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PERSISTENCE OF EBOLA VIRUS RNA IN SOME BODY FLUIDS OF EBOLA VIRUS DISEASE (EVD) SURVIVORS – THE NIGERIAN EXPERIENCE

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ABSTRACT

Introduction: **Ebola virus (EBOV) has been shown to persist in some body fluids of Ebola Virus Disease (EVD) survivors with implication for future transmission particularly in Nigeria where EVD was experienced for the first time in 2014. Thus, this paper was aimed at providing information on the duration of persistence of EBOV in Nigeria.**

Materials and Methods: **Ten consenting EVD survivors were enrolled. Baseline specimens; urine and semen (males), urine and high vaginal swab (HVS) (females) were obtained within one month after discharge from the Ebola Treatment Centre (ETC) and subsequently every fortnight. Samples were analyzed using quantitative Real-Star Filovirus Screen RT-PCR kit 1.0 at the National Reference Laboratory in Lagos.**

Results: **Ten EVD survivors comprising 4 (40%) males and 6 (60%) females with age ranges of 28 to >33 years (mean age: 33.0 ± 6.9 years) were evaluated. EBOV RNA was not detected in the urine of all the participants and HVS from the females. However, EBOV RNA was detected in the semen of all 4 (100%) male participants at baseline, and at 2 months after discharge from the ETC. Two men were still positive for EBOV RNA 4 months after discharge from the ETC despite persistent negative vireamia.**

Conclusions: **Our data confirm that a negative viremia in the convalescent period is not predictive of the absence of the virus in semen. Despite an early clearance of the virus from the urine and HVS, there was persistence of EBOV RNA in semen of male survivors 4 months after recovery.**

INTRODUCTION

The outbreak of Ebola virus disease (EVD) in the West African sub-region between years 2014 to 2016 is the largest to date, with more than 28, 600 cases and 11,300 deaths according to reports [1, 2]. In Nigeria, during the outbreak, twenty cases of EVD were identified with a case fatality rate of 40% [3]. Although, the outbreak is over, Ebola Virus (EBOV) is an understudied infection and many aspects of viral transmission remain unclear. During acute infection, EBOV RNA is present in the bloodstream and several body fluids and fomites such as saliva, sweat, vomitus and excreta while the virus may persist for longer periods of time within immune-privileged sites [4, 5]. Direct contact with blood and body fluids is considered the primary transmission mode, but other modes may be possible [6, 7, 8].

A growing body of evidence suggests that EBOV may persist in some body fluids and sanctuary sites such as male gonads, eye and the central nervous system (CNS) after clinical recovery, clearance of the virus from blood and discharge from an Ebola Treatment Centre (ETC) [3, 8, 9, 10, 11, 12], although limited data are still available. For instance, among convalescent patients, EBOV RNA has been detected in breast milk up to 15 days after the onset of the disease, in vaginal secretions up to 33 days after onset, in ocular aqueous humor up to 98 days after onset, and in semen up to 101 days after onset [4, 10, 13, 14]. In addition, EBOV has been cultured from semen samples that were collected 40, 61, and 82 days after disease onset when EBOV was cleared from the blood [14, 15]. This long-term persistence may provide an opportunity for the transmission of EBOV from survivors even after the official end of an outbreak, which is currently defined by the World Health Organization (WHO) as 42 days after the last direct contact with a patient or involved in EBOV corpse burial (i.e., two incubation periods after blood samples from the last patient with confirmed disease have tested negative twice for the virus) [14].

However, more information is required to establish the persistence of EBOV in various body fluids and the duration of persistence. Thus, this paper reports the persistence of EBOV RNA in different body fluids from a cohort of Nigerian EVD survivors at their clinic follow-up. It is intended to better inform behavioural modification advice for EVD survivors, necessary protective measures for health-care providers and public health decisions relating to future EVD outbreaks.

MATERIALS AND METHODS

Study Population and Sampling: The sampling population consisted of 10 of 12 EVD survivors with two consecutive negative quantitative reverse-transcriptase–polymerase-chain-reaction (qRT-PCR) results as criteria of clearance of viral RNA from their blood. They were enrolled at different times after discharge from the ETC after giving an informed consent.

Baseline specimens; urine and semen (males), urine and high vaginal swab (females) were obtained from each of the participants within one month (31 days) after their negative serum EBOV quantitative reverse-transcriptase- polymerase-chain-reaction (qRT-PCR). Subsequent specimens were collected every fortnight for the duration of the study. Samples were tested within 2 hours by means of a qRT-PCR Assay targeting the L-gene sequence of EBOV. Two consecutive negative results were used as criteria for clearance of EBOV RNA from specimen collected.

Ethical considerations: No ethics approval was obtained for this study. This investigation was performed as part of the EVD public health response in Nigeria and was considered not to be research on human subjects but an analysis of public health service data, as documented in Otto *et al*., [16]. All data were completely anonymised before analysis.

Data Collection and Counseling: The socio-demographic characteristics, EVD timelines at the point of enrolment and other laboratory information of all participants were extracted from our database. The EVD survivor certificates were used to ascertain the date of discharge from the Ebola Treatment Centre. Participants received pre–Ebola-test and post–Ebola test counselling at the baseline and when they received their individual qRT-PCR results. The counselling highlights information about the test performed, the meaning of the results, and education about sexual risk-reduction practices, including appropriate condom use and disposal.

Specimen Collection and Laboratory Analyses: All samples (urine, semen/ high vaginal swab) collected were transported to the laboratory using triple level packaging. All packages were decontaminated using 10% hypochlorite solution in a glove box. Aliquots of samples used for viral assays were immediately inactivated for 10mins inside the Glove box using guanidinium-thiocyanate lysis buffer with ethanol for viral nucleic acid extraction (Qiagen, Germantown, Maryland, United States). After the inactivation process, all samples were handled in a Biosafety Level-2 (BSL-2) facility and tested within 2 hours using the quantitative Real-Star Filovirus Screen RT-PCR kit 1.0.

The test kit targets the highly conserved filovirus L-gene sequence and allows differentiation of all relevant filoviruses down to the species level. The details of the assay compositions are confidential intellectual property of Altona Diagnostics [17]. Ebola virus species are detected in the FAM detection channel, Marburg virus species in the Cy5 detection channel, and the internal control in the JOE detection channel. A sample was considered to be positive for Ebola virus if the FAM and JOE detection targets were both detected within 23 to 40 cycles of replication, and the results were considered to be indeterminate if one of the FAM and JOE detection targets was detected but not both. Two consecutive negative results were used as criteria for clearance of viral RNA from any sample during the study. All demographic characteristics were analyzed using Statistical Package for Social Sciences (SPSS) version 20 and the level of significance for any observed association or difference was set at 0.05.

RESULTS

Ten (10) EVD survivors amongst the 12 survivors in Nigeria were recruited for this evaluation. There were 4 (40%) males and 6 (60%) females. The age of these survivors ranged from 28 to >33 years with a mean age of 33.0 ± 6.9 years. Other demographic characteristics and duration of Ebola virus disease are as highlighted in Table 1, with no significant associations found among the demographic variables collated from the participants.

Table 1
Characteristics of Ebola Virus Disease Survivors in Lagos, Nigeria

VARIABLES	CHARACTER	NUMBER OF PARTICIPANTS (%)
Sex	Male	4 (40)
	Female	6 (60)
Age Distribution (Yr)	28 – 30	3 (30)
	30– 33	6 (60)
	>33	1 (10)
Mean Age: 33.0 ± 6.9 yrs		
Marital Status	Single	3 (30)
	Married	5 (50)
	Widowed	2 (20)
Educational Status	Graduate	9 (90)
	Postgraduate	1 (10)
Duration of Disease	5 – 10 days	4 (40)
	11 – 16 days	5 (50)
	>16 days	1 (10)
Mean Duration of Disease: 11.8 ± 3.8 days		

Ebola virus RNA was not detected in the urine specimens of both male and female participants. Also, no EBOV RNA was detected in any of the high vagina swab specimens from the female participants from baseline and for the duration of the study. However, EBOV RNA was detected in the semen specimens of all 4 (100%) male participants at baseline and one month (31 days) after recovery during the study period. The mean cycle threshold (ct) value of the RT-qPCR was 23.17.

Two months (61 days) after discharge from the ETC, all 4 semen samples (100%) from the male participants still tested positive for EBOV RNA with a mean ct value of 28.08. Further evaluation of the semen samples revealed that only 3 (75%) and only 2 (50%) had detectable EBOV RNA at 3 (92 days) and 4 (123 days) months respectively. It is to be noted that patients were discharged from the ETC after a negative viraemia. Further analysis of the semen sample could not be done due to the exit of the remaining 2 participants after 4 months.

DISCUSSION

All the 10 EVD survivors (100%) included in the study presented on admission at the ETC with history of fever with a temperature range of between 37.6^{0}C and 39.0^{0}C, and an average temperature of 38.2^{0}C. Coupled with the fever, major signs and symptoms at presentation were anorexia/loss of appetite, vomiting/nausea, general weakness and diarrhea with 90%, 60%, 60% and 50% prevalence respectively. Other signs and symptoms seen in this set of individuals at presentation were muscle/joint pain (30%), headache (20%), chest pain and eye pain with 10% prevalence respectively.

Data from this study showed no EBOV RNA detected in the urine and high vaginal swab samples of all participants within one month after discharge from the ETC. All four male participants who provided semen specimens during the first 2 months (62 days) after discharge from the ETC had positive quantitative RT-PCR results for EBOV RNA. This finding was unswerving with those of previous studies involving male survivors of the Ebola and Marburg virus diseases [5, 7, 9, 12, 14]. The percentage of male participants with positive semen results declined with the increased time between the date of discharge and the date of enrolment in the study. Although, four months (123 days) after discharge, 50% of these male participants still had positive semen results. The longitudinal analysis of EBOV RNA shedding in semen beyond the 4th month (123 days) after discharge from the ETC could not be ascertained due to the opting out of these participants. Thus, we do not know how long beyond 123 days EBOV RNA shedding in semen continued in the remaining 2 (50%) of the participants in this study. Understanding the actual duration of Ebola virus shedding in EVD survivors remains an essential for ultimately controlling the EVD outbreaks in the West African sub-region.

The quantitative RT-PCR assays employed in this study to test urine, high vaginal swab and semen specimens are the same that were used to test blood specimens obtained from patients with suspected EVD [17]. The median cycle-threshold values for the EBOV gene targets increased during the analysis of semen samples obtained from participants that were positive 2, 3 and 4 months after being discharged from the ETC. This indicates the lowering of the EBOV RNA loads in these participants based on longer duration between the dates of discharge as also seen in other studies [18 – 21].

The data presented in this evaluation of the shedding of EBOV RNA in semen of male participants up to 4 months (123 days) after discharge from the ETC adds to a sparse body of literature about the shedding of the Ebola virus after recovery from EVD. However, the potential infectivity of the EBOV shedding of these EVD survivors after discharge from the ETC was not evaluated in this study. This finding supports the use of standard universal precautions and personal protective equipment (PPE) when attending and caring for EVD survivors after being discharged till all body fluids; urine, high vaginal swab and particularly semen tested negative for EBOV RNA.

Although, the detection of EBOV RNA in semen of survivors does not automatically indicate the presence of infectious virus. However, given the high case fatality and uncertainties surrounding the transmission dynamics of this virus, individuals who recovered from EVD should still be considered to be a potential source of transmission for some months after recovery [21]. Thus, the World Health Organization (WHO) interim advice on the sexual transmission of the EVD, that recommends repeat PCR testing of semen until it is negative or the use of condoms for 12 months after initial symptom onset is also strongly recommended as a means of breaking and stopping the transmission. EVD survivors and their immediate contacts should also be informed about these risks, as those who are said to have recovered from EVD may need to abstain from sex for at least 9 months or should use condoms until their semen tests are negative as previously documented [14, 22, 23].

CONCLUSION

EBOV remains on the horizon of the West African sub-continent and data availability on its persistence in different body fluids is limited by the minimal number of survivors tested. The present study has provided more evidence confirming the persistence of EBOV in different body fluids even up to 4 months (123 days) in semen. There is a need for further research on persistence of EBOV in body fluids, and approach to infection control guidelines in individuals who recovered from the disease.

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