



Persistence of Ebola virus in the semen of male survivors

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Introduction

The 2014–2016 epidemic of Ebola virus (EBOV) in West Africa was the longest and geographically most widespread Ebola outbreak ever recorded. According to the World Health Organization, it caused more than 28,616 human cases and 11,310 fatalities, but the actual case numbers are assumed to be even higher due to undetected and unreported cases. The unprecedented dimension of the outbreak has resulted in thousands of survivors of Ebola virus disease (EVD), many of whom suffer from physical and mental health problems (1-3). In addition, there is evidence that EBOV can persist for an extended period of time during the convalescent phase in specific body compartments of EVD survivors, including the central nervous system, eyes, amniotic fluid, placenta, breast milk and seminal fluid (4-11). Transmission of EBOV during convalescence may potentially lead to reinfections within families and local communities where the virus had seemingly been eradicated before. Given the large number of male EVD survivors in West Africa, particularly the presence of EBOV in semen raised concerns of EBOV transmission through sexual intercourse.

At present, only limited data is available for how long viable EBOV particles naturally persist in the seminal and other body fluids of EVD survivors. Such information is important to understand the mechanism of EBOV persistence and its shedding in various body fluids, including semen, in order to estimate and mitigate the risk of transmission from EVD survivors. Studies from previous EBOV outbreaks have shown that infectious EBOV can be isolated from the semen of survivors up to 82 days after onset of disease symptoms (9). Data from the recent West African

outbreak even demonstrated the presence of EBOV genetic material (RNA) in the semen of survivors for up to 9 months after disease onset, with individual reports showing viral RNA persistence for more than 1.5 years (6,12,13). Detection of EBOV nucleic acid, however, may only reflect the presence of residual viral RNA and therefore does not provide information about the presence of viable, infectious virions as well as the contagiousness of body fluids from survivors. Although in most studies the relationship between prolonged detection of viral RNA in semen and virus infectivity based on cell culture or animal models has yet to be determined, documented cases of sexual transmission of EBOV in Liberia and Guinea up to 470 days after onset of illness indicate that transmissible infectious virus can persist in the seminal fluid for a longer time than previously recognized (12,14). While available epidemiological information indicates that sexual transmission is rather rare, sexual partners of male survivors can be at risk of contracting EVD due to virus persistence and seminal shedding of EBOV. This highlights the need for continued post-outbreak surveillance and preventive measure activities aiming at reducing the potential risk of sexual transmission of persisting EBOV.

Liberia's Men's Health Screening Program (MHSP)

A very recent publication by Soka *et al.* in the "*The Lancet Global Health*" (15) reports on experiences and findings that were gained in the context of Liberia's Men's Health Screening Program (MHSP), the first and ongoing national semen testing program for EBOV survivors operated at three hospitals. Males ≥ 15 years with an Ebola hospital

discharge certificate were eligible for the program which includes semen sample testing for EBOV RNA (sequences specific for the first and third gene, NP and VP40) as well as counseling on safe sexual practices. After two consecutive negative semen tests, participants are released from the program. Information on sociodemographics and sexual behaviour was gathered at all appointments. For 38 of the 429 participants, EBOV RNA was detectable in at least one semen specimen, of which 24 had recovered from the disease at least 1 year ago and 1.5 years as the longest interval. Men aged >40 years had a higher incidence of their semen sample tested positive. Many participants, who declared no condom use at enrolment or stated that they were sexually active, reported using condoms or being abstinent at their first follow-up visit. Thus, the program makes EBOV survivors successfully aware of their individual risk and motivates them to protect their sexual partners.

Liberia's MHSP again conclusively illustrates the importance of aftercare (post-outbreak surveillance) programs for epidemic diseases, which combine body fluid testing with inquiries and counseling on relevant social and behavioral aspects. Such programs should be firmly installed not only for EBOV but also for HIV, Zika, Dengue or Lassa infections. An important issue is an appropriate financial incentive for program participants, considering that such infections primarily hit poorer countries, and often participants have to master long and troublesome access routes to get to their screening unit. Also, mobile counselors that visit participants in their secluded home villages should be an important component of such programs to foster the compliance of participants.

Infectivity of viral RNA detected in body fluids

As already mentioned, a key question is whether viral RNA detected in semen samples represents infectious viral particles or non-infectious genomic fragments. Here, testing of aftercare semen samples in sensitive cell culture systems or animal models should be informative to answer this question. Also, future research efforts have to be dedicated to exploring the mechanistic basis of how EBOV succeed to survive in immune privileged organs, including the testis, for extended periods. Exosomes may play a role in these processes, as detailed below.

Exosomes and viral infections

Many different viruses use exosomes as a kind of Trojan

horse which protects them from host immune responses, thus increasing their persistence [for review see (16)]. Furthermore, exosomes have been identified as vehicles for virus transmission, a strategy that may broaden the number of cell types which can be infected. Exosomal vesicles, which originate from multivesicular bodies (MVBs) and can be released from nearly all types of cells, play an important role in various biological processes such as intercellular communication as well as immune regulation and signaling. They can be isolated from various body fluids including saliva, plasma, serum, breast milk, seminal fluid and bronchial alveolar lavage. Exosomes can cross biological barriers such as the blood-brain, blood-ocular or blood-testis barrier, thus enabling viral access to immune-privileged tissues and organs (16).

For hepatitis C virus (HCV), a member of the *Flaviviridae*, it was shown that its RNA genome can remain in endosome-derived intraluminal vesicles to maintain infectious particles which can be secreted via exosomes (17). Indeed, exosomes isolated from sera of chronic HCV-infected patients contained replication-competent HCV RNA genomes (18). Moreover, it could be shown that exosome-mediated transmission of HCV can establish a productive infection (19). The picornavirus hepatitis A virus seems to use a similar route for virus transmission, as the virus hijacks endosomal membranes for encapsulation and subsequent release; such enveloped, fully infectious viruses resemble exosomes (20). It was also shown that Bunyaviruses such as the Rift Valley fever virus (RVFV) or the SFTS (severe fever with thrombocytopenia syndrome) virus store both viral RNA and viral proteins in exosomes, and such exosomes are able to infect host cells via receptor-independent transmission (21,22). Very recently, it has been shown that the Ebola matrix protein VP40 is packaged into exosomes that are taken up by T- and myeloid immune cells; in such recipient cells, VP40 is able to induce apoptosis and to downregulate the RNAi machinery, thereby weakening the host's adaptive immune system and its defense against the viral infection (23).

At present, our understanding how EBOV maintains viral reservoirs is only fragmentary. The isolation of EBOV RNA from immune-privileged sites several hundred days after recovery from infection is disturbing and needs to be analyzed in much more detail to understand how EBOV persists and is able to spread its infectious components over such long time periods. Thus, research on cell and tissue tropism in immune-privileged organs as well as the possible role of exosomes in EBOV infection is an urgent

requirement towards a better mechanistic understanding of EBOV persistence and virus transmission long after recovery from acute illness.

Concluding remarks

Despite open scientific questions, Liberia's MHSP is a paradigm for a successful post-outbreak surveillance program that combines body fluid testing with inquiries and counseling on relevant social and behavioral aspects. The installation and permanent maintenance of comparable programs is strongly recommended not only for EBOV but also for other sexually transmissible viral pathogens with great public health impact.

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