

PEER REVIEW HISTORY

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ARTICLE DETAILS

TITLE (PROVISIONAL)	A systematic literature review of viral persistence and sexual transmission from recovered Ebola survivors – Evidence and recommendations
AUTHORS	Thorson, Anna; Formenty, Pierre; Lofthouse, Clare; Broutet, Nathalie

VERSION 1 - REVIEW

REVIEWER	Ian M Mackay Queensland Health & The University of Queensland, Australia
REVIEW RETURNED	23-Jun-2015

GENERAL COMMENTS	<p>The authors present the most comprehensive review of the subject I have seen. At times the wording is difficult to interpret-and I have tried to highlight these areas in the Specific comments below.</p> <p>General</p> <ul style="list-style-type: none"> • Can Ebola virus disease be defined as EVD early on, and then EVD used instead of “Ebola disease” or “Ebola” when relating to EVD • Please maintain the term “viable “ in terms of infectious virus , rather than “live” • Could the authors define what “grey literature” is? • Please consider adding the recent Mora-Rillo paper, http://www.thelancet.com/journals/lanres/article/PIIS2213-2600(15)00180-0/abstract, which detected RNA from a vaginal swab • Please consider adding a more recent EBOC stability paper to the Survival paragraph: http://wwwnc.cdc.gov/eid/article/21/7/15-0253_article • The authors mention anal sex and used condom handling-they should consider noting oral sex and masturbation as sources of contact with potentially infectious fluids also • Given your review cannot show evidence that condoms likely provide “some protection” but just how effectively remains unclear, could the authors consider more strongly leaning on the abstinence aspect of their conclusion in Pg15Ln46-49. Given how one case can cause such damage when occurring in eh right setting, even small risks should be avoided and certainly very clearly documented, however inconvenient that process of risk reduction may be <p>Specific (Page numbering refers to those numbers on the 26 page PDF)</p> <p>Pg4Ln23 case=cases</p>
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	<p>Pg4Ln48 Please rephrase "lifted forward"</p> <p>Pg4Ln57 "...and to condom..?"</p> <p>Pg5Ln43 4 Please define "scoping literature review"</p> <p>Pg6Ln9 Please clarify this sentence or break in two</p> <p>Pg6Ln17 "The recent" to "a recent"?</p> <p>Pg6Ln35-57 Just "methods to identify Ebola virus in the sample" suitably encompasses both culture and molecular I believe</p> <p>Pg11Ln36 Please re-write this sentence to clarify meaning</p> <p>Pg12Ln19-24 Please clarify of both condoms had equal rates of pore leakage</p> <p>Pg13Ln8 "at" or "for anal sex"?</p> <p>Pg16Ln3-5 Not just men –men and women should practice safe sex.</p>
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REVIEWER	Onder Ergonul Koc University, School of Medicine, Istanbul, Turkey
REVIEW RETURNED	25-Jun-2015

GENERAL COMMENTS	This is an important review for sexual transmission of Ebola. In this area, there are few limited studies, because it is very difficult to perform such a study. This limitation of the area makes this report more important. It worths to be published.
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REVIEWER	Daniel J Park Group Leader, Viral Computational Genomics Broad Institute of Harvard and MIT USA
REVIEW RETURNED	29-Jul-2015

GENERAL COMMENTS	<p>In this manuscript, the WHO-based authors perform a literature review on the topic of sexual transmission of Ebola virus from convalescent patients. They also combine conclusions from a number of studies to infer the effectiveness of condoms in preventing sexual transmission of Ebola. Based on these findings, the authors make policy recommendations on sexual practices for Ebola survivors.</p> <p>The authors note that the published literature on this topic is somewhat limited, and they are careful to word their conclusions very modestly: "we conclude that the risk of sexual transmission ... cannot be ruled out" and "we conclude that ... condoms offer some protection." I appreciate this conservative wording as it is an appropriate depiction of what is known in the field.</p> <p>Based on their review of the literature, the authors make recommendations regarding abstinence, condom usage, and follow-up semen testing for EVD survivors. I think it might be helpful to a very brief explanation for how each number was chosen. For example, the "3 month" guideline of abstinence, condoms, and PCR-testing appears to be based on the fact that most of their data points in Figure 1 / Table 1 test PCR-negative in semen by 3 months. Is this particular number subject to change with new data as it becomes available? As for the "6 month" guideline, I'm not sure how that was chosen. Is it just twice the "3 month" guideline? Is it roughly the maximum value seen in Figure 1 / Table 1 (but then it should be "7 months", right)? If new data became available (and Table 1 grew), would this always be the maximum value (or twice the median, or</p>
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	<p>however this is being chosen)? Also, for the "abstinence" recommendation, I infer that this is because the literature does not definitively prove the effectiveness of condoms in preventing EBOV transmission. Would a condom leak study at the 80nm range, combined with a more thorough characterization of EBOV infectious dose, change this recommendation, or do the authors feel that abstinence would always be preferred due to the highly lethal nature of EBOV? I assume that the recommendation for RT-PCR-based semen testing (over virus isolation) is based mostly on practical and safety considerations?</p> <p>I do wish the authors had made more specific recommendations for future research. The final paragraph of the paper is where I find myself looking for these proposals, but it is very brief. The first sentence which calls for "in-depth investigations" and "in-depth analyses" does not make it clear to me how a future study might improve upon, for example, the Christie, et al report of putative sexual transmission in Liberia in the spring of 2015 (#15 in their reference list). If the Christie, et al study had a more definitive genomic result (and "putative" was removed from the title and throughout the text of that report), would Thorson, et al, lengthen the durations of their condom/abstinence/testing recommendations? What are the criteria for proving a definitive case of sexual transmission (something that has never been proven yet for Ebola)? Would a single thoroughly characterized example of sexual transmission be preferred over a larger set (dozens) of inconclusive/putative sexual transmissions? If no further sexual transmission events were identified in the current epidemic, are survivor studies of viral persistence in body fluids sufficient to learn most of what we need to know? Will these studies require full genomics or isolation of live virus or large numbers to be thorough enough?</p> <p>These authors have exhaustively reviewed the body of knowledge published thus far and found it lacking, but it would be helpful to know how the previous studies could have been improved to more definitively answer these questions--or whether entirely different studies are truly needed to answer the questions more directly. I think the biggest way this paper could advance the research field is to be specific about how the field falls short, and specific about what need to happen next to fill in some very important gaps in knowledge that have significant public health implications. Without this, I fear that some research group will add yet another persistence-in-fluids or putative-sexual-transmission paper to the body of literature without substantially improving it.</p> <p>Minor notes:</p> <p>There are a number of papers included in this review (detailed in the first two paragraphs of the results section) that appear to refer to Sudan virus (SUDV), not Ebola virus (EBOV), though the wording in this section seems to be quite clear that it is meant to refer only to Ebola virus (EBOV). Given that the ebolaviruses (including EBOV, SUDV, RESTV, TAFV, BDBV) have a wide spectrum of clinical presentation (from asymptomatic to fatal) and are otherwise very distinct viruses, it seems like a distraction to discuss them extensively in this paper. If included at all in Fig 1 / Table 1 for the purposes of having reference points from other species, they should be very clearly marked as (SUDV) and perhaps grouped separately (same for the MARV data point included here).</p>
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	<p>Although I have little familiarity with the literature about condom effectiveness, I was able to follow most of the discussion with a few small exceptions, which I'll note here. On page 12, it was unclear to me what "condom effectiveness for reducing the risk of HIV transmission" stated as a percentage meant. What are the numerator and denominator? Also, in the same paragraph, does "80% less likely to become HIV-infected" mean a five-fold reduction in probability of infection over the lifetime of the relationship? At the top of page 12, the authors warn that "for diseases where an infectious dose is present in <0.1 mL of semen," intact condoms may not prevent transmission. Are the authors implying that this statement is true for EBOV? If so, it should be stated ("for diseases, like EBOV, where an infectious dose"). If this is not what the authors intended to say, is there anything that can be said about what the infectious dose of EBOV is, and what concentrations of EBOV are thought to exist in convalescent semen?</p>
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REVIEWER	Lisa Bebell Massachusetts General Hospital, United States
REVIEW RETURNED	29-Jul-2015

GENERAL COMMENTS	<p>Summary:</p> <p>The authors present a review of sexual transmission of Ebola and recommendations for preventing such transmission. This is a timely and important topic and has been relatively neglected in the literature. This manuscript highlights the importance of examining so-called 'sanctuary' sites where Ebola virus may be harbored and from which it may be transmitted, even after patient convalescence. The review is thorough and reasonably well-organized, though some sections of the manuscript seem isolated and do not connect well with sections prior to and following. The authors draw the conclusion that the possibility of Ebola sexual transmission cannot be ruled-out, which is reasonable based on the evidence. The authors' recommendations appear to be monthly semen testing and sexual abstinence for semen RT-PCR positive men, and condom use up to 6 months for men who are unable to be tested. While the recommendation to use condoms is reasonable, the 6-month cutoff is not evidence-based and may be too short for a highly lethal illness without established treatment. Monthly semen testing is truly impractical in Ebola-affected countries. Recommending abstinence for 3 months after convalescence or first negative blood EBOV PCR test (given that live Ebola virus has been recovered up to 80 days after symptom onset) followed by 4 additional months of condom use would be a safer recommendation given that the authors describe a patient who is PCR positive at day 199 (6.5 months) after symptom onset. Although this is an important and timely topic, I do think the authors' conclusions and recommendations need to be revised to be stronger and more clear. In addition, the grammar and sentence structure used throughout make the data difficult to assimilate and make the manuscript hard to read. Prior to publication, I recommend the manuscript be read closely and revised by a native English speaker to ensure readability. Lastly, I have some concerns with the methods used in the literature search, as these were not described comprehensively, especially the inclusion of unpublished reports, and should be revised and updated to include current literature.</p>
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	<p>Major Comments:</p> <p>Disagreements between tenses and plurality appear throughout the manuscript. Though these are not egregious, they do distract from the message of the manuscript. I recommend the manuscript be edited by a native English speaker to correct the grammar mistakes.</p> <p>The paragraph in the Methods section under 'Search Strategies' (Lines 18-39) are not specific enough to understand how the search was conducted, nor to independently repeat the search, especially with respect to so-called 'grey papers' of unpublished literature. Search terms are listed in the appendix, but the appendix is not referenced in the text. I recommend inserting a comment (see Appendix 1) in the text of line 25 after the phrase "and sexual transmission." The number of articles returned is included, but it would be helpful to have a figure showing which of the 121 articles were disqualified for which reasons. The criteria for including so-called 'grey papers' (which I believe are called 'unpublished reports') is also not defined, nor is 'grey paper' itself defined, a terminology unfamiliar to many readers but used in the abstract. In my opinion, the way the search strategy as described is not rigorous enough to merit the label 'systematic review' until further explanation is provided on the inclusion of unpublished data. In lines 43-45, a 'scoping review' is discussed but the definition of a scoping review nor the specific strategy are clarified. This manuscript could be titled a 'review' of the literature, or the methods could be revised to be more consistent with a truly systematic approach. On Page 6, Lines 6-7, there is reference made to (app 1), which should be revised to say (see Appendix 1) to be clearer. In addition, the included searches were last performed in December 2014 and would benefit from additional literature published since that time. Performing the search again while clarifying the search criteria, updating the results and recommendations could strengthen the paper considerably.</p> <p>Page 7, Line 25 – Page 8, Line 24: This section of the results reads like a 'laundry list' and does not give a good summary of viral persistence in semen. This needs to be reorganized to highlight the similarities and differences between epidemics or to somehow organize the data into a digestible format.</p> <p>Page 9-10, section on Evidence of persisting Ebola virus in faeces, saliva, sweat and urine: The reason for including this presentation of data and review of the literature is unclear. If there is a rationale as to why data on persistence of EBOV in feces or urine is important, it should be described. For example, if these are included because sexual acts often involve mucosal contact of one partner with the feces or urine of a previously infected partner, this should be stated. Otherwise, this section should be removed because it is not clearly relevant.</p> <p>Table 1: This is one idea for graphical presentation of the data. Looking at Figure 1, this appears to be similar to what I am suggesting. However, this represents a duplication of the data presented in Table 1 and therefore Table 1 should be removed and Figure 1 could be made neater.</p> <p>Figure 2: This does not add to what is described in the text and should be eliminated.</p> <p>Table 2: The data in this table are not well-presented and do not work well with a table format. I would suggest instead using a plot,</p>
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	<p>with a horizontal line for each patient/case. The X-axis would be days and the Y axis would be individual cases. A line for each patient could be centered at zero, the day of first positive semen test. To the left of zero, the line could extend back to the onset of symptoms. To the right of zero, the line could extend to the date of negative testing, or a question mark for those where no negative test was done. A hash mark on the line at the appropriate day could indicate an additional positive test date. Patterns or colors could separate samples from different anatomic sites, and the graph could be divided vertically to separate RT-PCR from viral culture methods or to separate different sample times.</p> <p>Minor Comments:</p> <p>Page 2, Abstract, line 14: I would personally change this to state "Additionally, we aim to provide" adding the words 'we aim.'</p> <p>Page 2, Abstract, line 32-33: The use of the terminology 'grey literature' many not be clear to some readers. I recommend eliminating this term altogether, or describing what this terminology means at the time it is first used.</p> <p>Page 2, Abstract, line 57: The conclusion sentence is wordy. I would suggest it be revised to state "Based on evidence reviewed, we conclude that male and female latex condoms offer some protection against EBOV compared to no condom use."</p> <p>Page 3, Strengths/Limitations, lines 21-22: I believe the authors mean to say "viruses" instead of "virus." Regardless, this sentence is not grammatically correct and should be corrected.</p> <p>Page 3, Strengths/Limitations, lines 31-35: This could be stated more clearly as "The primary limitation of this review is the scarcity of published and unpublished evidence of viral presence in body fluids of survivors over time, including a paucity of thorough investigations into suspect sexual transmission chains."</p> <p>Page 4, Introduction, lines 23-24: This sentence is not grammatically correct. I recommend re-wording it to say "While the initial Ebola response was targeted at slowing transmission amidst the overwhelming needs of weak health systems, the focus has now shifted to ending the epidemic with zero new cases."</p> <p>Page 4, Line 35: please remove the comma</p> <p>Page 4, Line 41 and 42: please remove the commas</p> <p>Page 4, Lines 46-53: The current sentence reads "While the issue of unknown risks of sexual transmission has been lifted forward, there is a lack of a systematic approach that also addresses the evidence of potential protection against sexual transmission of Ebola." The statement is unclear, especially the phrase 'lifted forward.' Please re-word this more clearly.</p> <p>Page 4, Line 58 into Page 5, line 5: This sentence currently reads: "Additionally, to provide evidence-based recommendations, to circumvent what may constitute a threat to the goal of ending the Ebola epidemic." Please also re-word this to be clearer. I might suggest "In addition, we provide evidence-based recommendations to prevent sexual transmission of Ebola and hasten the end of the current epidemic."</p> <p>Page 6, Line 16-17: please change this to 'a recent report from Liberia' and cite the report you mention.</p> <p>Page 6, Line 50-Page 7, line 5: The description of Ebola virus detection methods "Virus isolation requires a cold-chain, is time consuming (3 to 5 weeks), and involves handling of live virus in a BSL4-level, high-security laboratory and handling may be challenging in low-income settings (8). The reverse transcription PCR (RT-PCR) detects copies of viral RNA, but does not distinguish between live, infectious, virus and RNA remains" could be removed</p>
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	<p>from this section, as it does not fit well here. Could this be moved to the results to describe differences between RT-PCR and viral 'isolation'? I would suggest placing it on page 7 line 28, before the sentence beginning "One man in England . . ." A description of what 'viral isolation' is needs to be included (is this viral culture?) to help the reader understand the results.</p> <p>Page 7, Lines 25-28: This sentence currently reads: "Evidence of persisting EBOV in semen has been presented from four epidemic settings including the ongoing. (11) (9) (10, 12, 13) (14, 15)," but should be revised. I suggest "Evidence of persistent EBOV in semen has been found in four different Ebola outbreaks, including the current epidemic." In addition, the references are not in order and should be combined.</p> <p>Page 9, Line 49-52: These sentences are not well worded. They currently read "While RT-PCR was positive in all specimen from patients with acute Ebola, antigen detection was not. The authors conclude this may be related that transudation of the antigen to the oral fluid is low." This should be reworded. I recommend "While RT-PCR was positive in all specimens from patients with acute Ebola, no antigen was not detected from the same specimens. The authors conclude that this may be the result of minimal passage of antigen from blood to saliva."</p> <p>Page 11, Lines 27-34: This discussion of sexual transmission by other viruses is only biologically relevant with Marburg virus, another filovirus. I would recommend removing the statement about Crimean-Congo Hemorrhagic Fever.</p> <p>Page 12, Line 9: What are 'ISO standards'?</p> <p>Page 12, Lines 8-27: This description of condom testing does not add to the argument below. I recommend you eliminate it.</p> <p>Page 12, Lines 31-39: Currently, this reads "Most STD virus of interest are smaller than what is captured in a water leak test. HIV has a diameter of 120 nm, HBV 42nm and EBOV has a diameter of 80 nanometers. Hence the importance of leakage through smaller pores to transmission of small-sized test virus or bacteriophages in in-vitro settings has been examined (24-27)." I would modify it to read "EBOV has a diameter of 80 nanometers. The virus may be transmitted sexually by leaking through small pores in latex condoms. In-vitro testing has examined this possibility using small test viruses and bacteriophages (24-27)"</p> <p>Page 12, Line 35 to Page 13, Line 10: This section on 'Male and Female latex condom effectiveness' should be shortened. Would eliminate this section and shorten to "No studies on condom effectiveness in EBOV have been performed. However, a Cochrane report examined the effectiveness of male condom use on HIV transmission in sero-discordant heterosexual couples, and estimated that HIV-negative partners were 80% less likely to become HIV-infected than persons in similar relationships in which condoms were not used. Rates of prevention of HIV transmission are similar for female condoms. The effectiveness of condoms in reducing transmission of other STDs is less well studied, but thought to be lower."</p> <p>Page 13, Lines 13-23: I recommend eliminating this section on 'Survival outside host, implications for transmission' as it does not add significantly to the content of the manuscript.</p> <p>Discussion, Page 13, Lines 30-44: This currently reads "There is evidence that viable Ebola virus can persist in semen for at least 82 days after</p>
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	<p>symptom onset. How long the live virus did remain in semen in this case is not known, since follow-up was not done until some 700 days after symptom onset. Viral RNA has been shown in the semen of one male survivor in Liberia some 199 days post symptom onset, results from virus isolation are not yet available. We found no study that shows presence of live EBOV in vaginal secretions, but Ebola RNA was detected on day 33, in vaginal secretions of one woman out of six tested in the Kikwit epidemic. (9, 10)” I do not think this wording is clear. I would edit it to read: “Viable Ebola virus can persist in semen for at least 82 days after symptom onset, and may persist for much longer. Follow-up testing on the same case demonstrated no Ebola virus 700 days after symptom onset. Non-viable viral RNA has been found in semen up to 199 days post symptom onset, though it is not yet known if viable virus was also present at that time. No viable EBOV has been reported in vaginal secretions, but non-viable RNA was detected on day 33 in the vaginal secretions of one of six women tested in the Kikwit epidemic. (9, 10)”</p> <p>Page 14, Lines 14-26: Currently, this section reads “The earlier mentioned male survivor in Liberia with a positive rt-PCR analysis of his semen some 199 days post symptom onset, was discovered when his wife fell ill with Ebola, and it is strongly suspected to have been a case of sexual transmission, adding also matching genetic sequences to the extent that these have been possible to analyze (15, 34).” Please revise and clarify this statement—are you saying that the genetic sequences were possible to analyze in this case? Or not?</p> <p>Page 14, Lines 28-29: Please eliminate the word ‘of’.</p> <p>Page 14, Lines 46-53: This paragraph is not directly relevant. Please revise to incorporate it into the paragraph above or eliminate it.</p> <p>Page 15, Lines 3-6: This is currently very wordy and difficult to understand. It reads “The reviewed condom studies have applied conservative conditions for estimations of efficacy, still it is difficult to assess efficacy of protection against Ebola.” Please revise it. I recommend “Despite conservative estimates of condom efficacy, it is difficult to assess the efficacy of condoms in preventing sexual transmission of Ebola based on the studies reviewed here.”</p> <p>Page 15, Lines 17-19: “Thought through” should not be used here. I recommend “thoughtful”.</p> <p>Page 15, Lines 26-28: This sentence does not contribute to your argument and should be eliminated “Transactional sex has been described as common and associated with unprotected sex, and difficulties for women to negotiate safe sex. (42)”</p> <p>Page 15, Lines 35-37: I recommend altering your main conclusions sentence to be more powerful. It currently reads “Based on the evidence reviewed, we conclude that the risk of sexual transmission from</p> <p>Pages 15-16, Lines 55-56: Your recommendations are buried in the conclusion here and should be highlighted more. You state “In the meantime testing semen of survivors after 3 months and every month until it is RT-PCR negative, is recommended. If this is not possible, men should practice safe sex until 6 months post symptom</p>
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	<p>debut." I think you should move this to a more prominent position, state your recommendation more clearly (monthly semen testing for survivors and abstinence until semen is negative, 100% condom use if testing is not possible). Also, 'safe sex' means different things to different readers and must be clarified if you choose to use that term.</p> <p>convalescent patients cannot be ruled out." I would edit it to read "Based on evidence reviewed here, we conclude that there is risk of sexual transmission of Ebola from convalescent patients."</p> <p>Pages 24-27, References: All references should be edited to ensure a consistent format. In addition, references should be updated to reflect dates of publication and access, e.g. reference 1, which is stated as accessed January 30th 2015, but the report is dated 25th February 2015. How can the date-specific update be accessed 25 days in advance? References 21, 35 and 43 are unclear and may be improperly cited, please revise.</p>
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REVIEWER	Emanuele Sozzi University of Brighton, UK
REVIEW RETURNED	14-Aug-2015

GENERAL COMMENTS	<p>General comments</p> <p>The paper discusses an important global public health topic and the methodology is solid. On the other hand, although the English is good, it seems that the paper has been written a bit 'too quickly' and lot of work needs to be done to match the expectations from the readers of a very good journal.</p> <p>A careful web-literature search made by the reviewer was not able to spot any important paper which was missed by the authors.</p> <p>The opinion of the reviewer is that this is a paper which has the potential to be published, the idea is good, the methodology fine and a gap of knowledge has been identified. But a significant amount of work needs to be done prior to publication.</p> <p>Major recommendations</p> <p>1) The tables are fairly basic, but I appreciate that is difficult to do more than descriptive analysis with the very limited available data. Much more needs to be said about the figures: they frankly seem a bit chaotic and could have been done much better. Not only graphically but especially in term of conceptual design.</p> <p>A reader who needs to quickly go through the paper (speed-read), even when expert about the topic, could in my opinion get easily lost between the (many) numbers provided if the figures don't support him/her. The included figures are important, therefore no need to cut any of them. But to support the speed-reader, I'd personally suggest one or two more figures similar to 'Fig. 1'.</p> <p>The BMJ Open instructions for authors state that it is possible to include "up to five figures and tables. This is flexible, but exceeding this will impact upon the paper's 'readability'". There is definitely room for at least one more figure, if not even two.</p> <p>The Fig. 1 is covering the seminal fluid aspect. I couldn't see anything to help the reader to structure in his/her mind the data reg. 1) feaces, 2) saliva, 3) sweat and 4) urine. Frankly this aspect of the paper looks a bit chaotic. Without better designed figures and tables, a potentially good and important paper runs the risk of simply becoming a list of data. Sorry. Happy to help to make it more readable if I can.</p>
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	<p>2) Under the section: “Male and female latex condom efficacy” is mentioned that: “Quality standards by the U.S FDA are met when less than 99.6% of manufactured male latex condoms show water leakage.” Something is wrong here. If this was the case, the vast majority of male condoms would leak. What the author mean is probably following: “Quality standards by the U.S FDA are met when AT LEAST 99.6% of manufactured male latex condoms DO NOT show water leakage.” More details can be found here:</p> <p>http://www.fda.gov/RegulatoryInformation/Guidances/ucm073947.htm</p> <p>3) Following point should not be seen as a request for revision, but rather necessary for the reviewer/editor to understand if something went missing. It was mentioned in the abstract that the paper would have included important grey literature within the comprehensive review performed. But then the references to the grey/unpublished literature are not many. The authors included the search terms for their report, which is good, but only provided few details about the grey/unpublished literature. Which of the presented numbers come from grey literature? Are we talking only about reference no. 14, 18, 34 (NYT) and 38 (Reuters)? Or are there more data that are ultimately coming from the grey /unpublished literature, but are missing in the list of references. Sorry: this is not a criticism, simply a request for clarifications</p> <p>4) Although I am not a native speaker myself, I'd suggest to check following sentence which is part of the “Male and female latex condom efficacy”section: “A female latex condom has been approved by the US FDA and the WHO/UNFPA, with manufacturing standards and efficacy that to a large extent matches the male latex condom (22, 23).” New suggested version: “A female latex condom has been approved by the US FDA and the WHO/UNFPA, with manufacturing standards and efficacy that, [comma] to a large extent [comma], MATCH the standards [the ones] of the male latex condom (22, 23).”</p> <p>5) Would it be possible to see the PRISMA check list with all answers? The checklist can be downloaded here:</p> <p>http://www.prisma-statement.org/2.1.2%20-%20PRISMA%202009%20Checklist.pdf</p> <p>6) The authors don't really emphasize the difficulties of behavioral change: 3 months of sexual abstinence requires a lot of work in terms of behavioral change. Although the reviewer is aware that this is a scientific paper written for a small audience and not a WHO manual, I'd consider the opportunity of adding a bit of extra thought here. If this means one extra sentence in the last section or an entire paragraph, this is up to the authors. But I'd definitely add little more.</p> <p>7) Similar considerations could be done regarding condom use and consequent behavioral change/stigma: the point has been addressed by one line in the conclusions only. I'd have said a bit more. Happy to help with further more detailed suggestions.</p> <p>8) It is stated in the conclusion that “If this is not possible, men should practice safe sex until 6 months post symptom debut.” Why ‘six months’? Where does this number come from?</p>
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	<p>9) It is very good that the authors quoted Piercy et al. and Sagripanti et al. study. Personally I think that Piercy study is sometimes a bit 'over-referred to'. This has the consequence of creating a bit too much alarm about the POTENTIAL survival of EBOV. Yes, it is true that Piercy and other authors documented (or we should probably say "managed to achieve"?) long survival for EBOV and other similar viruses. But EBOV is a fragile virus, these (incredibly long) survival times are not consistent and sometimes take place under conditions that have been - on purpose - made absolutely ideal for the virus to survive!</p> <p>This would rarely be the case when the virus is – for example – on a condom and this, in my opinion, should be made very clear in the discussion section. Again: it is good to quote these survival studies, but one extra sentence should perhaps be added with the aim to put the numbers into the right context/perspective. With this and similar additional observation, this paper could go beyond the simple academic exercise of putting data together, but rather become a more critical review of the available evidences.</p> <p>Minor editorial corrections</p> <p>1) The second Fig. 1 is named "Fig. 1", but should be named "Fig. 2" instead.</p> <p>2) The flow chart with shadows looks in my modest opinion a bit 'unprofessional'. As above, happy to help with more suggestion to improve it.</p> <p>3) The reference 29 needs re-formatting</p> <p>4) The reference 35 needs re-formatting</p> <p>5) The reference 36 needs to be checked</p> <p>6) The reference 43 needs to be checked</p>
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VERSION 1 – AUTHOR RESPONSE

Reviewer: 1

Comments

The authors present the most comprehensive review of the subject I have seen. At times the wording is difficult to interpret-and I have tried to highlight these areas in the Specific comments below.

Thank you for your comments. We have taken them all on board and present a revised version. See detailed responses below.

General

1. Can Ebola virus disease be defined as EVD early on, and then EVD used instead of "Ebola disease" or "Ebola" when relating to EVD

Yes, please see track changes.

2. Pleas maintain the term "viable " in terms of infectious virus ,rather than "live"

Thanks, please see track changes.

Authors feedback

Thank you for your comments. We have taken them all on board and present a revised version. See responses below.

General

1. Can Ebola virus disease be defined as EVD early on, and then EVD used instead of "Ebola disease" or "Ebola" when relating to EVD

Yes, please see track changes.

1.

2. Please maintain the term “viable” in terms of infectious virus, rather than “live”

Thanks, please see track changes. 2. Yes, please see track changes.

3. Could the authors define what “grey literature” is?

This term has been changed to “unpublished” or the document stated 3.

4. Please consider adding the recent Mora-Rillo paper,

[http://www.thelancet.com/journals/lanres/article/PIIS2213-2600\(15\)00180-0/abstract](http://www.thelancet.com/journals/lanres/article/PIIS2213-2600(15)00180-0/abstract) which detected RNA from a vaginal swab

Thank you the findings have been added and referenced to in the discussion. 4. Anna?

5. Please consider adding a more recent EBOC stability paper to the Survival paragraph:

http://wwwnc.cdc.gov/eid/article/21/7/15-0253_article

Thank you the findings have been added and referenced to in the survival para.

6. The authors mention anal sex and used condom handling—they should consider noting oral sex and masturbation as sources of contact with potentially infectious fluids also

This has been included. See sections on “Survival outside host, implications to transmission”

7. Given your review cannot show evidence that condoms likely provide “some protection” but just how effectively remains unclear, could the authors consider more strongly leaning on the abstinence aspect of their conclusion in Pg15Ln46-49. Given how one case can cause such damage when occurring in the right setting, even small risks should be avoided and certainly very clearly documented, however inconvenient that process of risk reduction may be

Please see the revised conclusions

Specific

(Page numbering refers to those numbers on the 26 page PDF)

Pg4Ln23 case=cases

Pg4Ln48 Please rephrase “lifted forward”

Pg4Ln57 “...and to condom...”?

Pg5Ln43 4 Please define “scoping literature review”

Pg6Ln9 Please clarify this sentence or break in two

Pg6Ln17 “The recent” to “a recent”?

Pg6Ln35-57 Just “methods to identify Ebola virus in the sample” suitably encompasses both culture and molecular I believe

Pg11Ln36 Please re-write this sentence to clarify meaning

Pg12Ln19-24 Please clarify if both condoms had equal rates of pore leakage

Pg13Ln8 “at” or “for anal sex”?

Pg16Ln3-5 Not just men –men and women should practice safe sex.

Thank you, we agree and have edited all these accordingly.

Reviewer: 2

Comments

This is an important review for sexual transmission of Ebola. In this area, there are few limited studies, because it is very difficult to perform such a study. This limitation of the area makes this report more important. It is worth to be published.

Thank you for your feedback!

Reviewer 3

8. The authors note that the published literature on this topic is somewhat limited, and they are careful to word their conclusions very modestly: "we conclude that the risk of sexual transmission ... cannot be ruled out" and "we conclude that ... condoms offer some protection." I appreciate this conservative wording as it is an appropriate depiction of what is known in the field.

Thank you!

9. A) Based on their review of the literature, the authors make recommendations regarding abstinence, condom usage, and follow-up semen testing for EVD survivors. I think it might be helpful to a very brief explanation for how each number was chosen. For example, the "3 month" guideline of abstinence, condoms, and PCR-testing appears to be based on the fact that most of their data points in Figure 1 / Table 1 test PCR-negative in semen by 3 months. Is this particular number subject to change with new data as it becomes available?

9A) Thank you, yes it is correct that the 3 months recommendation was based on the finding of a positive virus isolation in semen on day 82 in one man (Kikwit). As new evidence is available, these recommendations have changed. This has been added and clarified in the paper.

9B) As for the "6 month" guideline, I'm not sure how that was chosen. Is it just twice the "3 month" guideline? Is it roughly the maximum value seen in Figure 1 / Table 1 (but then it should be "7 months", right)? If new data became available (and Table 1 grew), would this always be the maximum value (or twice the median, or however this is being chosen)? Also, for the "abstinence" recommendation, I infer that this is because the literature does not definitively prove the effectiveness of condoms in preventing EBOV transmission.

9B) Currently the correlation between rt-PCR positivity and virus isolation for Ebola is not known, ie it is not clear whether the positive PCR findings represent live virus or fragmented dead virus. Hence we have today little knowledge of the actual risk of transmission involved following a positive PCR result in semen. The change to a 6 months recommendation came about as a results of the initial findings of semen PCR-positivity at 6 months in one man in Liberia, and the suspect case of transmission there. There is no standardised plan for increasing these, but to update accordingly in relation to new findings. The current recommendations state that primarily semen testing should be offered, if not available abstinence, or if not possible, condoms, should be used for at least 6 months. It is correct that if semen testing is not available, abstinence is recommended as the first option, given that we know very little about the actual effectiveness of condoms. We have edited the discussion to be clearer on all these components.

9C) Would a condom leak study at the 80nm range, combined with a more thorough characterization of EBOV infectious dose, change this recommendation, or do the authors feel that abstinence would always be preferred due to the highly lethal nature of EBOV? I assume that the recommendation for RT-PCR-based semen testing (over virus isolation) is based mostly on practical and safety considerations?

9c) Thank you, if more evidence would be available in relation to condom leaking, infectious dose, and (if any) transmission risks involved with handling condoms, it would be taken into consideration in relation to the recommendations.

Yes, the RT-PCR based semen testing can be done within the highly affected Ebola countries, whereas the virus isolation requires BSL4 and can only be done after transportation to laboratories outside the countries in question.

10. A) I do wish the authors had made more specific recommendations for future research. The final paragraph of the paper is where I find myself looking for these proposals, but it is very brief. The first sentence which calls for "in-depth investigations" and "in-depth analyses" does not make it clear to me how a future study might improve upon, for example, the Christie, et al report of putative sexual transmission in Liberia in the spring of 2015 (#15 in their reference list).

A) Thank you, we have expanded the research section in the conclusions.

10 B) bearing this in mind. If the Christie, et al study had a more definitive genomic result (and "putative" was removed from the title and throughout the text of that report), would Thorson, et al, lengthen the durations of their condom/abstinence/testing recommendations? What are the criteria for proving a definitive case of sexual transmission (something that has never been proven yet for Ebola)? Would a single thoroughly characterized example of sexual transmission be preferred over a larger set (dozens) of inconclusive/putative sexual transmissions?

B) We recommend primarily regular semen testing until negativity, and programme activities are under way in several of the affected countries. When that is not in place, abstinence or condom use is recommended for 'at least 6 months' post onset. This has been updated and further clarified in the paper. The Christie paper reports of a suspect sexual transmission to have occurred 6 months post symptom onset of the survivor, matching this updated recommendation.

Given limitations in in-country laboratory capacity in affected countries, it has not been possible to perform the necessary analyses in full for all suspect sexual transmission cases. Virus isolation and full genetic sequencing have not been done. Still we agree that the Christie report presents a strong suspicion of sexual transmission. We have edited language around the study and have clarified that the study has added to the evidence feeding into the recommendations, of semen testing programmes or else abstinence/condom use for at least 6 months.

10C) If no further sexual transmission events were identified in the current epidemic, are survivor studies of viral persistence in body fluids sufficient to learn most of what we need to know? Will these studies require full genomics or isolation of live virus or large numbers to be thorough enough?

C) Thank you, yes we believe that more information on the correlation between RT-PCR positivity and virus isolation as well as genetic sequencing is necessary in order to evaluate the transmission risk from semen (as well as vaginal and other bodily fluids). We also believe a combined bio-behavioural approach with sample sizes following traditional rules for inference is needed in order to assess both transmission and potential epidemic impact.

11. These authors have exhaustively reviewed the body of knowledge published thus far and found it lacking, but it would be helpful to know how the previous studies could have been improved to more definitively answer these questions--or whether entirely different studies are truly needed to answer the questions more directly. I think the biggest way this paper could advance the research field is to be specific about how the field falls short, and specific about what need to happen next to fill in some very important gaps in knowledge that have significant public health implications. Without this, I fear that some research group will add yet another persistence-in-fluids or putative-sexual-transmission paper to the body of literature without substantially improving it.

11. Thank you, we agree and have accordingly expanded on the research recommendations in the conclusion.

Minor notes:

12. There are a number of papers included in this review (detailed in the first two paragraphs of the results section) that appear to refer to Sudan virus (SUDV), not Ebola virus (EBOV), though the wording in this section seems to be quite clear that it is meant to refer only to Ebola virus (EBOV). Given that the ebolaviruses (including EBOV, SUDV, RESTV, TAFV, BDBV) have a wide spectrum of clinical presentation (from asymptomatic to fatal) and are otherwise very distinct viruses, it seems like a distraction to discuss them extensively in this paper. If included at all in Fig 1 / Table 1 for the purposes of having reference points from other species, they should be very clearly marked as (SUDV) and perhaps grouped separately (same for the MARV data point included here). Thank you. Given the limited evidence base, and the hypothesis that there may be some similarities between the different ebolaviruses, we chose to present these findings as well. We have clarified the wording and marked SUDV and EBOV etc where appropriate.

13. Although I have little familiarity with the literature about condom effectiveness, I was able to follow most of the discussion with a few small exceptions, which I'll note here. On page 12, it was unclear to me what "condom effectiveness for reducing the risk of HIV transmission" stated as a percentage meant. What are the numerator and denominator? Also, in the same paragraph, does "80% less likely to become HIV-infected" mean a five-fold reduction in probability of infection over the lifetime of the relationship? At the top of page 12, the authors warn that "for diseases where an infectious dose is present in <0.1 mL of semen," intact condoms may not prevent transmission. Are the authors implying that this statement is true for EBOV? If so, it should be stated ("for diseases, like EBOV, where an infectious dose"). If this is not what the authors intended to say, is there anything that can be said about what the infectious dose of EBOV is, and what concentrations of EBOV are thought to exist in convalescent semen?

13) This whole paragraph has been re-written for clarity in order with the above. The 80% indeed refers to that HIV negative partners in discordant couples using condoms were 80% less likely to become HIV-infected as compared to those discordant couples who did not use it. We report on what is known for the infectious dose of Ebola, and we have clarified language to reflect we do not have knowledge of Ebola concentrations in semen.

Reviewer: 4

Comments

Summary:

The authors present a review of sexual transmission of Ebola and recommendations for preventing such transmission. This is a timely and important topic and has been relatively neglected in the literature. This manuscript highlights the importance of examining so-called 'sanctuary' sites where Ebola virus may be harboured and from which it may be transmitted, even after patient convalescence. The review is thorough and reasonably well-organized, though some sections of the manuscript seem isolated and do not connect well with sections prior to and following. The authors draw the conclusion that the possibility of Ebola sexual transmission cannot be ruled-out, which is reasonable based on the evidence. The authors' recommendations appear to be monthly semen testing and sexual abstinence for semen RT-PCR positive men and condom use up to 6 months for men who are unable to be tested. While the recommendation to use condoms is reasonable, the 6-month cut-off is not evidence-based and may be too short for a highly lethal illness without established treatment. Monthly semen testing is truly impractical in Ebola-affected countries. Recommending abstinence for 3 months after convalescence or first negative blood EBOV PCR test (given that live Ebola virus has been recovered up to 80 days after symptom onset) followed by 4 additional months of condom use would be a safer recommendation given that the authors describe a patient who is PCR positive at day 199 (6.5 months) after symptom onset. Although this is an important and timely topic, I do think the authors' conclusions and recommendations need to be

revised to be stronger and more clear. In addition, the grammar and sentence structure used throughout make the data difficult to assimilate and make the manuscript hard to read. Prior to publication, I recommend the manuscript be read closely and revised by a native English speaker to ensure readability. Lastly, I have some concerns with the methods used in the literature search, as these were not described comprehensively, especially the inclusion of unpublished reports, and should be revised and updated to include current literature.

Thank you. The change to an 'at least 6 months' recommendation came about as a result of the initial findings of a suspect transmission case and semen PCR-positivity at 6 months in one man in Liberia. The current recommendations state that primarily semen testing should be offered, if not available abstinence, or if not possible, condoms, should be used for at least 6 months. We agree semen testing programming is a very big undertaking, but we also recognise that plans for this are currently discussed in the affected countries.

The text has been revised to clarify and reflect updates in methods and recommendations. The text has been revised by an English editor, please see also further details in relation to the revised methods section below.

Major Comments:

14. Disagreements between tenses and plurality appear throughout the manuscript. Though these are not egregious, they do distract from the message of the manuscript. I recommend the manuscript be edited by a native English speaker to correct the grammar mistakes.

This has been revised.

15. A) The paragraph in the Methods section under 'Search Strategies' (Lines 18-39) are not specific enough to understand how the search was conducted, nor to independently repeat the search, especially with respect to so-called 'grey papers' of unpublished literature. Search terms are listed in the appendix, but the appendix is not referenced in the text. I recommend inserting a comment (see Appendix 1) in the text of line 25 after the phrase "and sexual transmission." The number of articles returned is included, but it would be helpful to have a figure showing which of the 121 articles were disqualified for which reasons.

A) Thank you, this has been added and a PRISMA checklist has been added as well to describe the process in detail.

B) The criteria for including so-called 'grey papers' (which I believe are called 'unpublished reports') is also not defined, nor is 'grey paper' itself defined, a terminology unfamiliar to many readers but used in the abstract. In my opinion, the way the search strategy as described is not rigorous enough to merit the label 'systematic review' until further explanation is provided on the inclusion of unpublished data. In lines 43-45, a 'scoping review' is discussed but the definition of a scoping review nor the specific strategy are clarified. This manuscript could be titled a 'review' of the literature, or the methods could be revised to be more consistent with a truly systematic approach.

B) We have changed wording around grey literature accordingly. Additionally we have re-structured and changed the methods section to highlight that this was a systematic review of evidence in relation to viral persistence and sexual transmission of Ebola. We have further clarified the additional reviewing of evidence done in relation to efficacy and effectiveness of condoms in the case of Ebola.

C) On Page 6, Lines 6-7, there is reference made to (app 1), which should be revised to say (see Appendix 1) to be clearer.

In addition, the included searches were last performed in December 2014 and would benefit from additional literature published since that time. Performing the search again while clarifying the search criteria, updating the results and recommendations could strengthen the paper considerably.

c) Thank you the reference to appendix 1 has been added. We have also clarified in the text that the

search actually has been repeated. The conclusions have also been updated in line with the findings.

16. Page 7, Line 25 – Page 8, Line 24: This section of the results reads like a 'laundry list' and does not give a good summary of viral persistence in semen. This needs to be reorganized to highlight the similarities and differences between epidemics or to somehow organize the data into a digestible format.

Thank you the section has been revised.

17. Page 9-10, section on Evidence of persisting Ebola virus in faeces, saliva, sweat and urine: The reason for including this presentation of data and review of the literature is unclear. If there is a rationale as to why data on persistence of EBOV in feces or urine is important, it should be described. For example, if these are included because sexual acts often involve mucosal contact of one partner with the faeces or urine of a previously infected partner, this should be stated. Otherwise, this section should be removed because it is not clearly relevant.

Thank you, please see amendments to this section to clarify the relations to sexual transmission risks.

18. Table 1: This is one idea for graphical presentation of the data. Looking at Figure 1, this appears to be similar to what I am suggesting. However, this represents a duplication of the data presented in Table 1 and therefore Table 1 should be removed and Figure 1 could be made neater.

Thank you, we have revised both table 1 and figure 1 for clarity. We are open to removing table 1 as needed, but have left it for the time, given the range of comments from the different reviewers.

19. Figure 2: This does not add to what is described in the text and should be eliminated.
Thank you, we agree it duplicates to some extent what is in the text, but we have left it given the range of comments from the different reviewers

20. Table 2: The data in this table are not well-presented and do not work well with a table format. I would suggest instead using a plot, with a horizontal line for each patient/case. The X-axis would be days and the Y axis would be individual cases. A line for each patient could be centered at zero, the day of first positive semen test. To the left of zero, the line could extend back to the onset of symptoms. To the right of zero, the line could extend to the date of negative testing, or a question mark for those where no negative test was done. A hash mark on the line at the appropriate day could indicate an additional positive test date. Patterns or colours could separate samples from different anatomic sites, and the graph could be divided vertically to separate RT-PCR from viral culture methods or to separate different sample times.

Thank you we opted to revise the table.

Minor Comments:

Page 2, Abstract, line 14: I would personally change this to state "Additionally, we aim to provide" adding the words 'we aim.'

Page 2, Abstract, line 32-33: The use of the terminology 'grey literature' may not be clear to some readers. I recommend eliminating this term altogether, or describing what this terminology means at the time it is first used.

Page 2, Abstract, line 57: The conclusion sentence is wordy. I would suggest it be revised to state "Based on evidence reviewed, we conclude that male and female latex condoms offer some protection against EBOV compared to no condom use."

Page 3, Strengths/Limitations, lines 21-22: I believe the authors mean to say "viruses" instead of "virus." Regardless, this sentence is not grammatically correct and should be corrected.

Page 3, Strengths/Limitations, lines 31-35: This could be stated more clearly as “The primary limitation of this review is the scarcity of published and unpublished evidence of viral presence in body fluids of survivors over time, including a paucity of thorough investigations into suspect sexual transmission chains.”

Page 4, Introduction, lines 23-24: This sentence is not grammatically correct. I recommend re-wording it to say “While the initial Ebola response was targeted at slowing transmission amidst the overwhelming needs of weak health systems, the focus has now shifted to ending the epidemic with zero new cases.”

Page 4, Line 35: please remove the comma Page 4, Line 41 and 42: please remove the commas

Page 4, Lines 46-53: The current sentence reads “While the issue of unknown risks of sexual transmission has been lifted forward, there is a lack of a systematic approach that also addresses the evidence of potential protection against sexual transmission of Ebola.” The statement is unclear, especially the phrase ‘lifted forward.’ Please re-word this more clearly.

Page 4, Line 58 into Page 5, line 5: This sentence currently reads: “Additionally, to provide evidence-based recommendations, to circumvent what may constitute a threat to the goal of ending the Ebola epidemic.” Please also re-word this to be clearer. I might suggest “In addition, we provide evidence-based recommendations to prevent sexual transmission of Ebola and hasten the end of the current epidemic.”

Page 6, Line 16-17: please change this to ‘a recent report from Liberia’ and cite the report you mention.

Page 6, Line 50-Page 7, line 5: The description of Ebola virus detection methods “Virus isolation requires a cold-chain, is time consuming (3 to 5 weeks), and involves handling of live virus in a BSL4-level, high-security laboratory and handling may be challenging in low-income settings (8). The reverse transcription PCR (RT-PCR) detects copies of viral RNA, but does not distinguish between live, infectious, virus and RNA remains” could be removed from this section, as it does not fit well here. Could this be moved to the results to describe differences between RT-PCR and viral ‘isolation’? I would suggest placing it on page 7 line 28, before the sentence beginning “One man in England . . .” A description of what ‘viral isolation’ is needs to be included (is this viral culture?) to help the reader understand the results.

Page 7, Lines 25-28: This sentence currently reads: “Evidence of persisting EBOV in semen has been presented from four epidemic settings including the ongoing. (11) (9) (10, 12, 13) (14, 15),” but should be revised. I suggest “Evidence of persistent EBOV in semen has been found in four different Ebola outbreaks, including the current epidemic.” In addition, the references are not in order and should be combined.

Page 9, Line 49-52: These sentences are not well worded. They currently read “While RT-PCR was positive in all specimen from patients with acute Ebola, antigen detection was not. The authors conclude this may be related that transudation of the antigen to the oral fluid is low.” This should be reworded. I recommend “While RT-PCR was positive in all specimens from patients with acute Ebola, no antigen was not detected from the same specimens. The authors conclude that this may be the result of minimal passage of antigen from blood to saliva.”

Thank you these comments have been taken aboard as a whole.

Page 11, Lines 27-34: This discussion of sexual transmission by other viruses is only biologically relevant with Marburg virus, another filovirus. I would recommend removing the statement about Crimean-Congo Haemorrhagic Fever.

Thank you this has been removed.

Page 12, Line 9: What are ‘ISO standards’?

International Organization of Standardization (ISO), this has been spelled out in the text.

Page 12, Lines 8-27: This description of condom testing does not add to the argument below. I recommend you eliminate it.

Thank you, this has been done.

Page 12, Lines 31-39: Currently, this reads “Most STD virus of interest are smaller than what is captured in a water leak test. HIV has a diameter of 120 nm, HBV 42nm and EBOV has a diameter of 80 nanometres. Hence the importance of leakage through smaller pores to transmission of small-sized test virus or bacteriophages in in-vitro settings has been examined (24-27).” I would modify it to read “EBOV has a diameter of 80 nanometres. The virus may be transmitted sexually by leaking through small pores in latex condoms. In-vitro testing has examined this possibility using small test viruses and bacteriophages (24-27)”

Page 12, Line 35 to Page 13, Line 10: This section on ‘Male and Female latex condom effectiveness’ should be shortened. Would eliminate this section and shorten to “No studies on condom effectiveness in EBOV have been performed. However, a Cochrane report examined the effectiveness of male condom use on HIV transmission in sero-discordant heterosexual couples, and estimated that HIV-negative partners were 80% less likely to become HIV-infected than persons in similar relationships in which condoms were not used. Rates of prevention of HIV transmission are similar for female condoms. The effectiveness of condoms in reducing transmission of other STDs is less well studied, but thought to be lower.”

Thank you, these comments have been taken onboard and the text amended.

Page 13, Lines 13-23: I recommend eliminating this section on ‘Survival outside host, implications for transmission’ as it does not add significantly to the content of the manuscript.

Thank you, we think however that this may be of interest in relation to handling of used condoms and would like to suggest to keep it.

Discussion, Page 13, Lines 30-44: This currently reads “There is evidence that viable Ebola virus can persist in semen for at least 82 days after symptom onset. How long the live virus did remain in semen in this case is not known, since follow-up was not done until some 700 days after symptom onset. Viral RNA has been shown in the semen of one male survivor in Liberia some 199 days post symptom onset, results from virus isolation are not yet available. We found no study that shows presence of live EBOV in vaginal secretions, but Ebola RNA was detected on day 33, in vaginal secretions of one woman out of six tested in the Kikwit epidemic. (9, 10)” I do not think this wording is clear. I would edit it to read: “Viable Ebola virus can persist in semen for at least 82 days after symptom onset, and may persist for much longer. Follow-up testing on the same case demonstrated no Ebola virus 700 days after symptom onset. Non-viable viral RNA has been found in semen up to 199 days post symptom onset, though it is not yet known if viable virus was also present at that time. No viable EBOV has been reported in vaginal secretions, but non-viable RNA was detected on day 33 in the vaginal secretions of one of six women tested in the Kikwit epidemic. (9, 10)”

Thank you, the text has been revised in accordance with this and the full text has been edited by a native English speaker to clarify further.

Page 14, Lines 14-26: Currently, this section reads “The earlier mentioned male survivor in Liberia with a positive rt-PCR analysis of his semen some 199 days post symptom onset, was discovered when his wife fell ill with Ebola, and it is strongly suspected to have been a case of sexual transmission, adding also matching genetic sequences to the extent that these have been possible to

analyse (15, 34).” Please revise and clarify this statement—are you saying that the genetic sequences were possible to analyse in this case? Or not?

Thank you, the genetic sequences that were available were matching. Since virus were not isolated/cultured the whole sequencing could not be done. The wording has been clarified in the text.

Page 14, Lines 28-29: Please eliminate the word ‘of’.

Page 14, Lines 46-53: This paragraph is not directly relevant. Please revise to incorporate it into the paragraph above or eliminate it.

Page 15, Lines 3-6: This is currently very wordy and difficult to understand. It reads “The reviewed condom studies have applied conservative conditions for estimations of efficacy, still it is difficult to assess efficacy of protection against Ebola.” Please revise it. I recommend “Despite conservative estimates of condom efficacy, it is difficult to assess the efficacy of condoms in preventing sexual transmission of Ebola based on the studies reviewed here.”

Page 15, Lines 17-19: “Thought through” should not be used here. I recommend “thoughtful”.

Thank you this text has been edited.

Page 15, Lines 26-28: This sentence does not contribute to your argument and should be eliminated “Transactional sex has been described as common and associated with unprotected sex, and difficulties for women to negotiate safe sex. (42)”

Thank you, we have revised this to fit in with the paragraph.

Page 15, Lines 35-37: I recommend altering your main conclusions sentence to be more powerful. It currently reads “Based on the evidence reviewed, we conclude that the risk of sexual transmission from convalescent patients cannot be ruled out.” I would edit it to read “Based on evidence reviewed here, we conclude that there is risk of sexual transmission of Ebola from convalescent patients.”

Pages 15-16, Lines 55-56: Your recommendations are buried in the conclusion here and should be highlighted more. You state “In the meantime testing semen of survivors after 3 months and every month until it is RT-PCR negative, is recommended. If this is not possible, men should practice safe sex until 6 months post symptom debut.” I think you should move this to a more prominent position, state your recommendation more clearly (monthly semen testing for survivors and abstinence until semen is negative, 100% condom use if testing is not possible). Also, ‘safe sex’ means different things to different readers and must be clarified if you choose to use that term.

Pages 24-27, References: All references should be edited to ensure a consistent format. In addition, references should be updated to reflect dates of publication and access, e.g. reference 1, which is stated as accessed January 30th 2015, but the report is dated 25th February 2015. How can the date-specific update be accessed 25 days in advance? References 21, 35 and 43 are unclear and may be improperly cited, please revise.

Thank you these sections have all been amended accordingly.

Reviewer: 5

Comments

The paper discusses an important global public health topic and the methodology is solid. On the other hand, although the English is good, it seems that the paper has been written a bit ‘too quickly’ and lot of work needs to be done to match the expectations from the readers of a very good journal. A careful web-literature search made by the reviewer was not able to spot any important paper which was missed by the authors.

The opinion of the reviewer is that this is a paper which has the potential to be published, the idea is good, the methodology fine and a gap of knowledge has been identified. But a significant amount of work needs to be done prior to publication.

Thank you for your comments and feedback. We have acknowledged them all.

Major recommendations

21. The tables are fairly basic, but I appreciate that is difficult to do more than descriptive analysis with the very limited available data. Much more needs to be said about the figures: they frankly seem a bit chaotic and could have been done much better. Not only graphically but especially in term of conceptual design.

- A reader who needs to quickly go through the paper (speed-read), even when expert about the topic, could in my opinion get easily lost between the (many) numbers provided if the figures don't support him/her. The included figures are important, therefore no need to cut any of them. But to support the speed-reader, I'd personally suggest one or two more figures similar to 'Fig. 1'.

- The BMJ Open instructions for authors state that it is possible to include "up to five figures and tables. This is flexible, but exceeding this will impact upon the paper's 'readability'. There is definitely room for at least one more figure, if not even two.

- The Fig. 1 is covering the seminal fluid aspect. I couldn't see anything to help the reader to structure in his/her mind the data reg. 1) faeces, 2) saliva, 3) sweat and 4) urine. Frankly this aspect of the paper looks a bit chaotic. Without better designed figures and tables, a potentially good and important paper runs the risk of simply becoming a list of data. Sorry. Happy to help to make it more readable if I can.

Thank you, given the range of advise from reviewers we have opted to keep the figures that are included but to improve these in line with your comments.

22. Under the section: "Male and female latex condom efficacy" is mentioned that: "Quality standards by the U.S FDA are met when less than 99.6% of manufactured male latex condoms show water leakage." Something is wrong here. If this was the case, the vast majority of male condoms would leak. What the author mean is probably following: "Quality standards by the U.S FDA are met when AT LEAST 99.6% of manufactured male latex condoms DO NOT show water leakage." More details can be found here: <http://www.fda.gov/RegulatoryInformation/Guidances/ucm073947.htm>

Thank you this was a mistake/typo, the text has been corrected.

23. Following point should not be seen as a request for revision, but rather necessary for the reviewer/editor to understand if something went missing. It was mentioned in the abstract that the paper would have included important grey literature within the comprehensive review performed. But then the references to the grey/unpublished literature are not many. The authors included the search terms for their report, which is good, but only provided few details about the grey/unpublished literature. Which of the presented numbers come from grey literature? Are we talking only about reference no. 14, 18, 34 (NYT) and 38 (Reuters)? Or are there more data that are ultimately coming from the grey /unpublished literature, but are missing in the list of references. Sorry: this is not a criticism, simply a request for clarifications

We agree the amount of unpublished literature is not big, which may be reflecting the fact that during the acute epidemic response, any suspect cases of sexual transmission will be less evident given the vast amount of cases infected by other acutely ill cases or from exposure at funerals. We have further clarified the process and inclusion of internal reports that we did review.

24. Although I am not a native speaker myself, I'd suggest to check following sentence which is part of the "Male and female latex condom efficacy" section:

- "A female latex condom has been approved by the US FDA and the WHO/UNFPA, with

manufacturing standards and efficacy that to a large extent matches the male latex condom (22, 23)."

– New suggested version:

– "A female latex condom has been approved by the US FDA and the WHO/UNFPA, with manufacturing standards and efficacy that, [comma] to a large extent [comma], MATCH the standards [the ones] of the male latex condom (22, 23)."

Thank you this has been changed.

25. Would it be possible to see the PRISMA check list with all answers? The checklist can be downloaded here: <http://www.prisma-statement.org/2.1.2%20-%20PRISMA%202009%20Checklist.pdf>

Thank you, this is now included.

26 The authors don't really emphasize the difficulties of behavioural change: 3 months of sexual abstinence requires a lot of work in terms of behavioural change. Although the reviewer is aware that this is a scientific paper written for a small audience and not a WHO manual, I'd consider the opportunity of adding a bit of extra thought here. If this means one extra sentence in the last section or an entire paragraph, this is up to the authors. But I'd definitely add little more
Thank you, this is important we have added a short comment on this.

27 Similar considerations could be done regarding condom use and consequent behavioural change/stigma: the point has been addressed by one line in the conclusions only. I'd have said a bit more. Happy to help with further more detailed suggestions.

Thank you the point has been taken in and the text amended.

28 It is stated in the conclusion that "If this is not possible, men should practice safe sex until 6 months post symptom debut." Why 'six months'? Where does this number come from?

The current WHO recommendation states at least 6 months, this is reflected in this review, and is building on the Liberia case where a case of sexual transmission was suspected to have happened at 6 months, matching the man's semen RT-PCR positivity at the same time.

29 It is very good that the authors quoted Piercy et al. and Sagripanti et al. study. Personally I think that Piercy study is sometimes a bit 'over-referred to'. This has the consequence of creating a bit too much alarm about the POTENTIAL survival of EBOV. Yes, it is true that Piercy and other authors documented (or we should probably say "managed to achieve"?) long survival for EBOV and other similar viruses. But EBOV is a fragile virus, these (incredibly long) survival times are not consistent and sometimes take place under conditions that have been - on purpose - made absolutely ideal for the virus to survive!

o This would rarely be the case when the virus is – for example – on a condom and this, in my opinion, should be made very clear in the discussion section. Again: it is good to quote these survival studies, but one extra sentence should perhaps be added with the aim to put the numbers into the right context/perspective. With this and similar additional observation, this paper could go beyond the simple academic exercise of putting data together, but rather become a more critical review of the available evidences.

Thank you, with the above in mind we have revised and modified the survival paragraph.

Minor editorial corrections

i. The second Fig. 1 is named "Fig. 1", but should be named "Fig. 2" instead.

ii. The flow chart with shadows looks in my modest opinion a bit 'unprofessional'. As above, happy to help with more suggestion to improve it.

- iii. The reference 29 needs re-formatting
- iv. The reference 35 needs re-formatting
- v. The reference 36 needs to be checked
- vi. The reference 43 needs to be checked

VERSION 2 – REVIEW

REVIEWER	Ian M Mackay Supervising Scientist Public and Environmental Health - Virology Forensic & Scientific Services Queensland Department of Health Queensland AUSTRALIA
REVIEW RETURNED	01-Oct-2015

GENERAL COMMENTS	<p>_Suggest replacing "of one man" with "from one man", ln8, pg9</p> <p>_Suggest "specimen by vaginal swabs" be replaced with "vaginal swab specimens", ln38, pg9</p> <p>_Suggest replacing "in a total" with "from a total" ln17, pg10</p> <p>_Please specify "Ebola" further at ln24, pg10 (virus or virus disease)</p> <p>_Sentence starting ln40, pg11 - suggest deleting "has"</p> <p>_Sentence starting ln29, pg13 does not make sense. Is an "and" missing?</p>
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REVIEWER	Lisa Bebell Massachusetts General Hospital, USA
REVIEW RETURNED	08-Oct-2015

GENERAL COMMENTS	<p>Summary:</p> <p>The authors present a revised review of sexual transmission of Ebola and recommendations for preventing such transmission. This is a timely and important topic and has been relatively neglected in the literature. The authors have incorporated feedback from five reviewers in revising their manuscript. The changes are significant, and have led to a clearer presentation of this important information.</p> <p>The authors' conclusions remain the same, that the possibility of Ebola sexual transmission cannot be ruled-out, and condoms may provide benefit over no condom use. The conclusions are reasonable, and the timelines have been clarified. Lastly, methods used in the literature search are now described comprehensively and the inclusion of unpublished reports has been clarified.</p> <p>Major Comments:</p> <p>Table 1: This table represents a duplication of the data presented in Figure 1 and therefore Table 1 should be removed.</p> <p>Minor Comments:</p> <p>Page 6, Lines 23-24: The search was performed December, 2014. However, an additional search was later performed to update the references, correct? Please include this information in this section of your manuscript to clarify what is written in lines 40-46 of this section.</p> <p>Page 11, Lines 36-41: Please revise this sentence grammatically to clarify your point.</p>
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	<p>Page 13, Line 50-Page 14, Line 6: These two new sentences were recently added and should be revised grammatically to clarify your point. Are you saying that dried blood is more likely found in a hospital setting? That the hot climates of West Africa will lead to suboptimal conditions for ex-vitro ebolavirus to persist?</p> <p>Page 14, Lines 13-16: Please clarify these sentences. They currently read "Viable Ebola virus can persist in semen for at least 82 days after symptom onset, and may persist for much longer. Follow-up testing on the same case was delayed until 700 days after symptom onset when the specimen was negative." I suggest re-wording to say "Viable Ebola virus can persist in semen for at least 82 days and may persist for much longer, as demonstrated in one case from the 2014-2015 EVD epidemic. Though viable ebolavirus was detectable at 82 days post symptom onset in this patient, follow-up testing of the semen was delayed until 700 days after symptom onset, at which time ebolavirus was not detected."</p> <p>References: The format is inconsistent and should be revised.</p> <p>Throughout the manuscript: There continue to be minor grammatical errors as well as typesetting errors. These are minor and will hopefully be addressed by the journal's editorial staff.</p>
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REVIEWER	Emanuele Sozzi University of Brighton (UK) and Gilling School of Global Public Health - University of North Carolina (US)
REVIEW RETURNED	22-Oct-2015

GENERAL COMMENTS	<p>Plenty of work has been done since the 1st submitted manuscript. The paper is much better now and closer to completion. The methodology is solid and an important gap of knowledge has been identified. Only little work needs to be done before publication. Almost there ;-)</p> <p>o Excellent the addition of a Prisma Flowchart and the fact that the Prisma checklist has been added</p> <p>o Figure 1: questions</p> <p>1) Why have the data points regarding "Negative PCR or VI test" been removed? They are important. Please explain rationale of this decision;</p> <p>2) Patient 'Liberia (India) 2014' (3rd from the bottom): it was 140 days, now it is 120. I tried to go through the comments and 'track-back' the rationale for this change, but couldn't find any. I'm sure there is a reason for it and everything is fine. But please double check and explain your rationale</p> <p>3) In my previous review I pointed out following references to be checked or re-formatted:</p> <p>a) Ref. 29: needs reformatting</p> <p>b) Ref. 35: needs reformatting</p> <p>c) Ref. 36: needs to be checked</p> <p>d) Ref. 43: needs to be checked</p> <p>The authors commented my review point as follows: "Thank you, these points have all been corrected and amended." Although the accuracy of the reference list is never the first priority and checking these things is boring, it is worth to point out that NONE of the four references have been changed. See manuscript in 'track change' mode. Is this an issue with the track change? If not, this is frankly a bit surprising. If a review point has been taken into consideration, then the author should either 1) do something about it; 2) explain why the review comment makes no sense and was therefore</p>
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	<p>ignored ;-)</p> <p>o At pag. 5 (Intro) it is also stated: "each and every chain of transmission can be tracked and terminated". As stated at pag. 6 (Methodology): "Viral persistence in faeces, saliva, sweat and urine were included as sexual acts can involve mucosal contact of one partner with another's body fluids. As such they are deemed of interest in discussions on possible routes for sexual transmission." Excellent point. This is exactly the reason why it is legitimate to ask following question: why are there no figures reg. the available data on 1) vaginal secretions 2) faeces, 3) saliva, 4) sweat and 5) urine? Why are data on seminal fluid summarized by a figure and all data reg. other fluids are only in the appendix? This good paper is not focusing on semen only, which is good. But the figures unfortunately are! And figures are important as many readers only look at abstract, figures and conclusions. Please add one more image or explain the rationale of your choice ;-)</p> <p>All the rest is fine. Almost there!</p>
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VERSION 2 – AUTHOR RESPONSE

Response to Reviewers' comments

We would like to thank the reviewers for all the constructive comments that have assisted us in improving our paper!

Our answers following directly after reviewers' comments:

- _Suggest replacing "of one man" with "from one man", ln8, pg9
- _Suggest "specimen by vaginal swabs" be replced with "vaginal swab specimens", ln38, pg9
- _Suggest replacing "in a total" with "from a total" ln17, pg10
- _Please specifiy "Ebola" further at ln24, pg10 (virus or virus disease)
- _Sentence starting ln40, pg11 - suggest deleting "has"
- _Sentence starting ln29, pg13 does not make sense. Is an "and" missing?

- Thank you so much for these suggested corrections, changes have been made accordingly.

Major Comments:

Table 1: This table represents a duplication of the data presented in Figure 1 and therefore Table 1 should be removed.

- Thank you, we agree and have removed table 1. (Should the Editor want to make the table available as an appendix please let us know)

Minor Comments:

Page 6, Lines 23-24: The search was performed December, 2014. However, an additional search was later performed to update the references, correct? Please include this information in this section of your manuscript to clarify what is written in lines 40-46 of this section.

- Thank you, added!

Page 11, Lines 36-41: Please revise this sentence grammatically to clarify your point.

- Corrected.

Page 13, Line 50-Page 14, Line 6: These two new sentences were recently added and should be revised grammatically to clarify your point. Are you saying that dried blood is more likely found in a hospital setting? That the hot climates of West Africa will lead to suboptimal conditions for ex-vitro ebolavirus to persist?

- Thank you, this addition was done upon request from one of the reviewers, We have aimed to clarify the above point further, which seems to be the hypothesis the authors of the paper want to put forward.

Page 14, Lines 13-16: Please clarify these sentences. They currently read “Viable Ebola virus can persist in semen for at least 82 days after symptom onset, and may persist for much longer. Follow-up testing on the same case was delayed until 700 days after symptom onset when the specimen was negative.” I suggest re-wording to say “Viable Ebola virus can persist in semen for at least 82 days and may persist for much longer, as demonstrated in one case from the 2014-2015 EVD epidemic. Though viable ebolavirus was detectable at 82 days post symptom onset in this patient, follow-up testing of the semen was delayed until 700 days after symptom onset, at which time ebolavirus was not detected.”

- Thank you, we have revised the sentence to clarify. We would however like to still separate the findings of EBOV by virus isolation on day 82, from the cases with detected viral RNA by RT-PCR, which is why we have separated the information into two sentences.

References: The format is inconsistent and should be revised.

- Thank you we have revised the references.

Throughout the manuscript: There continue to be minor grammatical errors as well as typesetting errors. These are minor and will hopefully be addressed by the journal's editorial staff.

Thank you, we have corrected the errors we have been able to find, and our native English speaking co-author has reviewed the language.

o Figure 1: questions

1) Why have the data points regarding “Negative PCR or VI test” been removed? They are important. Please explain rationale of this decision;

1. Thank you, we agree fully and have added the day of negative sampling to the current version of figure 1.

1) Patient ‘Liberia (India) 2014’ (3rd from the bottom): it was 140 days, now it is 120. I tried to go through the comments and ‘track-back’ the rationale for this change, but couldn’t find any. I’m sure there is a reason for it and everything is fine. But please double check and explain your rationale

2. Thank you for alerting us to this mistake! It should be 140 days and this has been corrected accordingly.

3) In my previous review I pointed out following references to be checked or re-formatted:

a) Ref. 29: needs reformatting

- b) Ref. 35: needs reformatting
- c) Ref. 36: needs to be checked
- d) Ref. 43: needs to be checked

The authors commented my review point as follows: "Thank you, these points have all been corrected and amended." Although the accuracy of the reference list is never the first priority and checking these things is boring, it is worth to point out that NONE of the four references have been changed. See manuscript in 'track change' mode. Is this an issue with the track change? If not, this is frankly a bit surprising. If a review point has been taken into consideration, then the author should either 1) do something about it; 2) explain why the review comment makes no sense and was therefore ignored ;-)

3, We are indeed very sorry for this mistake and are grateful of the reviewer who spotted this, which we missed ourselves. The explanation is the use of a reference manager where the reference texts in question were changed, to be followed by an update to the bibliography in the doc, the last step of running the update was however not done in the version submitted, our mistake!

o At pag. 5 (Intro) it is also stated: "each and every chain of transmission can be tracked and terminated". As stated at pag. 6 (Methodology): "Viral persistence in faeces, saliva, sweat and urine were included as sexual acts can involve mucosal contact of one partner with another's body fluids. As such they are deemed of interest in discussions on possible routes for sexual transmission." Excellent point. This is exactly the reason why it is legitimate to ask following question: why are there no figures reg. the available data on 1) vaginal secretions 2) faeces, 3) saliva, 4) sweat and 5) urine? Why are data on seminal fluid summarized by a figure and all data reg. other fluids are only in the appendix? This good paper is not focusing on semen only, which is good. But the figures unfortunately are! And figures are important as many readers only look at abstract, figures and conclusions. Please add one more image or explain the rationale of your choice ;-)

Thank you, we understand your concern and we see the point! However, the big difference between the other body fluids as compared to semen (and vaginal fluids, fig 2) is that evidence is so far (even more) scanty, with very few cases analysed, and also very few of these being positive. While we agree visualisation is an important tool, we think it would be difficult to accurately reflect this in a figure and hence we opted for a table instead.

Correction

Thorson A, Formenty P, Lofthouse C, *et al*. Systematic review of the literature on viral persistence and sexual transmission from recovered Ebola survivors: evidence and recommendations. *BMJ Open* 2016;6:e008859. A formatting error occurred in figure 1 of this paper. The information for patient F, which should be the green circle with 62 (referring to a negative test at 62 days) is superimposed on patient E, ie one line higher than where it should be placed, on the line below for patient F. The corrected figure is below.

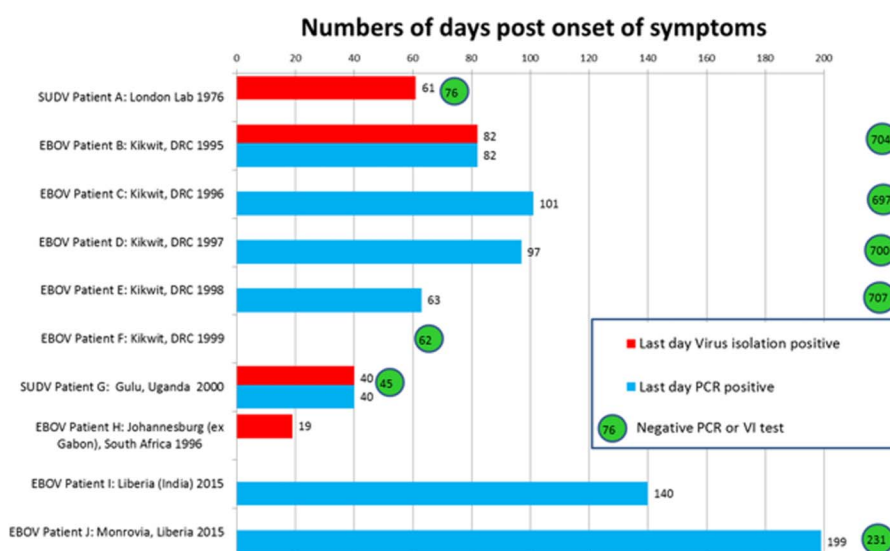


Figure 1

BMJ Open 2016;6:e008859corr1. doi:10.1136/bmjopen-2015-008859corr1



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