

## Ebola Virus Persistence in Semen of Male Survivors

Timothy M. Uyeki,<sup>1,a</sup> Bobbie Rae Erickson,<sup>1,a</sup> Shelley Brown,<sup>1</sup> Anita K. McElroy,<sup>1,2</sup> Deborah Cannon,<sup>1</sup> Aridith Gibbons,<sup>1</sup> Tara Sealy,<sup>1</sup> Markus H. Kainulainen,<sup>1</sup> Amy J. Schuh,<sup>1</sup> Colleen S. Kraft,<sup>2</sup> Aneesh K. Mehta,<sup>2</sup> G. Marshall Lyon III,<sup>2</sup> Jay B. Varkey,<sup>2</sup> Bruce S. Ribner,<sup>2</sup> Richard T. Ellison III,<sup>3</sup> Ellie Carmody,<sup>4</sup> Gerard J. Nau,<sup>5</sup> Christina Spiropoulou,<sup>1</sup> Stuart T. Nichol,<sup>1</sup> and Ute Ströher<sup>1</sup>

<sup>1</sup>Centers for Disease Control and Prevention, and <sup>2</sup>Emory University Hospital, Atlanta, Georgia; <sup>3</sup>Division of Infectious Diseases, University of Massachusetts Medical School, Worcester; <sup>4</sup>Division of Infectious Diseases, NYU School of Medicine, Bellevue Hospital Center, New York; and <sup>5</sup>Division of Infectious Diseases, Rhode Island Hospital, The Miriam Hospital, and the Warren Alpert Medical School of Brown University, Providence

We investigated the duration of Ebola virus (EBOV) RNA and infectious EBOV in semen specimens of 5 Ebola virus disease (EVD) survivors. EBOV RNA and infectious EBOV was detected by real-time RT-PCR and virus culture out to 290 days and 70 days, respectively, after EVD onset.

**Keywords.** Ebola virus; semen; sexual transmission.

Recently, molecular evidence of sexual transmission of Ebola virus (EBOV) from a male survivor of Ebola virus disease (EVD) to a female partner that occurred 179 days after EVD illness onset was reported in Liberia [1]. A semen specimen from the survivor collected 199 days after illness onset had detectable EBOV (*Zaire ebolavirus* species) RNA, and the virus isolation attempt was negative [1]. This case highlights the urgency of understanding EBOV persistence in semen and assessing potential infectiousness to inform the risk of transmission of EBOV from male survivors to their sexual partners.

Limited data from testing of semen specimens collected from a small number of male survivors of previous EBOV associated EVD outbreaks indicated that EBOV RNA can be detected up to 101 days after illness onset [2–4]. Recently, detection of EBOV RNA in semen was reported up to 284 days after illness onset [5]. However, the longest period from illness onset that infectious EBOV has been recovered by viral culture of semen was from a male survivor at 82 days after EVD symptoms began [2–4]. Given these data, it was important to investigate the duration and infectiousness of EBOV in available semen specimens

voluntarily submitted by a small number of adult male survivors of EVD in the United States.

### METHODS

Semen specimens from 5 survivors who had experienced moderately severe to critical illness with EVD, all of whom received different investigational therapeutics in addition to supportive clinical management during their hospitalization [6], were tested after recovery during 2014–2015. These survivors submitted semen specimens at different times when convenient after recovery from EVD for virologic testing at the Centers for Disease Control and Prevention (CDC) as part of their follow-up clinical management during the post-EVD convalescent period. Survivors were counseled by their physicians to abstain from sex or to use condoms until there was no evidence of EBOV RNA detected. Semen specimens were collected and transported as soon as possible or maintained at 4 degrees Celsius and shipped overnight on frozen cold packs to CDC. The majority of the specimens were processed within 0–3 days; however a few were processed 5–8 days after collection. Only one specimen that was collected 290 days post symptom onset was frozen prior to virus culture. Semen specimens were processed at CDC under biosafety level 4 (BSL4) conditions. Nucleic acid was extracted and tested by quantitative reverse-transcription polymerase chain reaction (qRT-PCR) for EBOV nucleoprotein (NP) as well as an internal cellular control gene (beta 2-microglobulin (b2m) (Thermo Fisher Scientific, #4326319E) [7]. The NP assay has an estimated limit of detection of 400 TCID<sub>50</sub>/mL based on several unpublished studies and from a similar performance profile of the NP EUA assay (<http://www.fda.gov/EmergencyPreparedness/Counterterrorism/MedicalCountermeasures/MCMLegalRegulatoryandPolicyFramework/ucm182568.htm#ebola>). Copies per mL that correspond with virus load cannot be calculated because the assay detects genomic, antigenomic, and mRNA; therefore, positive and negative controls are performed for each qRT-PCR run, but no standard curve is generated. Cycle threshold (Ct) values <40 were considered positive. Interpretation of qRT-PCR results includes analysis of the Ct values, curves, and the b2m results for each specimen. Given the specimen type, studies are ongoing to determine the distribution of the internal control to provide information on specimen quality. To detect infectious virus, cell culture virus isolation was attempted as previously described [2].

### RESULTS

The results of EBOV RNA detection in available semen specimens over time from illness onset are shown in Figure 1. EBOV RNA was detected in semen out to 290 days after symptom

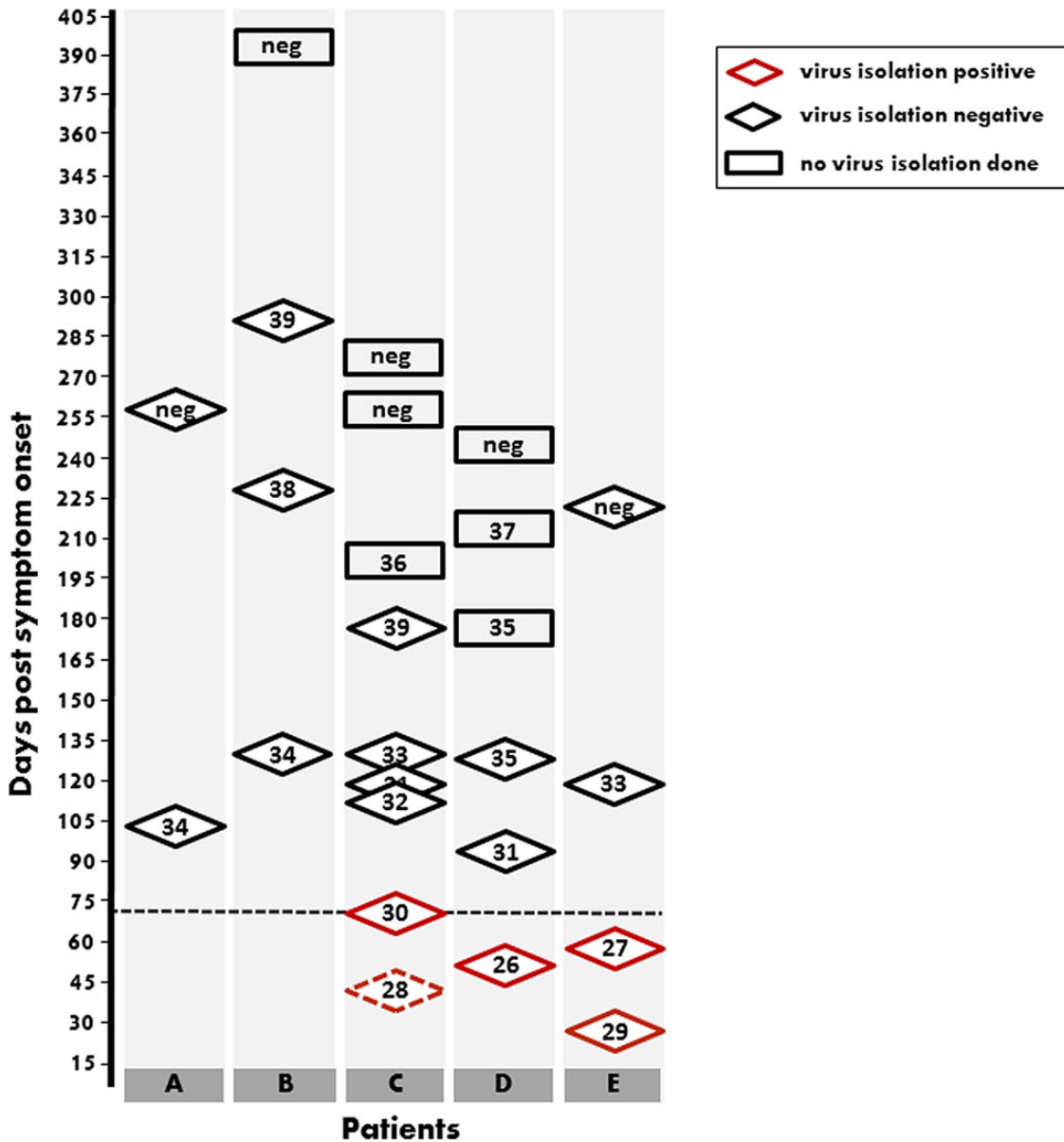
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<sup>a</sup>T. M. U. and B. R. E. contributed equally to this work.

Correspondence: T. M. Uyeki, Centers for Disease Control and Prevention, Mailstop A-20, 1600 Clifton Rd, NE, Atlanta, GA 30329 (tuyeki@cdc.gov).

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**Figure 1.** Ebola virus nucleoprotein (NP) quantitative reverse-transcription polymerase chain reaction (qRT-PCR) and virus isolation results on semen specimens. Each diamond represents an independent semen specimen. NP qRT-PCR results are included inside the diamonds and rectangles as either the cycle threshold value or neg for negative results. Red and black diamonds represent positive and negative virus isolation, respectively. The rectangles denote no virus isolation was attempted. The red dashed diamond indicates a contaminated isolation attempt and infectious Ebola virus could not be confirmed. The black dashed line signifies the last day post onset of symptoms when virus was isolated.

onset (272 days after the first negative blood specimen result) (Supplementary Table 1). The first negative EBOV RNA result in semen ranged from 222 to 393 days after illness onset. EBOV was isolated from semen specimens collected from 3 survivors and out to 70 days after illness onset (34 days after the first negative blood specimen result). The NP Ct values for semen specimens from which EBOV was isolated by viral culture ranged from 26 to 30 (Supplementary Table 1). (Higher Ct values indicate lower concentration of the target sequence).

## DISCUSSION

We describe the detection of EBOV RNA and infectious virus in semen specimens collected from 5 male patients who were hospitalized for EVD in the United States during 2014 and recovered. EBOV RNA was detected out to 290 days after illness onset, whereas infectious EBOV was isolated from semen collected out to 70 days after illness onset. This is similar to the reported timeframe of 82 days for isolating infectious virus from semen after illness onset in a previous EVD outbreak [2].

Infection of the testes by EBOV likely occurs through viremia during acute EVD. EBOV antigens were identified in seminiferous tubules from one fatal EVD case [8], and inflammation was reported with identification of EBOV particles in interstitial cells, endothelium, and monocytes of testicular tissue of experimentally infected nonhuman primates [9]. EBOV in the testes may be shielded from the immune response, and therefore active viral replication can continue for prolonged periods. Much more research is needed to understand the pathogenesis and maintenance of EBOV in immune-privileged organs. The impact of any investigational therapeutics administered during clinical management of EVD upon EBOV persistence in the testes during the post-EVD recovery period is unknown, although the half-life of these experimental therapies is likely far shorter than the period for which EBOV could be detected in semen specimens.

Until recently, the World Health Organization (WHO) and CDC recommended abstinence or condom use for at least 3 months after recovery from EVD [1]. Based upon uncertainty about the duration of infectious EBOV in semen and concern of EBOV sexual transmission through exposure to semen of a recovered male EVD survivor, WHO now recommends that male EVD survivors should abstain from sex or practice safe sex with correct condom use until semen has tested negative twice with at least a 1-week interval by RT-PCR [10]. Information about the limitations of the tests should also be discussed when providing results. WHO also recommends that if semen has not been tested, safe sex should be practiced for at least 12 months after onset of EVD. CDC recommends that until more information is known about the persistence of EBOV in semen, contact with semen from a recovered EVD patient should be avoided [1, 11].

Our findings are limited by the overall small number of semen specimens that were submitted for Ebola virus testing at different time intervals from illness onset. The longevity of EBOV RNA detection in semen of male EVD survivors requires further investigation to determine if there is a correlation between viral RNA detection and infectious virus. It may be that higher viral loads during the acute phase of the illness leads to increased seeding of these immune privileged sites and therefore prolonged presence of both infectious and non-infectious EBOV. The sensitivity of virus isolation from semen specimens could be affected by several factors such as storage time and temperature, host factors, fungal or bacterial contaminants, or presence of unknown inhibitors. Efforts were made to minimize the time from specimen collection to cell culture inoculation. Processing of a few specimens were delayed because of transportation issues, but even at day 5 post collection, a positive isolate was obtained. The case of suspected EBOV sexual transmission 179 days from symptom onset has raised some questions about the sensitivity of virus isolation [1], but because the specimen that tested positive by qRT-PCR and negative by virus culture was collected 20 days after the suspected transmission

event, it is possible that infectious virus was no longer present in semen by the time of sampling.

In conclusion, the reported results support previous data suggesting the potential for sexual transmission of EBOV through semen exposure to a susceptible sexual partner from a recovered adolescent or adult male EVD survivor out to at least 82 days after EVD illness onset. Given the case report of suspected EBOV sexual transmission 179 days after EVD onset [1], the detection of EBOV RNA in semen for 4–6 months after EVD onset in 43% of survivors in a cross-sectional study [5], and our findings, it seems prudent to adhere to the WHO recommendation for male survivors to practice abstinence or safe sex with condom use for up to 6 months after EVD onset if semen cannot be tested. Counseling of all adolescent and adult male survivors and their sexual partners about the potential for sexual transmission of EBOV through semen exposure is essential. Broader epidemiological and scientific studies are needed to assess the risk of sexual transmission of EBOV from semen as well as vaginal fluid of EVD survivors to their partners, compliance with WHO and CDC recommendations, and effectiveness of condoms to reduce sexual transmission of EBOV.

#### Supplementary Data

Supplementary materials are available at <http://cid.oxfordjournals.org>. 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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