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SUPPLEMENT ARTICLE

Ebola Virus Transmission Caused by Persistently Infected Survivors of the 2014–2016 Outbreak in West Africa

Lorenzo Subissi,[1] Mory Keita,[2] Samuel Mesfin,[2] Giovanni Rezza,[3] Boubacar Diallo,[4] Steven Van Gucht,[1] Emmanuel Onuche Musa,[4] Zabulon Yoti,[4] Sakoba Keita,[5] Mamoudou Harouna Djingarey,[2] Amadou Bailo Diallo,[2] and Ibrahima Soce Fall[4]

[1]Sciensano, Brussels, Belgium; [2]World Health Organization Country Office, Conakry, Guinea; [3]Department of Infectious Diseases, Istituto Superiore di Sanità, Rome, Italy; [4]World Health Organization Regional Office for Africa, Brazzaville, Republic of Congo; [5]National Agency for Health Security, Conakry, Guinea

The 2014–2016 Ebola virus (EBOV) disease outbreak affected over 29 000 people and left behind the biggest cohort (over 17 000 individuals) of Ebola survivors in history. Although the persistence of EBOV in body fluids of survivors was reported before the recent outbreak, new evidence revealed that the virus can be detected up to 18 months in the semen, which represents the biggest risk of Ebola resurgence in affected communities. In this study, we review the knowledge on the Ebola flare-ups that occurred after the peak of the 2014–2016 Ebola epidemic in West Africa.

Keywords: Ebola; re-emergence; resurgence; survivor; transmission.

Before 2014, Ebola virus (EBOV) was known to circulate in animal reservoirs mainly in Central Africa, where it used to cause sporadic and self-limiting human outbreaks. Between 2014 and 2016, the largest EBOV disease (EVD) outbreak of all time unexpectedly challenged the health systems of several countries, particularly those of Guinea, Liberia, and Sierra Leone, causing over 29 000 cases and 11 310 deaths in a total of 10 countries worldwide [1].

During the Ebola outbreak in West Africa, the investigation of each confirmed EVD case had 2 main components: (1) epidemiological investigation, which aimed at finding the link of acute EVD cases with ongoing chains of transmission, identifying high- and low-risk contacts and performing contact tracing (ie, following them up for 21 days and isolating them as soon as they manifest EVD-like symptoms) [2, 3]; (2) molecular investigation, which utilized whole-genome sequencing to identify cluster of infections by mapping genetic relationships between virus variants from different patients, particularly when a clear epidemiological link could not be established [4–6].

The conditions set by the World Health Organization (WHO) to start the 42-day countdown—corresponding to twice the maximal incubation period of the disease—to declare a country Ebola-free were as follows: (1) all listed contacts of an EVD case must have completed the follow-up period (21 days), and (2) all EVD cases who survived must have been released from the Ebola treatment unit (ETU) [7]. Forty-two days after those conditions were simultaneously met, a country was declared Ebola-free, and a 90-day enhanced surveillance was started [8]. If, at any point during this process, an EVD case was confirmed, the 2 above-mentioned conditions were no longer met and the process had to start again from the beginning.

The first declaration of the end of the 2014–2016 Ebola outbreak was issued for Liberia on May 9, 2015. At that time, Sierra Leone and Guinea reported also a decline in new cases. However, the real end of the West African Ebola outbreak was registered only in June 2016, when the WHO declared Liberia and Guinea—for the fourth and second time, respectively—Ebola-free [1].

Multiple episodes of Ebola re-emergence at the tail end of the outbreak were inconsistent with the typical pattern of human-to-human transmission, which involved direct contact with an acutely infected person, and the epidemiological investigations soon excluded contacts with infectious animals [1].

Ebola virus asymptomatic infections were reported in human beings, but their role in transmission is still unclear [9]. A cross-sectional study in Ebola-affected households in Sierra Leone found that asymptomatic EBOV infections were uncommon (2.6%, 10 of 388), and therefore they were unlikely to significantly contribute to Ebola transmission [10].

In March 2015, Mate et al [11] reported the first molecular evidence that EBOV could be sexually transmitted through the infectious semen of a survivor. Later on, a few other studies reported that EBOV can persist in immune-privileged sites and be detectable in corresponding body fluids, which include aqueous humor, breast milk, and semen [12–19]. In 2005, during the Marburg virus disease outbreak in Angola, 3 of 3 breast milk samples also tested positive for Marburg virus [20]. It is interesting to note that EBOV ribonucleic acid (RNA) was detected in semen by

Correspondence: L. Subissi, PhD, Viral Disease Service, Sciensano, Rue Juliette Wytsman 14, 1050 Ixelles, Belgium (lorenzo.subissi@sciensano.be).

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reverse transcription-polymerase chain reaction up to 18 months after recovery [19, 21–25]. Deen et al [19] detected EBOV RNA in the semen of 100% (7 of 7) of the men with a sample obtained within 3 months from ETU release, 62% (26 of 42) with a sample obtained at 4–6 months, 25% (15 of 60) with a sample obtained at 7–9 months, 15% (4 of 26) with a sample obtained at 10–12 months, 11% (4 of 38) with a sample obtained at 13–15 months, and 4% (1 of 25) with a sample obtained at 16–18 months.

Further evidence can be found from previous outbreaks: a study that analyzed samples from an Ebola outbreak in Kikwit (Democratic Republic of Congo) in 1995 also reported persistence of infectious EBOV in the semen as long as 3 months after onset of disease [26, 27]. It is interesting to note that the first case of male-to-female sexual transmission of a filovirus dates back to the 1967 Marburg virus disease outbreak, in the city of Marburg (Germany), after which the virus—at that time still unknown—was later named [28].

In this study, we reviewed and discussed the reported Ebola flare-ups that occurred towards the end of the epidemic in West Africa and give recommendations for the early implementation of future outbreak responses.

METHODS

We created a systematic approach using the following string search: (Ebola OR Marburg OR filo*) AND (resurgence OR recrudescence OR re-emergence OR recurrence OR cluster OR transmission OR flare-up) AND survivor (last search: March 27, 2018). We searched for additional information in the gray literature, ie, the WHO Ebola situation reports and other relevant reports.

We considered an Ebola flare-up as any new Ebola case or cluster of cases occurring since January 2015—ie, when the number of EVD cases started to decline—with no epidemiological link with ongoing chains of transmission at the time of confirmation and for which detailed information could be retrieved from the literature.

EBOLA FLARE-UPS OF THE 2014–2016 OUTBREAK

We describe here a total of 8 flare ups in the 3 affected countries extending up to 11 months after the first declaration of the Ebola-free status (Table 1).

First Flare-up

On March 20, 2015, an EVD case was confirmed in the Montserrado County, Liberia. The woman did not have an epidemiological link with patients belonging to ongoing chains of transmission. She declared to have had unprotected vaginal intercourse on March 7, 2015 with a male survivor, discharged from the ETU on October 7, 2014. The one nucleotide difference in the near-full genome sequence isolated from the blood sample of the woman and the semen sample (taken on March 27, 2015) of the survivor confirmed likely sexual transmission [11, 29].

Second Flare-up

The WHO declared Liberia Ebola-free for the first time on May 9, 2015. On June 29, 2015, a postmortem oral swab from a 17-year-old boy (index case) tested positive for EBOV RNA. Molecular data revealed that this virus originated from a chain of transmission stopped in August 2014. Retrospective sequencing of the virus from a leftover sample of the only known Ebola survivor

Table 1. Summary of the Episodes of EVD Re-Emergence From Persistently Infected Survivors

Flare-up	Country	Date of Confirmation of Index Case	Size of Cluster	Days After Ebola-Free Declaration	Months From ETU Release (Recovery) of Survivor	Most Suspected Body Fluid	Route of Transmission	Virus Isolation	Reference
1	Liberia	March 20, 2015	1	N/A	5	Semen	Sex	Unsuccessful	[11]
2	Liberia	June 29, 2015	7	51	10[c]	Unidentified	Possibly sex	N/A	[30]
3	Guinea	August 25, 2015	1	N/A	Unknown	Breast milk	Mother-to-child	Unattempted	[15]
4a[a]	Sierra Leone	August 29, 2015	6 (total)	N/A	1.5	Semen	Sex	Unknown	[5]
4b[a]	Sierra Leone	September 3, 2015				Semen	Close contact with body fluids		[5]
5	Guinea	October 13, 2015	1	N/A	11.5	Semen	Close contact with body fluids	Unattempted	[31]
6	Liberia	November 22, 2015	3	80	Unknown	Unidentified	Unidentified	Unknown	[32, 33]
7	Sierra Leone	January 14, 2016	2	68	14[c]	Unidentified	Unidentified	N/A	[34]
8[b]	Guinea (Liberia)	March 16, 2016 (April 1, 2016)	10 3	78 (78)	17	Semen	Sex	Unattempted	[35]

Abbreviations: EBOV, Ebola virus; ETU, Ebola treatment unit; EVD, EBOV disease; N/A, not applicable.

[a]This flare-up is subdivided into 2 because the same survivor is likely to have simultaneously infected 2 relatives (ie, 2 index cases).

[b]Liberia is in brackets because EVD reappearance in the country was not due to EBOV transmission from persistently infected survivor but EVD cases from the Guinean cluster moving to Liberia.

[c]The persistently infected survivor could not be identified: the number of months refers to the time between the dates of EBOV confirmation of the sample with closest EBOV sequence and the sample of the index case.

of the 2014 chain revealed that he was unlikely to be the EBOV source because his viral strain was not closely related to that of the index case [30]. Another woman, who reported a miscarriage that followed untreated Ebola-like symptoms, was suspected to be the source of the EBOV resurgence. She was a component of the same household as the 17-year-old boy index case. Because her EBOV infection was never confirmed, another unidentified survivor could also have been the cause of this flare-up. This episode of resurgence gave rise to a total of 7 EVD-confirmed cases; 5 of them, together with other members of the community, reported having consumed meat of a slaughtered dog [30].

Third Flare-up

On August 25, 2015, the oral swab of a deceased 9-month-old baby tested positive for EBOV in Dubreka district, Guinea. Three days after they received vaccination against Ebola, serology of the parents revealed that both were immunoglobulin (Ig)M negative and IgG positive, despite having not reported Ebola-like symptoms and having had no contact with known EVD cases and no family deaths. Although blood and urine samples of both parents tested negative, breast milk of the mother and semen of the father were positive for EBOV, and molecular analyses revealed that the EBOV genome from the breast milk sample of the mother and that from the oral swab of the baby were closely related. However, the EBOV genome from the semen sample of the father was not [15]. IgM-negative results suggest that both parents were likely to be undiagnosed survivors, as a result of a past unrecognized infection.

Fourth Flare-up

On August 29, 2015, Sierra Leone reported an EBOV-positive postmortem swab in Kambia district, where the last EVD-confirmed cases were dated 50 days previously. Whole-genome sequencing revealed that the EBOV genome from the oral swab only differed by 3 nucleotides to the genome sequenced from the blood and semen samples of a survivor, released from the ETU on July, 18, 2015. It is interesting to note that the daughter of the deceased woman also tested positive for EBOV on September 3, 2015, and the corresponding EBOV genome was exactly the same as that isolated from the survivor. The dates of disease onset of the woman and the daughter—very close to each other—were compatible with human-to-human transmission from the survivor to both the woman and the daughter. Epidemiological investigation confirmed sexual contact between the survivor and the woman. In the case of the daughter, EBOV transmission may have occurred through exposure to contaminated body fluids or secretions while giving care to the mother. This re-emerged chain of transmission gave rise to a total of 6 confirmed cases [5].

Fifth Flare-up

On October 13, 2015, a blood sample of a sick patient tested positive for EBOV in Conakry, Guinea. The patient had no epidemiological link with ongoing Ebola clusters in Guinea and Sierra Leone and was the brother-in-law of a male Ebola survivor. Semen sample of the survivor tested negative on October 17, 2015, but the near-full virus genome sequenced in the blood sample of the survivor during his disease, on November 27, 2014, differed by only 6 nucleotides. In this case, sexual transmission may have occurred, and then the wife of the Ebola survivor had an unrecognized episode of illness, during which her brother may have been exposed to infected body fluids while taking care of her. In fact, the 3 individuals lived in the same household in poor hygienic conditions. Alternatively, a third unidentified individual was the link in the transmission from the Ebola survivor to the patient. The latter was the only confirmed case of this chain of transmission [31].

Sixth Flare-up

On November, 22, 2015, less than 3 months after the second declaration of the end of the outbreak in Liberia (September 3, 2015), the blood sample of a 15-year-old boy from Montserrado County, who died 1 day later, was EBOV-positive. Although further details were not found, the cluster was associated to the resurgence of EBOV from a persistently infected individual. The re-emerged chain of transmission gave rise to a total of 3 confirmed cases [32, 33].

Seventh Flare-up

On January 14, 2016, an oral swab of a deceased 22-year-old woman tested positive for EBOV in Tonkolili district in Sierra Leone, 2 months after the WHO declared Sierra Leone Ebola-free for the first time (November 7, 2015). Whole-genome sequencing linked the EBOV genome sequenced from the woman's oral swab to 2 viral genomes from Western Area, Sierra Leone (1 and 2 nucleotide differences). However, no known survivors could be definitely linked to the woman [34].

Eighth Flare-up

The WHO declared Guinea Ebola-free for the first time on December 29, 2015. On March 16, 2016, 3 deaths in the same community were classified as probable EVD cases, and soon after EBOV RNA was detected in blood samples of contacts attending funerals of the index cases. In total, the cluster included 13 probable and confirmed Ebola cases, 3 of whom traveled to Liberia on April 1, 2016, after the WHO declared the country Ebola-free for the third time (January 14, 2016). The EBOV source of the cluster was clearly found to be a survivor, sexual partner of the first community death, in whose semen EBOV persisted for almost 17 months [35].

DISCUSSION

We reviewed the literature on reported Ebola flare-ups. Ebola flare-ups were defined as any new Ebola case or cluster of cases occurring from January 2015 onwards, ie, after the peak of the 2014–2016 Ebola outbreak in West Africa, deviating from usual

human-to-human transmission, which involves an acutely infected individual. In principle, most molecular and epidemiological evidence on these episodes of EVD re-emergence point to EBOV transmission from a survivor that experienced an acute episode of EVD in the past and became persistently and subclinically infected after recovery. In fact, transmission from persistently infected EVD survivors may have contributed to chains of transmission occurring earlier in the epidemic. This may have gone unnoticed because of the limited scientific evidence on EBOV persistence available before the outbreak. In addition, coordinating investigations and contact-tracing activities during the first part of the outbreak has been challenging because of the overwhelming number of EVD cases.

All flare-ups had persistently infected survivors as a confirmed or likely source of EBOV transmission. For almost all flare-ups, zoonotic events were excluded. The only suspicion was raised by the report of the second flare-up in Liberia (June 2015), which reported a common meal of 5 of the new EVD cases consisting of the meat of a slaughtered dog. However, a leftover sample of the dog carcass tested negative for EBOV [30], and the evidence that dogs can be an EBOV reservoir is still not conclusive [36].

Sexual intercourse was the most likely route of transmission from persistently infected male survivors, confirmed or suspected in half of the flare-ups (Table 1). It is interesting to note that EBOV female-to-male sexual transmission was not identified and remains undescribed to date. The case reports from the fifth flare-up (Guinea) and the fourth flare-up (Sierra Leone) suggest that survivors' infectious semen may have infected humans without sexual contact, eg, through small lesions. In fact, molecular data pointed at transmission through infectious semen, although epidemiological data were inconsistent with direct sexual transmission [5, 31]; the role of fomites in EBOV transmission needs further investigation. One study found that the risk of EBOV transmission through fomites is low in an isolation ward, when infection control measures are in place; however, this does not exclude their possible role in nonprotected settings, such as a survivor's household, which remains to be elucidated [37].

Molecular data were essential to link person-to-person transmission, which often would have been impossible solely based on epidemiological data. A common feature of the molecular data from the investigations of the Ebola flare-ups is a decrease in the evolutionary rate of Ebola virus, when persisting in semen [5, 11, 30, 31, 34]. Based on this observation, Arias et al [5] proposed that other infections from the early phase of the epidemic could have potentially been acquired from persistently infected survivors rather than acutely infected individuals. Because there are no known mechanisms for persistence in the absence of replication for RNA viruses (except retroviruses), reduced rates in viral replication is the most likely explanation. Indeed, a recent study illustrated that EBOV persistence may act as a viral reservoir, representing a balance between natural selection and genetic drift in a new intrahost niche [38].

The case reports from the third flare-up (Guinea) and the seventh flare-up (Sierra Leone) suggested that the number of survivors with persisting EBOV in semen and other body fluids may have been underestimated. The Guinea case report revealed undiagnosed EBOV infection in a man with viral shedding in semen and in a women with viral shedding in breast milk.

This study only reports known EVD re-emergence episodes that happened from January 2015 onwards, at the tail end of the epidemic or after the declaration of the end of the outbreak. Therefore, this study only partially covers the West African EVD outbreak, and such events have surely happened and gone unnoticed in 2014 and in early 2015, when they were much more difficult to recognize because of the higher number of EVD cases and the lack of knowledge on EBOV persistance.

The fact that the virus is rarely isolated in other body fluids than semen does not mean they are not potentially infectious, as suggested by the findings from the third flare-up [15]. It is clear that other body fluids, such as breast milk or aqueous humour, are a less likely source of transmission than semen, but their monitoring becomes essential when every single potential new case has to be prevented, as painfully demonstrated by the course of the tail end of the West African epidemic. Because scientific evidence of persistent infection and its possible consequences is now available, counseling should be prioritized, because often survivors of the West African outbreak were unaware of the risk they represented to their relatives [39].

CONCLUSIONS

Fortunately, sexual transmission is a rare mechanism of EBOV transmission and could be observed few times considering the unprecedented size of the cohort of West African survivors. Biological monitoring of survivors' body fluids and survivors' counseling programs must be implemented as soon as survivors leave the ETU and continued for up to at least 18 months after their release from the ETU or until their body fluids tested negative at least twice. Sexual education programs and condom use reinforcement for discharged patients is also of great importance to prevent episodes of Ebola resurgence. Among other prevention measures, vaccination of contacts of EVD cases before their release from the ETU is highly recommended. In addition, blood and semen donations should be systematically tested or restricted in contexts where EBOV circulates. Studies aiming at estimating the risk of EBOV transmission from fomites are warranted. Working groups lead by Ebola experts and coordinated by WHO are ongoing to update recommendations on how to best structure and implement such follow-up programs.

Notes

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