

Lactating mothers infected with Ebola virus: EBOV RT-PCR of blood only may be insufficient

M Moreau (mil.moreau@gmail.com)^{1,2}, C Spencer^{2,3}, J G Gozalbes², R Colebunders^{4,5}, A Lefevre^{2,6}, S Gryseels^{7,8}, B Borremans^{7,8}, S Gunther^{7,9}, D Becker^{7,10}, J A Bore⁷, F R Koundouno⁷, A Di Caro^{7,11}, R Wölfel^{7,12}, T Decroo¹³, M Van Herp¹³, L Peetermans¹⁴, A M Camara¹⁵

1. Department of Emergency Medicine, Centre Hospitalier Chrétien, Liège, Belgium
2. Médecins Sans Frontières, Operational Centre Brussels, Guéckédou, Guinea
3. Department of Emergency Medicine, Columbia University College of Physicians & Surgeons, New York, United States of America
4. Department of Epidemiology and Social Medicine, University of Antwerp, Antwerp, Belgium
5. Department of Clinical Sciences, Institute of Tropical Medicine, Antwerp, Belgium
6. Department of Pediatrics, Infectious and Tropical Diseases, CHR Citadelle, University of Liège, Liège, Belgium
7. European Mobile Laboratory (EMLab), Hamburg, Germany
8. Evolutionary Ecology Group, University of Antwerp, Antwerp, Belgium
9. Bernhard-Nocht-Institute for Tropical Medicine, WHO Collaborating Centre for Arboviruses and Hemorrhagic Fever Reference and Research, Hamburg, Germany
10. Institute of Virology, Philipps University of Marburg, Marburg, Germany
11. Microbiology laboratory and Infectious Diseases Biorepository, L. Spallanzani National Institute for Infectious Diseases, Rome, Italy
12. Bundeswehr Institute of Microbiology, Munich, Germany
13. Médecins Sans Frontières, Operational Centre Brussels, Medical Department, Brussels, Belgium
14. Department of Pediatrics, Neonatology, Cliniques Universitaires Saint-Luc, University Catholique de Louvain, Brussels, Belgium
15. Department of Infectious and Tropical Diseases, Centre Hospitalier Universitaire Donka, Conakry, Guinea

Citation style for this article:

Moreau M, Spencer C, Gozalbes JG, Colebunders R, Lefevre A, Gryseels S, Borremans B, Gunther S, Becker D, Bore JA, Koundouno FR, Di Caro A, Wölfel R, Decroo T, Van Herp M, Peetermans L, Camara AM. Lactating mothers infected with Ebola virus: EBOV RT-PCR of blood only may be insufficient. *Euro Surveill.* 2015;20(3):pii=21017. Available online: <http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=21017>

Article submitted on 1 January 2015 / published on 22 January 2015

We describe two Ebola virus (EBOV) RT-PCR discordant mother–child pairs. In the first, blood from the breast-feeding mother, recovering from EBOV infection, tested negative twice but her urine tested positive. Her child became infected by EBOV and died. In the second, the breastfed child remained EBOV-negative, although the mother’s blood tested positive. We highlight possible benefits of EBOV RT-PCR testing in urine and breast milk and the need for hygiene counselling when those fluids are EBOV-positive.

We report two Ebola virus (EBOV) RT-PCR discordant mother-child pairs that illustrate that EBOV RT-PCR testing of relevant fluids in addition to blood, such as urine and breast milk, may be useful, in certain instances.

Background

The current West African Ebola virus disease (EVD) epidemic is different from all previous EVD outbreaks [1]. Because of its regional and international distribution, the massive strain on the local health systems in the affected countries and the very large number of persons infected, the current outbreak has evolved into a major humanitarian crisis [2].

Offering patient care to breastfeeding Ebola virus (EBOV) infected women and their children in such a setting can be particularly challenging because evidence-based guidelines about breastfeeding are lacking. This

report of two EBOV RT-PCR discordant mother-child pairs illustrates possible benefits of EBOV RT-PCR testing in urine and breast milk, not just in blood.

Case 1: mother-child pair

In early October 2014, a woman in her late 30s was referred to the Ebola Treatment Centre (ETC) of Médecins Sans Frontières (MSF) in Guéckédou, Guinea because of general malaise and myalgia. She was accompanied by her asymptomatic, almost exclusively breastfed, six-month-old infant.

The patient had taken care of a relative who had developed symptoms compatible with EBOV in early September and had died 12 days after symptom onset. The patient had also organised the funeral. Two days after the relative’s death, she developed high fever, intense fatigue, headache, muscle and abdominal pain, vomiting and diarrhoea. She was admitted to a local hospital where she received oral and intravenous empirical anti-malaria treatment and antibiotics for three days. The diagnosis was unclear. Although she had symptoms compatible with EBOV infection, she was not tested for EVD as EBOV RT-PCR tests were not available.

After three days in hospital, 13 days after the onset of her symptoms, the patient was referred to the ETC of MSF for persistent malaise and myalgia. Upon admission, she was afebrile. Given the clinical history and

the high-risk contact, the patient was admitted to the ETC in the 'suspect' zone of the 'high-risk' area. Her asymptomatic child was housed in a nursery next to the ETC and breastfeeding was stopped. On day 14 of illness, the patient's EBOV RT-PCR blood test (Realstar Filovirus Screen, RT-PCR Kit 1.0, Altona Diagnostics, Hamburg) as well as a rapid malaria test (SD BIOLINE Malaria Ag P.f, Standard Diagnostics Inc.) were negative.

On the same day (day 14 of illness of the mother), the child developed fever (39.1°C), diarrhoea and severe weakness; a malaria rapid test was negative but EBOV RT-PCR test was positive (cycle threshold (CT) value 19.80; CT values <20 are highly positive whereas >35 are weakly positive).

A second EBOV RT-PCR blood test of the mother, 16 days after symptom onset, remained negative but the urine EBOV RT-PCR test from the same day was positive (CT value 29.09). EBOV RT-PCR test of breast milk performed on day 17 after symptom onset was negative and breastfeeding was restarted. The patient had recovered well and was discharged on the same day but the child passed away three days later.

Case 2: mother-child pair

A woman in her mid-20s developed a febrile syndrome four days after having given birth to a healthy baby and was admitted to an MSF ETC in Guéckédou five days later. We note that a close relative of the patient who was present during the delivery, developed symptoms compatible with EVD on the day following the delivery and died one week later. The patient had taken care of this relative.

Upon admission, the patient's temperature was 39°C and she had severe weakness, myalgia, arthralgia, anorexia, dysphagia, hiccups, abdominal pain and diarrhoea. Minor bloody vaginal discharge was noted. An oral antibiotic (cefixime) and anti-malaria treatment were started empirically. On day 6 after onset of illness, a rapid malaria test was negative but an EBOV RT-PCR blood test was positive (CT value 23.92). The clinical course of the patient was favourable and she was declared cured 12 days later (day 18 after onset of illness). After two negative EBOV RT-PCR blood tests, 24 hours apart, she was discharged from hospital. No EBOV RT-PCR of the breast milk was performed.

Upon admission, her infant was 10 days old and had been breastfed since birth. The child was immediately separated from the mother and breastfeeding was stopped. Six days later, the child developed fever (38.9°C). Ceftriaxone and gentamicin were started. Artesunate was also given but stopped after a negative malaria test. EBOV RT-PCR blood tests were negative on day 1 and 3 after onset of fever. Gentamicin was stopped after two days but ceftriaxone continued for eight days with a favourable clinical outcome. The infant rapidly became asymptomatic and was followed

up for 21 days after the last contact with the sick mother. The child did not develop EVD.

Discussion

We describe two EBOV RT-PCR discordant mother-child pairs that illustrate the complexity of taking care of patients with EBOV infection.

If a lactating mother's blood is EBOV RT-PCR negative and has an EBOV-positive breastfed child (Case 1), healthcare workers should investigate whether the mother recently recovered from a confirmed or suspected EBOV infection. The mother's urine and breast milk should be tested by EBOV RT-PCR for shedding of EBOV even after the virus becomes undetectable in the blood [3,4]. The child in Case 1 described, was most likely infected by the mother, however, whether the child became infected through breast milk or through contact with another bodily fluid, remains unknown. We cannot fully rule out the possibility that the source of the child's infection was the relative who was taken care of by the child's mother but this would mean the incubation period of the child was at least 16 days which is long given the average incubation period of 8 to 10 days [5].

Data on how long infective EBOV can be present in other body fluids such as saliva, tears, urine, stool, breast milk, vaginal and amniotic fluid and seminal fluids, are still limited [4]. We do know that in the 36-year-old patient with EVD who was evacuated in August 2014 to an isolation facility in Hamburg, Germany, infective EBOV was still isolated from urine samples on day 26 of his illness, nine days after the clearance of EBOV from plasma [3]. We also know that EBOV can be isolated from convalescent patients in semen up to 82 days after disease onset [6]. However, in a study by Bausch et al., EBOV could not be cultured from the urine in 11 cases, but this might have been caused by virus degradation from breaks in the cold chain during sample collection, storage and shipping [4].

Detection for long periods of time in urine is known for other viruses, such as the West Nile virus [7] but poorly documented for EVD. The added value of EBOV testing of the urine of convalescent patients remains to be determined. Indeed, a positive PCR test does not mean the urine is still infectious and it would be impossible to keep patients with positive EBOV RT-PCR urine or semen tests for months in isolation.

EBOV has been detected in breast milk previously [4] but the timing of EBOV appearance, how long it remains in breast milk in an EBOV-infected lactating mother and the exact risk for a child to become infected through breastfeeding, remain poorly understood. EBOV was isolated from the breast milk of one lactating woman 15 days after disease onset, and after EBOV was already cleared from the blood [4]. We will need prospective studies of mother and child pairs, combining PCR testing with virus culture of breast milk to finally come

up with evidence-based recommendations regarding breastfeeding in cases of lactating mothers with EVD. Although high levels of actively produced IgA in breast milk have been shown to provide limited local mucosal protection for breastfed children against influenza virus infection [8], further studies are needed to determine the cellular and immunologic effects of breast milk-secreted antibodies in EVD patients.

These two cases demonstrate that when caring for mother-child pairs, healthcare workers should consider the potential role of testing relevant body fluids in addition to blood, such as urine and breast milk.

In case of discordant RT-PCR results between an EBOV-positive mother and her EBOV-negative breastfed child, ideally, breastfeeding should be stopped if safe replacement for breastfeeding is available [9]. Otherwise, feeding the child with heat-treated expressed breast milk [10] could be considered. Where a mother has survived EVD, ideally, her breast milk should be confirmed negative for EBOV before resuming breastfeeding. If EBOV RT-PCR diagnostic is not available, it is advised to avoid breastfeeding by EVD-surviving mothers [9].

The possibility of prolonged EBOV shedding in urine and breast milk means that counselling about hygiene in handling those fluids should be an important component of health promotion at the time of discharge from the ETC.

Acknowledgements

We thank the National and MSF staff of the Guéckédou ETC for their support in taking care of these and many other patients.

Conflicts of interest

None declared.

Authors' contributions

Michel Moreau, Craig Spencer, Julia Garcia Gozalbes, Alseny Modey Camara were involved in the care of patients at the ETC in Guéckédou. Sophie Gryseels and Benny Borremans performed the PCR testing. Michel Moreau and Robert Colebunders wrote the first draft. Michel Van Herp, Tom Decroo, Annabelle Lefevre, Antonino Di Caro, Roman Wölfel, Dirk Becker, Stephan Günther, Joseph Bore, Raymond Koundouno, Leentje Peetermans, all reviewed the paper, and their comments were incorporated.

References

1. Feldmann H, Geisbert TW. Ebola haemorrhagic fever. *Lancet*. 2011;377(9768):849-62. [http://dx.doi.org/10.1016/S0140-6736\(10\)60667-8](http://dx.doi.org/10.1016/S0140-6736(10)60667-8)
2. WHO Ebola Response Team. Ebola virus disease in West Africa—the first 9 months of the epidemic and forward projections. *N Engl J Med*. 2014;371(16):1481-95. <http://dx.doi.org/10.1056/NEJMoa1411100> PMID:25244186

3. Kreuels B, Wichmann D, Emmerich P, Schmidt-Chanasit J, de Heer G, Kluge S, et al. A case of severe Ebola virus infection complicated by gram-negative septicemia. *N Engl J Med*. 2014;371(25):2394-401;371(25):2394-401. <http://dx.doi.org/10.1056/NEJMoa1411677> PMID:25337633
4. Bausch DG, Towner JS, Dowell SF, Kaducu F, Lukwiya M, Sanchez A, et al. Assessment of the risk of Ebola virus transmission from bodily fluids and fomites. *J Infect Dis*. 2007;196(s2) Suppl 2:S142-7. <http://dx.doi.org/10.1086/520545> PMID:17940942
5. Centers for Disease Control and Prevention (CDC). Ebola (Ebola Virus Disease). Signs and Symptoms. Atlanta: CDC. [Accessed 22 Jan 2015]. Available from: <http://www.cdc.gov/vhf/ebola/symptoms/index.html>
6. Rowe AK, Bertolli J, Khan AS, Mukunu R, Muyembe-Tamfum JJ, Bressler D, et al. Clinical, virologic, and immunologic follow-up of convalescent Ebola hemorrhagic fever patients and their household contacts, Kikwit, Democratic Republic of the Congo. *Commission de Lutte contre les Epidémies à Kikwit. J Infect Dis*. 1999;179(s1) Suppl 1:S28-35. <http://dx.doi.org/10.1086/514318> PMID:9988162
7. Murray K, Walker C, Herrington E, Lewis JA, McCormick J, Beasley DW, et al. Persistent infection with West Nile virus years after initial infection. *J Infect Dis*. 2010;201(1):2-4. <http://dx.doi.org/10.1086/648731>
8. Schlaudecker EP, Steinhoff MC, Omer SB, McNeal MM, Roy E, Arifeen SE, et al. IgA and neutralizing antibodies to influenza A virus in human milk: a randomized trial of antenatal influenza immunization. *PLoS ONE*. 2013;8(8):e70867. <http://dx.doi.org/10.1371/journal.pone.0070867> PMID:23967126
9. UNICEF. World Health Organization (WHO). Centers for Disease Control and Prevention (CDC). Emergency Nutrition Network (ENN). Infant feeding in the context of Ebola – Updated guidance. UNICEF. WHO. CDC. ENN. 19 Sep 2014. Available from: http://files.enonline.net/attachments/2176/DC-Infant-feeding-and-Ebola-further-clarification-of-guidance_190914.pdf
10. Jeffery BS, Webber L, Mokhondo KR, Erasmus D. Determination of the effectiveness of inactivation of human immunodeficiency virus by Pretoria pasteurization. *J Trop Pediatr*. 2001;47(6):345-9. <http://dx.doi.org/10.1093/tropej/47.6.345> PMID:11827302