The Journal of Infectious Diseases

BRIEF REPORT

New Evidence of Long-lasting Persistence of Ebola Virus Genetic Material in Semen of Survivors

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Ninety-eight semen specimens were obtained for Ebola virus (EBOV) RNA screening from 68 men in Guinea during the convalescent phase of EBOV infection. Ten samples from 8 men were positive for EBOV up to 9 months after onset of the disease, with decreasing trends in the proportion of positive samples and the level of viral RNA. Safe sex practices should be observed after discharge from treatment centers.

Keywords. Ebola virus; survivors; semen; Guinea; virus persistence; outbreak.

On 7 May 2015, the World Health Organization (WHO) issued a technical note on the criteria for declaring the end of the current Ebola outbreak in West Africa and acknowledged that the frequency of a possible sexual transmission, as well as the period of potential infectivity of the virus found in the seminal fluid, are unknown [1]. In addition, for 132 days (calculated as a 42-day interval after 2 blood specimens from the last individual with confirmed Ebola tested negative plus a 90-day interval of heightened surveillance) after the last confirmed or probable case, the WHO recommends offering testing of semen specimens from survivors, followed by monthly testing of individuals with Ebola virus–positive specimens, until negative results are obtained for 2 specimens.

On 8 May 2015, a report from Liberia indicated a confirmed case of Ebola in a woman 30 days after the latest patient was notified that was possibly sexual transmitted by a survivor whose semen was positive for Ebola virus by polymerase chain reaction (PCR) analysis (cycle threshold, 32) 199 days after onset of the disease [2]. The sexual transmission has been confirmed by genome sequencing [3].

In Guinea, as of 9 December 2015, 3351 cases have been confirmed, with 1268 survivors [4]. Here, we report new evidence of long-term persistence of Ebola virus RNA in semen of male survivors.

In March 2015, we started to enroll survivors in a follow-up study in Conakry and Macenta, Guinea, with a target cohort size of 450 patients and a follow-up duration of 1 year (referred to as the Postebogui cohort). Recruitment is ongoing, and enrollment is conducted at various times after discharge from Ebola treatment centers. After subjects provide informed consent, clinical examination, psychological assessment, and social assessment are performed, and semen specimens are obtained from adults for Ebola virus RNA detection. At Conakry/Donka National Hospital, a reverse transcription PCR (RT-PCR) developed by Weidman et al [5] and specific for Ebola virus nucleoprotein was used. In the Pasteur Laboratory in Macenta, both the RealStar Filovirus Screen RT-PCR kit 1.0 (Altona Diagnostics, Hamburg, Germany) and an additional in-house real-time RT-PCR kit, which has a higher sensitivity and targets the viral nucleoprotein, were used [6].

As of 29 October 2015, we have screened 98 semen specimens (76 were collected in Conakry, and 22 were collected in Macenta) from 68 survivors (52 underwent specimen collection in Conakry, 16 underwent collection in Macenta). PCR results were positive for 10 samples collected from 8 men up to 276 days (9 months) after disease onset (Table 1). The proportion of specimens testing positive by PCR decreased with time since the onset of disease, with positive results for 28.5% of specimens (4 of 14) obtained between 1 and 3 months after disease onset, 16.5% (3 of 18) obtained between 4 and 6 months, 6.5% (2 of 31) obtained between 7 and 9 months, 3.5% (1 of 29) obtained between 10 and 12 months, and 0% (0 of 6) obtained >12 months.

The viral RNA load seems to decrease with time after onset of the disease, showing long-term viral clearance. The positive results for semen samples from patients G and H were only observed using the highly sensitive Pasteur Institute in-house PCR. The viral RNA load in patient D's semen decreased 21-fold between days 48 and 84 after discharge and by 17-fold in the following 77 days. These results suggest that viral RNA could still be detected 8 months after discharge in semen and underline the benefit of a using a highly sensitive RT-PCR technique to ascertain the full clearance of the virus in the semen. However, semen probably does not remain infectious during the whole period, and viral isolation and sequencing will soon

Received 9 November 2015; accepted 29 December 2015.

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The Journal of Infectious Diseases®

DOI: 10.1093/infdis/jiw078

Table 1. Persistence of Ebola Virus in Semen From 68 Survivors of the Ebola Outbreak in Guinea, 2014–2015

Patient	Age at Enrollment, y	Date of Onset	Time After Disease Onset, d (mo)	Date of Discharge From ETC	Time After Discharge From ETC, d (mo)	RT-PCR CT[a] (Viral Load, Copies/mL)	RT-PCR CT[b]	RT-PCR CT[c]	Recruitment Site
A	28	15 Jul 2015	30 (1.0)	3 Aug 2015	11 (0.3)	26.7 (24 700)	. . .	. . .	Conakry
B	27	26 Mar 2015	56 (1.8)	8 Apr 2015	43 (1.3)	31.5 (835)	. . .	. . .	Conakry
C	27	31 Mar 2015	61 (2.0)	9 Apr 2015	42 (1.3)	33.9 (150)	. . .	. . .	Conakry
D									
Specimen 1	48	10 Mar 2015	69 (2.3)	31 Mar 2015	48 (1.5)	. . .	26.7	30	Macenta
Specimen 2	. . .	. . .	105 (3.5)	. . .	84 (2.7)	. . .	29.5	34.4	. . .
Specimen 3	. . .	. . .	182 (6)	. . .	161 (5.3)	. . .	33	38.5	. . .
E	19	22 Feb 2015	93 (3.0)	9 Mar 2015	78 (2.5)	32.5 (406)	. . .	. . .	Conakry
F	37	22 Mar 2015	99 (3.2)	2 Apr 2015	76 (2.5)	34.9 (74)	. . .	. . .	Conakry
G	58	22 Oct 2014	218 (7.1)	13 Nov 2014	196 (6.4)	. . .	Negative	39.6	Macenta
H	48	10 Nov 2014	276 (9.0)	15 Dec 2014	241 (7.9)	. . .	Negative	40.6	Macenta

Abbreviations: CT, cycle threshold; ETC, Ebola treatment center; RT-PCR, reverse transcription polymerase chain reaction.

[a] Viral nucleoprotein (Weidmann et al [5]); in-house.

[b] RealStar Filovirus Screen kit 1.0 (Altona Diagnostics).

[c] Viral nucleoprotein (Huang et al [6]); in-house.

be performed in a biosafety level 4 laboratory to determine the infectivity of samples.

A limitation of our study is the use of different RT-PCR kits. Indeed, we used the diagnostic facilities already in place in Guinea when we started to enroll the survivors in the cohort. Therefore, we cannot compare the cycle threshold between the techniques.

The WHO note is based on the 1995 Ebola outbreak in Kikwit, in which an infectious Ebola virus was isolated in seminal fluid specimens from a survivor up to 82 days after onset of the disease and viral RNA was detected up to 101 days, and was updated after the Liberia report [2, 6, 7]. The latest report on the persistence of genetic material of Ebola virus in semen specimens from survivors came from Sierra Leone, where viral RNA has been found up to 284 days (9 months) after onset of disease, with a decreasing trend in both in proportion of PCR-positive semen specimens and viral quantity over time [8].

Our report adds further evidence of long-term persistence of viral RNA for up to 9 months among survivors, with the same decreasing trend over time. As in Sierra Leone, we cannot yet make conclusions about the infectivity of the semen. In the absence of evidence on noninfectivity, these preliminary results from Guinea, as well as findings from the Liberian case and the Sierra Leone report, should reinforce the importance of safe sex practices among Ebola survivors, as recommended by the WHO [9].

STUDY GROUP MEMBERS

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Notes

Acknowledgments. We thank the study participants who consented to provide specimens; the Guinean associations of Ebola survivors; the personnel of the Postebogui cohort; S. Keita, the Ebola response national coordinator; and the staff at Ebola treatment centers, for their help in identifying and tracing survivors.

Financial support. This work was supported by the French Ebola Task Force Interministérielle (J. F. Delfraissy, Y. Yazdanpanah), the Institut National de la Santé et de la Recherche Médicale (Y. Lévy), and the Institut de Recherche pour le Développement.

Potential conflicts of interest. All authors: No reported conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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