Transmission of yellow fever vaccine virus from breastfeeding mothers to their infants: reporting of yellow fever virus (YFV) RNA detection in milk specimens [version 4; peer review: 1 approved, 2 approved with reservations]

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Abstract

Background

Because of yellow fever's serious impact on health, vaccination is the principal strategy to control the disease. Administration of the yellow fever vaccine to breastfeeding women should be before they complete 9 months post-delivery, in order to prevent transmission of the yellow fever vaccine virus to their infants through breast feeding. This study aimed to confirm whether the excretion of yellow fever vaccine virus is in milk of vaccinated breastfeeding mothers and to confirm the probable transmission to their infants through breast milk.

Methods

Samples were taken as follows: one serum specimen was taken 3-14 days after the date of the vaccination, and breast milk specimens were taken at four different time points between 3-4 days apart. Specimens were obtained from eight nursing mothers, who received the YFV vaccine (17DD). Mothers were asymptomatic before and after the
vaccine administration but their infants developed symptoms after administration. Maternal serum samples were tested for YFV specific IgM antibodies through immuno-fluorescent assay (IFA). RNA was extracted from serum and breast milk specimens and YFV RNA screened using real-time polymerase chain reaction (RT-PCR).

Results

In total, five mothers (62.5%) were positive for YFV and two mothers (25%) had YFV RNA in serum. Among milk specimens, YFV RNA was detected during the four different mentioned collection times as follows (positive milk specimens/total milk specimens): 3/8 (37.5%), 4/6 (66.6%) and 1/4(25%). RNA was completely undetectable in the last collection time.

Conclusions

YFV transmission from mothers to their babies through breast-feeding was highly probable indicated by the temporal relationship to mother’s YF vaccination.

Keywords

YFV, Vaccine, Milk, breast feeding mothers, Khartoum-Sudan

This article is included in the Emerging Diseases and Outbreaks gateway.

This article is included in the Pathogens gateway.
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Introduction

The yellow fever virus is a mosquito-borne flavivirus that causes yellow fever, a viral hemorrhagic fever that now occurs only in Africa, Central and South America, but historically has had many wide outbreaks in Europe and North America. Approximately 200,000 cases of yellow fever occur annually; 90% of them in Africa. Yellow fever severity ranges from self-limited fever illness to hemorrhagic syndrome and death. Most yellow fever patients are asymptomatic, but 15% develop severe illness, which appears as a systemic illness that affects the liver (jaundice and necrosis), renal system, and myocardial system resulting in hemorrhage and shock. Among the 15% of patients that develop severe illness, the mortality rate is 20%–60%. Reports show that yellow fever is responsible for 29,000 to 60,000 deaths in South America and Africa every year, and it’s the most severe form of mosquito-borne diseases in tropical areas.

Because yellow fever is a potentially fatal disease, vaccination is the principal strategy to control the disease. An effective yellow fever 17DD vaccine was established in 1937, but there are still currently over 400 million unvaccinated people in the areas of high infection risk.

Live attenuated 17DD vaccine confers protection in more than 95% of recipients within a month after single dose vaccination. Its protection is attributed to both innate and adaptive immunity through production of neutralizing antibodies directly against the envelope protein. 17DD vaccine administration provides immunity for at least 10 years and probably can extend to give life-long immunity.

The YFV vaccine can cause adverse side effects after its administration that range from mild to severe. The mild effects are headache, myalgia, and pain at the injection site, while severe effects can include anaphylactic shock, neurological diseases, and viscerotropic disease. The YFV vaccine is recommended to be administered after 9 months of age and from 6 months in endemic areas. Administration of the vaccine to breastfeeding women before 9 months post-delivery can transmit the yellow fever vaccine virus to their infants with high risk of neurological diseases. There are three case reports of confirmed transmission of the YFV vaccine strain from mothers to their infants through breastfeeding. The first case reported that the mother received the yellow fever vaccine 15 days after her delivery, and she had symptoms of headache, malaise, and fever after 20-22 days. The other two cases reported that the mothers received a yellow fever vaccine, and their infants had developed encephalitis 3-4 weeks later.

Many studies have reported the transmission and presence of other flaviviruses RNA in breast milk such as West Nile virus (WNV), Zika virus, dengue and chikungunya; however, no previous study has reported the detection of YFV RNA in breast milk samples from vaccinated breast-feeding mothers in Sudan. Therefore, this study aimed to detect the yellow fever virus in breast milk and serum samples from vaccinated breastfeeding women whose infants got yellow fever illness to confirm whether the yellow fever vaccine virus is excreted and transmitted through breast milk. The mothers received the vaccine during an intensive YF vaccination campaign that involved millions of people in Khartoum State.

Methods

Ethical considerations

The study approved by the ministry of health, Sudan (approval number 5688-2019). Verbal consent was taken from mothers due to some being unlettered and some afraid of providing written consent. This was deemed adequate and approved by the Ministry of health.

Study design and population

This study involved YFV testing in serum and breast milk specimens from eight nursing mothers (aged from 20-33 years) from Khartoum, Sudan in November 2019. The mothers had received the YFV vaccine (17DD) (Bio-Manguinhos/Fiocruz) during the 2019 YFV vaccination program in Khartoum within 9 months of delivery. They were not showing any symptoms of YFV before they got the vaccines; however, their infants (ages from 45 days to 8 months) developed symptoms of fever, diarrhea, jaundice, vomiting, and/or skin rashes after one week from their mother received the vaccines. Participants were approached via telephone through reports provided to the Ministry of Health to follow-up individuals who experience complications after getting vaccinated.
Sample collection
The samples were collected by Ministry of health medical staff from participants in different hospital vaccination points. Blood samples were collected from mothers in a plain blood container, then serum separated from the blood sample through centrifugation at 1100 rpm for 15 minutes using centrifuge (Hettich- ZENTRIFUGEN), then serum samples pipetted into clean Eppendorf tubes and stored at -80°C until their use. Milk specimens were collected in a clean glass jar by hand expression whilst the infant was nursing on the other breast and vice versa. The specimens from the breasts (right and left) were expressed into separate clean glass jars. Collection of milk specimens performed on four occasions: the first collection time was 3-14 days from the date of the vaccination. The three following collections were 3-4 days apart. Milk specimens were collected on all occasions, but serum samples were collected only in the first collection.

Serology
Maternal serum samples were tested for YFV specific IgM antibodies using immuno-fluorescent assay (IFA) according to manufacturer instructions (Yellow fever virus IIFT (IgM), EUROIMMUN, Germany, catalogue number Fl 26651005 M).

RNA extraction
RNA was extracted from serum and breast milk specimens using a commercial RNA extraction kit (QIAamp viral RNA mini kit) according to the manufacturer instructions (Qiagen viral RNA, Germany). The extracted RNA was stored at −80°C until use.

Real-time polymerase chain reaction (PCR)
Detection of YFV RNA was performed using real-time PCR (Rotor 5 plex real-time PCR machine Qiagen, Germany). Commercial kit which developed to detect all YFV strains including vaccine strain (RealStar® Yellow Fever Virus RT-PCR Kit 1.0, Germany) was used according to manufactures protocol. The PCR program consisted of 55°C for 20 min, 95°C for 2 min, followed by 45 cycles consisting of 95°C for 15 sec, 55°C for 45 sec and 72°C for 15 sec.

Statistical methods
No statistical analysis was needed. Data from participants were documented in an Excel sheet containing data for each mother and their infants. Rotor 5 plex real-time PCR thermo cycler software used to create Figure 3 while Figures 1 and 4 were created using word.

Results
The eight breast feeding mothers in Khartoum state were aged between 20 to 33 years old. Participation per each phase of the study shown in Figure 1.

![Figure 1. Participation per each phase of the study.](image-url)
Results of the first collection
Five mothers (62.5%) showed IgM antibodies against YFV using IFA technique (Figure 2). YFV RNA was detected by using real time PCR in 2/8 serum samples (25%) and 3/8 in breast milk (37.5%) (Table 1). Results of the PCR are shown in Figure 3.

Results of the second collection
In this phase, only 6 samples were obtained, among them YFV RNA was demonstrated in 4/6 (66.6%) breast milk specimens (Table 1).

Results of the third collection
Four mothers were enrolled in this occasion. Only 1/4 (25%) of the milk samples (25%) was positive for YFV RNA (Table 1).

Results of the fourth collection
Milk specimens were obtained from 3 mothers, all specimens showed negative result for YFV RNAs (Table 1). Minimal clearance of the viral RNA in milk was 11 day after vaccination and the maximum time needed for the clearance was 24 days after vaccine administration.

Real-time PCR results showed in Figure 1 and results of yellow fever virus (YFV) detection among different collection dates showed in Figure 4.

Discussion
According to the applied recommendations for YF vaccine, breast-feeding mothers and their infants who are aged less than 9 months should avoid YF vaccination. However, when breastfeeding mothers must travel to a yellow fever-endemic area, these women should be vaccinated, and its recommended to temporary interrupt breastfeeding for at least 2 weeks after YFV vaccination while in contrast, this study show that needed time to insure complete interrupted shedding of the virus in milk is up 24 days after vaccine administration. In a CDC report, the prenatal transmission of vaccine strain was demonstrated without RNA detection in the milk samples, in other side our study proves the transmission that by RNA detection in milk samples.
Table 1. Results of serological and molecular detection of YFV in milk and serum specimens.

| Patient number | Phase between vaccination and first collection (days) | Time since delivery | IFA-IgM serum | RT-PCR (No of cycles/40) Milk | PCR = polymerase chain reaction; YFV = yellow fever virus.
|----------------|------------------------------------------------------|--------------------|---------------|----------------------------| *Means no sample obtained.
| 1              | 13                                                   | 2.5 month          | +             | + (36.2)                  | - | - | - | - | - | - | - | - | - | - | - |
| 2              | 12                                                   | 2 months           | +             | + (34.8)                  | + (40.0) | - | - | - | - | - | - | - | - | - | - | - |
| 3              | 10                                                   | 7 months           | -             | + (39.7)                  | + (38.9) | + (34.6) | - | - | - | - | - | - | - | - | - | - | - |
| 4              | 11                                                   | 45 day             | +             | -                         | - | - | - | - | - | NA* | NA* | NA* | NA* | NA* | NA* | NA* | NA* | NA* | NA* | NA* | NA* |
| 5              | 4                                                    | 5 months           | -             | + (37.3)                  | + (36.4) | - | - | - | - | - | NA* | NA* | NA* | NA* | NA* | NA* | NA* | NA* | NA* | NA* | NA* |
| 6              | 5                                                    | 2 months           | -             | -                         | - | - | - | - | - | NA* | NA* | NA* | NA* | NA* | NA* | NA* | NA* | NA* | NA* | NA* |
| 7              | 12                                                   | 8 months           | +             | -                         | - | + (34.2) | NA* | NA* | NA* | NA* | NA* | NA* | NA* | NA* | NA* | NA* | NA* |
| 8              | 14                                                   | 7 months           | +             | + (32.8)                  | - | - | NA* | NA* | NA* | NA* | NA* | NA* | NA* | NA* | NA* | NA* | NA* | NA* | NA* | NA* | NA* | NA* |
This current study was the first study to detect YFV through detection of RNA in milk samples from vaccinated nursing mothers. In contrast a study by Eder Fernandes (2020), reported that YFV RNA was not detected in serum and milk samples from vaccinated lactating mother.\textsuperscript{19}

Between 2009 and 2020, YFV RNA was usually detected in samples other than milk such as CSF and serum. On the other hand, in April 2009 the first case of YF vaccine strain transmission through breast milk was reported in a Brazilian infant; yellow fever-specific IgM antibodies were detected in serum and CSF and yellow fever vaccine strain viral RNA was found in CSF of the infant. However, no breast milk or maternal serum was collected for yellow fever virus testing.

On March 2011, another case report study showed a baby was developing encephalitis after his mother had received the YFV vaccine when he was 10 days old.\textsuperscript{10} The authors claimed the probable transmission of the vaccine virus from the mother to her baby by detecting YF IgM in the infant’s serum and CSF. The clinical presentation, temporal relationship to

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**Figure 3.** Real time polymerase chain reaction (PCR) result for yellow fever virus (YFV) positive milk specimens.

| Collection Type | YFV RNA in Breast | YFV RNA in Serum | YFV IgM from Serum |
|-----------------|-------------------|------------------|-------------------|
| First collection [8 Mothers] | 2 (25%) | 3 (37.5%) | 5 (62.2%) |
| Second : 4 (66.6%) | | | |
| Third : 1 (25%) | | | |
| Forth : 0 Not detected | | | |

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**Figure 4.** Result of yellow fever virus (YFV) detection among different collection dates.
the mother’s vaccination, and lack of other alternative pathogens were also strongly supportive of acute central nervous system infection with the vaccine strain of yellow fever.10

Yet another study reported on detection of YFV IgM in a 38 days-old infant’s serum and CSF who was exclusively being breast-fed. The baby suffered from meningoencephalitis 3 to 4 weeks after the YFV vaccine administration to his mother.9,10 The baby was discharged after the convulsive crises were controlled.

Many studies have reported on the detection of flaviviruses RNA (Zika, West Nile, dengue, and chikungunya) in human milk.7 A study in 2017 described detection of viral RNA in serum and milk of three symptomatic breast-feeding mothers who were infected with Zika virus.13 And in another study WNV RNA and IgM antibodies were detected in breast milk samples from mothers whose babies developed West Nile virus.14 In one case of vertical transmission of dengue infection, the RNA of the virus was detected in blood samples from a mother and her child as well as in the mother’s breast milk.11 Furthermore, another study reported that chikungunya virus RNA was detected in serum, urine and milk samples of a breast-feeding mother at third day of symptoms onset.12

According to the previous reports of Yellow fever, all 3 reported cases of yellow fever were engaged to the vaccine virus strain, and in all reported cases RNA was not detected in breast milk. None of the reported cases was confirm detection of the virus in breast milk specimen, the only confirmed infection through viral RNA PCR detection was in cerebrospinal fluid of infant after his mother received vaccine, while in the other cases; serological detection of YFV IgM antibodies was performed in serum and cerebrospinal fluid.20 Also Eder Gatti (2020), reported that YFV RNA was not detected in serum and milk samples from vaccinated lactating mother while specific IgM was detected.19 It is important to mention that human milk’s cellular composition is dynamic, and a variety of circumstances, including the stage of lactation, health, and child feeding, can alter the quantity of various cell types during the lactation period.21

This current study was the first study to confirm detection of YFV vaccine strain, through detection of RNA in milk samples from vaccinated nursing mothers. In contrast a case study reported by Ana Freitas (2020), showed detection of wild type YFV RNA genome in breast milk specimens from the mother.20 Through searching it was un-able to find a report showing YFV vaccine strain presence in milk as we report.

This study has some limitations; because objections by mothers, no samples were collected from the infants in order to exclude other causes of the observed illness and to rule out an asymptomatic transmission of the virus. Also serum samples need to be collected through the other phases in order to follow the viral existence. Another limitation is that the detected YFV RNA must be sequenced to confirm the identity as 17DD vaccine strain.

Conclusion
Despite limitations, this study proves that YFV transmission from mothers to their babies through breast-feeding was highly probable indicated by the temporal relationship to mother’s YF vaccination. This also represents the first report on the detection of YFV RNA in human milk after YF vaccination in Sudan.

Data availability
Underlying data
Figshare: Transmission of yellow fever vaccine virus from breast feeding mothers to their infants: reporting of yellow fever virus (YFV) RNA detection in milk specimens. https://doi.org/10.6084/m9.figshare.17206640.15

Data are available under the terms of the Creative Commons Attribution 4.0 International license (CC-BY 4.0).

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One of the limitations is the sometimes very low concentrations of RNA. It is clear that for some of the samples, the detection threshold is reached between 30 and 35 cycles, while for other samples it is between 35 and 40 cycles. This means that the RNA concentrations are very low, at a level that contamination might be an issue. It is also questionable if such low concentrations are biologically meaningful. It would be good if the authors could provide the number of cycles necessary to reach the threshold for each sample. Is there exact information on the day the samples were collected? Please provide this information in Table 1. Please also provide information on the stages of breastfeeding. At the moment there is only mention of “within 9 months of delivery”, please provide at least the number of months since delivery for each mother. If the serum is negative for the RT-PCR test and negative for the IgM assay, it becomes difficult to understand why or how the milk became positive.

The authors should also elaborate on the limitation section. It is important to note that the lactation changes and that the amount and type of cells and material in the milk will change during the whole lactation period. It could be that in early lactation the change of viral RNA getting into the milk is higher than in the later stages of lactation. Also, highlight that the concentration of RNA needs to be taken into account when assessing any risks for the infant.

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Partly

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Not applicable

Are all the source data underlying the results available to ensure full reproducibility?
Partly

Are the conclusions drawn adequately supported by the results?
Partly

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** lactation research and analytical chemistry

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

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**Version 2**

Reviewer Report 20 May 2022

https://doi.org/10.5256/f1000research.122300.r129788

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Ralph Huits
Department of Infectious Tropical diseases and Microbiology, IRCCS Ospedale Sacro Cuore Don Calabria, Negrar, Italy

This manuscript reports on the testing of maternal serum for YFV specific IgM antibodies and YFV RNA, and serial breast milk samples for detectable YFV RNA, in eight nursing mothers, whose infants developed symptoms after the mothers received YFV vaccine. The authors found, for the first time, YFV RNA in breastmilk in five out of eight mothers 4-24 days after receiving 17DD vaccine. The detectable shedding of YFV (probably vaccine strain) in breast milk is the main message of this manuscript. This is an important finding that contributes to the evidence base for current YFV vaccination practices.

Major comments:

How were the mothers selected? It appears they were examined because their infants fell ill. This should be stated unambiguously, as it may underestimate the YFV RNA detection (and transmissibility) in breast milk.

Strictly speaking, transmission was not confirmed, because no diagnostic data was presented for
the infants.

Minor comments:

Introduction: (End of 1st paragraph): ‘the most severe form (?)’, suggest rephrasing, e.g., ‘…, making it the most severe of mosquito-borne diseases.’

The section on published case reports should include ref. 18.

(Also note: ref. 9 = ref. 20, cited twice)

Fig. 1: check spelling

Results section: list the percentages after the nouns (e.g., 2 serum samples (25%)); In reporting the PCR results obtained in the second, third and fourth collections of breast milk, I would favour expressing the positive results as a fraction with the number of patients tested per timepoint in the denominator (e.g., 3/8, 4/6, etc.), rather than percentages that (falsely) lead back to the original number of patients (n=8).

Table 1: Column headings: ‘Sample number’ should be ‘patient number’; ‘Date’ should be date of YFV vaccination; For clarity it is probably more relevant to list the number of days between vaccination and timing of first sample collection, instead of the columns 2 & 3. Do ‘Right’/ ‘Left’ column headings refer to the right/ left mamma? Please explain in legend.

Fig. 3: check spelling; data in ‘YFV RNA in breast milk’ and ‘… in serum’ are not consistent with Table 1.

Discussion: It is hard to ascertain whether recommendations are ‘universally applied’; the statement needs a reference. The reference for a 2-week interruption of breastfeeding before YFV vaccination is expert opinion. Could the authors comment on this duration based on their findings? (Detectable RNA in breastmilk up to 24 days post vaccination?)

The CDC report cited (ref. 18) does confirm detection of YFV vaccine in the infant’s CSF. I suggest rephrasing. I have 2 comments on the statement ‘This current study was the first…’: this study was not the first, see ref. 18 above and in the next paragraph of the text- ref. 18 was not a probable, but a confirmed case.

For Eder Gatti (2020): their surname is Fernandes. (Repeating findings from this paper on p. 8 is redundant).

Is the work clearly and accurately presented and does it cite the current literature?
Partly

Is the study design appropriate and is the work technically sound?
Partly

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Not applicable

Are all the source data underlying the results available to ensure full reproducibility?
No source data required

Are the conclusions drawn adequately supported by the results?
Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Arbovirus infections, malaria, tropical medicine, diagnostics evaluation.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 08 Jun 2022

Dina Naser

Dear Ralph Huits,

Major comment:
The selected mothers had received the YFV vaccine within 9 months of delivery. They were not showing any symptoms of YVF before they got the vaccines; however, their infants developed symptoms of fever, diarrhea, jaundice, vomiting, and/or skin rashes after one week from their mother received the vaccines.

Transmission to infants was confirmed through that their mothers get vaccinated week before developing symptoms and also through the detected YFV RNA in milk specimens from these same mothers during the infant's appeared symptoms.

Minor comments:

Result:

Do ‘Right’/ ‘Left’ column headings refer to the right/left mamma?

Yes, milk samples collected from both breasts in order to get the most excreted YFV in milk and also to study if there is any relation or correlation between breast side and YFV shedding in milk.

*Other reviewer edits are done in the new submitted version.*
**Competing Interests:** No competing interest.

Author Response 22 Jun 2022

**Dina Naser**

In addition, some changes have been made in table, these changes are topical to that addressed online by the reviewer.

**Competing Interests:** No competing interest.

Reviewer Report 22 March 2022

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**Leo G. Visser**

Department of Infectious Diseases, Leiden Medical University Center, Leiden, The Netherlands

The authors have addressed the concerns raised in the peer review report appropriately.

**Is the work clearly and accurately presented and does it cite the current literature?**
Partly

**Is the study design appropriate and is the work technically sound?**
Partly

**Are sufficient details of methods and analysis provided to allow replication by others?**
Partly

**If applicable, is the statistical analysis and its interpretation appropriate?**
Partly

**Are all the source data underlying the results available to ensure full reproducibility?**
Partly

**Are the conclusions drawn adequately supported by the results?**
Partly

**Competing Interests:** No competing interests were disclosed.
**Reviewer Expertise:** Vaccine immunology, alternative vaccination routes, yellow fever vaccine, rabies vaccine, mRNA 1273

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

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**Leo G. Visser**
Department of Infectious Diseases, Leiden Medical University Center, Leiden, The Netherlands

The authors report for the first time the presence of YVF RNA in breast milk specimens of five out of eight woman, three to fourteen days after receiving 17DD during vaccination campaign in Khartoum, Sudan.

**Major comment**

Although transmission of YFV-17DD to the neonate through breastfeeding has been demonstrated before,\(^1\) attempts to isolate the vaccine strain from breast milk have been unsuccessful.\(^2\)

On the other hand, wild YFV has been detected in breast milk before.\(^3\) Although there were no reports of a yellow fever outbreak in Khartoum state in November 2019, final proof would require to demonstrate that the nucleotide sequence of the amplified PCR product in the breast milk was identical to 17DD yellow fever vaccine virus. This limitation should be addressed in the discussion.

**Minor comments**

*Introduction:*

The line “Administration of the vaccine to breastfeeding women before 9 months post-delivery can transmit the yellow fever vaccine virus to their infants through breast milk.” is confusing: breastfeeding is a risk factor for transmission irrespective of time post-delivery. However, the risk of YFV-associated neurological disease after transmission may occur in infants less than 6-9 months of age. The sentence should be corrected accordingly.

*Method:*
What were the inclusion criteria? How were these women selected? What is the cut-off CT-value for q-PCR result to be considered positive? Was a negative control included to check for contamination? What was the positive control for the YFV PCR?

**Results**

Presentation of the results in a single table would increase readability. The data provided in Figures 1 and 4, and Tables 1 and 2 could be compiled into one table (time and specimen in the upper row, and subjects in left column). Figure 2 is redundant.

In Figure 3 it is not clear from which specimen the amplification curves are presented? Is this serum or breast milk? The CT-values of 32 - 40 indicate very low levels of viral RNA. Maybe the authors could provide some more information on the CT-values in serum and breast milk.

**Discussion**

Could the authors elaborate on how many weeks breast feeding should be interrupted to prevent transmission to the infant?  
The authors should discuss on the lack of confirmation that the nucleotide sequence of the amplified PCR product in the breast milk was identical to 17DD yellow fever vaccine virus.

**References**

1. Centers for Disease Control and Prevention CDC: Transmission of yellow fever vaccine virus through breast-feeding - Brazil, 2009. *MMWR Morb Mortal Wkly Rep.* 2010; 59 (5): 130-2  
2. Fernandes EG, Nogueira JS, Porto VBG, Sato HK: The search for yellow fever virus vaccine in breast milk of inadvertently vaccinated women in Brazil. *Rev Inst Med Trop Sao Paulo.* 2020; 62: e33
3. Ribeiro AF, Brasil LMC, Prada RM, Nogueira JS, et al.: Detection of Wild-type Yellow Fever Virus in Breast Milk. *Pediatr Infect Dis J.* 39 (1): 68-69

**Is the work clearly and accurately presented and does it cite the current literature?**  
Yes

**Is the study design appropriate and is the work technically sound?**  
Partly

**Are sufficient details of methods and analysis provided to allow replication by others?**  
Yes

**If applicable, is the statistical analysis and its interpretation appropriate?**  
Not applicable

**Are all the source data underlying the results available to ensure full reproducibility?**  
No source data required

**Are the conclusions drawn adequately supported by the results?**
Partly

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** Vaccine immunology, alternative vaccination routes, yellow fever vaccine, rabies vaccine, mRNA 1273

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

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**Comments on this article**

**Version 1**

Author Response 09 Mar 2022

Dina Naser

**Comments**

**Major comment:**
The mentioned edits has been reviewed and corrected in the draft. At that time in 2019, there were different arboviral outbreaks in many regions in Sudan, as a response to that, the ministry of health start a preventive vaccination campaign against YVF.

**Minor comments:**

- **Introduction:**

Done

- **Method**

1. What were the inclusion criteria? How were these woman selected?

   Eight nursing mothers who had received the YFV vaccine (17DD) during the 2019 YFV vaccination program in Khartoum within 9 months of delivery.

2. What is the cut-off CT-value for q-PCR result to be considered positive?

   Results obtained according to the used rt-PCR kit.

3. Was a negative control included to check for contamination?
Yes of course.

4. What was the positive control for the YFV PCR?

Positive controls already included with the kits reagent.

- Results

Results showed in tables presented into single table, while it will be difficult to understand results showed in figures if they presented in one figure.

- Discussion

Changes have been made in the draft.

**Competing Interests:** No competing interests

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