Breast Milk as a Possible Route of Vertical Transmission of Dengue Virus?

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We report a case of vertical transmission of dengue infection. The virus was detected and quantified by reverse-transcription polymerase chain reaction in sequential blood samples from mother and child as well as in breast milk, but not in cord blood. This case poses questions about the risk of breastfeeding transmission of dengue virus.

Keywords. dengue virus; breast milk; vertical transmission; breastfeeding; newborn.

Dengue fever is an emerging viral disease caused by 4 dengue virus (DENV) serotypes of the genus Flavivirus and is a major cause of morbidity in tropical and subtropical areas [1]. DENV is transmitted between human hosts by mosquito vectors. Other transmission modes have been described through blood [2], mucocutaneous [3], and maternal-fetal [4] routes. We report a case of vertical DENV transmission that illustrates the evolution of viremia in a newborn as well as the presence of DENV in breast milk.

CASE REPORT

A Polynesian 23-year-old pregnant woman (gravida 2, para 1) presented to the Noumea hospital (New Caledonia) in July 2012 with preterm labor at 30 weeks and 4 days of gestation. Upon admission (day 0), the mother had a 2-day history of fever without any associated symptoms. Blood tests showed normal white cell count (5.3 × 10^9/L), anemia (82 g/L), and thrombocytopenia (122 × 10^9/L). C-reactive protein was elevated at 18 mg/L. Vaginal and blood cultures were negative. Despite tocolytic treatment, a live male infant was delivered vaginally a couple of hours after admission. The 2450-g healthy newborn (Apgar score 10/10) was transferred to the neonatal intensive care unit (NICU) and placed in an incubator.

After delivery (day 0), the mother had persistent fever, and her anemia (64 g/L) and thrombocytopenia (104 × 10^9/L) worsened. She received 2 units of packed red blood cells, antibiotics, and antipyretics. On day 2, she still had fever but had normal arterial pressure and no bleeding. On day 3, her biological tests revealed a drop in platelet count to 38 × 10^9/L. Because of an epidemic context, she was tested for DENV infection, and reverse-transcription polymerase chain reaction (RT-PCR) of blood samples showed positive results. She was placed in a single room and was advised to use insect repellent.

Starting from day 2, the infant was fed with expressed breast milk, then breastfed. Of note, the mother did not show any breast lesion. Upon a pediatrician’s request, DENV was tested in breast milk. Because RT-PCR was positive, breastfeeding was stopped on day 4.

On day 4, the infant developed low-grade fever at 37.9°C, and his serum tested positive for DENV. He then developed thrombocytopenia as low as 34 × 10^9/L on day 9 without any sign of discomfort or severity. Both mother and infant evolved favorably and were discharged home on day 6 and day 25, respectively.

Virological investigations were retrospectively and prospectively done after detection of DENV in breast milk. Results are presented in Figure 1. DENV was detected by real-time RT-PCR in sequential blood samples taken from the mother (day 0 to day 6), the infant (day 4 to day 13), and in breast milk (day 4 and day 6). Cord blood (day 0) tested negative for DENV as well as the infant’s blood samples collected from day 0 and day 2. Negative serum samples were tested twice in 2 different runs.

Quantification of DENV was performed on all positive samples. Viral loads from both patients’ blood samples were high and peaked between 10^7 and 10^8 copies/mL. We describe kinetics of the infant’s DENV infection starting before the onset of symptoms, which showed a viral load increase over 3 days, a plateau phase during 2 days, and a slow decrease over >4 days. Breast milk viral loads were significant (>10^7 and >10^5 copies/mL) and in the same range as the ones found in the mother’s blood that same day.
DENV detected in blood samples from the mother and the infant as well as in breast milk were typed as DENV-1. Breast milk culture allowed us to confirm that the virus was readily cultivable. Sequences and phylogenetic analysis of the E gene (Supplementary Figure) from breast milk and the mother and infant’s blood samples showed a 100% identity of the strains at the nucleotide level (GenBank accession numbers: KC741438 to KC741440).

Serological diagnosis was performed showing immunoglobulin M (IgM) seroconversion in both patients. Mother and infant tested negative on day 0 and day 4 and positive on day 6 and day 25, respectively. Non-serotype specific immunoglobulin G (IgG) was positive on day 0 in the mother’s blood samples, reflecting a former dengue infection.

We received the mother’s written informed consent to use her and her baby’s samples for research and publication. Blood samples were collected regularly during hospitalization and kept at 4°C. Total nucleic acids were purified on the automated MagNA Pure LC2.0 using the Total Nucleic Acid Isolation Kit (Roche Diagnostics, Auckland, New Zealand) from 200 µL of sample into 50 µL eluates. DENV detection was performed using a duplex DENV-human GAPDH real time RT-PCR on a LightCycler 480 II (Roche Diagnostics, Auckland, New Zealand) using positive and negative controls in each run. This RT-PCR uses the technique published by Warrilow et al [5], who described, for DENV-1, a 100% sensitivity compared to cell culture. Viral loads were quantified using real-time RT-PCR and a standard curve was generated from dilutions of a reference sample. DENV serotyping was performed using a fourplex RT-PCR as described by Johnson et al [6]. Cell culture was performed using an Aedes albopictus C6/36 cell line. DENV-1 E gene sequences analysis (M. Dupont-Rouzeyrol, M. Aubry, O. O’Connor, C. Roche, V. M. Cao-Lormeau, unpublished data) were performed directly on serum sample or on cell supernatant eluates. Enzyme-linked immunosorbent assay detection of anti-DENV IgG and IgM (Panbio Diagnostics, Alere, Australia) was performed on an Elispeed Duo (BioAdvance, Bussy Saint Martin, France) analyzer.

### DISCUSSION

Our patient presented to the hospital with preterm labor as described in other cases of DENV infection during pregnancy [7] and gave birth to a premature but healthy newborn. Both mother and infant experienced nonsevere acute dengue infection with fever and severe thrombocytopenia but no sign of hemorrhage or plasma leakage. Analyzing the infant’s samples taken before the onset of symptoms allowed us to describe kinetics of viral load before fever, a figure rarely reported. In this case, both patients’ peak viral loads were similar, notably showing no difference between the mother’s secondary and the infant’s primary infection. Moreover, the viremic period was prolonged (≥10 days) in the newborn. The child’s prematurity might have been a factor favoring extended viremic period and high viral loads, sometimes reported as lower in primary infections [8].

Vertical transmission of DENV has been reported [2, 4], and the mechanisms of infection transmission remain unclear in the peripartum period. This case provides evidence, for the first time to our knowledge, of the presence of DENV in breast milk during acute dengue infection. Though prenatal or perinatal infection cannot be strictly excluded, our results suggest that breast milk may be a possible route of DENV transmission from a mother to a child. Indeed, DENV was not detected from cord blood or from the infant’s blood samples on day 0 or day 2. Whereas the presence of a few viral copies on samples from day 0 could fall under the technique’s low detection limit, it seems unlikely that dengue viral load would still be undetectable 2 days later. However, other routes of transmission through placenta or amniotic fluid cannot be ruled out. Transmission to the child through mosquito bite is regarded as impossible in the NICU, considering the strict sanitary precautions and the newborn being placed in an incubator.

Transmission through breastfeeding has been described for other flaviviruses such as West Nile virus. Nucleic acid of this virus was found in breast milk, and a case of breastfeeding mother–to-child transmission has been reported [9]. Concerning yellow fever virus, another Flavivirus, cases of breastfeeding transmission have been described [10, 11], and recommendations have been made to stop breastfeeding during the viremic phase after vaccination using a live attenuated virus [12]. To the best of our knowledge, transmission of DENV has been described through blood and during pregnancy [2] but never through breastfeeding. One case [3] reported DENV transmission through...
mucosa with the face, mouth, nose, and eyes coming into contact with viremic blood. In our case, we evidenced dengue viral loads in breast milk as high as the ones found in the mother’s blood sample on the same day. The infant’s extended contact time with milk during feeding and the large volume of intake compared to very small blood volumes reported as infectious make transmission through breastfeeding plausible. Should breast milk be the route of transmission, the short incubation period in our case might be related to a high infective dose.

To summarize, we report a case of DENV vertical transmission with a detailed clinical, biological, and virological description. Moreover, we report the presence of DENV in breast milk. Significant breast milk viral loads and the breastfeeding transmission route described for other flaviviruses make DENV transmission through breastfeeding plausible. The case report raises concern about infants being breastfed by mothers presenting acute dengue infection. Such cases could be numerous as epidemics are large, affecting tropical and subtropical countries where natality rates are high and breastfeeding is widespread. Further work is clearly required to evaluate the risk of transmission of DENV through breast milk.

### Supplementary Data

Supplementary materials are available at Clinical Infectious Diseases online (http://cid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

### Notes

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