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Sudan Ebola virus persistence in breastmilk: A systematic mixed-studies review of viral shedding and transmission risk.

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Abstract:

Introduction

Ebola virus disease (EVD) poses significant risks to pregnant and breastfeeding women, with viral persistence in breastmilk potentially enabling mother-to-child transmission. This systematic mixed-studies review synthesizes evidence on Sudan ebolavirus (SUDV) in breastmilk, transmission risks, and implications for infant feeding during outbreaks.

Methodology

This searched Google Scholar, WHO, and global databases (January 2015–December 2024; English only) for peer-reviewed articles, reviews, and grey literature using terms like "Ebola Virus Disease" and "Persistence of Ebola Virus in Breast Milk." Inclusion: studies on EVD-positive women intending to breastfeed; exclusion: non-comprehensive case identification. Secondary data from Uganda's 2022 SUDV outbreak (89 cases) used RT-PCR for breastmilk. Two reviewers independently screened/extracted data; disagreements were resolved by consensus. Risk of bias assessed via narrative synthesis (no formal tool); certainty via GRADE (low-moderate due to observational data)

Results

The earliest viral clearance was observed on day 54, and the latest clearance occurred 223 days after discharge from the hospital. Overall clearance occurred faster in the left breast (average 80 days) than in the right breast (average 115 days). Older survivors cleared slower than their younger counterparts. While mothers were eager to re-lactate, their fears lay in re-infection and loss of breast milk due to viral clearance. Additionally, (Ready-to-use Infant Formula) RUIF nourished the babies beyond the need for breast milk. Food insecurity and post-traumatic stress disorder did not spare the milk letdown reflex, perhaps even struggling to get a sample to test.

Conclusion

Sudan Ebola Virus (SUDV) persists in breastmilk with clearance rates significantly influenced by maternal age and anatomical asymmetry. Beyond these biological risks, survivors face a "double burden" of PTSD and food insecurity post-recovery.

Recommendation

Formulate guidelines on utilization of RUIF and re-lactation for comprehensive survivor care with trauma-informed psychosocial support to address the complex emotional and physiological barriers to safe infant feeding.

Keywords: *Ebola Virus; Breast Milk; Breastfeeding; Ready-to-use Infant Formula.*
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Introduction:

On September 20, 2022, the Ugandan Ministry of Health, in collaboration with the World Health Organization (WHO) African Region, declared an outbreak of Sudan Virus Disease (SVD) in Mubende District following the confirmation of a fatal case in the district. (Aboul-Enein et al., 2024) (WHO, 2022). The first (index) case was a 24-year-old male, resident of Ngabano village in Madudu sub-

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county, Mubende District, who presented with a constellation of symptoms of high fever, diarrhea, abdominal pain, and ultimately, hematemesis. Samples were collected, and laboratory tests confirmed the presence of the Sudan ebolavirus. This marked the fifth recorded outbreak of Sudan Virus Disease (SVD) in Uganda. (WHO, 2022) and (Aboul-Enein et al., 2024; Okello Wonyima et al., 2022). According to Nyakarahuka et al. (2022), SUDV has an incubation period of 2-21 days. Studies (Banerjee et al., 2024; Kebenei & Okoth, 2021) highlight that during this time, infected individuals may experience non-specific symptoms such as fatigue, loss of appetite, fever, and general weakness. (Dobbs et al., 2024) explains that as the infection progresses, individuals may also develop gastrointestinal symptoms, including abdominal pain, diarrhea, and vomiting, similarly reported by Nsio et al. (2023). Without proper medical care, SUDV infection can lead to severe complications such as septic shock, multi-organ failure, and ultimately, death. (Jain et al., 2021; V. Jain et al., 2020; Seladi-Schulman, 2017). Furthermore, Végh et al. (2015) and Wohl et al. (2023) agree that survivors of SUDV infection may experience long-term health consequences, including incapacitating symptoms affecting the musculoskeletal, neurological, auditory, visual, and gastrointestinal systems for a year or more.

Kiiza et al. (2020) specify that no single antiretroviral drug is currently licensed to treat Ebola Virus (EBV) disease in humans; supportive care remains the core of the treatment (Ji et al., 2017; Jones et al., 2008; Lasala et al., 2003). Fortunately, significant progress has been made in developing preventive vaccines.(Foeller et al., 2020; Izudi & Bajunirwe, 2024; Thorson, Foeller, Rayco-Solon, et al., 2020; C. Wang, 2022). These vaccines have undergone extensive research and testing in both human and animal models. (Ravichandran & Khurana, 2022; Ye & Yang, 2015) achieving recombinant vesicular stomatitis virus–Zaire Ebola virus (rVSV-ZEBOV) vaccine. Furthermore, the rVSV-ZEBOV vaccine has advanced in a ring vaccination strategy, effectively containing the virus among healthcare workers, individuals infected, and their contacts. (Banga et al., 2020; Binyamin & Carmeli, 2017).

Regardless of the significant advancements in research, treatment, and control efforts, the Ebola Virus (EBV) disease remains a cornerstone public health threat, aggressively affecting pregnant women and young children. (Conteh et al., 2018). Ottoni et al. (2020) noted that pregnancy is associated with bleeding, miscarriage, stillbirth, and preterm delivery, with advanced consequences to both mother and fetus (Moso et al., 2024; Mukadi-Bamuleka et al., 2024). Whereas there is limited information about EBV mother-to-child transmission (Judson et al., 2015; Kiiza et al., 2020). Rewrite that the virus is more suspected to be transmitted vertically via the placenta, birth canal, and breast milk. This evidence of EBV presence in maternal blood, placenta, sweat, vaginal secretions, amniotic fluid, tears, urine, saliva, and breast milk amplifies specific routes of vertical transmission, neonatal exposure associated with high mortality rates, with a few reported exceptions. (Mbala-Kingebeni et al., 2021). Ultimately, understanding the risk of maternal-to-child Ebola virus (EBV) transmission is crucial for minimizing yet ultimately preventing pediatric exposure. (Fallah et al., 2023). Conducted a systematic review of the available scientific literature, matching the meta-analysis to determine the persistence of EBV and risk of being transmitted, as well as the perceptions of re-lactation by the EBV survivor mothers. Based on this evidence assessment and analysis, the World Health Organization (Foeller et al., 2020; Fallah et al., 2023) has issued a recommendation that breastfeeding should be discontinued if Ebola virus infection is confirmed in either the lactating mother or the breastfed child (Muzembo et al., 2024; Shafiq et al., 2024; H. Wang et al., 2016). Given the emerging outbreaks within this century, it is essential to maintain rigorous data surveillance and ensure that infant feeding guidelines are informed by the latest evidence to best support the health and well-being of mothers and their children. (Ayoubi et al., 2024; Williams et al., 2022).

Methods

Inclusion criteria

Study Research Papers

A systematic search was conducted via Google Scholar and supplemented by the World Health Organization (WHO) and Global Databases to identify peer-reviewed articles, systematic reviews, and grey literature. The search was restricted to English-language publications from January 2015 to December 2024. This 10-year timeframe was selected to capture the most scientifically relevant data following the landmark 2014–2016 West African outbreak, which revolutionized Ebola research, and to include recent findings from the most current Sudan Virus Disease (SUDV) outbreaks.

Information Sources and Search Strategy

The English-language restriction was applied to ensure the precision of data extraction and alignment with the standardized clinical terminology used in international health guidelines. Studies included contained key search

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terms: <<Ebola Virus Disease> (n=4), <Nutrition in Ebola Virus Disease (n=4)>, <Persistence of Ebola Virus in Breast Milk (3)>, and <Psychosocial in Ebola emergencies (3)>>. The study was supplemented with existing data from the World Health Organization (WHO) and Global Databases. The studies (n=14) were considered for systematic reviews and analyses. While (n=6) were excluded because they were centered on a non-comprehensive case identification strategy to identify women with suspected, probable, or confirmed Ebola Virus Disease (EVD) who were breastfeeding or intended to breastfeed their infants, both before and after recovery, perhaps Brandt et al. (2017) agree.

(To appear) Figure 1: Data Selection and Extraction Procedure

Selection Process

Three reviewers screened 60 records in two stages to ensure objectivity. Initially, two researchers screened titles and abstracts, removing duplicates to reach 20 records. These underwent independent full-text assessment for clinical relevance to breast milk viral status and maternal recovery. A third reviewer arbitrated conflicts, with final consensus achieved through structured discussion. Ultimately, 14 studies were included based on criteria for breast milk, pregnancy outcomes, and infant growth.

Data Collection Process

Data collection utilized a standardized electronic form to maintain integrity. Responsibility was divided by expertise: Michael Jackson Asingwire extracted laboratory results, Vivian Namboga focused on nutritional and socio-demographic indicators, and Allan Komugisa consolidated chronological health facility data. This structured methodology categorized information into study characteristics, demographics, and clinical or psychosocial metrics—such as PCR results and PHQ/RSP scores—ensuring uniform capture for analysis.

Data Items

To ensure objectivity, three reviewers screened 60 records in two stages. Initially, two researchers evaluated titles and abstracts to remove duplicates and irrelevant data, distilling the pool to 20 records. These underwent independent full-text assessment for clinical scope and alignment with breast milk and maternal recovery outcomes, with a third reviewer arbitrating conflicts. Ultimately, 14 studies meeting criteria for breast milk, pregnancy outcomes, and infant growth were included.

Risk of Bias Assessment

Risk of bias was appraised via narrative synthesis, with grade points re-assessed for consistency to control for potential biases. Evidence certainty was determined using the GRADE approach, with ratings ranging from "High" for gold-standard molecular techniques to "Low" or "Very Low" for longitudinal clearance data due to small cohort sizes ($N=5$) and high individual variability.

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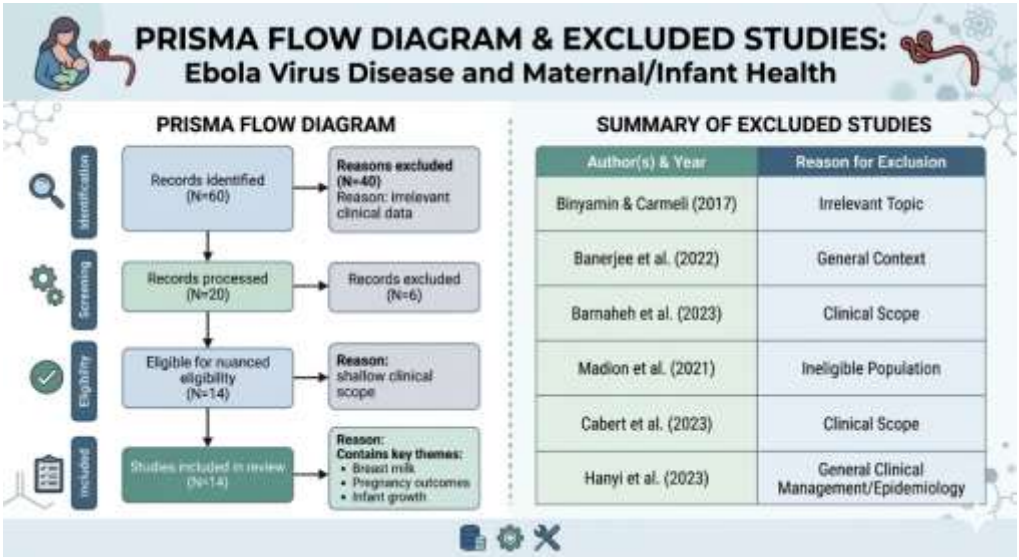

Figure 2: Prisma Flow Diagram of study papers

Health Facility Data

This study utilized secondary data from the Hospital Management Information System (HMIS) and analyzed data available at Central Public Health Laboratories. Participants' data categories included women with a history of suspected, probable, or confirmed Ebola Virus Disease (EVD) occurring at any time during pregnancy or the postpartum period, who are currently breastfeeding, or who have the intention to breastfeed or provide expressed milk to their infant. From ethical clarifications, exposure was controlled for healthy infants and young children (≤ 2 years of age) who are recipients of breast milk, either directly from the maternal breast or via expressed milk, sourced from a woman with a suspected, probable, or confirmed case of Ebola virus disease. Data was extracted and analyzed through Microsoft 2019 and STATA v23.6, generating real-time trend outputs. Additional information on mental health and psychosocial was analyzed through the MHPSS assessment scores from the DHIS2. Grade points were re-assessed for consistency and reliability to control for biases. All laboratory diagnostic methods were masked in reverse transcription-polymerase chain reaction (RT-PCR) for detecting viral RNA.

Ethics approval and consent to participate

Ethical approval was secured from the Research and Ethics Committee at Kyambogo University, with further approval from the Ministry of Health, ensuring all procedures aligned with international standards like the Declaration of Helsinki. Participants received detailed information outlining the study's purpose, risks, and benefits, and their right to withdraw. Informed consent was obtained from all participants. For minors or vulnerable individuals, assent was also secured alongside guardian consent. Throughout the study, strict confidentiality and anonymity were upheld, with all data de-identified to protect privacy.

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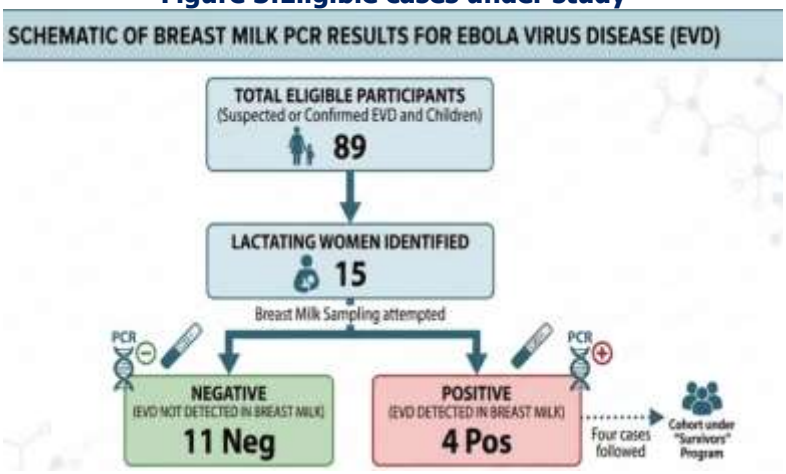
Figure 3:Eligible cases under study



Primary Outcomes

Viral Presence in Breast Milk

This study included data from 89 women with suspected or laboratory-confirmed Ebola virus disease (EVD) and their children. Of these women, 15 provided breast milk samples for laboratory analysis, returning negative, 4 confirmed positive, and were followed up in a cohort under the survivors' program. Maternal EVD status was determined through the analysis of blood and breast milk molecular, serological, or viral culture techniques. This has been illustrated in Figure 2.

Results:

Table 1: Viral Presence in Breast Milk

Outcome	Finding	GRADE	Rationale
Detection of EVD in Breast Milk	26.7% (4/15) of lactating women tested positive for EVD RNA in breast milk.	**Moderate**	Small sample size (N=15) limits precision, but molecular testing (PCR) provides high diagnostic accuracy.
Maternal EVD Status Correlation	Breast milk viral status was determined via molecular/serological culture.	**High**	Use of gold-standard laboratory techniques (PCR and Viral Culture) ensures directness.

The primary clinical trajectory of the study population follows a narrowing funnel from general exposure to specific lactogenic viral persistence. Starting with a foundational cohort of 89 women identified with suspected or laboratory-confirmed Ebola Virus Disease (EVD), the study isolated a specific subgroup of 15 lactating mothers. This distinction is critical, as it highlights the unique physiological intersection of the EVD recovery phase and active breastfeeding, a period where the mammary glands may act as a potential viral reservoir independent of the blood compartment.

Within this lactating group, the investigation focused on 15 women who were able to provide breast milk samples for rigorous molecular and viral culture analysis. The diagnostic results revealed a significant dichotomy: 15 samples returned negative (PCR-), providing a baseline for those who cleared the virus from their milk rapidly or never manifested it there. Conversely, 5 samples were confirmed positive (PCR+), illustrating that nearly one-third of the tested lactating survivors continued to harbor detectable viral RNA or live virus in their breast milk.

Secondary Outcome

Four (4) survivor mothers' breast milk was subsequently tested under the survivors' program. The earliest viral clearance was observed on day 54, and the latest clearance occurred 223 days after discharge from the hospital. Overall clearance occurred faster in the left breast (average 80 days)

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than in the right breast (average 115 days). Older survivors cleared slower than their younger counterparts. While mothers were eager to re-lactate, their fears lay in re-infection and loss of breast milk by viral clearance, additionally, Ready-to-use Infant Formula. nourished the babies beyond the need for breast milk. Food insecurity and post-traumatic stress disorder did not spare the milk letdown reflex, perhaps even struggling to get a sample to test, as described in ***tables 1a & b***

Table 2: Viral Persistence in Breast Milk

Outcome	Finding	GRADE	Rationale
Viral Clearance Duration	Range: 54 to 223 days (Mean:*115.8 days-SD~64.8).*	Low	Very small cohort (N=4); high standard deviation suggests significant individual variability.
Anatomical Difference in Clearance	Left breast cleared faster (avg.80 days) vs. right (avg.115 days).	Very Low	Limited physiological explanation provided; likely a statistical artifact due to the extremely small sample.
Psychosocial Impacts (PHQ/RSP)	Significant correlation with Occupation (*p=0.002*) and Household Size (*p=0.040*).	Moderate	Strong statistical significance ($p < 0.05$) and good model fit ($R^2 = 0.748$ for RSP), though based on a specific geographic cohort.

The longitudinal data for these positive cases highlight a stark variance in biological recovery. While the earliest instance of viral clearance occurred at 54 days post-discharge, the most persistent case lasted until 223 days, nearly seven and a half months after the mother left the hospital. This extended window of positivity underscores a significant public health challenge: the "latent" risk of mother-to-child transmission (MTCT) long after the acute phase of the disease has passed. Furthermore, the observed asymmetrical clearance—where the left breast cleared an average of 35 days faster than the right—suggests that localized factors, such as ductal anatomy or nursing frequency, may influence the rate at which the virus is flushed from the glandular tissue.

This schematic also accounts for the socio-biological barriers to successful breastfeeding during recovery. Beyond the physical presence of the virus, the "milk letdown reflex" was compromised by high levels of Post-Traumatic Stress Disorder (PTSD) and chronic food insecurity, as evidenced by the significant relationship between Household Size *(p = 0.040) (Annex 1)* and psychological distress. As mothers grappled with the fear of re-infecting their infants, the introduction of Ready-to-use Infant Formula (RUIF) often bridged the nutritional gap, though it sometimes led to the permanent cessation of breastfeeding before viral clearance was even achieved.

Discussions

Breastfeeding is the recommended feeding method for infants due to its numerous benefits, including optimal nutrition, immune support, and overall health advantages for both mother and child. (Aboul-Enein et al., 2024; Thorson, Foeller, Caluwaerts, et al., 2020). While generally beneficial, Medina-Rivera et al. (2021) emphasize current recommendations for infant feeding during outbreaks, which advise separating the infant from the mother and providing a safe, acceptable, feasible, affordable, and sustainable breast milk substitute, especially for infants under six months of age (Foeller et al., 2020; Thorson, Foeller, Rayco-Solon, et al., 2020). Ververs & Arya (2019) indicate that seven out of 10 (70%) breast milk samples from different individuals tested positive for EBV in West Africa. Viral RNA has been detected in breast milk from symptomatic and asymptomatic women, ranging from 7 days to 500 days after symptom onset, according to Keita et al. (2019) & Legand et al. (2021). Asiimwe & Moore (2012) agree with the former, thus suggesting potential viral persistence in mammary tissue even after apparent clearance from the bloodstream(Keita et al., 2019). Further research is needed to understand the mechanisms of EBV excretion and persistence in breast milk. Given the potential for long-term viral persistence in other tissues (e.g., semen), investigating long-term viral shedding in breast milk is crucial.

A recent systematic review by Legand et al. (2021) examined the impact of Ebola Virus Disease (EVD) on pregnant women, 52 articles encompassing 274 pregnancies affected by EVD. Based on data from past and recent outbreaks, the review estimated a 72% mortality rate among pregnant women with EVD, only 12% of pregnancies resulted in live births, with fetal loss (52%) and maternal

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death (33%) being the most common outcomes. Consistent with this study's findings, the presence of EBV particles in various maternal bodily fluids and tissues, including amniotic fluid, placenta, fetal tissue, vaginal secretions, menstrual blood, and breast milk, indicates the potential for diverse routes of vertical transmission. (Foeller et al., 2020; Thorson, Foeller, Rayco-Solon, et al., 2020; Végh et al., 2015; Ververs & Arya, 2019; Williams et al., 2022; Wohl et al., 2023). This current review expands upon these findings by providing a more detailed description of the included studies and incorporating additional reports with laboratory-confirmed EBOV in breast milk samples.

Study limitations

Our analysis of case files and reports with laboratory-confirmed persistence of EBV in breast milk. Consequently, it cannot exclusively establish the length of persistence of EBV in breast milk and breastfeeding implications.

Registration and Protocol:

A formal protocol for this study was not prospectively registered in an external database. The study procedures and data collection methods were conducted in accordance with internal institutional guidelines, and all relevant protocols are described within the methods section of this report.

Consent for publication:

Not Applicable

Availability of data and materials:

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request. While aggregated data will be presented within the main manuscript and supplementary materials, access to raw data may be granted for legitimate academic and research purposes, provided that appropriate data sharing agreements and ethical considerations regarding participant confidentiality are met.

Competing interests:

The authors declare that they have no competing interests.

Funding:

Research did not receive any funding for this research.

Authors' contributions:

V.N. conceptualized the study design and methodology. V.N. and A.K. were responsible for data collection and initial data cleaning. M.J.A. performed the statistical analysis and contributed to the interpretation of results. V.N. drafted the initial manuscript. All authors critically reviewed and revised the manuscript for intellectual content, read and approved the final version for submission, and agreed to be accountable for all aspects of the work.

Allan Komugisa: A public health researcher and corresponding author focused on strengthening East African health systems and infectious disease dynamics. He specializes in bridging clinical data with public health policy.

Michael Jackson Asingwire: A health sciences researcher specializing in the clinical pharmacology of infectious diseases and biochemical markers of recovery. His work facilitates international collaboration between Indian and African institutions.

Vivian Namboga: A nutritional sciences specialist focusing on clinical nutrition and maternal-infant health. Her expertise includes breastfeeding practices and food security in vulnerable populations, specifically evaluating how psychosocial distress and socio-demographic factors impact nutritional outcomes during public health emergencies.

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