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BRIEF REPORT

# Ebola Virus Persistence in Breast Milk After No Reported Illness: A Likely Source of Virus Transmission From Mother to Child

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A 9-month-old infant died from Ebola virus (EBOV) disease with unknown epidemiological link. While her parents did not report previous illness, laboratory investigations revealed persisting EBOV RNA in the mother's breast milk and the father's seminal fluid. Genomic analysis strongly suggests EBOV transmission to the child through breastfeeding.

**Keywords.** Ebola virus; mother-to-child transmission; real-time sequencing; breast milk; asymptomatic carriage.

On 18 August 2015, a 9-month-old breastfed female infant from Dubréka, Guinea, developed fever (38°C), diarrhea, vomiting, and cough. The father, a trained nurse, administered erythromycin, paracetamol (acetaminophen), amodiaquine, albendazole, and metopimazine. During the following 5 days, the clinical status of the child remained relatively stable. On 24 August, her condition rapidly deteriorated, with severe vomiting and diarrhea. The family attended a primary healthcare center in Dubréka, which referred them to the University Hospital in Conakry. On the way to the hospital, the infant developed respiratory distress and died. A buccal swab tested positive for Ebola virus (EBOV) by reverse transcription polymerase chain reaction (RT-PCR) on 25 August (Figure 1A). The National Committee of Ethics in Medical Research of Guinea approved the use of diagnostic leftover samples and corresponding patient data for this study (permit number 11/CNERS/14). All necessary consents required by applicable law from the patients whose information is included in the article have been obtained in writing.

An epidemiological investigation did not yield a known source of infection. Contact with known Ebola virus disease (EVD) patients or survivors could not be identified. The child was the first EVD case in >42 days in the area where the family was living (Supplementary Figure 1). She had never presented at a healthcare facility and did not receive routine vaccinations, as the mother feared contact with EVD patients in health centers. In addition, the family members reported few social contacts with the exception of contacts to the 4-year-old stepbrother and the grandmother.

In an attempt to trace the source of infection by molecular epidemiology, the EBOV-positive RNA of the child was transferred to our sequencing facility at the Ebola Treatment Center in Coyah on 30 August and the viral genome was sequenced using MinION technology in Guinea (Oxford Nanopore, United Kingdom) [1]. Phylogenetic analysis revealed that the virus (European Nucleotide Archive [ENA] accession number LT630606) belonged to the Sierra-Leone 3 (SL3) lineage and clustered with strains of the large Conakry–Dubréka sublineage that were circulating between May and July 2015 in these prefectures (Figure 1B) [1]. We identified a unique genomic signature consisting of 3 nucleotide polymorphisms at positions 6027, 12290, and 15660, which were not found elsewhere in the EBOV database.

Due to EVD confirmation in the child, the parents received the recombinant vesicular stomatitis virus (rVSV)-based vaccine expressing the glycoprotein of Zaire Ebola virus (rVSV-ZEBOV) vaccine on 27 August (Figure 1A) [2]. On 30 August, both tested positive for EBOV-specific immunoglobulin G but not immunoglobulin M in enzyme-linked immunosorbent assay, which was interpreted as a sign of past EBOV infection and raised the suspicion of asymptomatic virus carriage. Therefore, body fluids of the parents were tested for EBOV RNA using the RealStar ZEBOV RT-PCR kit (Altona, Germany) on a Rotor-Gene instrument (Qiagen) [3]. On 9

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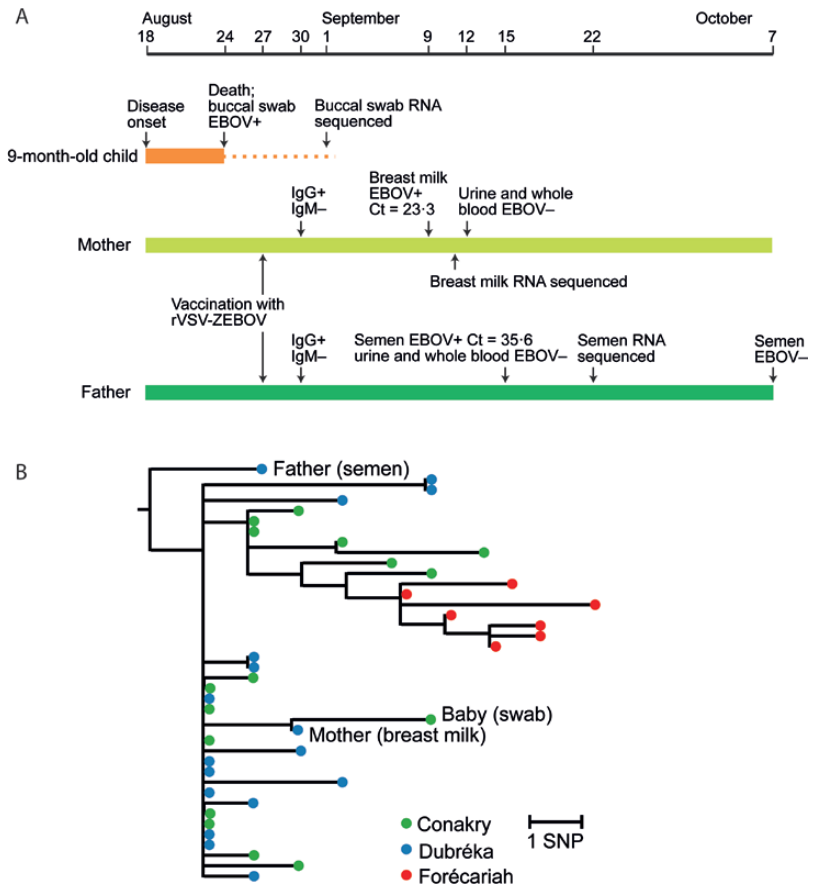


**Figure 1.** Timeline of clinical events, interventions, and laboratory investigations and phylogenetic tree of the Ebola virus sublineage containing the sequences from the family. A, The 3 cases are depicted by horizontal bars with events indicated by arrows. Results of laboratory investigations are indicated as positive (+) and negative (–). For positive real-time polymerase chain reaction results, the cycle threshold is given, which is an indirect measure of viral RNA concentration. B, Phylogenetic reconstruction was performed by maximum likelihood under the GTR + Gamma model using RAxML as described [1, 12]. The subtree shown in the figure depicts the sequences that share a common ancestor with the sequences from the mother and father; the complete tree is shown in [1]. The geographic origin of the cases is indicated by color. European Nucleotide Archive accession numbers are LT630562 for the father's semen, LT630561 for the mother's breast milk, and LT630605 for the child's swab. The length of the branches roughly corresponds to the number of single-nucleotide polymorphisms in the respective strain(s) that distinguish them from the ancestral virus. Abbreviations: Ct, cycle threshold; EBOV, Ebola virus; IgG, immunoglobulin G; IgM, immunoglobulin M; rVSV-ZEBOV, recombinant vesicular stomatitis virus (rVSV)-based vaccine expressing the glycoprotein of Zaire Ebola virus; SNP, single-nucleotide polymorphism.

September, the mother's breast milk tested EBOV RNA positive with a cycle threshold (Ct) value of 23.3, whereas urine and whole blood were negative (Figure 1A). Sequencing and phylogenetic analysis demonstrated that the viruses from child and breast milk (ENA accession number LT630561) are closely related and share 2 of the unique single-nucleotide polymorphisms (Figure 1B). In the phylogenetic tree, the virus from the breast milk appears ancestral to that of the infant. On 15 September, a semen sample from the father tested EBOV RNA positive with a Ct value of 35.6, whereas urine and whole blood were negative (Figure 1A). The presence of EBOV RNA in the semen sample was confirmed by repeat RNA extraction and RT-PCR testing as well as genomic sequencing. The virus sequenced from the semen (ENA accession number LT630562) was also part of the SL3 lineage, but ancestral to the Conakry–Dubréka cluster and without close link to the viruses of mother and child (Figure 1B). On 7 October, the father's semen tested negative for EBOV.

Epidemiological investigation could not reveal source, locality, or time period of EBOV infection in the parents. They did not report a severe febrile illness and denied contact with known EVD cases or family deaths, or attending funerals since the beginning of the Ebola outbreak. The father, a 28-year-old nurse, had worked as community healthcare worker in the district of Mali in the northern part of Guinea from February 2014 to March 2015. Only 5 EVD cases have been reported in this district throughout the outbreak. From March to August 2015, he was unemployed and lived in Dubréka in a family with 4 members. With the exception of a motorcycle accident that required dental reconstructive surgery in a private clinic in Conakry, he denied any noticeable health episode in the past. The mother, a 23-year-old college student, did not report any



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medical episode except chronic headaches. She traveled daily between Dubréka and her school in Conakry by collective taxi.

The described family cluster highlights peculiarities of EVD, which are poorly understood and difficult to study, including EBOV infection in the absence of classical risk factors and virus persistence in patients with a mild or asymptomatic course of EVD associated with risk of virus transmission.

The molecular epidemiological data indicate that mother and father became infected in Conakry or Dubréka and that both infections are not directly linked. The lack of known risk factors suggests that sources and routes of transmission exist, which are not yet understood. The signs and symptoms of EVD in the parents are also difficult to assess, as intermittent febrile illness may be considered the norm in rural populations in Africa. In the absence of objective clinical investigations, we describe the disease here as "mild or asymptomatic." Whether there is an association between this specific clinical manifestation and the (unknown) source and route of infection is a matter of speculation.

Mild or asymptomatic EBOV infections have been described [4]. However, it is not known whether this clinical manifestation, like severe nonfatal EVD, is associated with virus persistence [5, 6]. The cases of mother and father show that EBOV may persist in breast milk and seminal fluid in survivors with mild disease or asymptomatic infection. This raises the possibility of virus transmission by individuals who were not diagnosed with the disease (and thus do not know they had EVD) and are not listed in the national survivors databases, like the 2 parents.

The closely related EBOV sequences in mother and child, the ancestral position of the mother's virus relative to the child's virus in the phylogram, the epidemiological link between mother and child, and the absence of contact of the child to other EVD cases in the community or the hospital suggest that the mother transmitted the virus to the child via breastfeeding. Our findings are in line with the case of an asymptomatic mother whose breast milk tested positive and who may have transmitted EBOV to her 13-month-old child [7]. In addition, there are anecdotal descriptions on presence of EBOV or Sudan virus RNA in breast milk of mothers with symptomatic EVD [8, 9]. The timing of events in our case remains speculative. Given that the child was breastfed for 9 months before she became infected, it is plausible to assume that the infection in the mother occurred rather recently before the child's infection. However, due to the mild or asymptomatic course, the accumulation of EBOV in the mammary gland may have been delayed relative to blood.

The detection of EBOV RNA in seminal fluid of the father suggests that the number of survivors with persisting EBOV in seminal fluid, as predicted from the incidence of hospitalized cases only [10], may be an underestimate. The mechanisms of EBOV persistence in immunologically privileged or glandular tissue, in particular in the presence of circulating antibodies against EBOV, are not well understood [5, 11]. The cases described here at least suggest a lack of correlation between severity of disease and virus persistence. Whether persistence is associated with reduced levels of neutralizing antibodies or poor T-cell memory response remains to be studied.

In conclusion, the described findings call for efforts to estimate the prevalence of EVD survivors who had not been diagnosed during the acute phase and improve counseling for this group of survivors. Fortunately, transmission of virus via breastfeeding many months after recovery is likely to be an extremely rare phenomenon. Among the high number of female survivors, there are presumably hundreds of mothers who have given birth and nursed healthy babies in the postoutbreak period.

## Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the author to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the author, so questions or comments should be addressed to the author.

## Notes

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***Author contributions.*** D. S., M. K., B. D., N. A., D. L. F., and B. A. D. performed the epidemiological investigations. J. A. B., F. R. K., K. S., S. M., T. E., A. M., V. A., O. F., N. M., and S. D. performed the laboratory investigations. N. J. L. performed the sequence analysis. A. A. S., M. W. C., X. A., D. M., P. F., R. B. A., S. K., M. H. D., S. G., and S. D. coordinated case management, laboratory investigations, and fieldwork. D. S., S. D., and S. G. wrote the manuscript. All authors reviewed the final draft. The corresponding author had full access to all of the data in the study and had final responsibility for the decision to submit for publication.

***Disclaimer.*** The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the US Centers for Disease Control and Prevention.

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