

# Prevention of sexual transmission of Ebola in Liberia through a national semen testing and counselling programme for survivors: an analysis of Ebola virus RNA results and behavioural data



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## Summary

**Background** Ebola virus has been detected in semen of Ebola virus disease survivors after recovery. Liberia's Men's Health Screening Program (MHSP) offers Ebola virus disease survivors semen testing for Ebola virus. We present preliminary results and behavioural outcomes from the first national semen testing programme for Ebola virus.

**Methods** The MHSP operates out of three locations in Liberia: Redemption Hospital in Montserrado County, Phebe Hospital in Bong County, and Tellewoyan Hospital in Lofa County. Men aged 15 years and older who had an Ebola treatment unit discharge certificate are eligible for inclusion. Participants' semen samples were tested for Ebola virus RNA by real-time RT-PCR and participants received counselling on safe sexual practices. Participants graduated after receiving two consecutive negative semen tests. Counsellors collected information on sociodemographics and sexual behaviours using questionnaires administered at enrolment, follow up, and graduation visits. Because the programme is ongoing, data analysis was restricted to data obtained from July 7, 2015, to May 6, 2016.

**Findings** As of May 6, 2016, 466 Ebola virus disease survivors had enrolled in the programme; real-time RT-PCR results were available from 429 participants. 38 participants (9%) produced at least one semen specimen that tested positive for Ebola virus RNA. Of these, 24 (63%) provided semen specimens that tested positive 12 months or longer after Ebola virus disease recovery. The longest interval between discharge from an Ebola treatment unit and collection of a positive semen sample was 565 days. Among participants who enrolled and provided specimens more than 90 days since their Ebola treatment unit discharge, men older than 40 years were more likely to have a semen sample test positive than were men aged 40 years or younger ( $p=0.0004$ ). 84 (74%) of 113 participants who reported not using a condom at enrolment reported using condoms at their first follow-up visit ( $p<0.0001$ ). 176 (46%) of 385 participants who reported being sexually active at enrolment reported abstinence at their follow-up visit ( $p<0.0001$ ).

**Interpretation** Duration of detection of Ebola virus RNA by real-time RT-PCR varies by individual and might be associated with age. By combining behavioural counselling and laboratory testing, the Men's Health Screening Program helps male Ebola virus disease survivors understand their individual risk and take appropriate measures to protect their sexual partners.

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## Introduction

In March, 2015, a 44-year-old female from Monrovia, Liberia, contracted Ebola virus disease and died in an Ebola treatment unit (ETU). An extensive investigation revealed one epidemiological link to Ebola virus exposure: unprotected sexual intercourse with a male

Ebola virus disease survivor.<sup>1</sup> A semen specimen collected from the Ebola virus disease survivor tested positive for Ebola virus RNA by real-time RT-PCR (rRT-PCR) 199 days after he first became ill with Ebola virus disease. Although no infectious virus was isolated from the semen, genetic analysis of the Ebola virus collected

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### Research in context

#### Evidence before this study

We searched PubMed and MEDLINE for the following search terms: “Ebola” and “sexual transmission”, “semen”, and “viral persistence”. The search was done from Nov 15, 2015, to May 30, 2016. Search results were then limited to research studies using RT-PCR or virus culture, or both, to detect the presence of Ebola virus in semen of survivors of Ebola virus disease. This search yielded seven articles. Four publications reported results for Ebola virus disease survivors of the 2014 west Africa Ebola virus disease outbreak treated in west Africa. Of these, the largest cohort of Ebola virus disease survivors tested was 100. The longest period of time between disease onset and the detection of Ebola virus disease RNA by RT-PCR was 276 days. Viral culture results were not reported in the four articles reporting semen test results of Ebola virus disease survivors cared for in west Africa. One article reported semen test results from five male Ebola virus disease survivors cared for in the USA. The longest period of time between disease onset to the detection of Ebola virus disease RNA by RT-PCR in these men was 290 days. The highest cycle threshold value for the nucleoprotein gene target for semen specimens from which Ebola virus disease was isolated by viral culture was 30.

#### Added value of this study

We describe the preliminary test results and behavioural outcomes for, to our knowledge, the first national semen testing and counselling public health programme for Ebola

virus disease survivors. We present serial RT-PCR semen test results for 429 Ebola virus disease survivors in Liberia, and report a possible association of age and the duration of detection of Ebola virus RNA by RT-PCR. We also report, to our knowledge, the longest interval between discharge from the Ebola treatment unit and the collection of a positive semen sample (565 days). We found that counselling paired with laboratory testing favourably affected reported condom use by men enrolled in the programme, with 74% of participants who reported not using a condom at enrolment subsequently reporting using a condom at their last sexual encounter.

#### Implications of all the available evidence

We found that the duration for which Ebola virus is detected in the semen of Ebola virus disease survivors varies by individual. As such, semen testing programmes that combine behavioural counselling and laboratory testing can play an important part in educating male survivors of Ebola virus disease of their risk of transmitting Ebola virus through sex and could potentially mitigate future outbreaks associated with sexual transmission. The Men’s Health Screening Program can serve as a model for future semen testing programmes for Ebola virus. We also found that the duration in which Ebola virus is detected in the semen of Ebola virus disease survivors might be associated with age. Future studies should be designed to investigate this possible association and to identify other factors that might be associated with prolonged viral persistence in semen.

from the semen of the Ebola virus disease survivor closely matched the Ebola virus recovered from the female patient.<sup>2</sup>

Based on virus-isolation results from previous Ebola virus disease and Marburg virus disease survivors,<sup>3–5</sup> Ebola virus disease survivors were encouraged to practice abstinence or use condoms for 90 days after recovering from the disease. However, the possibility of infectious Ebola virus persisting in the semen of survivors beyond this timeframe prompted WHO to issue new guidance. In May, 2015, WHO released interim guidance for male Ebola virus disease survivors.<sup>6</sup> This interim guidance recommended the following: (1) in addition to receiving condoms and sexual risk reduction counselling at ETU discharge, all male survivors should be offered semen testing for Ebola virus RNA by rRT-PCR until their semen tests negative twice for Ebola virus RNA; (2) male survivors and their sexual partners should be provided with condoms and receive counselling to ensure safe sexual practices until their semen has twice tested negative; and (3) if a survivor’s semen has not been tested, he should practise safe sex for at least 6 months after onset of symptoms.

At the time WHO released their interim guidance, semen testing for Ebola virus RNA was not widely available in Liberia. To address this gap, on July 7, 2015,

the Liberian Ministry of Health, in collaboration with WHO, the Academic Consortium Combating Ebola in Liberia, and the US Centers for Disease Control and Prevention (CDC), launched the Men’s Health Screening Program (MHSP). Based on the principles outlined in WHO’s interim guidance,<sup>6</sup> the MHSP provides male Ebola virus disease survivors with semen testing for Ebola virus RNA by rRT-PCR and behavioural counselling on safe sex practices. We describe Liberia’s national semen testing programme for Ebola virus, present preliminary semen testing results, and report sexual risk behaviours.

## Methods

### Study design and participants

The MHSP operations manual is provided in the appendix (pp 2–34). The MHSP operates out of three locations in Liberia: Redemption Hospital in Montserrado County, Phebe Hospital in Bong County, and Tellewoyan Hospital in Lofa County (figure 1). Men are eligible to enrol if they are aged 15 years or older and can provide an ETU discharge certificate. Due to insufficient laboratory capacity and challenges in specimen transportation during the height of the Ebola virus disease outbreak in Liberia, laboratory confirmation of Ebola virus infection was not available for all patients with suspected Ebola

See Online for appendix

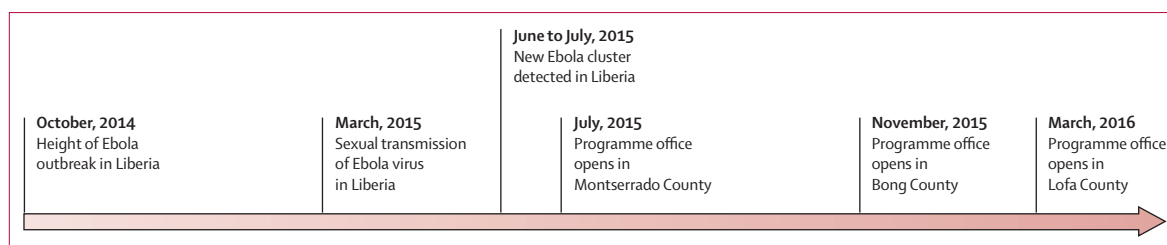


Figure 1: Programme implementation timeline

virus disease.<sup>7-9</sup> As such, possession of an ETU discharge certificate was used as proof of survival from the disease.

Potential programme participants are identified through two primary methods: informational events held in conjunction with Ebola virus disease survivor association meetings and through the national Ebola virus disease survivor registry. Maintained by the Liberia Ministry of Health, as of June 17, 2015, the national Ebola virus disease survivor registry listed 1541 laboratory-confirmed Ebola virus disease survivors, 534 of whom were males individuals aged 15 years or older.

Created to implement a WHO-recommended semen testing programme for male Ebola virus disease survivors, the MHSP was granted a non-research determination by the CDC. On June 18, 2015, the Liberian Ministry of Health officially adopted the MHSP as a public health programme of Liberia. Programme participation was voluntary and written informed consent was obtained from participants before programme enrolment.

### Procedures

Upon programme enrolment, trained counsellors collect information from enrollees on sociodemographics and sexual behaviours since ETU discharge of the enrollee using a standardised baseline questionnaire (panel; appendix pp 35–47); provide counselling on safe sexual practices (appendix pp 48–69); and provide condoms and instruction on condom use. Date of discharge from an ETU is established from the participant's discharge certificate. Additionally, all participants receive a brochure listing health-care facilities that provide clinical care services to Ebola virus disease survivors. Participants presenting with physical or psychological complaints are referred for health-care services as needed or if requested. In keeping with the MHSP's non-research status, data collection was limited to those items that directly affected programme delivery and services. As such, participants were not asked about any pre-existing medical disorders (eg, hypertension, diabetes, or HIV).

Participants were also asked to provide a semen sample for testing by rRT-PCR. After collection of the first semen sample, the frequency of subsequent semen tests was dependent on the test result of the previous sample (figure 2). Participants whose previous semen sample tested positive for the presence of Ebola virus RNA were

### Panel: Selected questionnaire data fields

#### Baseline questionnaire

Whether he (the patient) has resumed sexual activity  
 Frequency of sexual intercourse  
 Condom use at last sexual encounter; where he procured the condoms  
 Signs and symptoms of a sexually transmitted infection\*  
 Age  
 Highest level of education attained  
 Marital status  
 Date of ETU admission or discharge  
 Did he receive counselling on when they were safe to resume sexual activity; if so, who provided this counselling?  
 After ETU discharge, did he discuss with his sexual partner when to resume sexual activity?

#### Follow-up questionnaire

Whether he has had sex since the last visit  
 Frequency of sexual intercourse  
 Condom use at last sexual encounter  
 Signs and symptoms of a sexually transmitted infection\*

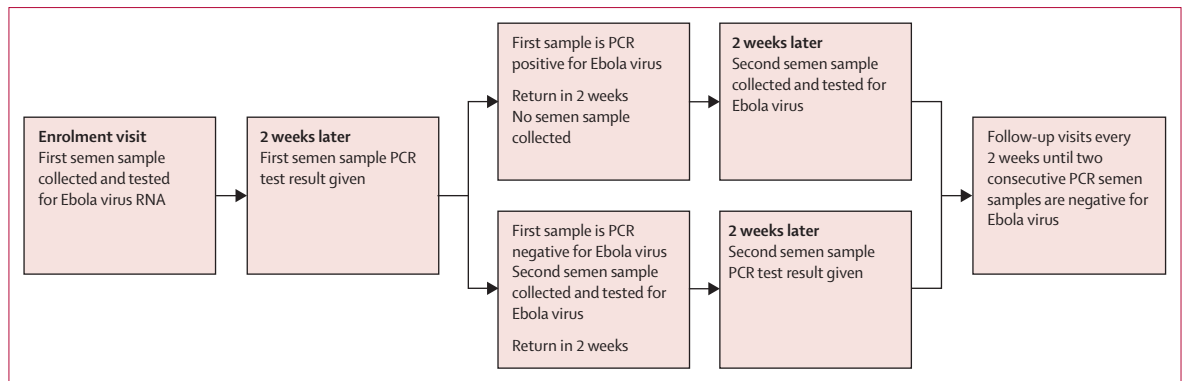
#### Graduation questionnaire

Whether he has had sex since the last visit  
 Frequency of sexual intercourse  
 Condom use at last sexual encounter  
 Signs and symptoms of a sexually transmitted infection\*  
 Whether he plans to share his semen test results with his sexual partner or partners  
 Confidence in correctly putting on and taking off a condom  
 Participant satisfaction with programme services  
 Why he choose to receive services from the clinic or mobile team

ETU=Ebola treatment unit. \*Dysuria, penile discharge, testicular pain or swelling, or genital sores or blisters.

tested once a month. Participants whose previous semen sample did not detect Ebola virus RNA were tested every 2 weeks, which is the minimum turnaround time for specimen transport and processing. In accordance with WHO guidelines,<sup>6,10</sup> participants graduate from the programme after receiving two consecutive semen test results that do not detect the presence of Ebola virus RNA by rRT-PCR. Participants who are repeatedly unable to produce a specimen (at least three unsuccessful attempts) are referred for health-care services but may remain enrolled in the programme.

During follow-up visits, participants are informed of their individual rRT-PCR results, asked about their sexual



**Figure 2: Semen testing algorithm**

Frequency of semen collection is dependent on the previous semen test result: if positive, the next sample is taken 4 weeks later; if negative, the next sample is taken 2 weeks later.

practices since their last visit using a standardised follow-up questionnaire (panel), counselled on safe sexual practices, and provided with condoms. In addition to questions about their sexual practices, questions were added on Oct 20, 2015, to assess graduating participants' satisfaction with programme services, and their confidence in correctly putting on and taking off a condom (graduation questionnaire; panel). Consistent with other semen testing services for Ebola virus disease survivors in Liberia, MHSP participants were given US\$5 to cover transportation costs and \$20 for semen samples.

The primary location for service delivery for both the enrolment and follow-up visits was the programme clinic. However, Ebola virus disease survivors who were unable to travel to the programme clinic were offered services at a location of their choosing by a two-person mobile team composed of a counsellor and a semen technician. In addition to the services offered in the clinic setting, participants who receive services from the mobile team were offered the opportunity to include their sexual partners in their counselling sessions.

The risk reduction behavioural counselling used in the MHSP was adapted from the Ebola virus disease Viral Persistence Study in Sierra Leone.<sup>11</sup> The goal of the counselling programme was to (1) encourage abstinence or condom use among programme participants until they had received two consecutive semen test results that did not detect the presence of Ebola virus RNA by rRT-PCR; and (2) refer programme participants to available Ebola virus disease survivor services in the community as needed for other health concerns.

Behavioural counselling to reduce the risk of spreading Ebola virus disease was provided at each visit (appendix pp 48–69). At the first visit, counsellors introduced the participant to the session; provided information about the semen rRT-PCR test; conveyed the importance of abstinence or condom use, or both, to reduce risk of transmitting Ebola virus; engaged the participant in a conversation regarding his sexual behaviour and intimate relationships; negotiated risk reduction steps with the

participant; and demonstrated how to safely use and dispose of condoms using a wooden penis model. At each subsequent visit, counsellors delivered the semen test results from the previous visit, explained what the test results mean, and provided education on ways to reduce the risk of sexual transmission of the virus. These processes continued until the participant was discharged from the programme after having two consecutive negative semen test results. At all counselling sessions, counsellors asked participants to report any medical, psychosocial, or other issues they were experiencing. Counsellors referred these participants to appropriate health-care services.

All self-collected semen specimens submitted by the MHSP were stored and transported at  $-20^{\circ}\text{C}$  or colder to the Tappita Ebola virus disease laboratory in Nimba County. Upon receipt in the laboratory, specimens were maintained at  $-20^{\circ}\text{C}$  or colder until testing with the CDC's Ebola Virus NP and VP40 Real-Time RT-PCR Assays (CDC, Atlanta, GA, USA).<sup>12</sup> Briefly, semen specimens were allowed to thaw for up to 30 min before total nucleic acid isolation using the MagMAX Pathogen RNA/DNA Kit (Applied Biosystems, Foster City, CA, USA). Inactivation of the semen specimen with MagMax lysis buffer (ThermoFisher Scientific, Waltham, MA, USA) was done in a glove box. After proper exterior decontamination of the vial, automated nucleic acid extraction was done using the BeadRetriever platform (Applied Biosystems). rRT-PCR was done on the Bio-Rad CFX96 Touch (Hercules, CA, USA) instrument as per the Emergency Use Authorization protocols.<sup>12</sup> The combined Emergency Use Authorization assays detected specific sequences of the Ebola virus' nucleoprotein and viral matrix protein (VP40) genes and the human RNase P gene that controlled for nucleic extraction and specimen quality. Samples of the extracted nucleic acids were stored at  $-70^{\circ}\text{C}$  or colder for potential retesting.

Interpretation of the rRT-PCR results was done in a similar way to that described previously.<sup>11</sup> Semen test

results were reported to the MHSP with target PCR cycle threshold values (ie, the number of cycles needed for the fluorescent signal to exceed the background level) and interpretation. Specimens were deemed positive if both targets (nucleoprotein and VP40) were amplified within 40 cycles of replication. Specimens were deemed negative if neither of the Ebola virus targets was amplified within 40 cycles of replication and the RNase P target yielded amplification in less than 30 cycles of replication. Specimens were considered indeterminate if only one of the Ebola targets showed positive amplification. Specimens without amplification of either Ebola virus target and RNase P amplification of over 30 cycles of replication were judged to be of poor quality and re-collection was requested. Testing of semen samples was limited to rRT-PCR because undertaking virus isolation was not possible in Liberia.

### Statistical analysis

Data were collected through the use of questionnaires at baseline and all follow-up visits and were entered into a Microsoft Access database. We used the  $\chi^2$  test to test for an association between categorical variables and the Kruskal-Wallis test to test for a difference between the median of non-normally distributed numerical variables across categorical groups. McNemar's test was used to test for differences between paired nominal data between baseline and the first follow-up visit. We used SAS version 9.3 for data analysis. Because the programme is ongoing, data analysis was restricted to data obtained from July 7, 2015, to May 6, 2016.

### Results

As of May 6, 2016, 466 Ebola virus disease survivors had enrolled in the programme. Median time from ETU discharge to programme enrolment was 384 days (range 7–697; table 1); four survivors who were a part of an Ebola virus disease cluster in July, 2015 (figure 1), were enrolled within 2 weeks of ETU discharge. The median age of participants was 33 years (range 15–79), 266 (57%) were residents of Montserrado County, and 79 (17%) chose to receive services from the mobile team.

rRT-PCR results were available for 429 of 466 programme participants. Semen test results for nine participants were not available at the time of data analysis for this manuscript (May 6, 2016); five cited personal or religious objections to masturbation; seven were unable to provide a semen specimen because of erectile dysfunction; two reported being unable to produce a specimen as a result of ongoing medical issues, one because he was recovering from a hernia operation and the other because of testicular pain; eight were lost to follow-up according to the programme protocol after missing several follow-up appointments despite several attempts to re-engage them; and six were unable to produce a specimen at enrolment and had follow-up appointments scheduled after the cutoff date for data analysis for this report (May 6, 2016).

	Participants (n=466)
Age (years)	33 (15–79)
County of residence	
Montserrado	266 (57%)
Margibi	67 (14%)
Bong	49 (11%)
Lofa	49 (11%)
Other	35 (8%)
Point of service delivery	
Montserrado Program Office	273 (59%)
Bong Program Office	64 (14%)
Lofa Program Office	50 (11%)
Mobile team	79 (17%)
Time from ETU discharge to programme enrolment (days)	384 (7–697)
Sexually active at time of programme enrolment	424 (91%)
Reported sexual frequency at programme enrolment	
Twice a week or more	126/454 (28%)
More than once a month but less than twice a week	195/454 (43%)
Once a month or less	133/454 (29%)
Reported using a condom the last time they had sex	190/422 (45%)
Reported experiencing at least one sign or symptom of a sexually transmitted disease at programme enrolment*	118/463 (25%)
Data are median (range), number (%), or n/N (%). Some percentages do not add up to 100 because of rounding. ETU=Ebola treatment unit. *Dysuria, penile discharge, testicular pain or swelling, or genital sores or blisters.	

Table 1: Participant characteristics

Among the 429 programme participants with rRT-PCR results, 38 (9%) had at least one semen sample test positive for Ebola virus RNA by rRT-PCR. The median age for those with positive Ebola virus results was 40 years (range 18–68) compared with 32 years (15–70) for those who never had a positive test (table 2). The proportion of men reporting at least one sign or symptom of a sexually transmitted disease did not differ between those who had at least one semen sample test positive for Ebola virus RNA (42%) and those who never had a semen sample test positive for Ebola virus RNA (39%;  $p=0.74$ ).

24 (6%) of 429 participants provided semen specimens that tested positive for Ebola virus RNA at least 12 months after their ETU discharge. The longest interval between ETU discharge and the collection of a positive specimen was 18 months (565 days). One participant whose semen tested rRT-PCR positive more than 12 months after ETU discharge self-disclosed that he was diagnosed with HIV infection in 2009 and was taking antiretroviral therapy.

Ebola virus RNA was detected in the initial semen specimen of all four participants who were enrolled within 90 days of their ETU discharge, eight (22%) of 37 enrolled within 181–270 days, ten (8%) of 123 enrolled within 271–360 days, 11 (8%) of 133 enrolled within

	Participants with at least one positive semen test for EBOV (n=38)	Participants who never had a positive semen test for EBOV (n=391)	p value
Participants with detectable Ebola virus in semen ≥12 months after ETU discharge	24 (63%)	NA	NA
Age (years)	40 (18–68)	32 (15–70)	0.0002*
Reported ≥1 sign or symptom of a sexually transmitted infection	16 (42%)	154 (39%)	0.74†
Reported sexual frequency at baseline			
Twice a week or more	11 (29%)	106/381 (28%)	0.45†
More than once a month but less than twice a week	13 (34%)	167/381 (44%)	..
One a month or less	14 (37%)	108/381 (28%)	..

Data are number (%), median (range). EBOV=Ebola virus RNA. ETU=Ebola treatment unit. NA=not applicable. \*Kruskal-Wallis. †χ<sup>2</sup> test.

**Table 2: Participant characteristics by semen test result**

361–450 days, and five (7%) of 72 enrolled within 451–540 days (table 3). No participants were enrolled 91–180 days after ETU discharge.

In semen specimens collected within 90 days of ETU discharge, the mean cycle threshold value was 32.28 (range 27.55–36.38) for VP40 and 33.37 (29.17–37.10) for nucleoprotein (table 4). After 90 days, mean cycle threshold values for both gene targets plateaued (VP40 range of means 35.91–36.82, nucleoprotein 36.99–38.70).

Among participants who enrolled and provided specimens more than 90 days since their ETU discharge, men older than 40 years were more likely to have at least one semen sample test positive for Ebola virus RNA by rRT-PCR than were men aged 15–40 years (p=0.0004; table 5). Also, reported sexual frequency did not differ between the two age groups (p=0.45). Participants who were tested within 90 days of ETU discharge were excluded from this analysis because Ebola virus persists in semen during this time period.<sup>3,5</sup>

427 (92%) of 466 participants reported being counselled by ETU staff to either abstain from sex or to use condoms for 90 days after recovering from Ebola virus disease. At enrolment, 424 (91%) participants reported having resumed sexual activity; 42 (9%) reported current abstinence (table 1). Of the 410 participants who reported the date they resumed sexual activity, 363 (89%) waited 90 days or more after their ETU discharge before resuming sex. Frequency of sexual intercourse was not associated with age (p=0.41; appendix p 69).

Of the 424 participants who reported resuming sexual activity on their initial enrolment questionnaire, 190 (45%) reported using a condom the last time they had sexual intercourse. At the first follow-up visit, 84 (74%) of 113 participants who reported not using condoms at enrolment reported use at their last sexual encounter (intrasubject comparison between enrolment and follow-up visit: p<0.0001). At graduation, 80 (76%) of 105 participants who reported

	MHSP*	Sierra Leone <sup>††</sup>	Guinea <sup>‡‡</sup>	USA <sup>§§</sup>
1–90 days	4/4 (100%)	9/9 (100%)	4/14 (29%)	4/4 (100%)
91–180 days	0	26/40 (65%)	3/18 (17%)	3/4 (75%)
181–270 days	8/37 (22%)	11/43 (26%)	2/31 (6%)	2/5 (40%)
271–360 days	10/123 (8%)	NA	1/29 (3%)	0/1 (0%)
361–450 days	11/133 (8%)	NA	0/6 (0%)	0/1 (0%)
451–540 days	5/72 (7%)	NA	NA	NA
541–630 days	0/1 (0%)	NA	NA	NA

Data are n/N (%). MHSP=Liberia's Men's Health Screening Program. ETU=Ebola treatment unit. NA=not applicable. \*Days from acute illness calculated from ETU discharge. †Days from acute illness calculated from symptom onset. ‡Days from acute illness calculated from disease onset. §Days from acute illness calculated from first blood sample negative for Ebola virus on PCR.

**Table 3: Proportion of patients with initial samples positive for Ebola virus on qualitative RT-PCR by days from acute illness**

not using condoms at enrolment reported use at their last sexual encounter (intrasubject comparison between enrolment and graduation: p<0.0001). Additionally, 176 (46%) of 385 participants for whom follow-up data were available who reported being sexually active at enrolment reported abstinence at the first follow-up visit (intrasubject comparison between enrolment and first follow-up: p<0.0001). At graduation, 109 (36%) of 300 participants who reported being sexually active at enrolment and for whom graduation data were available reported abstinence (intrasubject comparison between enrolment and graduation visit: p<0.0001).

326 participants graduated from the programme after two consecutive semen samples tested negative for Ebola virus RNA. 24 (63%) of 38 participants who had at least one semen sample that tested positive for the presence of Ebola virus RNA have graduated from the programme. The median time from programme enrolment to graduation was 50 days (range 24–226). Of the 299 programme graduates who were asked, 290 (97%) reported that they would refer a family member or friend to participate in the semen testing programme and 257 (86%) shared or planned to share their semen tests results with their sexual partners. Of the 294 programme graduates who provided a response to the graduation questionnaire question about confidence with condom use, 220 (75%) reported feeling “very confident” about knowing how to correctly put on and take off a condom, 52 (18%) reported feeling “somewhat confident”, and 22 (7%) reported feeling “not confident” or “not at all confident”.

### Discussion

Among participants enrolled in the MHSP, 38 (9%) of 429 produced at least one semen sample that tested positive for Ebola virus RNA by rRT-PCR. Of these, 24 provided semen specimens that tested positive for the presence of Ebola virus RNA at least 12 months after ETU discharge. The longest interval between ETU

discharge to the collection of a positive semen sample among MHSP participants was 565 days, which exceeds previously reported time intervals.<sup>11,13,14</sup> However, detection of Ebola virus RNA by rRT-PCR does not necessarily indicate the presence of infectious virus.<sup>3,5,9,11</sup>

Consistent with findings from previous studies,<sup>11,14</sup> the lowest mean rRT-PCR values for both gene targets occurred in MHSP participants who were within 3 months of ETU discharge. Also consistent with findings from previous studies,<sup>11,13,14</sup> the total number of MHSP participants whose semen tested positive for the presence of Ebola virus RNA declined over time. Unlike findings from previous studies,<sup>11,14</sup> the mean cycle threshold values for both gene targets for MHSP participants seemed to plateau. Although some of these differences probably occurred because this study had a larger sample size and longer follow-up period than previous studies, this finding also suggests that heterogeneity exists in Ebola virus persistence in semen among survivors of Ebola virus disease.

Among men for whom more than 90 days had passed since their ETU discharge, age older than 40 years seemed to be a factor in the likelihood of having at least one semen sample test positive for Ebola virus RNA by rRT-PCR. This possible association, to our knowledge, has not been reported previously and the reason for this finding is not clear. Also, for men enrolled in the programme, reported sexual frequency was not associated with age. In view of these findings, differences in the persistence of Ebola virus RNA in semen might be a result of age-related factors such as changes in semen composition<sup>15,16</sup> or age-related changes in immune function. Because participants are not asked about pre-existing medical disorders, sustained viral persistence in semen could also be a result of the presence of other immunocompromising disorders, such as HIV or diabetes.

91% of men reported having resumed sexual activity at the time of programme enrolment. This finding is not surprising because the programme launched 9 months after the peak of the Ebola virus disease outbreak in Liberia. Initial examination of condom use and abstinence showed that counselling, paired with laboratory testing, led to a reduction in reported high-risk sexual behaviours. This reduction was noted at the first follow-up visit and at programme graduation. However, because these measures are all self-reported, the implications of these findings are limited by social desirability bias.

Preliminary data analysis of participants enrolled in the MHSP suggests that duration of detection of Ebola virus RNA by rRT-PCR varies by individual and might be associated with age. In view of this finding, semen testing programmes such as the MHSP, which combine behavioural counselling with laboratory testing, can play an important part in educating male Ebola virus disease survivors of their risk of transmitting Ebola virus through sex and could potentially mitigate future outbreaks associated with sexual transmission. However, our

	Number of semen samples testing positive for Ebola virus	Number of participants with at least one semen sample testing positive for Ebola virus*	VP40 mean (range)	Nucleoprotein mean (range)
1–90 days	8	4	32.28 (27.55–36.38)	33.37 (29.17–37.10)
91–180 days	3	2†	36.82 (36.38–37.62)	38.18 (37.43–39.22)
181–270 days	7	6	36.02 (30.58–38.39)	37.25 (32.00–39.54)
271–360 days	12	11	36.59 (34.31–39.09)	37.72 (34.87–39.58)
361–450 days	31	18	36.22 (31.40–39.50)	37.39 (32.94–39.68)
451–540 days	13	8	35.91 (31.58–39.74)	36.99 (32.14–39.74)
541–630 days	2	2	36.43 (35.20–37.66)	38.70 (38.48–38.91)

\*These numbers are cumulative and only include patients who had not yet graduated. †Two patients produced samples that were of poor quality and could not be tested.

**Table 4: Mean RT-PCR cycle threshold values by days from Ebola treatment unit discharge to collection of a positive semen sample**

	Participants with at least one semen sample testing positive for Ebola virus (n=34)	Participants who never had a semen sample test positive for Ebola virus (n=391)
Age ≤40 years	17 (50%)	302 (77%)
Age >40 years	17 (50%)	89 (23%)

Analysis excluded four participants who enrolled within 90 days of Ebola treatment unit discharge.  $\chi^2$  p=0.0004 for the difference between age groups.

**Table 5: Semen test results stratified by age**

experience shows that implementing and sustaining such a programme is time and resource intensive. Health-care facilities caring for survivors after ETU discharge should offer semen testing and behavioural counselling as part of a standard package of health-care services. In addition to fostering a holistic approach to survivor care, integration would probably reduce the time and costs associated with implementing a semen testing programme and ensure a better surveillance for potential future transmission events.

#### Contributors

MJS and MJC co-wrote the manuscript. AB, NM, MMA, JKo, PKB, AC, VL, SP, MMu, EG, KEV, SMZ, HHWG, BM, TGN, AG, BK, and DW developed the programme and wrote the programme description. SW, ER, CW, SD, OCS, JKp, SN, US, JAB, and PR wrote the laboratory methods section. LJP, SK, and MS did the data analysis and wrote the data analysis section. NA wrote the counselling section.

#### Declaration of interests

We declare no competing interests.

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