gao X, Wang S, Zeng W, Chen S, Wu J, Lin X, Liu Y, Sun Z, Feng L. Clinical and immunologic features among COVID-19-affected mother-infant pairs: antibodies to SARS-CoV-2 detected in breast milk. New Microbes New Infect. 2020 Sep;37:100752. doi: 10.1016/j.nmni.2020.100752. Epub 2020 Sep 1. PMID: 32904990; PMCID: PMC7462625.</p> <p>Costa S, Posteraro B, Marchetti S, Tamburrini E, Carducci B, Lanzone A, Valentini P, Buonsenso D, Sanguinetti M, Vento G, Cattani P. Excretion of SARS-CoV-2 in human breast milk. Clin Microbiol Infect. 2020 Oct;26(10):1430-1432. doi: 10.1016/j.cmi.2020.05.027. Epub 2020 Jun 2. PMID: 32502644; PMCID: PMC7266588.</p> <p>However, occasional case reports keep appearing suggesting that it occasionally is. So worth have more series reporting the negative.</p> <p>I'm not an expert of the PCR assay used.</p> <p>Minor points. Tables 1 and 2 should be removed.</p> <p>The figures are not labelled but suggest remove figures 2-6. they all repeat data in tables.</p>
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<b>REVIEWER</b>	<p>Reviewer name: Dr. Ryan Pace</p> <p>Institution and Country: University of Idaho, United States</p> <p>Competing interests: None</p>
<b>REVIEW RETURNED</b>	03-Apr-2021

<b>GENERAL COMMENTS</b>	<p><b>Summary</b></p> <p>I appreciate the opportunity to review the manuscript submitted by Prasad et al. In the submitted work the authors present their findings on whether SARS-CoV-2 is present in milk produced by 50 women with COVID-19 and the outcomes of their infants. Milk samples were tested for SARS-CoV-2 using RT-qPCR and all milk samples tested negative. Importantly, outcomes of infants that were breastfed were generally well. Overall, this is an interesting topic and to my knowledge this is the first report analyzing milk produced by women in India for the presence of SARS-CoV-2.</p> <p><b>General comments</b></p> <p>I have a couple comments on terminology used throughout the current manuscript that the authors may consider. I recommend that the authors substitute "human milk" in place of "breast milk" and "breastmilk" throughout the manuscript, as "human milk" is the preferred term among human milk and lactation researchers. There is also inconsistent usage of "breast milk", "breastmilk", breastfeeding, and "breast-feeding" throughout the manuscript. Additionally, the term "excretion" is used throughout the manuscript to refer to the putative presence of SARS-CoV-2 in human milk and I do not think that it is the correct word to use in this situation. First, the definition of "excretion" is the process of eliminating or expelling waste matter. Currently, there is no evidence that SARS-CoV-2 is excreted in milk (as supported by this study's findings). In contrast, most studies to date have failed to detect SARS-CoV-2 in</p>
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	<p>human milk, and in the only study to date to examine milk (that tested positive for SARS-CoV-2 RNA) for replication competent virus found no viable virus (doi: 10.1001/jama.2020.15580). Further, the use of "excretion" in the current title may mislead readers into presuming that the authors detected SARS-CoV-2 in the milk samples tested; however, this is not what the authors found or report in the manuscript. Perhaps a better word is "detection", as this relates to what the present work (and described in the objective of the abstract), or more specifically a "lack of detection" of SARS-CoV-2 in milk, e.g., "Lack of detection of SARS-CoV-2 in human milk of 50 infected mothers and outcome of neonates delivered and breast fed by them".</p> <p>The manuscript would be greatly improved by additional proof-reading and editing for spelling and grammar mistakes, as well as consistency in names (e.g., "health" in "World Health Organization" should be capitalized. Additionally, the current Introduction would be improved with some restructuring. For example, the second paragraph details that it is not well known whether transmission of SARS-CoV-2 occurs via human milk. This is then contradicted in the following paragraph that UNICEF has reported this not to be the case and that breastfeeding is recommended (and is also missing a reference).</p> <p>Many important details are also missing on how milk samples were collected and tested, e.g.:</p> <ol style="list-style-type: none"> <li>1) Did mothers wash their hands, wear gloves and/or masks during milk collections?</li> <li>2) How was the milk collected, via manual expression or with an electric pump?</li> <li>3) How were milk samples stored?</li> <li>4) How were milk samples tested? All that is stated is that detection of viral RNA was done via RT-PCR. Was the test validated for use with human milk?</li> </ol> <p>These are all critical pieces of information that is necessary to assess the results and conclusions of the study.</p> <p>A novel aspect of this work that the authors should also consider highlighting is that most studies on this topic have been on North American populations and that similar data from more diverse populations, including India, have been lacking.</p> <p>Should these revisions be made I believe this manuscript would be a valuable contribution to the literature and to our understanding of maternal and infant health.</p> <p>Minor comments</p> <p>After being first defined, WHO is used interchangeably with the full name (World Health Organization) throughout the manuscript; once defined it is appropriate to just use WHO.</p> <p>I would recommend not using "babies" and instead use "neonate" and/or "infant" instead.</p> <p>Figure 2 is very difficult to interpret as the size and resolution are very low. The resolution of Both table 1 and 2 is also too low.</p>
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## VERSION 1 – AUTHOR RESPONSE

Amazed to see such an extensive and meticulous review by 7 learned reviewers. Thanks to all of them for providing necessary comments for improving my manuscript draft. All the issues have been taken care of, as advised. We request you for an early publication.



Kindly find pointwise response to reviewers' comment-

Reviewer: 1

Dr. Jim Thornton

### Comments to the Author

Important topic. It has already been shown that SARS-CoV-2 is not (or rarely) transmitted in breast milk

1.Minor points. Tables 1 and 2 should be removed.

Thank you for the review. Tables 1 and 2 have been removed from the revised manuscript.

2.The figures are not labelled but suggest remove figures 2-6. they all repeat data in tables.

Thank you for the comment. The figures are labelled now. As the figures are very useful for the easy interpretation of the study, it has been decided to keep the figures in the revised manuscript.

Reviewer: 2

Dr. Stephanie Pfaender

### Comments to the Author

1.It is not clear if the mothers were tested positive at the time point of milk sampling.

Mothers coming with RT-PCR positive report in our dedicated COVID hospital, were tested again through RT-PCR for presence of viral RNA in their NPS, at the time of admission, as per our institutional protocol. Those tested positive were included in the study. All mothers delivered within a period of 3 days from the day they were admitted. All milk samples were obtained within 4 days of delivery. Considering the less time gap (Maximum 7 days) between the previous NPS collection milk sample collection, mothers were not tested again with NPS at the point of milk sampling.

2.The authors state that inclusion criteria involve diagnosed SARS-CoV-2 infection within 10 preceding days of delivery and collection of milk samples within 7 days of delivery, at which time point the infection could have been resolved. Please clarify and if necessary, re-phrase.

Kindly refer above paragraph for clarification. Re-phrasing has been done in the manuscript also as- "Informed consent for participation in the study was taken from expectant mothers, coming for delivery who were positive for the presence of viral ribonucleic acid (RNA) in their NPS by RT- PCR, within 10 preceding days of delivery and at the time of hospital admission".

3.Furthermore, there are no details given regarding the material and methods (RNA extraction, RT-PCR). As there are several technical issues associated with RNA extraction from milk samples, including decreased RNA recovery in the presence of milk, specific controls should be included to avoid false-negative reports. In addition, the viral loads of the infected mothers should be added to the table as this could potentially influence viral shedding.

RT-qPCR

Qualitative real time reverse transcriptase PCR was performed by using one step real time PCR reaction mixture and primer-probe mixture approved by Indian council of Medical research (ICMR), Govt of India. Different kits were used during the period of the study depending on the availability because of the huge turnover during the pandemic. However, all of the real time PCR kits used were capable to amplify at least one screening and one confirmatory gene. In addition, primer-probe detecting an internal control gene (human RNase P) was also included in all samples. All the patients' samples are run along with a positive control and a no template control for validation of the run. All real time RT PCR were performed in either Biorad CFX96 or ABI Quant studio 5 Dx machine with reaction protocol set as per instruction in the kit insert.

We could not include viral loads of mothers. This has been one the limitations of our study.

4.Minor points: The title does not support the conclusion of the study and should be re-phrased.

Many thanks for the suggestion. The title of the study is now re-phrased as "Lack of detection of SARS-CoV-2 in the breast milk of 50 infected mothers and clinical outcome of neonates breastfed by them: A prospective observational study"

5.Language editing throughout the manuscript would be required.

Thank you for the comment. The manuscript is thoroughly reviewed for language editing and necessary correction are done. If any editing is still required, kindly consider it doing from your end.



Reviewer: 3

Dr. Richa Mukhra

### Comments to the Author

1. The introduction part of the manuscript must be more elaborated on with the existing knowledge. The guidelines must be elaborated in brief to get an overview of the present scenario.

Thank you for the comment. It is considered to keep the same content in introduction with adequate details as per further review comments and suggestions,

2. In the case of twins, any difference was found in the transmission of the virus?

No, both twins were asymptomatic and negative for SARS-CoV-2 infection.

3. What were the criteria of the telephonic follow-up after discharge?

a. All those 50 alive neonates sent from our institute in any mode of discharge (Normal discharge, left against medical advice or Referral) were considered for telephonic follow-up.

b. All these neonates should be completed 1 month of age at the time of follow-up.

4. The manuscript states that after the follow-up of 37 respondents it was found that the neonates were found to be asymptomatic. What if they were COVID positive but asymptomatic? Were there any criteria to cover that part of the examination?

Thank you so much for raising the comment. We too have agreed to your view on asymptomatic COVID-19 infection in infants. But we have not tested them by RT-PCR again after discharge. It is one of the limitations of our study.

5. the references were found to be dated from August 2020. I would recommend updating the references with new references. Consider in-text and end-references thoroughly.

Thank you so much for the suggestion. Appropriate modifications were added in revised manuscript.

6. I also suggest passing the manuscript from the Plagiarism check software.

Many thanks for the suggestion. We have tested the study manuscript for Plagiarism after review.

Reviewer: 4

Dr. Harshil Bhatt, Goshen Hospital, Indiana University School of Medicine-South Bend

### Comments to the Author

1. Minor suggestion: The title might suggest that the study is about 50 mothers already positive with SARS-CoV-2 in the breastmilk. You can look into changing the title accordingly.

"Excretion of SARS-CoV-2 in breastmilk....." vs "Study for evaluating excretion....." or something similar. Many thanks for the suggestion. The title of the study is now re-phrased as "Lack of detection of SARS-CoV-2 in the breast milk of 50 infected mothers and clinical outcome of neonates breastfed by them: A prospective observational study".

2. Abstract – unexplained acronym in abstract – LSCS, consider writing the full name instead.

Many thanks for the comment. The acronym is explained in full form in the revised manuscript after considering the review.

3. 6/21, line 26- "We also assessed the health condition of babies at  $\geq 1$  month of their age.." The duration of this follow-up is missing from the abstract. You could mention in the abstract that follow-up was done at more than one month of age.

Many thanks for notifying me. We have added the duration of follow-up in the abstract in the revised manuscript.

4. Minor – SARS-CoV-2 is a correct word/format to use, SARS-Cov-2 is not.

Many thanks for the correction. It has been corrected with 'SARS-CoV-2' in the revised manuscript.

5. Minor - RT-PCR word should be described when using it for the first time in the manuscript.

Thank you so much for the comment. It has been explained in full form in the revised manuscript.

6. I believe readers will appreciate if in the abstract/methods it is mentioned that the newborns were also screened by RT-PCR, and also twice (within 24 hours and another after 24 hours).

Many thanks for the suggestion. It has been added to the abstract in the revised manuscript.

7. Neonates were fed with EBM from the mothers. (7/21, line 31) It means the neonates did not have any direct contact with their mothers (for breastfeeding). Mention if it is true.

Yes, it is true. The infants were physically isolated from their mothers from the time of birth till discharge of their mother from the hospital in an asymptomatic state.

8. 7/21, line 40 – Were mothers advised to continue expressed breast milk feeding only, or were they

told to do contact breastfeeding after discharge?

As per our institutional protocol, symptomatic mothers were advised for continuing expressed breast milk feeding till 7 days after hospital discharge with due precautions like maintaining hand hygiene by mothers, use of face masks while expressing breast milk, and breast hygiene just before and after manual expression of breast milk. Thereafter to continue exclusive breastfeeding, maintaining breast and hand hygiene. Infant's cot should be at least 1.5-2 meters away from the mother's bed during the isolation period. Asymptomatic mothers were advised to maintain home isolation for 2 weeks from the day first time they were detected to be RT-PCR positive.

9. Figures were not numbered at the end of the file, making it difficult to identify them according to the numbers mentioned in the text.

Thank you so much for the review. It has been corrected in the revised manuscript.

10. Discussion – Well-written with the examination of other studies and comparing the study with other authors. But still, some of the points mentioned in the discussion are more suitable for the results section and are already mentioned in the results. Hence, the discussion could be more streamlined to include only the inference of the findings and authors' interpretation but rather not the findings/results themselves with data/numbers (example – line 46 onwards).

Thank you so much for the suggestion. The necessary changes have been made in the revised manuscript.

11. "Findings of our study supports advocating the policy of breastfeeding by SARS-CoV-2 positive mothers, with due precaution....." Please specify what due precautions you are referring to – is it the safety measures, or using expressed breastmilk only, etc... Are you advocating for maternal-child contact for breastfeeding, or what would be your advice on that based on your research? Kindly specify. The findings of our study support advocating the policy of breastfeeding by SARS-CoV-2 positive mothers with due precautions like-the mother to maintain hand hygiene, use of face masks while expressing breast milk, breast hygiene just before and after manual expression of breast milk, infant's cot should be at least 1.5-2 meters away from mother's bed during the isolation period and after 7 days of isolation period from the time of hospital discharge. This has been included in the manuscript.

Reviewer: 5

Dr. Melisa Medina-Rivera, Cornell Univ

### **Comments to the Author**

#### **III. Major Concerns:**

1. From the manuscript it is not clear the timeline of when COVID-19 maternal symptom onset and diagnosis relative to the birth and breastmilk samples collection. This brings problems of interpretation of results and limitations concerning viral detection within a timeline where RNA, if present in the breastmilk sample, can be detected via RT-PCR. Moreover, even if detected by RT-PCR, defining RNA presence within a breastmilk sample is not sufficient to define breastmilk samples as infective. Other tests such as viral culturing are still needed.

Yes, agreed. This will be a limitation of our study.

2. There is an incomplete description of expressed milk collection and breastfeeding practices.

Thank you for the comment. The necessary details have been added in revised manuscript.

3. Other potential routes of SARS-CoV-2 transmission were not evaluated or considered.

This is one of the limitations of our study.

4. Disclosing abbreviations: LSCS

Thank you so much for the comment. It has been rectified in the revised manuscript.

5. Method of SARS-CoV-2 detection is missing in this section

Thank you so much for the comment. It has been added in the revised manuscript.

6. The description of sample collection is somewhat limited. Was the mother using recommended guidelines (as described in the introduction) to express her breastmilk? Were the breast pumps provided at the clinic? If so, cleaning between collections?

Yes, mothers were advised to strictly follow guidelines for expression of breast milk as per protocol which was monitored during hospitalization. Breast milk was manually expressed. No breast pumps were used.

7. Include a better description of the analytical performance of the RT-PCR assay employed. Was it commercially available or made at the clinic?

Thank you for the suggestion. It has been included in the revised manuscript. It is commercially

available

8. First table with demographic information is missing. General characteristics of study participants are included in two tables; however, it would be more useful to include a summary table. The existing tables could stay as supplementary data.

Thank you so much for the comment. It has been rectified in the revised manuscript.

9. I would suggest including a graphical representation that includes maternal symptom onset (if any) or suspected disease, and sample collection concerning birth.

Thank you so much for the comment. But, the related details have been not collected in our study and hence will be the limitation of the study.

10. How many neonates were breastfed during or after the hospital stay? Where there any mix-fed (formula + breastmilk)? If so, how many for how long?

As per the response received during follow-up, 37/50 caregivers of infants have strictly ensured breastfeeding. The details of the same are lacking in the rest of the infants due to loss to follow-up and are the limitation of our study.

11. I found it interesting that 2 out of the 50 infants that were positive for SARS-CoV-2 infection were receiving formula. Could the authors elaborate in the discussion of how these two children might have contracted COVID-19?

There are two possible routes for the transmission of infection.

a) Horizontal transmission-Any asymptomatic health care worker handling these neonates during hospitalization could be the possible source for the infection in those neonates. But it was not further evaluated as only symptomatic health care workers used to appear for RT-PCR testing as per institutional protocol.

b) Any other modes of vertical transmission, which are not evaluated in our study.

12. Please provide a better description of the follow-up interview/measurements. Also, during follow-up, was the child with the cold assessed for COVID-19?

Thank you for the comment. As it is already detailed in the questionnaire for the study, the follow-up was done to collect the details like any symptoms after discharge, information about the frequency of micturition was included to indirectly assess the adequacy of feeding. No assessment was done for the one infant with a history of a common cold which was self relieved over a short period.

13. If breastmilk samples are still available it would be interesting to also measure antibody levels within the samples.

Thank you so much for the comment. No EBM samples were stored for further evaluation our study

14. In the last update from the WHO (December 23, 2020) there were 67 published articles where breastmilk was assessed for the presence of SARS-CoV-2. These articles include a total of 413 breastmilk samples that were assessed via RT-PCR and 72 samples that were assessed for anti-SARS-CoV-2 antibodies via ELISA. The systematic analysis of this data found that only 13 out of the 423 breastmilk samples were positive, suggesting that vertical transmission of SARS-CoV-2 is not common. Noteworthy, a big limitation on interpreting this data was that many reports did not include information on breastfeeding practices, which is as important as measuring the presence or absence of the virus. Another limitation is the lack of measuring/reporting other possible routes of viral transmission. About this manuscript, this reviewer agrees with the authors in that the excretion of the virus through breastmilk could suggest potential vertical transmission. However, as the discussion is drafted it is misleading. RT-PCR data by itself is not sufficient evidence to support or discard the possibility of viral transmission through breastmilk.

- Study limitations should be expanded.

Thank you so much for the review. The necessary changes have been made in the revised manuscript.

14. Figures are missing, not clearly labeled, or too small for viewing.

Thank you so much for the comment. The necessary changes have been made in the revised manuscript.

15. Spelling SARS-Cov-2, breast milk, or breastmilk. Spelling misuse of capitalizing words throughout the document.

Thank you so much for the comment. The necessary changes have been made in the revised manuscript.

16. This reviewer suggests rephrasing the following sentence as it is misleading (symptoms are not COVID-19 related).

"Overall, 8/51 (16%) neonates were symptomatic, Birth asphyxia being most common symptom (4, 8%) followed by Neonatal hyperbilirubinemia (2, 4%), Abdominal distension due to Mesenteric cyst (1, 2%), and transient feed intolerance (1, 2%)."

Thank you so much for the suggestions. The sentence has been re-phrased in the revised manuscript.

17. Based on the narrative and the goals that the author set out to accomplish, this manuscript successfully describes the 50 pregnant women positive pregnant women with SARS-CoV-2, assesses their breastmilk for the presence of RNA, and observes infant outcomes. This manuscript, however, could be improved by establishing a clear timeline between disease onset, and sample collection between mothers and infants. Also, the result section could be significantly improved by providing a better description of breastfeeding practices, re-sizing the figures/labels, and, if still available, performing additional analysis on the samples. Further elaborating in these areas might help not only to put this study into context with relevant literature but also include a broader picture of potential viral transmission routes.

Many thanks for the suggestions. With available data, possible necessary changes have been done in the revised manuscript with due limitations of our study.

Reviewer: 6

Dr. Ryan Pace, University of Idaho

#### Comments to the Author

1. I have a couple of comments on the terminology used throughout the current manuscript that the authors may consider. I recommend that the authors substitute "human milk" in place of "breast milk" and "breastmilk" throughout the manuscript, as "human milk" is the preferred term among human milk and lactation researchers. There is also inconsistent usage of "breast milk", "breastmilk", breastfeeding, and "breast-feeding" throughout the manuscript. Additionally, the term "excretion" is used throughout the manuscript to refer to the putative presence of SARS-CoV-2 in human milk and I do not think that it is the correct word to use in this situation. First, the definition of "excretion" is the process of eliminating or expelling waste matter. Currently, there is no evidence that SARS-CoV-2 is excreted in milk (as supported by this study's findings). In contrast, most studies to date have failed to detect SARS-CoV-2 in human milk and are the only study to date to examine milk (that tested positive for SARS-CoV-2 RNA) for replication-competent virus found no viable virus (doi: 10.1001/jama.2020.15580). Further, the use of "excretion" in the current title may mislead readers into presuming that the authors detected SARS-CoV-2 in the milk samples tested; however, this is not what the authors found or report in the manuscript. Perhaps a better word is "detection", as this relates to what the present work (and described in the objective of the abstract), or more specifically a "lack of detection" of SARS-CoV-2 in milk, e.g., "Lack of detection of SARS-CoV-2 in human milk of 50 infected mothers and outcome of neonates delivered and breastfed by them".

Thank you so much for the comment and suggestions. Most acceptable changes have been made in the revised manuscript.

2. The manuscript would be greatly improved by additional proofreading and editing for spelling and grammar mistakes, as well as consistency in names (e.g., "health" in "World Health Organization" should be capitalized. Additionally, the current Introduction would be improved with some restructuring. For example, the second paragraph details that it is not well known whether transmission of SARS-CoV-2 occurs via human milk. This is then contradicted in the following paragraph that UNICEF has reported this not to be the case and that breastfeeding is recommended (and is also missing a reference). Thank you so much for the suggestions. The necessary changes have been made in the revised manuscript.

3. Many important details are also missing on how milk samples were collected and tested, e.g.:

1) Did mothers wash their hands, wear gloves and/or masks during milk collections?

Yes.

2) How was the milk collected, via manual expression or with an electric pump?

Manual expression.

3) How were milk samples stored?

Expressed breast milk was collected every 4-6 hours during hospitalization and was stored in the refrigerator.

4) How were milk samples tested? All that is stated is that detection of viral RNA was done via RT-PCR. Was the test validated for use with human milk?

Expressed breast milk collection

EBM samples collected manually in the clean and sterile container within 7 days of delivery, were transported in viral transport media (VTM) to the microbiology laboratory of our institute for the detection of viral RNA through RT-PCR. Samples were directly stored at -20°C.

RNA isolation

RNA was isolated from breast milk using the Qiagen Viral RNA Mini Kit (Qiagen, #52906) according to the manufacturer's instructions with slight modifications from the general protocol. Briefly, 280 µl samples were mixed with 1120 µl lysis buffer (AVL) and incubated 20 min at room temperature to ensure lysis. Then, 5.6 µg carrier RNA added to each sample, followed by vortexing and an additional 10 min incubation at room temperature. Ethanol (1120 µl) was added to it, vortexed and briefly centrifuged to remove droplets from the lid. The entire volume of lysate was then stepwise loaded onto columns. All subsequent steps were performed as instructed by the manufacturer. Viral RNA was eluted in 60 µl AVE buffer. The eluted RNA was stored at -80°C.

RT-qPCR

Qualitative real time reverse transcriptase PCR was performed by using one step real time PCR reaction mixture and primer-probe mixture approved by Indian council of Medical research (ICMR), Govt of India. Different kits were used during the period of the study depending on the availability because of the huge turnover during the pandemic. However all of the real time PCR kits used were capable to amplify at least one screening and one confirmatory gene. In addition primer-probe detecting an internal control gene (human RNase P) was also included in all samples. All the patients' samples are run along with a positive control and a no template control for validation of the run. All real time RT PCR were performed in either Biorad CFX96 or ABI Quant studio 5 Dx machine with reaction protocol set as per instruction in the kit insert.

5) A novel aspect of this work that the authors should also consider highlighting is that most studies on this topic have been on North American populations and that similar data from more diverse populations, including India, have been lacking.

Thank you so much for the suggestion. It will be considered in the revised manuscript.

6) After being first defined, WHO is used interchangeably with the full name (World "Health Organization) throughout the manuscript; once defined it is appropriate to just use WHO.

Thank you for the comment. The necessary changes have been made in the revised manuscript.

7) I would recommend not using "babies" and use "neonate" and/or "infant" instead.

Thank you so much for the suggestion. The necessary changes have been made in the revised manuscript.

8) Figure 2 is very difficult to interpret as the size and resolution are very low. The resolution of Both tables 1 and 2 is also too low.

Thank you so much for the comment. The necessary changes have been made in the revised manuscript by considering overall reviews.

Reviewer: 7

Dr. Aakash Pandita, Sanjay Gandhi Post Graduate Institute of Medical Sciences

#### **Comments to the Author**

A very good study from India but requires few clarifications

1. Testing breast milk sample once is not sufficient that to over a wide range of 7 days as we know that excretion in breast milk is intermittent

Thank you so much for the comment. It will be one of the limitations of our study.

2. Was it a prospective or a retrospective study?

This is a single-center, prospective observational study and the same thing is updated in the revised manuscript.

3. How many covid positive moms were delivered during the study period which is not mentioned?

Study period: 1st June 2020 to 31st January 2021

Total COVID-19 positive deliveries: 53

(Singleton alive deliveries: 49

Alive Twin delivery: 1

Singleton Intrauterine death :2

Twin Stillbirth:1)

4. Did you admit all neonates born to covid positive mothers?

Yes, as per our institutional protocol.

5. Bed strength of NICU?

5

6. Birth asphyxia being most common symptom (4, 8%) followed by Neonatal hyperbilirubinemia (2, 4%), Abdominal distension due to Mesenteric cyst (1, 2%).....these cannot be symptoms of neonatal covid

Yes, agreed. The sentence has been rephrased with necessary modification.

7. Were all the babies exclusively breastfed till discharge?

No, they were fed with expressed breast milk.

8. How many mothers were symptomatic and sick?

07/50 mothers were symptomatic and sick.

9. What happened to the feeding of such mothers?

Except for the one symptomatic and sick mother who was expired, the rest of the mothers continued with expressed breast milk feeding with due precautions.

10. No clinical characteristic of neonates are provided

Though all characteristics are not mentioned in the manuscript, most relevant details have been shared in the revised manuscript.

11. The manuscript needs a thorough revision for language and syntax errors

Thank you so much for the suggestion. The revised manuscript is tested for language and syntax errors.

Regards

Corresponding author