**Ebola virus disease**

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**[Au: I know I’ve asked before, but please make sure names (including initials) and affiliations are listed as you want them indexed (for example PubMed). We cannot accept corrections after publication, so please double check. All Affiliations should include Laboratory, Department, Institute, City, (State) and Country.]**

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**Author contributions**

Introduction (J.H.K.); Epidemiology (S.T.J., A.L.V. and J.H.K.); Mechanisms/pathophysiology (A.L.V., V.W., A.G. and J.H.K.); Diagnosis, screening and prevention (S.T.J., I.C., A.G. and J.H.K.); Management (S.T.J., I.C., W.A.F.II, A.H., C.K. and M.J.S., J.H.K.); Quality of life (S.T.J., I.C., W.A.F.II, A.H., C.K. and M.J.S., J.H.K.); Outlook (all authors); Overview of Primer (all authors).

**Competing interests**

All authors declare no competing interests.

**[Au: In general, the article reads very well. However, all our articles are thoroughly and heavily edited, taking into account clarity, language, scientific correctness, consistency and house style, to ensure they meet our high publication standards. The majority of changes have been made to bring the article in line with house style and to tighten up the narrative flow. These changes are just suggestions and you should feel free to discuss them with me. At places marked [Au:OK?], I’d be grateful if you could check especially carefully that the meaning of what you wrote has not been altered by the editing. I have also asked a few questions where I felt a bit more information or clarity was needed. Some general comments and instructions:**

* **Queries are meant to draw your attention to edits, inconsistencies or issues that are unclear. If we just ask you to confirm edits are correct, a simple yes/ok between the brackets will do [Au:OK? Is this what you meant? Edits OK? yes]. If questions are asked, please rephrase/update the manuscript text when addressing queries, so that the message is conveyed to the reader (do NOT just type your answer to our query). In most cases, one or two sentences would be sufficient. Sentences in green have been moved from elsewhere.**
* **The referees comments that you should address have been inserted in the text in purple: although it might seem that there are many or that they are extensive, in most cases I think that adding one-two sentences (with references as appropriate) or changing the text would be sufficient**
* **Whenever additional references are suggested by the referees, they are for your consideration only, as you are of course free to decide the literature that you wish to cite**
* **As we are already at ~250 references, if I (or the referees) have asked for an additional reference, please try to use one already included in the list, or consider if a recent review or just the most recent paper could be cited (under the assumption the other papers will be cited therein).**
* **Please don’t forget to refresh the references using your reference manager after the revisions**
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* **We only use significant in the context of statistics; hence changed or flagged throughout.**
* **Please note that our style is official nomenclature for protein (Uniprot) and gene (Entrez and italics) names and that they are styled according to species (capitals for humans, only first letter capital for mice).]**

**ABSTRACT [Au: edited to touch on the main points of the article, please feel free to edit further]**

Ebola virus disease (EVD) is a severe and frequently lethal disease caused by the filovirus Ebola virus. EVD outbreaks typically start from a single case of probable zoonotic transmission, followed by human-to-human transmission via direct contact or contact with contaminated bodily fluids or fomites. EVD has a high case-fatality rate; it is characterized by fever, gastrointestinal symptoms, and multi-organ dysfunction syndrome. Diagnosis requires a combination of case definition and laboratory tests, such as real-time PCR to detect viral RNA or rapid diagnostic tests based on immunoassays to detect anti-EBOV antibodies **[Au: added text on RDTs for completeness, ok?]** . No vaccines **[Au: I think this needs updating in light of the recent EMA approval, correct?]** or direct medical countermeasures are yet formally approved for the prevention and treatment of EVD, although preliminary results of clinical trials show survival benefits of cocktails of monoclonal antibodies against EBOV membrane glycoprotein. **[Au: added text on trials ok?]** The observations from the unprecedented international containment responses deployed for the 2013–2016 West Africa EVD outbreak (the largest in history, with almost 29,000 cases) substantially **[Au: in our style, we only use “significant” in statistical context, so I’ve changed it here to “substantially”, ok?]** improved the understanding of EVD including, importantly, clinical sequelae and virus persistence in survivors, and may lead to improved clinical outcomes in the near future.

 **[Au: some general referee comments:]**

 **[Referee comment: The article is a little short on some facets of the human factors involved in the spread and perpetuation of the recent outbreaks, and in turn how they might be approached in the control of the epidemic.]**

 **[Referee comment: On the human factors side of epidemiology, the risk of traditional funerals is mentioned, but the difficulties in controlling the disease in traditional rural areas and in less well educated groups is not covered, perhaps because it is rarely noted in the literature. There are however publications on the need for good anthropology in outbreaks, on how traditional healers may be useful or merely a focus for spreading disease and how scientific messages can be translated for a rural society. Some of the problems were discussed by politicians in private, but not published, such as the hiding of cases and families who would dig up safely buried corpses to perform cultural last rites. Nonetheless these are reflected in a few publications on the need for community engagement, not only through the local chiefs but also through traditional healers and the old women of the community who control social acceptance in a highly traditional society. MSF, WHO and some other groups now deploy anthropologists with outbreak response teams. Some suitable references include [Au: see suggested examples below] . An undocumented aspect of epidemiology is the changes in mobility and the widespread use of mobile phones. Better transport and wider vehicle ownership, combined with the means to notify relatives rapidly of a death allow more people to flock to funerals, as well as to facilitate spread across a country. Trade routes, and unofficial cross-border movements facilitate spread.**

**I do not think that more than a few sentences are needed here, but the story is not complete without them. ]**

 **[Au: I agree this is a very important topic, and I recommend to discuss it in a new text box (~300 words max), in a sort of mini-essay, which can be mentioned in the main text wherever appropriate. Perhaps in this context you could also mention this issue: https://www.nature.com/articles/d41586-019-01432-y?utm\_source=Nature+Briefing&utm\_campaign=d2ad678e85-briefing-dy-20190503&utm\_medium=email&utm\_term=0\_c9dfd39373-d2ad678e85-43380673 References suggested by the reviewers:]**

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**[H1] INTRODUCTION**

 **[Referee comment: Sections prior to the case management portion of the manuscript are not referenced as completely as would be expected and at times refer to books or clinical texts for important information. Since these textbooks are not readily available, referencing to these sources of information should be minimized to cases where no other option is available.]** **[Au: The referee provided some examples, which I have flagged with a query “Book reference”, please consider changing/adding references]**

To date, twelve distinct filoviruses have been described 1. The seven filoviruses that have been found in humans belong either to the genus *Ebolavirus* (Bundibugyo virus (BDBV), Ebola virus (EBOV), Reston virus (RESTV), Sudan virus (SUDV), and Taï Forest virus (TAFV); Figure 1) or the genus *Marburgvirus* (Marburg virus (MARV) and Ravn virus (RAVV)) 2. **[Referee comment: It is worth to mention the Bombali virus.]** **[Au: please comment on this virus for completeness]** The WHO **[Au: one of the few acronyms we don’t spell out in our style]** International Classification of Diseases Revision 11 (ICD-11) of 2018 recognizes two major subcategories of filovirus disease (FVD): **[Au: I have removed the ICD codes for brevity, as suggested by this referee comment: ICD codes are used by clinicians/governments for reimbursement and resource allocation but are not meaningful for the broad audience. Recommend this information be removed. I have also removed abbreviations that are used only once or twice, as this is our style]** Ebola disease ) caused by BDBV, EBOV, SUDV, or TAFV, and Marburg disease caused by MARV or RAVV. Ebola virus disease (EVD) is defined as a disease only caused by EBOV. This subcategorization is largely based on the increasing evidence of molecular differences between ebolaviruses and marburgviruses, as it may be hypothesized that such differences **[Au: edits ok?]** could influence virus host reservoir tropism, pathogenesis, and disease phenotype in accidental primate hosts 2.

 Since the discovery of filoviruses in 1967 (Ref3) **[Au: this is our style for references that follow a number]** , 43 FVD outbreaks (excluding at least 5 laboratory-acquired infections) have been recorded, either in or exported from Africa (updated from 4). **[Au: could you please provide the additional references for the outbreaks not included in reference 4, for completeness?]** The epidemiological definition of outbreak is ≥1 case above the known endemic prevalence. For example, the single case of TAFV infection recorded in a setting in which FVD had never been reported before (Côte d’Ivoire) 5 is still considered an outbreak . All FVD outbreaks, with exception of that caused by TAFV, were characterized by extremely high case-fatality ratios (CFRs). Until 2013, the most extensive outbreak, caused by SUDV, involved 425 cases and 224 deaths (CFR: 52.7%) 6. The overall limited numbers of FVD cases (1967–2013: 2,886 cases including 1,982 deaths; updated from 4), **[Au: similarly to my previous query, could you please provide the additional references?]** the typical remote and rural locations of outbreaks, and the often delayed announcement of new outbreaks to the international community 7 have not enabled the systematic study of clinical FVD in humans. Thus, the commonly used **[Au: instead of “boilerplate”, ok? or, please feel free to edit]** description of FVD was derived either from observation of small groups of patients in care settings that were not well-equipped for diagnosis, treatment, and certainly not for disease characterization, or from observations of even smaller samples, such as **[Au: edits ok?]** affected **[Au: added “affected”, ok? as these individuals were already sick when they were transferred, correct?]** individuals who were transferred from Equatorial Africa to developed countries or individuals who fell sick in developed countries after contracting the virus elsewhere **[Au: added “after…” for clarity, ok?]** . Pathological characterization of FVD via autopsies have been rare historical exceptions 4,7. **[Au: book reference]** In the absence of high-resolution **[Au: please explain what you mean by high-res in this context: reliable? Consistent?]** human clinical data, FVD could only be defined further via the use of experimental animal infections 8,9.

Until 2013, most EVD outbreaks originated from Central **[Au: instead of “Middle”, ok?]** Africa: Democratic Republic of the Congo (abbreviated to COD by the International Organization for Standardization (ISO)), Gabon, and Republic of the Congo (abbreviated to COG by the ISO). **[Au: I have added the explanation for the abbreviations used. However, I still have to consult with my copyeditor and proofreader colleagues to check whether such use is permitted by our house style, so we might have to change to the common “DRC” and “RC” abbreviations later. For now, please do not worry about it]** From late 2013 to early 2016, EBOV caused an outbreak that spread from Guinea to other countries in Western Africa, leading to 28,652 human infections and 11,325 deaths (Table 1, Figure 2) 10. The location and scale of the outbreak was entirely unexpected 11. Consequently, local, national, and international organizations were caught unprepared for an outbreak caused by what, until then, was considered an exotic pathogen of largely negligible consequence for global public health 12-14. After the WHO declared the outbreak a Public Health Emergency of International Concern, the global and local responses to the outbreak intensified and ultimately the outbreak was contained, leaving, however, great devastation to individuals, families, communities, health care systems, and economies in its wake. 15. In most affected countries, the response included the establishment of Ebola (EVD) Treatment Units (ETUs) 16-18, which enabled medical professionals and biomedical scientists to manage large cohorts of suspected and confirmed patients **[Au: edits ok?]** in controlled settings and to better understand a virus previously best known as a potential bioweapons agent 19,20.

Owing to observations from the Western Africa outbreak and, to a limited degree, from subsequent EVD outbreaks in COD (Table 1) 21-24, clinicians can now better describe predictable phases in the progression of EVD in humans. Typically, EVD begins with a non-specific febrile illness followed by severe gastrointestinal signs and, in highly viremic patients who often also have **[Au: edits ok?]** dysregulated immune responses, progresses to complex multi-system organ dysfunction that can be fatal. A subset of patients, usually with lower viremia, have less severe disease progression and organ dysfunction **[Referee comment: The relation between viral load and diseases severity needs to have a reference.]** and ultimately develop appropriate immune responses leading to clearance of viremia and a resolution phase; however, recovery can be complicated by long-lasting clinical sequelae and/or virus persistence in immune-privileged sites that can lead to disease flare-ups and even sexual transmission 4. In this Primer, we outline the current improved understanding of EVD based on the most recent published human clinical data.

**[H1] EPIDEMIOLOGY**

 **[Au: I have slightly reorganized the epidemiology section for flow, following this outline:**

* **Initial general data (classic epi)**
* **Description of outbreaks**
* **Risk factors**
* **CFR**
* **Case definition**
* **Molecular epidemiology**

**I have not tracked these changes in the interest of readability, but I have added subsection headings]**

**[H2] Classic epidemiology**

Since the discovery of EBOV in 1976 in COD (then Zaire) 25-27, at least 17 EVD outbreaks have originated in Gabon, Guinea, COG, and COD. At the time of writing, ~33,259 human EBOV infections, including 14,571 deaths (average CFR: 43.8%) are on record (updated from 4, although case numbers differ slightly from source to source). Of these cases, 28,652 infections and 11,325 deaths occurred during the single Western Africa EVD outbreak 10 (Table 1 and Figure 2). **[Referee comment: Epidemiology: a part regarding the current epidemic in DRC should be added. The DRC epidemic in fact is mentioned later (regarding the vaccine and the treatment) but considering the size of it, it should be mentioned already in this section]** **[Au: I agree that it’s an important addition for completeness]**

 **[H3] Outbreaks and transmission** **[Au: new subheading for flow, ok?]**

 **[Referee comment: I would recommend the epidemiology section mentions the geographic distribution of risk of spill-over events, based on previous outbreaks and assumptions about the reservoir species, and the risk of geographic extension by subsequent human-to-human transmission. This might also include a consideration of the role of changes in population density and movement in Africa on the fact that the two largest outbreaks in history have occurred in the last five years. Should also mention that the risk of widespread outbreaks in middle and high income settings is very low since effective R can readily be bought below 1 with fairly simple infection control and contact tracing measures.]**

 **[Au: another referee also commented along these lines:]**

 **[Referee comment: I recommend better presentation of information on transmission routes and risks. Paragraph starting on line 170 [Au: the next paragraph] should perhaps place greater emphasise on the role of healthcare settings and burials in amplification, and the small but important risk of sexual transmission (which is only mentioned later). See: Dean NE, Halloran ME, Yang Y, Longini IM. 2016. Transmissibility and pathogenicity of Ebola virus: a systematic review and meta-analysis of household secondary attack rate and asymptomatic infection. Clin. Infect. Dis. 62: 1277–86. Are vomitus and stool infectious?]**

Most outbreaks can be traced back to a single introduction of EBOV into the human population by unknown means. Subsequently, the virus is transmitted by direct, non-aerosol, human-to-human contact or contact with infected tissues or body fluids, or contaminated fomites (Figure 1) 7. **[Au: book reference]** Based on historical records, EBOV may have been transmitted from its natural reservoir host(s) to humans to cause disease only about 20–30 times (Table 1), although it is quite probable that limited EVD outbreaks may have been overlooked or not reported. Tracking EBOV within the human population after a zoonotic transmission event can be challenging. A strong risk factor linked to human-to-human EBOV propagation is contact with contaminated bodily fluids 43-45. Indeed, infectious EBOV was recovered from breast milk, saliva, urine, semen, and aqueous humor, in addition to blood or blood derivatives 39,46-49. **[Referee comment: Virus has been found in diarrhea samples in the referenced study (JID. 2016. 214.S177-S184) and likely vomitus? They should probably be included in the list of fluids that contain infectious virus.]** Taking care of an individual with EVD at home or in a healthcare facility or following traditional funeral practices, **[Au: here we could add a callout to the new box I (and the referees) have suggested]** which involve contact with the deceased’s body, substantially **[Au: in our style, we only use “significant” in statistical context, so I’ve changed it here to “substantially”, ok?]** increases the risk of acquiring infection — this is one of the reasons why women, who traditionally care for the sick in certain African regions, are at higher risk of acquiring EBOV than men.

 **[H3] Risk factors** **[Au: new subheading for flow, ok?]**

Demographic risk factors for contracting EVD, such as age, sex, or ethnicity, are not well-defined. By current understanding, men and women are equally susceptible, but women are at higher risk of being exposed to EBOV and the incidence increases almost linearly with age to a peak at 35–44 years **[Au: does this incidence trend apply to women only or both sexes?]** . **[Referee comment: Sex differences are known to associate with variance in susceptibility to infection and responses to infection/vaccination (Trends in Immunology. 2014. 35(3):97-104). It is possible that women have both a higher risk of exposure and are more/less susceptible to infection. References to support the statement that women are equally susceptible should be supported by Ebola-specific data or statement rephrased to fit what the referenced data supports.]** Although children typically constitute a disproportionately small number of EVD cases (even in countries with disproportionally **[Au: I’m not sure I understand this second “disproportionally”: the first one means “there are fewer cases of EVD in children than it would be expected in a paediatric population of that specific size” (correct?) but in this case what does it mean?]** high youth populations), they have shorter incubation periods, more rapid disease course, and higher risk of death than older populations, with children of <5 years of age at the highest risk 28-33. Possible explanations of the low incidence of EVD in children include behavioral factors that result in children deliberately being prevented from being exposed to infected individuals 30 and differences in susceptibility across age groups 34. **[Referee comment: Low incidence in children: This is in part contradicted by the current DRC epidemic where children are at risk probably due to nosocomial transmission.]** **[Referee comment: There are data in some studies that advanced age is linked to a higher risk of death. Should this be added as a special population at higher risk of death?]** Infected pregnant women are at high risk for miscarriage or stillbirths, and newborn babies **[Au: we cannot use “newborn” as a noun in our style]** of infected mothers rarely survive 35. Indeed, EBOV can be transmitted transplacentally 36or lead to fetal death related to placental insufficiency. Transmission of EBOV from infected pregnant women to their embryos or **[Au: we cannot have slashes in our style, so replaced with “or”, ok?]** fetuses or from infected mothers to their children occurs frequently and is associated with elevated *in utero* and neonatal lethality 35. **[Referee comment: In pregnancies that occur in women following recovery from infection, is there a higher risk of miscarriage?]** EBOV RNA has been detected at high concentrations in amniotic fluid, placenta, fetus **[Au: in fetal tissue?]** and breast milk 37-41. Molecular studies of specific host factors influencing the outcome of EBOV infection in particular human populations are absent, with the exception of one study that associated expression of killer cell immunoglobulin-like receptor (KIR) 2DS1 (KIR2DS1) **[Au: official uniprot name]** and KIR2DS3 with fatal outcome 42. **[Au: in what population?]**

 **[H3] Case fatality rate** **[Au: new subheading, ok?]**

 **[Referee comment: I recommend better presentation of CFR data. CFR is mentioned throughout (e.g. Epi section, special populations) but there is no single paragraph that gives a clear overall picture of the range of CFR by virus, outbreak, age and gender. Parts of the text on CFR are difficult to interpret without actual numbers (line 145-147). ]** **[Au: I agree this is an important point, and it would be good to elaborate on it a little (CFR by outbreak is already included in table 1, the referee might have missed it)]**

Although unpublished observations described varying disease clinical signs or levels of severity depending on the specific outbreak, these findings are not necessarily reflected in the published literature. Based on comparative statistics on CFRs, a fundamental difference of virulence between ebolaviruses that cause lethal human disease **[Au: so “ebolaviruses that cause Ebola disease” (BDBV, EBOV, SUDV and TAFV, as defined in the introduction)? Or not all of these cause lethal disease? We could clarify this in the introduction]** is not observed. **[Au: Please reference this statement.]** The oft-repeated notion that EBOV is the most virulent ebolavirus (let alone filovirus) is not supported by available data 4. **[Referee comment: Data not supporting EBOV not being as virulent amongst ebolavirus or filovirus in human is reference to a medical textbook chapter]** **[Au: another referee commented: Even with a reference, the statement should be followed by a brief description of the data to correct readers incorrect notion.]** The reasons for fluctuating CFR data **[Referee comment:** **Among the reasons for fluctuating CFR  I would add the different reporting system/capacity.]** are not truly understood but possibly include differences in health status (nutrition, immunity, co-infection status), genetics (ethnicity-dependent haplotypes or random polymorphisms), health seeking behavior, and the development and accessibility of health-care facilities providing supportive care in the affected African countries. **[Au: Please reference this statement.]**

**[H3] Case definitions.**

**[Au: I’ve edited this section for brevity and to reduce repetition, as the referees suggested, and to give you some room to expand the discussion as suggested by the refs. Thus, I have removed the description of the Sierra Leone example of modified case definitions (but kept the reference, so the interested reader can follow up) and created a new text box with the example of the retrospective cohort study]**

 **[Referee comment: Case definitions for public health surveillance purposes to account for things like community deaths and epidemiology is not discussed but should at least be acknowledged as a use. Case definitions used in public health surveillance are not meant to be used by clinicians for diagnosis. Public health surveillance case definitions are not discussed but the manuscript should make clear for readers that the type case definitions being discussed are not this type.]**

During an EVD outbreak, a robust case definition and accurate confirmatory testing are key to ensuring that individuals with suspected EBOV infection are efficiently identified and, upon admission to an ETU, isolated for confirmation of diagnosis and treatment **[Au: sentence edited based on the following referee comment, ok? “The identification of the suspects doesn’t happen at the ETC but in the community.”]** (Box 1). However, patient screening time should be minimized to limit exposure of uninfected individuals, including ETU staff, to potentially infected individuals **[Au: edits ok?]** .

 **[Au: new paragraph; sentence in green moved up from below for flow, as it’s a nice introduction]** Case definitions are rarely 100% sensitive and specific, and attempts to optimize either come at the expense of the other. A case definition with a low sensitivity will mislabel true EBOV-positive individuals as EBOV-negative, leading to an increased risk for discharge of EBOV-infected individuals back to the community, where EBOV transmission can be reinitiated. Particularly in a setting with a low community incidence of new EVD cases, the sensitivity of the case definition should be maximized. By contrast, a case definition with a low specificity might result in misclassification of true EBOV-negative individuals as EBOV-positive. Such individuals might be admitted to an ETU as suspected EVD cases, placing them at increased risk of EBOV exposure and nosocomial infection, especially when the probability that other suspect patients might be EBOV-positive is high. Thus, during a high incidence of EVD cases in the community, increased specificity in EVD case definition may be crucial.

Given these considerations, currently no EVD case definition is globally applied. Indeed, the EVD case definition can be revised during the course of an outbreak, and variations in case definition were used during the Western Africa outbreak (for example, in Sierra Leone50) as the outbreak evolved from high incidence to low incidence. **[Referee comment: Relative risk stratification into possible, probable and confirmed is not discussed except in passing on line 209. This impacts cohorting patients while awaiting diagnostic test results.]** In addition, some efforts have been made to evaluate the performance characteristics of the case definition (Box 2). **[Referee comment: Performance of case definitions is dependent on disease prevalence, which is not mentioned.]**

**[H2] Molecular epidemiology**

 **[Referee comment: the molecular epi section might better focus on virus evolution and clades, and the implications for disease behaviour and MCMs.]**

The 2013‒2016 Western Africa EVD outbreak was the first to be largely characterized by molecular-epidemiological evidence. Deep-sequencing efforts, often performed on site and in parallel by several groups, resulted in the determination of >1,600 near-complete or coding-complete EBOV genome sequences **[Au: just to be clear, you mean “near-complete genomes [coding+non-coding sequences] or complete exome sequences [all coding sequences only]”?]** directly from human patient samples 53-56. **[Au: does this mean 1 genome from each patient, or can multiple different genomes be isolated from the same patient (or the same genome be isolated from different patients)?]** Subsequent phylogenetic analyses traced EBOV movement through the human populations of all affected countries and even pinpointed multiple back-and-forth border crossings 54 (Figure 3). The genomic data confirmed the classical epidemiological model of filovirus infections: all 28,652 human infections of this outbreak occurred via direct human-to-human contact tracing back to a single human index case (probably due to zoonotic transmission) in Méliandou, Nzérékoré Region, Guinea. Such molecular-epidemiological investigations are now slowly becoming routine. During the most recent two EVD outbreaks in 2018 and 2018–present in COD, deep sequencing again revealed single spillover **[Au: added “spillover” for clarity, ok?]** EBOV transmission events into the human populations with subsequent person-to-person transmission 23,24. Molecular approaches further revealed that rarely sexual transmission of EBOV may occur from apparently healthy survivors of EVD and that EBOV may persist in survivors in various immune-privileged sites for extended periods of time **[Au: could you please provide a range, for context?]**, 57-59 and enabled progress in understanding of within-outbreak and within-host viral evolution 60.

**[H1] MECHANISMS/PATHOPHYSIOLOGY**

 **[Au: text in green moved up from the Host-pathogen determinants subsection as it’s a nice introduction to the Mechanisms section]**

Many outstanding questions still surround the pathophysiology of EVD. Findings from animal studies, *in vitro* work, and human clinical data are beginning to decipher the normal course of EVD in humans and to link disease progression to the molecular basis of EBOV pathogenesis. With these data, researchers may be able to identify the crucial pathways involved in effective immune responses to EBOV infection and the various candidate medical countermeasures (MCMs) that may be developed to augment any host response shortcomings.

 **[H2] Animal models**

 **[Au: one general introduction sentence deleted for brevity]** Exposure of immunocompetent laboratory mice, Syrian hamsters (*Mesocricetus auratus*), and domesticated guinea pigs (*Cavia porcellus*) to EBOV does not yield severe (or any) disease, and EBOV must be adapted via serial passages in rodents before lethal infection is achieved 61,62. Even when adapted viruses are used, these rodent models do not fully mimic human disease. Because nonhuman primates are evolutionarily much more closely related to humans than rodents, nonhuman primate models of EVD are often considered to be more useful for the study of human EBOV infection and disease. Indeed, much of the information on viral pathogenesis has been derived from studies with wild-type EBOV predominantly in crab-eating macaques (*Macaca fascicularis*) and rhesus macaques **[Au: I understand that “rhesus monkey” is also used, but macaque is more-accurate and therefore we should use it]** (*Macaca mulatta*) 8,9. Based on experimental animal data, two factors that may influence development and severity of human EVD may be EBOV exposure route and dose. Direct contact with infected biological materials or contaminated non-biological materials via cuts or scratches or via contact with mucosal membranes (oral or, theoretically, nasopharyngeal or conjunctival mucosa) is considered the most frequent mode of human-to-human EBOV transmission 63. However, these transmission pathways are difficult to simulate in experimental settings. Thus, animal models of EVD have been established using injection and aerosol methods of EBOV exposure to model accidental needlestick injury and respiratory routes of exposure, respectively, despite the lack of evidence that these exposure routes have any relevant **[Au: instead of “significant”, for style]** roles during natural EVD outbreaks 63.

 Most nonhuman primate studies rely on either intramuscular injection or small-particle aerosol exposure of 1,000 plaque forming units (pfu) of EBOV, a dose that ensures that all infected animals will develop a disease that is almost always lethal 64 and can be included in the analyses; thus, significant results can be achieved with overall low animal numbers. Interestingly, intramuscular infection of nonhuman primates with some EBOV variants results in a 100% lethal disease following injection of doses as low as a calculated dose of 0.01 pfu (corresponding to only 89 **[Au: can we say “approximately” 89?]** virus particles) 65. The lethality associated with this low virus dose suggests that very few virions may be required to initiate a lethal human disease course and that differences between viral variants may influence incubation period, disease course, and disease severity in subtle ways not necessarily captured in nonhuman primate models. **[Au: one sentence deleted as it was a bit repetitive]** **[Referee comment: A detailed logic of why the 0.01pfu causing lethal infection suggests that differences in viral variants influence incubation period, disease course or severity in ways not captured by NHP studies should be provided and include other relevant data that supports the claim.]**

 During the 2013–2016 Western Africa EVD outbreak, molecular-genomic analyses were used to observe EBOV evolution during human-to-human transmission. The comparison of the ≈1,600 near-complete EBOV genome sequences obtained during the 2013-2016 Western Africa EVD outbreak revealed several positively selected genomic mutations. A mutation leading to an amino acid residue change, A82V, in EBOV glycoprotein **[Au: do you mean glycoprotein GP1,2?]** occurred in viral genomes isolated from samples collected early in the Western African outbreak and remained present in genomes from all later samples 66-68. *In vitro*, this mutation enhances glycoprotein-mediated virus entry in human cells. **[Au: Please reference this statement.]** However, *in vivo* experiments have yet to unambiguously ascribe a phenotype to A82V and similar mutations. For instance, initial studies with IFNAR-/- **[Au: is this the murine gene? If so, please change to Ifnar as it is our style]** laboratory mice **[Au: are these mice immunodeficient as a result?]** and rhesus macaques did not demonstrate an effect of A82V on disease severity or virus shedding 69. Consequently, an explanation for positive selection of certain mutations in EBOV genomes over the course of the outbreak is still lacking. **[Referee comment: Possible explanations should be provided for why adaptations required for humans are not important to mice or NHP (e.g., mouse/NHP and human infection cofactors, immune responses, might be different)] [Au: another referee also commented “the authors mention the glycoprotein A82V mutation that emerged during this outbreak, but really has no bearing on the pathogenesis of the virus. These data are rather weak and could be omitted from the review” so I recommend to elaborate a little on this] [Au: another referee commented (on the mechanisms section in general, but perhaps this would be a good location to address this comment): Mechanisms/Pathophysiology: Publications that employ systems biology approaches to examine cellular responses to Ebola infection and resulting viral pathogenicity using samples obtained from the 2013–2016 should be added to this section [e.g., Kyle JE, et al. PNAS USA 116:3919–3928 (2019) and Eisfeld AJ, et al. Cell Host and Microbe 22:817–829 (2017)].]**

**[H2] Host-pathogen determinants of outcome [Au: had to remove “clinical” from heading for style (<39 characters)]**

 **[Referee comment: The following citation showing overexpression of chemokines (chemokine storm) in fatalities of the West Africa Ebola outbreak should be included: Reynard S et al. JCI Insight 2019. ]**

 **[Referee comment: I was looking for more information on mechanisms of acute disease, especially gastrointestinal symptoms and vascular leakage.]** **[Au: I agree, it’s important to provide mechanistic explanations for the clinical manifestations as much as possible]**

 EBOV tissue and cell tropism are determined by the EBOV glycoprotein GP1,2, host cell-surface GP1,2 attachment factors, and the intracellular GP1,2-binding NPC intracellular cholesterol transporter 1 (NPC1, also known as Niemann-Pick C1 protein) receptor 70,71. **[Referee comment: The portion of NPC1 that binds filoviruses is topologically exterior to the cell. NPC1 is located intracellular organelles (i.e., endosomes and lysosomes). This should be corrected.]** **[Au: we have added NPC1 in the figure, please check it’s correct]** **[Referee comment: Tissue and cell tropism are determined not only by the entry step but also by other steps of the EBOV life cycle such as transcription/replication and budding, which also involve interactions with many host proteins. This point should be considered.]** (Figure 4). Most human cells can become infected (exceptions are lymphocytes, myocytes, and neurons, although the reasons for this resistance to infection are unclear), **[Referee comment: Myocytes and neurons have been reported to be susceptible to Ebola virus (MacKenzie TC et al. Mol Ther 2002, McWilliams IL et al. Cell Rep 2019). The text should be modified to reflect this point.]** but mononuclear phagocytes (for example, Kupffer cells in the liver, macrophages and microglia) and dendritic cells are primary EBOV targets 64,72-78.

 **[Au: new paragraph]** As the primary target cells become infected, they facilitate further virus dissemination 73 and migrate to the regional lymph nodes and to the liver and spleen 75. Infected macrophages are activated by binding to EBOV glycoproteins **[Au: the secreted GPs? If so, please add “secreted” for clarity]** 79 to secrete pro-inflammatory cytokines, in particular interleukins IL-1B **[Au: do you mean IL-1β?]** , IL-6, CXCL8 **[Au: do you mean IL-8?]** , and tumor necrosis factor (TNF), resulting in the recruitment of additional EBOV-susceptible macrophages to the site of infection and, ultimately, breakdown of endothelial barriers. **[Referee comment: The wording of the sentence implies the referenced paper showed that the cytokines recruited macrophages to the site of infection and caused endothelial barrier breakdown in vivo. But the paper was in vitro only. The sentence should be modified to clarify this conclusion is presumed based on the in vitro assay and known literature (including their refs).]** In nonhuman primate models, this breakdown frequently causes third spacing (that is, excess movement of intravascular fluid into interstitial spaces), leading to edema and hypovolemic shock. This manifestation occurs less often in human patients 78,80-82. **[Referee comment: The sentence states that third spacing, edema and hypovolemic shock is less often seen in human patients. However, other parts of the manuscript mention third spacing (e.g., Line 390). Please clarify and unify text.]** **[Au: I’ve added a query in the diagnosis section where vascular leakage is mentioned]** Dendritic cells react to EBOV infection with partial suppression of MHC class II responses and expression of tissue factor and TNF ligand superfamily member 10 (TNFSF10) 83,84 . **[Referee comment: References #83 and #84 are not appropriate; these references do not describe MHC II and TNFSF10. In addition, infected DCs do not secrete pro-inflammatory cytokines, but they do produce high levels of chemokines such as MCP-1 (Mahanty S et al. J Immunol 2003, and Ref #83). This observation regarding infected DCs should be included.]** **[Referee comment: Please make sure it is clear when switching from in vitro to in vivo experiments and animal vs human data. The reference for TNFSF10 appears to be missing. It would be helpful to the reader to understand how the aberrant cytokines and TNFSF10 expression might be leading to cytokine death.]** The aberrant cytokine responses and TNFSF10 expression probably are key players in the extensive lymphocyte death (probably contributing to the susceptibility of patients with EVD to acquire secondary infections) 75,85, **[Referee comment: A recent study reported that EBOV GP directly induces T lymphocyte death (Lampietro M et al. PLoS Pathog 2017). This study should be included.]** hypotension, disseminated intravascular coagulation (DIC), and ultimately multiple organ dysfunction syndrome that is typical of EVD (Figure 5) 85-88.

**[H3] Immune responses [Au: new subheading to break up this long section, ok?]**

 **[Au: I have switched the order of the two paragraphs of this subsection so innate immunity is discussed before adaptive immunity]**

Although considerable progress has been made toward understanding the immune response to EBOV infection on a cellular level using *in vitro* testing, data are limited regarding the systemic immune response in humans following infection. Prior to the Western Africa EVD outbreak, there had been no opportunities to conduct thorough and contemporary **[Au: as in “modern”? or as in “simultaneous”? ]** immunological analyses of the human host response to EBOV infection. **[Au: there had been outbreaks before, though, do you mean that the scale and duration of the Western Africa outbreak enabled to conduct such studies?]**

 **[Referee comment on the next paragraph: Innate responses that are modified during EBOV infection is poorly developed relative to the other sections and should be expanded upon because innate immunosuppression impacts the development of adaptive immunity.]**

EBOV inhibits induction of intrinsic and innate host immune responses 97,98. **[Au: please briefly define intrinsic immunity]** This inhibition permits efficient replication in host cells, thereby accelerating viral spread. To this end, the virus invests a substantial amount of its genome coding capacity. Perhaps the best studied inhibitor is the EBOV polymerase cofactor VP35, which is also a type-I interferon antagonist. EBOV VP35 acts through multiple mechanisms; it directly binds double-stranded viral RNA during replication **[Au:OK?]** , thereby avoiding triggering the host’s innate antiviral response mediated by interferon-induced helicase C domain-containing protein 1 (IFIH1, also known as melanoma differentiation-associated protein 5 (MDA-5)) and probable ATP-dependent RNA helicase DDX58 (also known as retinoic acid-inducible gene 1 protein (RIG-I)) signalling. VP35 also binds and inhibits interferon induced protein kinase EIF2AK2 (PACT) **[Au: EIF2AK2 (**<https://www.uniprot.org/uniprot/P19525> **) and PACT (**<https://www.uniprot.org/uniprot/O75569>) **are two different proteins, as per the Uniprot links I’ve added for your convenience, which one do you mean?]** , thereby inhibiting the DDX58 pathway, and inhibitskinases that activate interferon regulatory factors 3 and 7 (Ref99-105) **[Au: this is our style for references that follow numbers]** . **[Referee comment: The paper “Mutual antagonism between the Ebola virus VP35 protein and the RIG-I activator PACT determines infection outcome by Luthra P et al.” should be included in the references. In addition, the recently published manuscript on VP35 by Woolsey C et al. (Cell Reports 28:3021–3046) should be included in this section.]** In addition, VP35 suppresses micro-RNA silencing (an important posttranslational regulatory pathway) **[Au: I’ve added the parenthesis for a bit of context, ok? please feel free to edit]** in the host cell 106, and VP35 antagonizes a cellular antiviral restriction factor, BST-2 (Ref107). **[Referee comment: the authors mistakenly state that “VP35 antagonizes a cellular antiviral factor, BST-2”. This statement is incorrect; the viral glycoprotein (GP) antagonizes this cellular protein.]** A second EBOV-encoded protein, VP24, also inhibits the antiviral response by preventing the nuclear accumulation of phosphorylated signal transducer and activator or transcription 1α/β (STAT1), which is induced by type I interferon and xxxx **[Au: please complete this sentence and explain the role of STAT1]** 108,109. **[Referee comment: EBOV VP40 in exosomes has been reported to cause immune cell disability (Pleet ML et al. Front Microbiol 2016, Pleet ML et al. DNA Cell Biol 2017). This VP40 function should be included.]**

Recent studies have confirmed that EBOV is immunosuppressive and often **[Au: added “often” because sometimes the host response is successful, ok?]** outpaces an effective host humoral immune response 89,90, even in vaccinated individuals, with the assumption that the vaccinated individual is outside the window for a vaccine-induced humoral response to be mounted (for example, ≈10 days for the vesicular stomatitis Indiana virus vaccine rVSVΔG-ZEBOV-GP) **[Au: Please reference this statement.]** . **[Referee comment: : It is advised to be cautious in comparing human EBOV infection in vaccinated and unvaccinated individuals. The numbers are likely to be small and would be difficult to interpret. Also, the text wording makes it difficult to determine what “outside the window” is referring to. Is the outside part >10 after vaccination or less than 10 days?]** Initial research on human immune responses to EBOV infection has focused largely on the detection of secreted pro-inflammatory mediators **[Au: such as? please provide 1-2 examples? ]** , the expression of **[Au: add “viral” here, for clarity? ]** RNA transcripts from peripheral blood mononuclear cells (PBMCs), and the appearance of EBOV-specific antibodies. Recent data both from EVD patients treated in the US and Western African cohorts suggest that robust **[Au: can this robust response occur even in non-survivors?]** adaptive immune activation that includes antigen-specific T- and B-cell responses occurs during acute illness **[Au: by acute, do you mean both early and peak phases as shown in figure 6?]** 90. Efforts are ongoing to define the characteristics of effective and ineffective B and T-cell responses during acute infection and over time 91,92. These limited results suggest a race between EBOV proliferation and the ability of the human host to mount an effective and regulated anti-EBOV immune response; no study **[Au: in humans?]** has been able to measure inoculation dose and its relationship with disease severity. The study of US patients with EVD **[Au: do you mean patients who were treated in the US?]** also revealed a second peak and persistence of T-cell activation during convalescence 90, which might implicate persistence of EBOV in tissue compartments (immune-privileged sites) after the viral nucleic acid is no longer detected in blood. Indeed, EBOV RNA has been detected by several groups in samples **[Au: what kinds? I’m guessing not blood, as it was negative]** from convalescent patients **[Au: from countries other than the US (or other non-African countries)?]** long after their blood samples tested negative for EBOV 93-96.

**[H1] DIAGNOSIS, SCREENING AND PREVENTION**

**[H2] Clinical manifestations of acute EVD**

Given the difficulties in identifying the exposure source and route of infection in most patients **[Au: added “in most patients”, ok?]**, data that confidently determine the time from exposure to symptoms onset are sparse. The most convincing data derive from situations characterized by a single definitive exposure event. An analysis of all published human exposure data determined that the mean incubation period of EVD is 6.22 ± 1.57 days **[Au: is this the standard deviation?]** for all routes of exposure, 5.85 ± 1.42 days for percutaneous exposure, and 7.34 ± 1.35 days for person-to-person contact or contact with infected animals 110. Rarely, asymptomatic or paucisymptomatic infection of individuals without known clinical manifestations of EVD has been described 111,112. A study of household contacts of survivors discharged from an ETU in Sierra Leone revealed that although a high proportion of contacts (47.6% of 481 contacts) had high level exposure **[Au: could you specify what a high level of exposure would be?]** to individuals diagnosed with EVD, an assay detecting anti-glycoprotein IgG from oral fluid samples tested positive only in 2.6% of contacts 113. **[Au: of all contacts or the 47% with high exposure?]** In general, patients with EVD have a predictable clinical course (Figure 6). During early infection (days 1–3 of following disease onset), EVD patients present with a non-specific febrile illness (symptoms may include anorexia, arthralgia, headache, malaise and myalgia) that progresses in the first week to severe gastrointestinal symptoms (nausea, vomiting and high volume diarrhoea). **[Au: rash is also mentioned in figure 6 as an early manifestation, please include it in the main text for consistency]** During the 2013–2016 Western Africa EVD outbreak, fatigue, anorexia, abdominal pain, diarrhoea, vomiting, fever, and myalgia **[Au: instead of muscle pain, for consistency]** were among the most common clinical signs and symptoms 114-119. However, whether the incidence of these signs and symptoms represents a difference to previous EVD outbreaks is unclear 118. **[Referee comment: The last sentence of the paragraph seems out of place because previous statements do not suggest to the Ebola inexperienced reader that the signs and symptoms described for West Africa patient were significantly different from expected.]** **[Au: were they different from expected?]**

 As the EBOV load increases, typically the severity of EVD clinical manifestations increases as well. **[Referee comment: While increases in viral load is mentioned to increase with disease, it would help the discussion to mention that the onset of detectable viremia is typically day 3.]** From days 4–8 following disease onset, patients may have persistent fever and increased gastrointestinal fluid losses and hypotension from dehydration and vascular leakage. **[Au: should this read something like “and, to a minor extent, vascular leakage”, as in the mechanisms sections it’s stated that third spacing occurs less frequently in humans than in non-human primates?]** Rhabdomyolysis (the release of the content of dead myofibres into the blood) **[Au: definition ok? please feel free to edit]** has also been observed 120. Despite EVD is still often referred to as a “viral haemorrhagic fever,” (now the use of this terminology is discouraged 2), not all patients have overt bleeding manifestations, nor is fever always present. **[Referee comment: The following citation concerning the absence of fever should be included: Rojek A et al. Lancet Infect Dis 2017.]** However, with observations of early consumptive coagulopathy **[Au: do you mean disseminated intravascular coagulation?]** followed by hypercoagulability in the recovery period, haematological dyscrasias **[Au: please define dyscrasias, or replace with a less technical term]** may be more common and complex than previously understood 121,122. During the terminal phase (days 7–12 following disease onset) tissue hypoperfusion and vascular leakage often in conjunction with dysregulated inflammation lead to multi-system organ dysfunction and/or damage, including renal and respiratory failure. **[Au: please briefly elaborate on renal and respiratory failure (eg, what are the symptoms?)]** A subset of patients develop central nervous system manifestations and encephalopathy; although there are several potential underlying causes, EBOV RNA has been detected in the cerebrospinal fluid of patients with EVD, suggesting that meningoencephalitis may be directly mediated by the virus 123,124. **[Au: some discussion of the characteristics of EVD in children could be added here (see also query in the last subsection of the Management)]**

 In humans, no EVD outbreak including more than a single case has ever resulted in a 100% CFR 4. However, the clinical correlates of outcome following filovirus **[Au: do you mean to include here ebolaviruses other than EBOV, and marburgviruses as well? If you mean EBOV, please specify]** infection have been difficult to discern owing to challenges in data collection, clinical follow-up, and limited laboratory services. Data from the Western Africa EVD outbreak showed that viral load or the RT-qPCR cycle threshold value (which is a proxy for the viral load), age, and signs of organ dysfunction (for example, creatinine or AST values), in this order, most reliably predict outcome. The cycle threshold value refers to the number of cycles of amplification during PCR required before viral RNA is detectable above a background threshold: a low value means that viral DNA can be detected in a short period of time, which suggests a high viral load, whereas a high value is associated with a low viral load. For instance, data from an ETU in Sierra Leone suggested poor prognosis for patients admitted with viral loads >10 million genome copies per ml of blood 125, **[Referee comment: Since many publications report Ct values in relation to risk of death, it would be helpful to provide an equivalent Ct value to the genomes per copy per ml of blood value provided.]** but mean-initial viral load decreased over the course of the outbreak, as did the CFR, although such decreases occurred in the setting of numerous factors. **[Referee comment: There is no reference given for this statement and this reviewer is not aware of studies showing that the initial viral load and the CFR decreased over the course of the outbreak. There might be observations at specific treatment units, but hardly across the whole outbreak.]** Other risk factors that have been linked with fatal outcome include age ≥45 years, fever > 38°C, weakness, dizziness, diarrhoea, conjunctivitis, difficulty breathing or swallowing, confusion or disorientation, coma, haemorrhagic signs, and laboratory evidence of hepatocellular damage (for example, increased concentration of blood aspartate transaminase) and impaired kidney function (for example, increased concentrations of blood urea nitrogen and creatinine) 29,126-128. These risk factors are an aggregate list from several distinct cohorts during the same outbreak; however, different associations were found in different cohorts, partially because the same factors were not measured across all cohorts. An increase in overall lethality has also been observed in patients co-infected with *Plasmodium falciparum* (the causative agent of malaria) **[Au: added the parenthesis for extra clarity, ok?]** and potentially other *Plasmodium* species 129,130.

**[H2] Diagnosis of acute EVD**

 **[Referee comment: When discussing diagnosis and management of patients at risk for Ebola it is important to consider pathogens that are in the differential diagnosis as an alternative diagnoses or coinfections. Since malaria is only mentioned in passing in the context of outcome (Lines 412-414). However, it should also be mentioned in the manuscript in the context of diagnosis and management. Please provide a short discussion in the management section on the role of testing and treating for malaria.]** **[Au: and other possible differential diagnoses (other infections). I have highlighte in green some text in the supportive care section that could be moved here to address this query]**

**[Referee comment on this paragraph: the authors state that a BSL4 laboratory is required for diagnosis and this creates delays in obtaining results. Whilst a BSL4 facility is necessary for viral culture, all the facilities deployed to W African and latterly to DRC were based on a BSL2 facility, with initial handling and viral inactivation taking place in glove boxes (European and Canadian facilities) or by staff wearing PPE in dedicated area (US facilities). Subsequent processing is at BSL2, and most facilities tested samples on the day of receipt. Problems occur** **with community samples where transport to the laboratory can take several days. Recent practice has been to include clinical chemistry and haematology analysis in the care process in field laboratories whenever possible, and correction of electrolytes and good fluid management dramatically improve survival e.g. Hunt L, Gupta-Wright A, Simms V, Tamba F, Knott V, Tamba K, et al. Clinical presentation, biochemical, and haematological parameters and their association with outcome in patients with Ebola virus disease: an observational cohort study. Lancet Infect Dis. 2015;15(11):1292-9. This should be mentioned in the overview of diagnostic provision and clinical care.]**

 **[Au: another referee noted:]**

 **[First, there was no need for biosafety level 4 laboratories for diagnostics in the West African outbreak. Molecular diagnostics requires a glove box or class III cabinet for sample inactivation and such equipment has been used in national laboratories or the 20+ field laboratories deployed to the outbreak area. This reviewer is not aware that field labs with glove box have been classified as BSL-4. Second, molecular diagnostics in field labs did not require 5 days. Turn-around time from** **sample reception to reporting has been in the range of some hours. The main delay between onset and diagnosis was due to late presentation of patients, which was in the range of 5-6 days after onset of symptoms. Transport of samples from a remote area to the lab might have contributed to delays as well. Please revise the paragraph.]**

Appropriate isolation of patients with laboratory-confirmed EVD not only requires optimization of a front-line clinician’s ability to rapidly identify a patient who fits the EVD case definition (see Epidemiology) but also necessitates that an EVD diagnosis is accurately confirmed with readily available laboratory tests. Until recently, field diagnosis of EVD during an outbreak has relied primarily on real-time reverse transcription (RT) **[Au: added the RT bit, ok?]** PCR assays. **[Referee comment: The sentence omits serologic testing as a previous method of diagnosis.]** Although PCR assays **[Au:OK?]** are accurate, factors such as cost, time to processing, availability, required level of expertise, and the need for a biosafety level 4 laboratory contributed to substantial delays (up to 5 days in some instances) in provision of rapid results during the Western Africa EVD outbreak. Improving the time to diagnosis may have a major effect on transmission dynamics during an outbreak. One simulation estimated that by decreasing the average time to diagnosis and isolation with PCR **[Au: do you mean “diagnosis with PCR and isolation of the patient”?]** from 5 days to 1 day in 60% of EBOV-infected patients, the virus attack rate (the proportion of people at risk of the infection who become infected, a measure of the speed of the infection spread) **[Au: definition ok? please feel free to edit]** would drop from 80% to nearly 0% 131. Consequently, in November 2014, the WHO issued a call for “rapid, sensitive, safe and simple EBOV diagnostic tests” 132.

Since then, several diagnostic tests, which range from hand held lateral flow assays to bench top PCR-based technologies, have been developed, some of which have been evaluated and used in the field (Table 2). A mathematical model was used to evaluate the effect on EVD CFR and EBOV transmission dynamics of incorporating different diagnostic strategies that did and did not include the introduction of novel rapid diagnostic tests (RDTs) in various scenarios 133. A strategy that coupled novel RDTs with confirmatory PCR testing was deemed superior to the use of either PCR assays or RDTs alone and would result in a reduction of the scale of an outbreak by one-third. **[Referee comment: What is the impact on the coupling rapid diagnostic tests (RDT) with PCR on false positives and negatives]** Notably, in this model it was assumed that RDTs performance characteristics were inferior to those of PCR assays for accurately diagnosing EVD. However, with improvement in RDTs performance, the model suggests that RDTs alone may replace PCR assays and alter transmission dynamics during future outbreaks. **[Referee comment: Should discuss the use of RDT assays outside of known outbreaks and risk of false positive when there is a low pre-test probability]** **[Au: Another referee commented: I agree overall with comments on RDTs, but limiting factors apart from sensitivity include a concept of sue and the need to train front-line workers to implement & read the tests whilst dressed in PPE. RDTs have to be validated in use in their final setting, when used by those workers who will use them as opposed to laboratory studies with scientific staff (Walker NF, Brown CS, Youkee D, Baker P, Williams N, Kalawa A, et al. Evaluation of a point-of-care blood test for identification of Ebola virus disease at Ebola holding units, Western Area, Sierra Leone, January to February 2015. Euro Surveill. 2015;20(12).)]**

Importantly, disease severity, disease acuity, and sample material need to be taken into consideration before choosing a particular diagnostic test. In general, blood is the sample material of choice for live patients, whereas swabs are useful for post-mortem diagnosis. **[Au: the sentence in green was the last one of this paragraph, but I moved it up as it’s a nice introduction]** Asymptomatic or pauci-symptomatic EBOV-infected people generally do not develop viremias levels that are high enough for detection by PCR assays, but typically have detectable IgG and IgM responses ~3 weeks after infection. Hence, RDTs using blood samples are the diagnostic tests of choice for these patient cohorts. **[Referee comment: There is no reference for the recommendation that an RDT is the test of choice in asymptomatic/pauci-symptomatic EBOV-infected patients at a subacute time-point. Also, there are many types of RDTs with different performance characteristics. The specific RDT or type of RDT (i.e., PCT, lateral flow, etc…) be recommended should be specified]** **[Referee comment: The authors should specify the type of RDT. However, there is no need for rapid diagnostics in RT-PCR negative asymptomatic or pauci-symptomatic cases. Any type of serological assay, including IgG/IgM RDT, may be the test of choice in these cases.]** Of note, antibody responses do not reliably develop in acutely symptomatic EVD patients. Thus, PCR tests are more likely to correctly diagnose EBOV infection than RDTs **[Au: which type(s)?]** in blood samples and, in contrast to RDTs, **[Au: which type(s)?]** can also detect EBOV RNA in amniotic fluid, breast milk, ocular fluid, saliva, seminal fluid, stool, sweat, tears, urine, and vaginal fluid even after blood samples begin to test negative40,46,49,96,134,135.

**[H2] Prevention**

 **[Referee comment on this section:** **the role of anthropologists in social & cultural engagement and training of local workers in terms to which they can relate should be mentioned again at this point. These considerations also apply to rehabilitation of Ebola survivors in the community (James PB, Wardle J, Steel A, Adams J. Pattern of health care utilization and traditional and complementary medicine use among Ebola survivors in Sierra Leone. PLoS One. 2019;14(9):e0223068.).]**

 **[Referee comment on this section: In this chapter it is lacking a part regarding the contact tracing and follow up that is a crucial component of the prevention (the lack of it is one of the main reason of the current failure in DRC).]**

The overall strategy for mitigating the spread of an ongoing EVD outbreak is to interrupt community and nosocomial transmission of EBOV from patients to EVD-susceptible individuals. **[Au: it seems to me that only the prevention of community transmission is discussed; could you please add a sentence or two on how to prevent nosocomial transmission?]** Effectively achieving this strategy depends upon the quality of measures in place; ideally, interruption of the chain of transmission in the community can be achieved by isolating individuals with suspected, probable, or confirmed EVD for care and treatment in an ETU or holding center. In a mathematical model assessing changes in EBOV transmission in 12 Sierra Leonean districts between June 2014 and February 2015, introduction of additional treatment beds in the ETU to isolate suspected or confirmed cases directly resulted in avoidance of ≈56,000 new EVD cases 136. **[Referee comment: Does this mean that additional treatment beds had been introduced in an ETU and subsequent modelling suggested that this measure avoided 56,000 cases? It would be of interest to understand how many beds had been introduced that supposedly caused such an enormous effect: 10, 20, 50?]**

 **[Au: please add here an introductory sentence explaining that discharge criteria are important to avoid reintroduging the virus in the community]** During the Western Africa EVD outbreak, the WHO recommended that clinicians consider discharging from healthcare facilities of patients diagnosed with EVD after resolution of clinical signs for ≥3 days, appreciable improvement in clinical condition, ability to perform activities of daily living, and a blood sample negative for EBOV RNA (detected with RT-PCR tests) from the third day of becoming asymptomatic. In the case of unresolved signs and symptoms, discharge should occur after two negative blood test results (48 h apart), and an alternative diagnosis should be sought that may explain lack of clinical improvement 137. Other health authorities have made recommendations for the discharge of patients under investigation, but none for patients diagnosed with EVD. **[Au: do you mean that the only recommendations on discarging pts with *confirmed* EVD are those from the WHO, but that other agencies recommend to discharge pts with *suspected* EVD? Whereas the WHO does not recommend to discharge pts with *suspected* EVD?]** In most EVD patients managed in the US and Europe during the Western Africa outbreak, repeatedly negative RT-PCR tests of blood samples was the primary criterion used for discharge, along with symptomatic improvement. However, several centers **[Au: do you mean centers in the field (i.e. not in US or Europe)? Or that these (US and European) centres used the other criteria listed *in addition* to those in the previous sentence?]** used other criteria, including RT-PCR tests of samples of other bodily fluids and EBOV cell culture under biosafety level 4 containment 138.

 **[Au: new paragraph]** After a scant historical experience **[Au: do you mean that until the Western Africa outbreak there were few reports of sexual transmission/EBOV in the semen of survivors?]** , the time-limited detection of infectious EBOV **[Au: do you mean the detection of infectious EBOV (virions?) during EVD?]** and long-term detection of EBOV RNA in the semen of male survivors of the Western Africa EVD outbreak has been increasingly characterized, as have rare but consequential sexual transmission events 47,59,93,139. Accordingly, WHO recommendations140 (currently being updated) for the prevention of sexual transmission from survivors include routine PCR testing of semen beginning at 3 months after discharge (as the semen should be assumed to be infectious for the first 3 months) and until two consecutive **[Au: how far apart? 48 hours?]** semen samples are negative. Abstinence or safe sexual practices should be implemented for the same period or for at least 1 year.

**[H3] Candidate vaccines.**

 **[Au: the referees had several important comments on this section and table 3 (see below), and of course the recent EMA approval of the rVSV-ZEBOV-GP vaccine (**<https://www.who.int/news-room/detail/18-10-2019-major-milestone-for-who-supported-ebola-vaccine> ) **should be discussed too:]**

 **[Referee comment: The manuscript will be substantially strengthened by including a few sentences on pre-exposure vaccination efforts (currently a conspicuous gap).]**

 **[Referee comment: It is important to distinguish between ring and pre-exposure vaccination because mechanisms of protection (i.e., innate immunity vs adaptive), efficacy and durability may not be the same for each strategy. For EBOV, human efficacy data are only available for ring vaccination. Information on mechanisms of protection and long-term durability is not known for ring vaccination. Because the manuscript and table do not clearly make the distinction, the reader may draw inappropriate inferences from ring vaccination to pre-exposure vaccination. Therefore, the review should clearly separate data and discussion for the two different vaccination strategies.]**

**[Referee comment: The manuscript does not contain discussion of durability or pre-exposure vaccination in the text/table - it is an important area to cover as health care providers and responders will want to be vaccinated in advance with a vaccine that offers safe and long-term durable protection. The pre-exposure vaccination Phase III trials listed in the table are closed and only evaluated safety and/or did not have sufficient statistical power to determine efficacy. Since ring vaccination is now a key component of outbreak responses, it will be very difficult to ethically set-up pre-exposure efficacy trials. Thus, it seems arbitrary to leave out candidates that remain under active development and which have undergone Phase I or II clinical trials, due to the absence of a Phase III trial in West Africa.]**

**[Referee comment: To better communicate the effectiveness of ring-vaccination, please include the number needed to treat (i.e., the number of vaccinations needed to prevent one case) for the trials discussed. This is more approachable and clinically meaningful than geographic area of risk.]**

**[Referee comment: A vaccine based on Chimpanzee adenovirus type 3 (ChAd3) that has demonstrated potent acute and durable protection in monkeys was part of an efficacy trial in Liberia. The trial did not fully enroll due to cessation of cases. This vaccine is in active development by Sabin Vaccines - since it is a level of development comparable to hAd26 the review is unbalanced if it excludes this vaccine but includes discussion of hAd26]**

Amid increasing concerns about unmitigated EVD **[Au: unmitigated *spread* of EVD?]** during the Western Africa EVD outbreak in mid-2014, stakeholder meetings held by the WHO resulted in a statement that urged acceleration of the development and evaluation of EVD candidate vaccines. As the EBOV glycoprotein (GP1,2) is the major viral immunogen, all candidate vaccines in advanced development are designed to stimulate a host immune response against this protein, among others **[Au: edits ok?]** . Several EVD candidate vaccines are being evaluated in phase III clinical trials (Table 3). As of February 2019, some candidate vaccines have been licensed in China and Russia 141. **[Au: any updates since then? This might be a good spot to discuss the recent EMA approval]** **[Au: As suggested by the referees, before discussing the details of the vaccines/trials, please add a short section describing the general principles of the trials (strategies, target populations, etc), so when the reader refers to table 3 he/she can understand it easily]**

US Food and Drug Administration-approved vaccines are still not available for use in EVD outbreaks. On the heels of the *Ebola Ça Suffit!* ring vaccination trial **[Au: what phase was this trial? What results prompted the current use in COD?]** in Guinea 142,143 the rVSVΔG-ZEBOV-GP, a live-attenuated recombinant vesiculovirus candidate vaccine, is actively administered under an Expanded Access/Compassionate Use protocol to help contain the currently ongoing EVD epidemic that started in Nord-Kivu Province of COD in 2018 **[Au: added the year for clarity, ok?]** . **[Au: should we add a comment on this:** <https://www.statnews.com/2019/09/23/vaccination-strategy-in-long-running-ebola-outbreak-comes-under-fire/?utm_source=STAT+Newsletters&utm_campaign=eabb389963-MR_COPY_10&utm_medium=email&utm_term=0_8cab1d7961-eabb389963-149588829> ?**]** Through a ring vaccination strategy, whereby contacts of infected individuals (primary ring) and contacts of those contacts (secondary ring) are vaccinated, this candidate vaccine has been administered **[Au: I’ve deleted after informed consent as it goes without saying, correct?]** to 211,620 people in Eastern COD as of September 2, 2019 (Ref144). **[Au: any updates since then?]** Preliminary analyses on data evaluating the first 93,965 vaccinated individuals **[Au: instead of “contacts and contacts of contacts”, ok?]** are promising and reveal a lower frequency of new EVD cases **[Au: so a “lower EVD incidence”?]** among individuals who were vaccinated (0.017%) than in unvaccinated individuals (0.656%). **[Au: this difference bewteen the incidences is really small, in both cases <1 person every 100 becomes infected, or am I missing something here? please clarify]** **[Au: Please reference this statement, is it the same ref as next sentence?]** True determination of vaccine efficacy cannot be done in the absence of a control comparator **[Au: what do you mean by this? isn’t vaccine efficacy measured as the reduction of the virus attack rate in vaccinated people compared with non vaccinated people? Then there is a comparator (the unvaccinated population), no?]** , though the WHO has reported an estimated vaccine efficacy of 97.5%, 95%CI [95.8-98.5%] 145. Notably, a model of the EBOV infection risk during the 2018 EVD outbreak in Équateur Province in COD found that the introduction of ring vaccination with rVSVΔG-ZEBOV-GP vaccine resulted in a decrease of 70.4% of the geographical area of risk and 70.1% of the level of EBOV infection risk **[Au: by level of infection risk do you mean the infection rate (the incidence)?]** . However, the size of this effect would be considerably diminished with delays (as little as 1 week) in the introduction of the ring vaccination 146. **[Au: do you mean delays in vaccinating contacts and contacts of contacts once the diagnosis is confirmed in the patient? Or do you mean delays in setting up the ring vaccination programme after the start of the outbreak?]** The same candidate vaccine is also used **[Au: in the ongoing COD outbreak?]** as emergency post-EBOV exposure prophylaxis in, for instance, health-care workers or people who have been exposed to EVD survivors with recrudescent infection.

**[H1] MANAGEMENT**

**[H2] Direct medical countermeasures**

 **[Referee comment: I would however have liked to see some consideration of host-directed therapies e.g. FX06.]** **[Au: is targeting the effects of the virus on the immune system a viable therapeutic option? Any data?]**

No US Food and Drug Administration-approved drugs are currently available to treat EVD, **[Referee comment: The manuscript refers to FDA approved of MCMs, but other regulatory bodies exist, so should talk more generally about 'regulatory approval']** **[Au: or are there any drugs that are approved by other agencies?]** although many experimental therapeutic agents have been evaluated in animal models. During the 2013–2016 Western Africa EVD outbreak, these agents were administered in an uncontrolled fashion to individual patients, usually through Emergency Use Authorization (a temporary authorization to use unapproved medications in public health emergencies) **[Au: definition ok? please feel free to edit]** , and, therefore, no scientifically valid conclusions could be drawn on their efficacy **[Au: efficacy added for clarity, ok?]** . In addition, several non-randomized clinical trials and one randomized controlled trial were performed in Africa 147. **[Au: do you mean that however, in addition to these uncoordinated efforts, several trials were also performed during the same outbreak?]**

 **[Au: new paragraph]** Results from one single-arm trial conducted in Guinea evaluating the viral RNA polymerase inhibitor favipiravir did not reach any efficacy conclusions. However, a non-significant trend towards improvement in CFR was observed in patients with a low viral load (EBOV RT-qPCR Ct values ≥20 cycles) treated with favipiravir (an RNA polymerase inhibitor) **[Au: mechanism of action ok?]** compared with historical controls 148 . **[Au: definition of Ct moved to the Diagnosis section where it’s first mentioned]** Similarly, results from another single-arm trial conducted in Sierra Leone with TKM-130803, a formulation of small interfering RNAs that target three EBOV proteins involved in suppression of the host’s immune system **[Au: please specify which proteins, I assume VP35, VP24, and?]** , did not demonstrate improvement in survival compared with historical controls 149. **[Au: paragraphs merged, so all the trials during the Western Africa outbreak are grouped together]** A randomized controlled trial was conducted in Guinea, Liberia, Sierra Leone, and the US to evaluate the safety and efficacy of ZMapp, a cocktail of monoclonal antibodies, added to optimized standard of care (oSOC) versus oSOC alone in the treatment of EVD. The trial was stopped early after enrolling only 71 patients because the numbers of incident cases had declined at that stage **[Au:OK?]** of the Western Africa EVD outbreak. However, a trend towards improved survival was reported in the oSOC plus ZMapp arm compared with the oSOC only arm (22.2% and 37.1%, respectively) but, at a 91.2% threshold for posterior probability, **[Au: could you please explain what the posterior probability means in this context?]** statistical significance (which required a 97.5% threshold) **[Au: edits ok?]** was not achieved 150. **[Referee comment: In the West African ZMapp trial, in some ETUs oSoc included favipiravir. This should be noted because it could have impacted the results.]**

 **[Au: new paragraph, as this is a different outbreak]** In early 2018, WHO led a panel of experts to evaluate the latest (human and animal) efficacy data on available therapeutics to inform the Monitored Emergency Use of Unregistered Investigational Interventions (MEURI), an ethical framework to guide access to investigational therapeutics during an EVD outbreak as a bridge to a clinical trial 151. This MEURI framework has been implemented during the 2018–present EVD outbreak in COD, whereby almost all patients admitted to ETUs since mid-August 2018 have received ZMapp, mAb114, REGN-EB3, or remdesivir (Table 4). **[Referee comment: Actually very few patients have received Zmapp under MEURI.]** **[Referee comment: Please reference the initial discovery papers and Phase I clinical trial publications for these treatments.]** **[Au: I think at least some of these references are already present in table 4, please also include them here]** The Pamoja Tulinde Maisha (PALM, which is Swahili for “together save lives” **[Au: parenthesis ok?]** ) study, a randomized controlled trial **[Au: phase?]** evaluating efficacy of these four candidate therapeutics, commenced in Eastern COD on November 20, 2018 (Ref151). In August 2019, the Data Safety Monitoring Board for the PALM study recommended halting of the trial because preliminary results suggested that individuals randomized to either the REGN-EB3 or mAb114 arms had a higher probability of survival than individuals randomized to the ZMapp or remdesivir arms 152. **[Au: Couldn’t just these two arms be suspended?]**

The transfusion of whole blood, plasma, or serum from convalescent individuals (passive immunization therapy) has also been studied as a therapeutic intervention against EVD and still remains a potential treatments when no effective alternatives, such as antivirals or antibodies, are available 153. However, results from one study **[Au: where? When? Please specify as this information is provided for other studies]** did not demonstrate a significant **[Au: statistically?]** improvement in survival in patients who had received plasma from convalescent survivors 154. In a continuation of the same study, titers of anti-EBOV IgG and neutralizing antibodies **[Au: can’t IgG be neutralizing too?]** in plasma from convalescent survivors were determined. The dose of anti-EBOV IgG antibodies was associated with increases in cycle threshold values following infusion, but no significant **[Au: statistically?]** difference in lethality **[Au: between patients who received the transfusion and those who didn’t, or do you mean that the dose of the antibodies does not correlate with lethality?]** was observed 155. A case of acute respiratory distress was reported in a repatriated individual, potentially as an adverse event associated with administration of plasma from convalescent individuals 156. As the availability and initial effectiveness of monoclonal antibody-based therapeutic strategies in ongoing research studies in COD increase **[Au: edited sentence ok?]** , it is unlikely that polyclonal passive immunization strategies will continue to be pursued. **[Au: also, as HIV infection is very prevalent in Africa, would there be a risk of transmission? Or is plasma/blood from survivors routinely tested before it’s given to patients? Could there be the risk of promoting the transmission of malaria? The patient receiving the infected transfusion would only have a few bouts of fever as long as the infected cells are in the circulation, he/she wouldn’t have malaria, as he/she wouldn’t have the parasites in the liver where they multiply; however, the patient could still spread the infection if he/she is bitten by mosquito while still infected, no?]**

**[H2] Supportive care for acute EVD**

 **[Au: in the Monitoring section it’s mentioned that hepatitis is very common, but there’s no mention of it in this section or the one on critical care: please briefly discuss it in the appropriate section]**

Aggressive supportive care is recommended to prevent EVD patients from undergoing shock **[Au: Hypovolemic?]** from profound dehydration and/or excessive vascular leak 157,158. **[Au: please see previous queries where it’s pointed out that vascular leak is mentioned in the mechanisms section as not occurring often in humans: should the text be modified here?]** In the early stages of disease when the patient is ambulatory and able to eat and drink without nausea and excessive vomiting, oral rehydration solutions (ORS) can be administered to replace gastrointestinal and insensible water **[Au:OK? or “fluid”?]** losses (insensible water loss is attributed to evaporation from the skin and respiratory tract). Establishing an intravenous access at the early phase **[Au:edit OK?]** is crucial for administration of balanced saline solutions (for example, Ringer’s lactate) as the patient’s condition worsens and can no longer tolerate drinking owing to increased nausea, vomiting, asthenia (weakness) **[Au: definition of asthenia ok? please feel free to edit]** , malaise, and lassitude (fatigue) **[Au: definition ok?]** . **[Referee comment: the article should address The role of IV hydration, where there is some controversy – see PMID 31050703]** In the Western Africa EVD outbreak, improved fluid optimization was demonstrated in groups using central venous catheters compared with those using peripherally inserted catheters 159. However, in resource-limited settings, establishing a peripheral intravenous access is often more logistically feasible than establishing central access. **[Au: I switched the order of the last two sentences of this paragraph for flow]**

As their illness progresses from early to more severe stages of illness **[Au: can we say the peak phase, for consistency with figure 6?]** predominated by increased gastrointestinal losses (secretory phase), patients with EVD may produce large amounts of emesis and stool. Stool volumes of 5–10 liters per day have been reported, leading to heavy fluid and electrolyte losses 160. Managing patients’ bodily fluids is also an important infection control modality in the healthcare environment that can be accomplished with physical and pharmacological controls. Anti-emetic medications (for example, metoclopramide and ondansetron) have been used to control nausea and vomiting. Also, anti-diarrhoeal agents (for example, loperamide) have been used to reduce the frequency of diarrhoea. With potential adverse events such as intestinal ileus (that is, obstruction) **[Au: definition ok?]** , the risk-benefit ratio of using this drug **[Au: loperamide?]** for inflammatory diarrhoea associated with EVD is uncertain 161. Examples of physical controls used in the hospital include emesis bags, bedside commodes, and fecal management systems (temporary containment devices composed of a catheter and a collection bag) **[Au: explanation ok? please feel free to edit]** for the non-ambulatory patient.

Although primary EVD-attributable respiratory disease is uncommon, patients who have respiratory symptoms **[Au: such as? please give 1-2 examples]** and hypoxia may require conservative treatment with supplemental oxygen, particularly those with pulmonary oedema as an iatrogenic effect of aggressive fluid replacement. Haemorrhagic complications can be treated with blood products when available, but clinicians should be aware of potential hypo-coagulable and hyper-coagulable states. **[Au: please elaborate a little on this point: what are these states? What causes them?]** Severe neurological manifestations, including meningitis, encephalitis, seizures, and coma, have been reported in patients with acute EVD 162. **[Au: sentence on delirium moved down a bit for flow (in green)]** Although poorly understood, viral encephalitis or encephalopathy has been circumstantially implicated in a patient with MRI findings of encephalomalacia (softening or loss of brain tissue) **[Au: definition ok?]** and seizures post-recovery who had evidence of previous haemorrhagic encephalitis **[Au: was this previous encephalitis not associated with EVD?]** (I.C., data unpublished). In Guinea, EBOV RNA has been detected in the cerebrospinal fluid of four patients with clinical signs of meningoencephalitis 123,124. Delirium or agitation can be a challenging feature of EVD. Benzodiazepines or other available sedating medications may be needed to keep patients from harming themselves, other patients, or healthcare providers. Fever and pain may be treated by acetaminophen. In tropical areas, where numerous febrile illnesses can mimic the presentation of EVD, testing or empirically treating parasitic (for example, *Plasmodium* spp.), viral (for example, Lassa virus), and bacterial (for example, *Salmonella* Typhi **[Au: I’m confused, why is Typhi not italics and with capital T? should it be typhimurium?]** ) diseases is important 130,163. **[Au: this text could be moved to the diagnosis section]**

**[H2] Critical care**

 **[Referee comment: in this chapter I would summarize better the 2 main problems in providing intensive care:**

**1. Feasibility (HR capacity, necessary instruments, adequate structures...)**

**2. Risk: invasive procedures put the HCWs at higher risk of infection]**

 **[Au: please touch on these points in the first introductory paragraph]**

Patients with EVD who progress to critical illness, including multi-organ dysfunction syndrome, may require advanced life support modalities. However, guidance on the critical care management of EVD remains limited, as aggressive interventions can be challenging to deliver in the resource-constrained settings where EVD outbreaks typically occur. The knowledge of modern critical care of patients with EVD stems from the care of several patients who acquired EBOV infection in Western Africa but were managed in the US and Europe 115,164-168, and limited experience in an ETU in Sierra Leone that was equipped with ICU capabilities 169. **[Au: merged first two paragraphs for flow]** , Substantial pre-planning was necessary to provide treatment for critically ill patients, to ensure the availability of physicians with experience in **[Au:OK?]** airway management, the necessary equipment, and appropriate personal protective equipment for potentially aerosol-generating procedures. **[Au: just to clarify, in these last paragraphs of this section you are referring to both patients treated in the US/Europe AND those in the ICU-equipped ETU in Sierra Leone, correct? or just patients in the field?]**

 **[Au: new paragraph, as now we start to discuss the specific procedures. However, I would delete this short paragraph on intubation, as it is not specific to EVD. If you wish to keep it, please address my queries ]** Intubation was accomplished via rapid sequence induction using neuromuscular blockade, followed by video laryngoscopy to provide direct visualization of the airway 166,167. **[Au: is this the standard method for intubation? If not, why was it chosen?]** Validation of correct endotracheal tube placement was often difficult, as some centers did not have the ability to auscultate **[Au: the lungs?]** or monitor end-tidal CO2 concentrations in their treatment units 166. **[Au: and what are the implications of this lack of validation?]**

For patients with EVD who developed acute kidney injury, continuous renal replacement therapy (CRRT) was performed 164,170. CRRT was chosen over intermittent haemodialysis to decrease the frequency of exposure to blood and body fluids during the initiation process. **[Au: do you mean “as the procedure is started”? also, is this the only reason why CRRT was preferred to intermittent haemodialysis? I’d think that in patients who already have altered volumes of fluids (owing to GI symptoms), CRRT would be less traumatic than intermittent haemodialysis]** Frequent laboratory monitoring **[Au: of several biomarkers?]** , including electrolytes, was performed while patients remained on CRRT, and regional citrate anticoagulation (that is anticoagulation is achieved by administering citrate, which reduces the blood concentration of calcium, a cofactor required to activate the coagulation cascade, and XXXX) **[Au: please complete this parenthesis: what is the “regional” part?]** was used in lieu of systemic anticoagulation 170. The safe and effective provision of CRRT in an isolation setting posed many challenges, including the need to minimize contact with blood and body fluids, the generation of effluent waste, the training of healthcare workers to operate equipment, and terminal cleaning of devices. In particular, the generation of effluent waste required additional consideration. Effluent waste was found to be negative for EBOV by RT-PCR on three separate occasions at one center, probably owing to the inability of EBOV particles to cross the dialyzer membrane. However, as the effluent waste was found to be positive for EBOV **[Au: by RT PCR?]** in one of three samples at another center, the CDC and some **[Au: “some” instead of “other”, as the CDC is not a clinician, ok?]** clinicians recommend that effluent waste be generally handled as potentially contaminated 166,171.

 **[Au: in figure 6 “Cardiac dysfunction” starts at the recovery phase, whereas here in the text it is discussed under critical care: should we move the bar in figure 6 leftwards?]** **[Au: one sentence listing the drugs used deleted for brevity]** Conflicting arguments exist **[Au: conflicting evidence as well?]**regarding the utility of advanced cardiac life support measures and cardiopulmonary resuscitation in EVD patients 172-174. Ultimately, cardiopulmonary resuscitation and other interventions, including defibrillation and cardioversion, should be assessed on an individual case-by-case basis. A careful risk-benefit assessment should occur prior to all critical care interventions to provide optimal care for the patient while reducing the risk of healthcare worker exposure. **[Au: the previous two sentences (especially the previous one) seem to imply that it could be ok not to do CPR on a patient if it puts the healthcare workers at risk, is it so?]** Of note, the CFR of 27 patients with EVD managed with aggressive supportive care measures in Europe and the US was only 18.5%, suggesting that availability of aggressive interventions may have a substantial effect on CFR for all patients during an EVD outbreak 164. **[Referee comment: In order to put case-fatality rates (CFR) in context, it would be helpful to provide CFRs for relevant control groups or for the given outbreak being discussed]** In the ongoing outbreak in COD, substantial efforts have been made to improve and optimize the supportive care provided to patients EVD in an African setting; these efforts has often occurred in innovatively designed ETU spaces that enable a higher degree of monitoring and care and have been catalyzed by newly developed protocols to support care delivery 175. **[Referee comment: The improvements in care infrastructure and care in the current outbreak did not improve overall mortality vs West Africa. This should be addressed, including the possible explanations (e.g., viral differences, # deaths outside of ETUs, etc…).]**

**[H3] Monitoring.**

 **[Au: this section is currently marked as a subsection of the Critical care section: please confirm that it ONLY applies to critically ill patients, otherwise we will need to change the heading level and title]**

**[Referee comment: The ‘Monitoring section is rather brief. What clinical lab tests are essential and why? K+, glucose? What about oximetry, ECG, coagulation? Is there any clinical value in monitoring LFTs?]**

 **[Referee comment: ‘Clinical laboratory testing;’ is a bit opaque, would be clearer to cite clinical chemistry, haematology, coagulation etc]**

 **[Au: I suggest to reorganize and expand this section along these lines: first, a general introduction on the role of clinical lab tests in monitoring, followed by the specific tests (grouped by organ/system), with a short explanation on why they are important. I’ve moved some text around with this in mind]**

The performance of clinical laboratory testing varies by the resources available, and a risk assessment should occur to ensure the safety of the laboratory staff who process the samples 176. Point-of-care testing **[Au: for what tests are POC tests available?]** can be useful if the temperature and humidity conditions do not affect performance. In settings where robust laboratory support is available, clinical parameters can be monitored closely, and therapies can be determined based on the daily values. In the setting of multi-organ failure and critical illness, frequent laboratory monitoring becomes a cornerstone to guide supportive therapy. In addition, viral load monitoring is also helpful as a surrogate of viral replication, immune containment, and (in retrospective studies **[Au: parenthesis ok?]** ) CFR, and may assist in risk stratification of patients with EVD.

 **[Au: new paragraph]** Profound gastrointestinal fluid **[Au:OK?]** losses and concomitant kidney injury require timely monitoring and replacement of electrolytes to prevent potentially lethal arrhythmias and fluid shifts 126,177,178. Also, hepatitis is very common during EVD and is associated with a disproportionate increase in aspartate aminotransferase (AST) over alanine aminotransferase (ALT) concentrations. Although mild abnormalities have been reported, mild aberrations in liver function tests (including bilirubin and international normalized ratio **[Au: please explain what this is]** ) are less common 177. **[Au: this last sentence is not very clear, don’t AST and ALT levels also indicate liver function?]**

**[H2] Complications**

 **[Referee comment: the article should address** **The role of anti-malarial. An MSF report on benefit for Ebola of artesunate-amodiaquine is not reported – PMID 26735991]**

Secondary infectious complications in patients with EVD, including sepsis induced by Gram-negative bacteria have been observed 48. Patients with EVD may be at high risk for bacterial translocation of the commensal gut microbiota into the blood stream, owing to substantial inflammation in the gastrointestinal tract 163. Patients with EVD treated in Western Africa **[Au: do you refer here to the Western Africa outbreak, or do you mean to distinguish these patients from those who were treated in the US/Europe?]** received empiric antibiotic therapy to prevent sepsis but also, particularly in children, to treat potentially other life-threatening infections **[Au: do you mean “other potentially life-threatening infections”, or “other life-threatening potential infections”?]** that can mimic clinical EVD signs **[Au: such as? Salmonella Tiphy infection as previously mentioned?]** . Initiation of broad-spectrum antibiotics is recommended in EVD patients who are critically ill 157,164. Since EVD patients may remain hospitalized for prolonged periods and may undergo invasive procedures, they should be monitored closely for the development of nosocomial infections such as central line-associated bloodstream infections, ventilator-associated pneumonia, and urinary tract infections. As performing blood cultures for full identification of the species of the causative bacterium and its antimicrobial susceptibility patterns **[Au: edits ok? ]** can be logistically challenging in treatment units **[Au: ETUs, for consistency?]** , broad-spectrum PCR-based methods may prove useful in some settings for rapid identification of secondary or nosocomial infections 179.

**[H2] Special populations**

Over 3,000 patients with EVD were <15 years of age during the Western Africa outbreak. Clinical characteristics of pediatric EVD patients have been described **[Au: not really, except for a sentence in the epidemiology section: could you please briefly discuss this point in the Diagnosis section? I’ve added a query where this text could be added]** , and CFR of 42–76% in this population **[Au:OK?]** were reported in the Western Africa outbreak 28,180-182. **[Referee comment (repeat): In order to put case-fatality rates (CFR) in context, it would be helpful to provide CFRs for relevant control groups or for the given outbreak being discussed.]** The clinical management of children with EVD introduces unique challenges, including the need for healthcare workers trained in paediatrics and the issue of parental presence and its associated benefits and risks 183. Similarly, the care of obstetrical patients also presents remarkable challenges, especially regarding infection control during the provision of surgical procedures such as cesarean section 184. Survival is rare for neonates born to EBOV-infected mothers, and the frequency of miscarriage and maternal CFR is extremely high 185-188. **[Referee comment: The CFR in pregnancy is said to be ‘extremely high’(line 696-697) whereas there are data to suggest the CFR is no higher than in non-pregnant women with EVD - see PMID 28379374.]** It remains unclear if the management of these patients in better resourced settings or with experimental therapeutic agents would have an effect on survival 189. To modify **[Au: reduce?]** healthcare worker risk, it is of high importance to create protocols to maintain the standards of infection control and to ensure the availability of staff who are trained to provide safe and effective care to these special patient populations 190.

**[H1] QUALITY OF LIFE**

Owing to the high CFR and overall low case numbers of EVD outbreaks prior to 2014, EVD survivors were rare and not followed-up systematically with modern clinical research methodology. Limited case-controlled data from an EVD outbreak in 1995 first suggested that convalescence could be complicated by substantial morbidity that might limit a survivor’s ability to resume a pre-EVD quality of life; reported sequelae **[Au:OK?]** included arthralgias, myalgias, visual and auditory changes, and extreme fatigue 191,192. Almost two-thirds of survivors continued to experience one or more of these symptoms for 2 years following disease onset, and many survivors reported that their capacity to work was decreased compared with their pre-EVD state 192. By contrast, during the Western Africa outbreak the percentage of survivors exceeded that of casualties , **[Au: I wouldn’t say that post-EVD symptoms were the rule rather than the exception, as the average CRF was still ~40%, so I edited accordingly]** and the majority of survivors who participated in observational cohort studies reported symptoms similar to those described for the 1995 outbreak. **[Au: Please reference this statement (or is it the same as the next two sentences?)]** In the largest controlled observational study **[Au:OK?]** of EVD survivors to date, certain symptoms (headache, joint and muscle pain, memory loss, fatigue and urinary frequency **[Au: increased or decreased? Or “changes in”, to include both scenarios?]** ) and signs (as revealed by abnormal abdominal, chest, neurological, musculoskeletal and ocular examinations) were significantly **[Au: please confirm this means statistically significant ]** more common in survivors than controls. In general, these conditions improved over time, with the exception of uveitis which increased **[Au: do you mean that the prevalence increased or that the severity was worse?]** slightly over follow-up in the study 193 (Figure 7).

**[H2] Clinical sequelae in survivors of EVD** **[Au: new subsection, heading ok?]**

 **[Au: text in green moved up from the end of the Physical sequelae subsection for flow, as it’s a nice introduction]** WHO treatment guidelines for the clinical management of EVD survivors were rapidly developed and published during the Western Africa outbreak. These guidelines, by necessity, are based on consensus expert opinion on the best management of a clinical disease that is still being characterized; clinical evaluation of therapeutic interventions have not yet been performed 204. With the exception of EVD-associated uveitis, **[Au: and what is the primary management goal for uveitis? Please add for completeness]** symptom alleviation focusing on pain management is a primary focus, with analgesics and NSAIDs recommended. Also, the diagnosis and appropriate management of ophthalmological and mental health conditions may be difficult given the overall low number of ophthalmologists **[Au: and phychiatrists/psychologists?]** and limited supplies of ophthalmological and psychotropic medications in Africa

**[H3] Physical sequelae [Au: new subsection, title ok?]**

Post-EVD arthralgias seem to be particularly prevalent and have been reported in up to 87% of survivors—with symmetric polyarticular involvement affecting (in order of decreasing frequency) the knees, back, hips, fingers, wrists, neck, shoulders, ankles, and elbows 192. The presence of arthralgias has been directly associated **[Au: based on the abstract of the cited reference, can we say “the severity of arthralgias has been negatively associated”, for clarity?]** with recovery of functional status 194. Although rarely described, physical findings are often unremarkable without overt erythema or swelling 192,194,195. Imaging of a limited number of joints has thus far been unrevealing, and, in the only joint arthrocentesis (aspiration of synovial fluid from a joint capsule) **[Au: definition ok?]** that has been reported, EBOV could not be detected by PCR in the synovial fluid 196. **[Au: edits ok?]**

 Ocular symptoms and signs, including retro-orbital pain, blurry vision, eye pain, sensitivity to light, and conjunctival injection **[Au: infection?]** also seem to complicate EVD recovery for a substantial proportion of adult (14–60%) and pediatric (32%) survivors 191,192,194,195,197-203. These signs and symptoms are most frequently due to uveitis, or intra-ocular inflammation, which has been reported most frequently within the first 12 weeks (but sometimes even after a year) following hospital/ETU discharge. **[Au: Please reference this statement.]** However, the true incidence and prevalence of ocular complications are uncertain, as diagnosis requires advanced ocular equipment, including a slit lamp, and ophthalmological expertise, both of which are rare in resource-limited settings. Careful characterization of the clinical phenotype and natural history of uveitis in EVD survivors is ongoing, but emerging reports suggest involvement of all anatomic locations (anterior uveitis **[Au: so affecting the front of the uvea, the iris?]** in 46–62%, posterior uveitis **[Au: affecting the choroid in the back?]** in 26%, and pan-uveitis in 21–25% of examined populations) 195,200. Although most clinical sequelae after EVD seem to improve over time, in the largest study of EVD survivors to date, the frequency of uveitis actually increased over study follow-up. **[Au: Please reference this statement.]** Patients may also develop structural ocular complications, most commonly cataracts, that require surgical intervention. In one study, 7 of 57 patients with post-EVD uveitis (12%) were also diagnosed with cataracts, and at least 3 of 57 **[Au: just to clarify, 7 were diagnosed at initial assessment, and in addition to these 7, 3 more developed cataract(s) during the course of the study (after being already diagnosed with uveitis)?]** developed cataract(s) following the onset of uveitis. These findings raise the concern for long term visual disability if complications of uveitis **[Au:OK?]** are not diagnosed and treated early 195. Timely diagnosis and early appropriate cycloplegia and anti-inflammatory treatment (topical or systemic steroids depending on severity) for uveitis **[Au: do you mean “Timely diagnosis and early appropriate treatment of uveitis (anti-inflammatory treatment with topical or systemic steroids depending on severity) and cycloplegia (the paralysis of the ciliary muscle, which impairs the ability to focus on nearby objects and can be treated with XXXX)”? or, please clarify]** as well recognition and management of complications are crucial to avoid long term visual disability. Recurrent uveitis has been described. **[Au: Please reference this statement.]**

Neurological issues (headache, memory loss, mental status changes, seizures and insomnia), psychiatric conditions (anxiety and depressive disorders and post-traumatic stress disorder, see next section) **[Au: I have separated the neurological from the psychiatric conditions, ok?]** , dermatologic disorders (alopecia and rashes), gastrointestinal issues (poorly defined abdominal pain syndromes) auditory issues (hearing loss and tinnitus), and generalized **[Au: instead of constitutional for consistency with figure 7, ok?]** symptoms, including severe and persistent fatigue, have also been reported in a substantial number of EVD survivors 192,194,195,197,198,201,202. Many of these conditions have important functional limitations; greater than one-third of survivors in a single study reported health problems lasting more than 1 year, and 29% indicated that their health problems limited their ability to walk or run 193. Additionally, gender-specific complications (for example, orchitis and amenorrhea) and sexual dysfunction in both women and men have been reported. Association of these complications with EVD and the consequences for future fertility remain unclear 191,194,195,201.

**[Au: subsection on mental issues moved up, as these are sequelae as well]**

**[H3] Mental health and psychosocial sequelae**

In addition to the physical complications of EVD, reports from the current epidemic indicate that psychological sequelae have a substantial effect on the lives of survivors. Survivors not only experienced a life-threatening event but often also the loss of immediate family members to EVD. In one study of 24 survivors, all reported losing at least one family member, and 67% of survivors reported witnessing the death 219. The loss of a family member was significantly **[Au: please confirm it means statistically]** associated with signs of depression (odds ratio 5.7; 95% CI 1.2–28.0) and an inability to concentrate (adjusted odds ratio 10.1; 95% CI 1.7–60.7) 220. Coping with such loss was probably compounded by an inability to observe traditional burial practices owing to infection control procedures designed to mitigate EBOV transmission 219,221. Furthermore, EVD has resulted in >16,000 orphans, **[Au: in what outbreak?]** further complicating the recovery of **[Au: these?]** children in particular, and affected communities in general 222,223.

Survival can be complicated by post-traumatic stress disorder symptoms and stigma. Within the first month of discharge, 71% of survivors experienced arousal reactions, such as racing heart, abdominal discomfort and dizziness, when reminded of their experiences. In addition, 21% of survivors reported distressing thoughts about their experiences and difficulty sleeping 219,220. . **[Au: sentence in green moved up for flow]** Furthermore, survivors have only limited access to psychiatrists or counselors who are trained in dealing with post-traumatic stress disorders. Additionally, almost a third of survivors reported experiencing stigma from their community upon return from an ETU, as manifested by social distancing by community members and even family members and by verbal abuse 219. Questions regarding viral persistence and possible shedding also contribute to social isolation and stigmatization

Compounding these emotional sequelae is the loss of employment opportunities. A substantial proportion of EVD survivors have lost their livelihoods. **[Au: Please reference this statement.]** Many healthcare workers who were occupationally exposed and infected have not been welcomed back to their previous positions. **[Au: do you mean healthcare workers from developed countries who travelled to the affected areas and then went back? Or also local workers?]** The loss of employment exacerbates social isolation, feelings of worthlessness, and poverty. Full recovery from the trauma of the EVD experience is not possible without full reintegration into society, including its work force.

**[H2] Mechanisms of sequelae after EVD [Au: heading slightly edited, ok?]**

 **[Referee comment: Sequelae section is thorough and well written but overly long compared to other sections (I am not keen on classifying sequalae as ‘phenotypes’)]**

 **[Au: I agree with this comment, so I have edited for brevity, please check carefully (this will also help to give you some extra room to address comments elsewhere)]**

Putative inflammatory and immune activation pathways may underly the pathogenesis of clinical sequelae in EVD survivors, but these pathways remain speculative and data to support specific therapeutic approaches are sparse. In a few survivors, an inflammatory component to joint and muscle pain is suggested by stiffness associated with inactivity and response to steroid therapy, although the classical signs of infectious arthritis (pain, redness and swelling) **[Au: heat too?]** are typically not present. **[Au: Please reference this statement.]** A significantly **[Au: statistically?]** increased **[Au: anti EBOV?]** IgG antibody titer in survivors with arthralgias compared with survivors without arthralgias supports a role for sustained immune activation and inflammation in the pathogenesis of post-EVD arthralgias 192, a hypothesis consistent with findings of polyarthropathies associated with other viral infections 205-207.

 Sustained immune activation 1 month after clearance of viremia has been found in four survivors of EVD, as evidenced by persistent **[Au: circulating in the blood?]** EBOV antigen-specific activated CD8+ and CD4+ T-cells 90.This sustained T-cell response support the persistence of viral antigen stimulation. For example, ocular disturbances suggest ongoing antigen presentation with intense immune responses leading to local inflammation. In one study, **[Au: high?]** viral load **[Au: during acute EVD?]** was associated with post-recovery uveitis 200. These findings suggest that direct viral injury and/or the immune response at the time of active infection **[Au: but immune response is active even 1 month after active infection, so the timing of the immune response is not relevant, no? please clarify]** contribute to post-EVD complications and that viral loads are markers of severe disease 193,200 **[Au: edits ok?]**

**[H3] Viral persistence**

Although EBOV RNA can be found in virtually any body fluid of individuals with acute EVD, **[Au: Please reference this statement.]** historically little is known about viral kinetics in body fluids other than blood. EBOV can persist in immune-privileged sites (including the central nervous system, eye, the urogenital system, the placenta, and potentially breast milk) and viral persistence may be associated with recrudescent generalized or organ-specific inflammatory disease (uveitis and meningoencephalitis). Two case reports have documented the development of encephalopathy and meningoencephalitis 13 days and 9 months after clearance of viraemia, and viable EBOV was detected in the cerebrospinal fluid in one case 212. **[Au: only one case report is referenced, please provide the reference for the other.]** Additionally, in a patient with severe unilateral uveitis, replication-competent EBOV was detected in the aqueous humor at high levels 9 weeks after clearance of viremia 208.

 **[Au: new paragraph]** Despite clearance of viraemia, some survivors continue to shed live EBOV in these immunologically-protected compartments, thereby posing a public health risk of sexual transmission and reignition of outbreaks 49,58,59,90,192,208-211. Studies have investigated clusters of EVD cases that occurred sporadically after the peak of the 2013–2016 Western Africa EVD outbreak , each thought to be initiated by a transmission event involving viral persistence in EVD survivors, typically in semen 213.

A few cases of EVD in Guinea in 2016 have been attributed to a sexual transmission event involving a survivor: EBOV RNA was detected in his semen ~ 500 days after EVD onset, although isolation of infectious virus was not possible 139. **[Au: sentence heavily edited for clarity, please check.]** These findings led to the implementation of new recommendations regarding the practice of safe sex for EVD survivors. **[Au: is there a reference we could cite here?]** Despite the detection of EBOV RNA in a few vaginal fluid swabs from female EVD survivors (up to 37 days after disease onset) 215, no studies have thoroughly evaluated the persistence of EBOV in cervical-vaginal fluid 214. Though rarely documented, viral persistence has been associated with transplacental transmission, and EBOV was detected in an infant stillborn to a mother whose blood was negative for EBOV RNA detected by PCR tests, but had detectable levels of anti-EBOV IgM and anti-EBOV IgG antibodies 216. EBOV RNA was also recovered from breastmilk following the death of an infant, suggesting transmission via breastfeeding 217. Of note, in many of these studies unvalidated diagnostic tests for the detection of EBOV RNA have been used, and, therefore, the limits of detection and the repeatability of the assays are unknown. EBOV persistence in immune-privileged sites has been reported in non-human primates who survive infection; these and future studies will provide crucial insights into the mechanisms of viral persistence 218.

 **[H1] OUTLOOK**

 **[Au: edited for brevity and reducing repetition]**

**[Referee comment: The Outlook section needs to cover the vaccine(s) in more detail given this has been the major advance. What is the epidemiological outlook? Are large scale and protracted outbreaks the ‘new norm’?]**

 **[Referee comment: I would wish to see a line on better understanding of culture, belief and the role of traditional healers to be included in future studies. ]**

The 2013–2016 Western Africa EVD outbreak considerably increased the knowledge base **[Au: do you mean the knowledge in the general global population? Can we say “awareness” instead?]** , attention, and the approach of the global community to this disease. During the outbreak, the rapid distribution, administration, and evaluation of medical countermeasures, appropriate supportive clinical care and preventive measures lacked coordination. **[Au: edited sentence ok?]** However, important lessons learned have been subsequently applied during smaller EVD outbreaks, including the ongoing second-largest outbreak in history in COD, where the capacity of response teams to understand and apply new strategies has been remarkably accelerated. Hopefully, multipronged prevention approaches (including for example ring vaccination and advanced supportive care) will not only interrupt transmission and prevent new cases but also decrease CFR.

 **[Au: new paragraph]** EBOV-specific therapeutics initially made available through a compassionate use protocol have been subsequently studied in the rapidly initiated PALM randomized clinical trial. Interim results suggest significant survival benefits for REGN-EB3 (a cocktail of three monoclonal antibodies) and the monoclonal antibody mAb114. **[Au: Please reference this statement.]** Despite these interventions, the CFR of patients presenting with high viral loads and late disease manifestations remains unacceptably high. **[Referee comment: the overall CFR is high in this epidemic, not only among those with high VL. It is important to underline this, because it is essential to understand the reasons of the current failure in the DRC.]** Medical countermeasure success is likely to be most dependent on EBOV serum titers, yet the relationship between the mechanisms of action and dosing of the therapeutic agents and viral kinetics is poorly understood. **[Au: edited sentence ok?]** **[Referee comment: There are no references provided to support the statement that the success of medical countermeasures will be dependent on EBOV serum titers? These should be provided. What about further optimization of medical care? For example, in the treatment of sepsis with appropriate antibiotics, patients still succumb to infection if they do not receive optimal care.]** Although the PALM trial will help to clarify this issue in humans, no study has systematically addressed which candidate antiviral may be preferred over another at particular time points during the disease, or whether particular countermeasures may be more effective to penetrate immune-privileged tissues than others. The potential utility of multiple EBOV-specific or host-directed therapeutics in combination has not been studied in humans, either.

Despite encouraging advances, supportive treatment of EVD remains largely based on logical assumptions borrowed from other disease syndromes (for example, septic shock) and from observations of very small numbers of patients; however, data to support or refute current supportive care approaches are scant and difficult to obtain given the difficulties in ethically exploring these approaches in the research setting in humans with EVD. Similarly, the influence of pre-existing, co-existing or super-infections with endemic pathogens (for example, *Plasmodium* spp., HIV-1, measles virus and mycobacteria) on the course and outcome of EVD remains to determined, as does the influence of genetic or other host factors in different ethnicities and regions.

The medical follow-up of EVD survivors is not trivial, either. For those with physical or psychological **[Au: added for completeness, ok?]** sequelae, even the most basic medications are scarce, and simple diagnostic testing is non-existent or prohibitively expensive. Ultimately, longitudinal research to inform care of EVD survivors is crucial to understand better the mechanisms underlying post-EVD complications to implement appropriate treatment, define the outer time limit of viral shedding to inform public health recommendations, and support the unprecedented number of survivors who continue to experience social isolation and stigma.

The typical setting of EVD outbreaks in poor or underdeveloped areas with limited access to medical infrastructure and in multiethnic populations characterized by vastly different mobility and health seeking behaviors, the presence of armed conflicts, and the rarity, unpredictability and typically limited size (dozens to a few hundred cases) of EVD outbreaks in general pose immense challenges to conducting clinical trials on both patients with acute EVD and EVD survivors. Clinical trials are also not necessarily welcomed by local populations, who, owing to the African history of colonialism, may be suspicious of outsiders’ intentions. In a very challenging setting of the current COD EVD outbreak, the clinical research response represents an important step forward but also emphasizes these challenges.

 On a positive note, EBOV diagnostics have greatly improved over recent years and are now relatively broadly available in countries with a history of EVD outbreaks. With local or international support, crucial infrastructures (for example, laboratories, field stations and treatment centers) have been build in several countries. National and international health agencies (including the WHO) and nongovernmental organization streamlined their interactions and communication strategies to be able to work together to curtail future outbreaks. Thus, whereas many problems remain to be tackled, we are hopeful that the response to future outbreaks will be even more rapid and more coordinated than before, and that future outbreaks will involve an increasing number of EVD-experienced health-care staff and of clinically successfully tested medical countermeasures to decrease CFR **[Au: instead of mortality, ok?]** and prevent or ameliorate sequelae.

 Zooming out from the outbreak setting, ecological and epidemiological uncertainties remain to be clarified, including the identity of the natural EBOV reservoir host and the determinants and dynamics of EBOV spillover and subsequent human-to-human transmission. Understanding the animal-human interface may prevent the introduction of EBOV into the human population. Finally, EBOV is not the only filovirus that causes severe human disease; indeed, MARV seems statistically to be even more lethal than EBOV. **[Au: Please reference this statement.]** By extrapolation, MARV could cause an outbreak of the scale of the 2013–2016 EVD outbreak. Our limited understanding of Marburg virus disease, let alone the diseases caused by other ebolaviruses and marburgviruses, includes large knowledge gaps in the entire spectrum from basic science to human clinical disease. Progress in filovirus disease, including nascent medical countermeasure and vaccine development endeavors, lag far behind the recent progress made in EVD.

**BOXES**

**Box 1. WHO and CDC EVD case definitions [Au: formatted for style]**

 **[Au: just to confirm, these are verbatim the WHO and CDC definitions, correct? I need to know because if they are not I can edit for style (e.g. diarrhoea instead of diarrhea). Also, why are some letters within square brackets?]**

[H1] WHO definition for a suspected case of Ebola virus disease (EVD)

* “a) **[Au: there’s no “b)” in this definition, is it missing or should we delete the “a)”?]** [a]ny person, alive or dead, suffering or having suffered from a sudden onset of high fever and having had contact with a suspected, probable or confirmed Ebola or Marburg case or a dead or sick animal; or
* [a]ny person with sudden onset of high fever and at least three of the following symptoms: headache, lethargy, anorexia / loss of appetite, aching muscles or joints, stomach pain, difficulty swallowing, vomiting, difficulty breathing, diarrhea, hiccups; or
* [a]ny person with inexplicable bleeding; or
* [a]ny sudden, inexplicable death” 224.

[H1] The US Centers for Disease Control and Prevention (CDC) definition for a person under investigation for EVD

* Someone with both “1. Elevated body temperature or subjective fever or symptoms, including severe headache, fatigue, muscle pain, vomiting, diarrhea, abdominal pain, or unexplained hemorrhage; **AND** 2. An epidemiologic risk factor within the 21 days before the onset of symptoms” 225.
* Such risk factors include direct contact with “[B]lood or body fluids (urine, saliva, sweat, feces, vomit, breast milk, and semen) of a person who is sick with or has died from…EVD”, **[Au: is there an omissis here?]** [O]bjects (such as needles and syringes) contaminated with body fluids from a person sick with EVD or the body of a person who died from EVD”, “[I]nfected fruit bats or nonhuman primates (such as apes and monkeys)”, and “[S]emen from a man who recovered from EVD (through oral, vaginal, or anal sex)” 226.

**Box 2: Case definition during an outbreak [Au: tentative title, please feel free to edit]**

A retrospective cohort study from a holding unit (a temporary holding facility for suspected or confirmed patients waiting for a bed in an ETU) in Freetown, Sierra Leone, reported the clinical characteristics of 724 individuals who underwent EBOV PCR testing between May and December 2014 (Ref51). The standard case definition adapted by the MOHS-SL from the existing WHO case definition had suboptimal performance, with a sensitivity of 57.8% and specificity of 70.8%. A subgroup analysis revealed that 15 (9%) of 161 confirmed EVD cases lacked two of the major criteria required to fulfill the EVD case definition, that is, history of fever and risk factor for EVD exposure. Additionally, an Ebola (virus disease) Prediction Score was developed to improve the performance characteristics of the existing case definition 52. **[Au: do you mean the MOHS-LS-adapted WHO definition?]** This score was derived from a retrospective analysis of clinical and epidemiological characteristics of 382 individuals presenting to a Liberian ETU from September 2014 to January 2015, and contained the following 6 predictors of EVD: contact with an individual with EVD **[Au: edits ok?]** , diarrhoea, anorexia, myalgia, dysphagia, and absence of abdominal pain. With a receiver-operator-curve **[Au: please explain what the ROC indicates]** of 0.75 (95% CI 0.70–0.80) for the prediction of laboratory-confirmed EVD, the prediction score was not found to be practically useful for determining EVD status without **[Au: additional?]** specific testing, although it may be useful in stratifying risk in future outbreak settings 52. **[Referee comment: There is no discussion of the potential reasons prediction scores might be difficult to develop (e.g., overlap of early symptoms with many infectious diseases, prevalence of other infectious diseases in area, etc..)]**

**FIGURE LEGENDS**

**Figure 1 Filovirus taxonomy and Ebola virus transmission**

A| Taxonomy of the genus *Ebolavirus*. Thus far, five ebolaviruses have been associated with human infections and four of them have been identified as pathogens. B| The natural reservoir host(s) of Ebola virus (EBOV) has(have) yet to be identified. **[Referee comment:** **Fig 1b: To the understanding of this reviewer, several EVD outbreaks had been initiated by contact of humans to infected monkeys or other wildlife. This mode of transmission from nature to humans should be represented.]** **[Au: I have asked our art editor to update this figure accordingly]** Multiple data indicate a direct or indirect role of bats in EBOV ecology, but to date, EBOV has not been isolated, nor a near-complete EBOV genome has been detected in any wild animal 227. However, it is tempting to speculate that EVD is a zoonosis (that is, an infectious disease caused by an agent transmitted between animals and humans **[Au: should this be “from animals to humans”?]** ) because retrospective epidemiological investigations have often been able to track down the probable index cases of EVD outbreaks. These individuals had been in contact with wild animals or had handled the carcass of a possible accidental EBOV host 7,228. **[Au: panels C and D from figure 5 moved here for layout reasons]** C | Scanning electron microscopic (SEM) image of EBOV particles (green) budding from grivet (African green monkey **[Au: I’ve added the alternative name to clarify that it’s a monkey, ok?]** ) cells. D | Transmission electron microscopic (TEM) image of EBOV particles (green) in grivet cells 1,229. **[Au: please provide the source of the images in panels C and D]**

**Figure 2. Ebola virus disease outbreaks. [Au: title slightly edited, ok?]**

The map shows the location and years of all reported EVD outbreaks. **[Au: added the first sentence to describe the figure (details on the colours are in the key), ok?]** Two cases of laboratory-acquired EVD occurred in Russia (not shown). Modified and updated from Ref 4, as of September 10, 2019. **[Au: please provide the figure number of the original figure in the book, as we’ll need it to apply for permission to re-used it]**

**Figure 3. Reconstructed EBOV transmission chains during the 2013**–**2016 Western African EVD outbreak. [Au: title shortened for style]** Molecular evidence using hundreds of individual Ebola virus (EBOV) genomes sequenced from individual patients indicates that the index case of the outbreak acquired EBOV by unknown means at the end of 2013 in or around Méliandou in Guinea. From there, person-to-person transmission enabled EBOV to spread (coloured lines) throughout the country, to cross borders and ultimately to affect a total of 15 countries **[Au: changed from 10 to 15, ok?]** (see also Figure 2). Modified from Ref 54. The direction of EBOV spread is represented by the lines and goes from the thick end to the thin end. **[Au: I’ve added the last sentence to explain the lines, ok?]**

**Figure 4. EBOV genome and lifecycle.**

 **[Referee comment: Might be helpful to show site of action of current MCMs in figure 4.]** **[Au: I like this suggestion, please indicate in the figure where we should add them]**

A | Ebola virus (EBOV) has a linear, non-segmented, negative-sense, single-stranded **[Au: added single-stranded for completeness, ok?]** RNA genome (≈19 kb) expressing 7 structural and several nonstructural proteins from seven genes: *NP* encodes nucleoprotein NP, *VP35* polymerase cofactor VP35, *VP40* matrix protein VP40 *GP* glycoprotein GP1,2 **[Au: so is this GP a structural GP, as opposed to the secreted ones?]** and secreted glycoproteins, *VP30* transcriptional activator VP30, *VP24* RNA complex-associated protein VP24 and *L* large protein L. ORF, open reading frame 1,229. **[Au: please explain what gene overlaps are (the genes do not actually overlap in the figure, should they?); What do the length and thickness of the ORFs represent?]**B | The binding of EBOV particle to the host cell-surface attachment factors is mediated by glycoprotein GP1,2, which is formed by two subunits, GP1 and GP2 (1) **[Au: added highlighted text for clarity and completeness, ok?]** . Binding to the host cell membrane triggers viral particle endocytosis (2). In the late endosome, GP1,2 is sequentially cleaved by cathepsin B (CatB) and cathepsin L **[Au: L1 or L2?]** (CatL)(3). A low-pH induces GP1 interaction with the EBOV receptor NPC1, with subsequent GP2-mediated fusion of the particle envelope with endosomal membrane and thereby expulsion of the ribonucleoprotein complex (RNA+ NP) into the cytosol (4). There, the filovirus genome is replicated (5) and the filovirus genes are transcribed into mRNAs (6). Viral proteins are translated (7), and GP1 and GP2 subunits and soluble GPs (sGPs) XXXXXXX **[Au: please complete this sentence and explain the different pathways of these proteins (step 8 in the figure)]** (8). Mature progeny ribonucleoprotein complexes and viral proteins **[Au:OK?]** are transported to the plasma membrane, where particle budding occurs (9). Panel A courtesy of Jiro Wada, IRF-Frederick, Fort Detrick, MD, USA; **[Au: is this correct?]** Panel B adapted from White, J., Schornberg, K. A new player in the puzzle of filovirus entry. *Nat Rev Microbiol* **10,**317–322 (2012) doi:10.1038/nrmicro2764 **[Au: please add this reference as a numbered entry in the bibliography]**

 **[Au: for layout reasons, panels C and D of figure 4 have been moved to figure 1]**

**Figure 5. Conceptualized EVD pathogenesis.**

EBOV particles enter the body through dermal injuries microscopic or macroscopic wounds) or via direct contact via mucosal membranes. Primary targets of infection are macrophages and dendritic cells. Infected macrophages and dendritic cells migrate to regional lymph nodes while producing progeny virions. Through suppression of intrinsic, innate, and adaptive immune responses, systemic distribution of progeny virions and infection of secondary target cells occur in almost all organs. **[Au: please add one-two sentences to explain why we focus in this figure on kidneys and liver]** Eventual lysis of infected cells leads to additional inflammatory reactions, which contribute to partial or complete organ impairment.

**Figure 6.** **Clinical acute EVD course in a conceptualized patient over time.**

The time course of the clinical manifestations (top), laboratory findings (middle) and immune response (bottom) in patients with EVD. . Haemorrhagic manifestations include oozing from venipuncture sites, hemoptysis, hematemesis, melena and vaginal bleeding. **[Au: please also briefly touch on respiratory and renal failure for completeness]** Neurological manifestations include meningoencephalitis and cerebrovascular accidents **[Au: what do you mean by accidents?]** . ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; CPK, creatine phosphokinase; Hb, haemoglobin; HCT, haematocrit; WBC, white blood cell count; PMNS, polymorphonuclear leukocytes; PLTS, platelets; PT, prothrombin time; PTT, partial thromboplastin time; ↑, increase; ↓ decrease. **[Au: Please provide the source of this figure; were any parts of this figure previously published?]**

**Figure 7. Clinical sequelae in survivorsof EVD.**

Clinical sequelae in survivors of Ebola virus disease (EVD) that are supported by evidence that includes physical examination of the invididuals. Patient-reported symptoms are not included in this figure. aIn the PREVAIL III clinical trial, a prospective, controlled studyassessing symptoms in survivors that had a >10% increase in prevalence compared with control close contacts, this symptom had an increased odds ratio **[Au:OK?]** (*p*<0.0001) compared with contact close controls **[Au: sentence edited for clarity, is it ok?]** ; bIn the PREVAIL III clinical trial, when symptoms in survivors were compared with symptoms in control close contacts (regardless of any increase in their prevalence in survivors), this symptom had an increased odds ratio **[Au:OK?]** (*p*<0.01) compared with control close  **[Au: sentence edited for clarity, is it ok?]**; cData from uncontrolled cohorts, case series or case reports; dMost common abnormalities in neurological exam are oculomotor, abnormal reflexes, **[Au: do you mean abnormal oculomotor reflexes?]** tremor, or sensory examination **[Au: do you mean abnormal sensory examination?]** ; eMost common abnormalities include irregular heart rate, cardiac murmur, decreased breath sounds, rales (crackling lung sounds) **[Au: definition ok?]** and wheezes; fMost common abnormalities include abdominal tenderness, mass, or distention; gMost common abnormalities include muscle tenderness and decreased range of motion. Based on **193,195,200,208,212,230-233. [Au: we cannot have the references in the title (for style), so I moved them at the end. However, it would be preferable if you could integrate them in the legend next to the appropriate words]**

**TABLES**

**Table 1 Ebola virus disease outbreaks statistics**

 **[Au: I’ve changed the country names to the currently used names, for clarity; I’ve also removed the last row (with average CRF and total cases) for style, as it would require separate headings etc; this information is already in the epidemiology section, so it’s not lost!]**

 **[Au: two referees commented on the reference for this table:]**

 **[Referee comment: Table 1 provides data on outbreaks, case numbers and CFRs, and references a medical textbook.]**

 **[Referee comment: The reference provided as a source of this data is a medical textbook chapter from 2018. The provided case numbers and CFRs differ from what is on the CDC website, including: Gabon (1994-1995)/(1996)/(2002), Zaire (1995), COG (2002)/(2005) & West Africa (2013-2016). COD 2014 & 2017 appear swapped.]**

|  |  |  |
| --- | --- | --- |
| **Country (year)** | **Case-fatality rate (%)** | **Number of cases** |
| COD (then Zaire) (1976) | 88.1 | 318 |
| COD (then Zaire) (1977) | 100.0 | 1 |
| Gabon (1994–1995) | 61.5 | 52 |
| COD (then Zaire) (1995) | 77.3 | 317 |
| Russiaa (1996) | 100.0 | 1 |
| Gabon (1996) | 67.7 | 31 |
| Gabon, also exported to South Africa (1996–1997) | 74.2 | 62 |
| Gabon, COG (2001–2002) | 78.2 | 124 |
| COG, also exported to Gabon (2002) | 90.9 | 11 |
| COG (2002–2003) | 89.5 | 143 |
| COG (2003–2004) | 82.9 | 35 |
| Russiaa (2004) | 100.0 | 1 |
| COG (2005) | 81.8 | 11 |
| COD (2007) | 70.5 | 264 |
| COD (2008–2009) | 46.9 | 32 |
| Guinea, also exported to Liberia, Mali, Senegal, Sierra Leone, and, from Liberia, **[Au: I’ve added the highlighted text so the table matches figure 2, ok?]** to France, Germany, Italy, , Netherlands, Nigeria, Norway, Spain, Switzerland, UK, and USA (2013–2016) | 39.5 | 28,652 |
| COD (2014) | 50.0 | 8 |
| COD (2017) | 71.0 | 69 |
| COD (2018) | 61.1 | 54 |
| COD, also exported to Uganda (2018–present) | 67.0 | 3,073 |

aLaboratory-acquired infection

COD, Democratic Republic of the Congo; COG, Republic of the Congo; Modified and updated from 4.

**Table 2. EBOV detection tests used in the field (adapted from 234) [Au: shortened title ok?]**

 **[Au: just to clarify, is this a complete list of all the tests? If not, we need to state so and clarify the reasons why some tests were left out]**

 **[Au: I have reorganized the table on the basis of the referees’ comments below; for readability, I have created a new one rather than editing the original table, which you can find after the revised one for your reference only.**

**Please fill all empty cells, as we cannot have any in our style; if no specificity data are available, please state so]**

 **[Referee comment: Avoid using RDT as this is a generic term and does describe the type of test it is. It is better to specify the assay type(i.e., type of PCR, lateral flow, etc…)]**

**[Referee comment: Under the LLOD/Sensitivity(%) column heading, please provide published sensitivity (ability of test to correctly identify those with the disease) and specificity (ability of the test to correctly identify those without disease) for each diagnostic. Recommend changing column heading to reflect the addition of specificity.]**

**[Referee comment: Specificity column heading name should be changed to something else as specificity has meaning for diagnostic testing]**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Test (Manufacturer)** | **Test type** | **Target** | **Samples** | **Sensitivity** | **Specificity** | **Viral species detected** |
| **RDTs** |
| Dual Path Platform (DPP) Ebola Antigen System (Chembio)a |  **[Au: how does this test work?]**  | Ebolavirus matrix protein **[Au:OK?]** VP40 | Whole blood and plasma | * Qualitative;
* Less sensitive than PCR;
* Requires confirmatory testing
 |  | Ebolaviruses |
| OraQuick Ebola Rapid Antigen Test (OraSure Technologies)a,b | immune-chromatographic immunoassay | VP40 antigen **[Au: is this the same as “Ebolavirus matrix protein VP40”? if so, please use terminology consistently]**  | Oral fluid and whole blood | * LLOD: 53,000 pg/ml **[Au: instead of pictogram, can we say 53 nanograms? Also, pg of VP40 or of viral material?]**
* 94% (from **[Au:OK?]** oral fluid from deceased patients **[Au: instead of “cadaveric”, ok?]** )
 | 100% **[Au: for EBOV?]** |  |
| SD Q Line Ebola Zaire Ag test (SD Biosensor)b | lateral flow immunoassay | GP **[Au: do you mean GP1,2?]** , NP and VP40 protein antigens | Plasma, serum and whole blood | 84.9% **[Au: from all samples?]**  | 99.7% **[Au: for EBOV?]** |  |
| **PCR-based tests** |
| Ebola Real Time RT-PCR Kit (Liferiver Bio-tech)b | Fluorescent real-time RT-PCR **[Au:OK?]**  | EBOV nucleic acids | Serum, whole blood, oral swab, body fluid **[Au: which one?]** and urine | LLOD: 23.9 copies of viral genome **[Au:OK?]** per reaction |  | Ebolaviruses **[Au: all of them? Because the target is EBOV]**  |
| EZ1 test (DOD)a |  **[Au: what kind of PCR is this?]**  | EBOV nucleic acids | Whole blood and plasma | * Qualitative;
* LLOD: 100‒1,000 pfu/ml depending on live **[Au: what do you mean by live? The virus doesn’t need to be viable for PCR, no?]** EBOV strain and cycler used
 |  | EBOV |
| FilmArray NGDS BT-E (BioFire)a | Fluorescent nested multiplex PCR **[Au: RT-PCR?]**  | EBOV nucleic acids | Whole blood, plasma and serum | LLOD: 1,000 pfu/ml or 4.36 x 103 genome equivalentsc **[Au: please define this in the footnote]** per ml for live virus |  | EBOV |
| FilmArray Biothreat-E (BioFire)a | Fluorescent nested multiplex PCR **[Au: RT-PCR?]**  | EBOV nucleic acids | Whole blood and urine; saliva has been tested **[Au: and? not approved yet as sample?]**  | * LLOD:4,059 copies **[Au: of viral genome? Or GEs?]** per ml
* 95%
 |  | EBOV, BDBV and TAFV; MARV not detected **[Au: can we omit MARV not detected? If a virus is not listed it means it’s not detected, no?]**  |
| Idylla Ebola Virus Triage Test (Biocartis)a | Fluorescent PCR **[Au:OK? should it be RT-PCR?]**  | EBOV nucleic acids | Whole blood | * LLOD: 465 pfu/ml or 178 copies/ml
* 97%
 |  | EBOV and SUDV |
| LightMix Ebola Zaire (TIB MolBio with Lightcycler (Roche))a | **[Au: what kind of PCR is this?]** | EBOV nucleic acids | Whole blood | * LLOD 4,781 pfu/ml
* 95%
 | 100% |  **[Au: EBOV?]**  |
| Ebola Virus NP Real-Time PCR (ThermoFisher (CDC))a | **[Au: what kind of PCR is this?]** | RNA **[Au: do you mean EBOV nucleic acids?]** , NP **[Au: NP antigen? Or do you mean that this test targets NP RNA only?]**  | Whole blood, serum and plasma | * LLOD: 62.5 copies/ml
* 99.80%
 |  | EBOV |
| RealStar Ebolavirus RT-PCR Kit (Altona Diagnostics)a,b  | Fluorescent PCR **[Au: RT?]**  | Ebolavirus **[Au: should this be plural?]** nucleic acids | Plasma | * LLOD: 1 pfu/ml
* >95%
 |  | Ebolaviruses |
| VP40 RT-PCR (CDC)a | Fluorescent PCR **[Au: RT?]**  | VP40 **[Au: RNA?]**  | Whole blood, serum, plasma and urine | LLOD: 400‒600 TCID50d**[Au: please define in the footnote]** per ml from whole blood; 600 TCID50/ml from urine; 250 TCID50/ml from human sera depending on extraction method **[Au: if the LLOD depends on the method, the number should be a range, not just “250”, no?]**  |  | EBOV |
| Xpert Ebola (Cepheid)a,b | **[Au: what kind of PCR is this?]** | RNA, NP and GP **[Au: do you mean NP and GP RNA only?]**  | Whole blood **[Au: should buccal swab be added here? (can we say oral instead, for consistency?)]**  | * LLOD: 82 copies **[Au: of viral genome?]** per reaction
* Whole blood, buccal swab: 100%
 | Whole blood: 99.5%; buccal swab 100% |  **[Au: EBOV?]**  |

aEmergency use authorization approved by the FDA

bEmergency use authorization approved by the WHO

cGenome equivalents are XXXXXXX **[Au: please complete]**

dTCID50 XXXXXXX **[Au: please complete]**

BDBV, Bundibugyo virus; CDC, US Centers for Disease Control and Prevention; DOD, US Department of Defense; EBOV, Ebola virus; FDA, US Food and Drug Administration; GP, glycoprotein; LLOD, lower limit of detection; MARV, Marburg virus; NP, nucleoprotein; PCR, polymerase chain reaction ; pfu: plaque forming units; RDT, rapid diagnostic test ; RT, reverse transcription; **[Au:OK?]** ; TAFV, Taï Forest virus; VP40, viral protein 40

 **[Au: original table below, *for your reference only*: please edit and complete the table above]**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Test** | **Test type** | **Manufacturer** | **Target** | **Acceptable Samples** | **LLOD/Sensitivity (%)** | **Specificity** | **Emergency Use Authorization approved by** |
| Dual Path Platform (DPP) Ebola Antigen System | RDT | Chembio | Ebolavirus VP40  | Whole blood, plasma | Qualitative; less sensitive than PCR; requires confirmatory testing | Ebolaviruses | FDA |
| Ebola Real Time RT-PCR Kit  | Fluorescent PCR | Liferiver Bio-tech | EBOV nucleic acids | Serum, whole blood, oral swab, body fluid, urine | LLOD: 23.9 copies/rxn | Ebolaviruses | WHO |
| EZ1 test | PCR | DOD | EBOV nucleic acids | Whole blood, plasma | Qualitative; LLOD: 100‒1,000 pfu/ml depending on live EBOV strain and cycler used | EBOV only | FDA |
| FilmArray NGDS BT-E | Fluorescent nested multiplex PCR | BioFire | EBOV nucleic acids  | Whole blood, plasma, serum | LLOD: 1,000 pfu/ml or 4.36 x 103 GE/ml for live virus | EBOV only | FDA |
| FilmArray Biothreat-E | Fluorescent nested multiplex PCR | BioFire | EBOV nucleic acids  | Whole blood, urine; saliva has been tested | * LLOD:4,059 copies/ml
* 95%
 | EBOV, BDBV, TAFV; MARV not detected | FDA |
| Idylla Ebola Virus Triage Test | PCR | Biocartis | EBOV nucleic acids detected via fluorescence | Whole blood; do not inactivate sample prior to testing | * LLOD: 465 pfu/ml or 178 copies/ml
* 97%
 | EBOV, SUDV | FDA |
| LightMix Ebola Zaire | PCR | TIB MolBio with Lightcycler (Roche) | EBOV nucleic acids | Whole blood | * LLOD 4,781 pfu/ml
* 95%
 | 100% | FDA |
| Ebola Virus NP Real-Time PCR | PCR | ThermoFisher (CDC) | RNA, NP | Whole blood, serum, plasma | * LLOD: 62.5 copies/ml
* 99.80%
 | EBOV | FDA |
| OraQuick Ebola Rapid Antigen Test | RDT immune-chromatographic immunoassay | OraSure Technologies | VP40 antigen | Oral fluid, whole blood | * LLOD: 53,000 pg/ml
* 94% cadaveric oral fluid
 | 100% | FDA, WHO  |
| RealStar Ebolavirus RT-PCR Kit  | Fluorescent PCR | Altona Diagnostics | Ebolavirus nucleic acids  | Plasma | * LLOD: 1 pfu/ml
* >95%
 | Ebolaviruses | FDA, WHO |
| SD Q Line Ebola Zaire Ag test  | RDT lateral flow immunoassay  | SD Biosensor | GP, NP, VP40 protein antigens | Plasma, serum, whole blood | 84.9% | 99.7% | WHO |
| VP40 RT-PCR | Fluorescent PCR | CDC | VP40 | Whole blood, serum, plasma, urine | LLOD: 400‒600 TCID50/ml whole blood depending on cycler; 600 TCID50/ml urine; 250 TCID50/ml of human sera depending on extraction method | EBOV | FDA |
| Xpert Ebola  | PCR | Cepheid | RNA, NP and GP | Whole blood | * LLOD: 82 copies/rxn
* Whole blood, buccal swab: 100%
 | Whole blood: 99.5%; buccal swab 100% | FDA, WHO |

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**Table 3. EVD candidate vaccines in phase III clinical trials**

 **[Au: the referees had sevareal comments on this table (see below), and to facilitate the revisions I have suggested a new, more-structured template, so we can clearly separate the trials and the various vaccines. I’ve made a start, please complete it also taking into account the referees’ comments.]**

 **[Referee comment: [the authors should] improve the balance in some areas, especially in the tables describing pros and cons of vaccines and therapeutics]**

**[Referee comment:** **Tables 3 and 4 should be updated based on known results from the current DRC outbreak in order keep this review current. For instance, the Janssen (J&J) vaccine is now Ad26 ZEBOV-GP prime with MVA-BN-Filo boost 56 days later.]**

**[Referee comment: information missing and recommended for inclusion on each trial:**

* **VSV Phase II/III trial (NCT02378753): Pre-exposure, Sierra Leone (at-risk) and no efficacy measure could be determined due to lack of EVD cases.**
* **VSV Phase IIIb trial (NCT03161366): Ring-vaccination, Efficacy info needed on table**
* **hAd26 Phase II/III trial (NCT02509494): Pre-exposure, Sierra Leone (at-risk) and outbreak ended prior to having the ability to determine an efficacy.**
* **hAd26 Phase III (NCT02543268): Pre-exposure, US population (no risk), safety & immunogenicity achieved**
* **MVA-BN Filo Phase III trial (NCT02509494): Pre-exposure, Sierra Leone (at-risk) and outbreak ended prior to having the ability to determine an efficacy.**
* **For VSV, there are publications suggesting a potential for neurotropism. (Cell Reports. 2019. 26(7):1718-1726. doi.org/10.1016/j.celrep.2019.01.069). Neurotropism may not have yet been seen due to insufficient number of people having received the vaccination and/or insufficient monitoring in outbreak environment. Should this be added as potential disadvantage?**
* **The VSV Ebola vaccination given as pre-exposure has only been shown to provide protection for one month after vaccination and there may be preclinical data to suggest that protection is lost in a few months. Given that the current outbreak has lasted more than a year and the vaccine has been given under ring-vaccination conditions, is it worth mentioning that the durability of protection is unknown as a potential disadvantage for VSV ring-vaccination?**
* **For hAd26, preexisting immunity against hAd26 (i.e., prior infection with hAd26) is high in Africa (Vaccine. 2010. 28:950-957) and it has been previously reported that prior immunity against vector reduces potency (J Virology. 2011. 85(9):4222-4233).**
* **For pre-exposure vaccination with hAd26 and VSV, durability of protection has not been shown in preclinical studies beyond 1-2 months.**
* **hAd26 or MVA-BN-Filo are both missing adverse event information. If there are no significant adverse events, then this should be stated]**

 **[Au: here is my suggested new template, original table (unedited) after it: please fill and complete this table using the information contained in the original one]**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Candidate vaccine(s)** | **Vaccine design** | **Study design** **[Au: arms and strategy]** | **Outcomes [** **Au: primary and secondary outcomes]** | **Results** | **Notes [Au: adverse effects, etc]**  | **Trial** |
| rVSVΔG-ZEBOV-GP (also known as BPSC-1001 and V920) | Replication-competent rVSIV expressing EBOV GP1,2 in place of VSIV glycoprotein |  |  |  |  | NCT03161366 |
| rVSVΔG-ZEBOV-GP (also known as BPSC-1001 and V920) | Replication-competent rVSIV expressing EBOV GP1,2 in place of VSIV glycoprotein |  |  |  |  | NCT02378753 |
| hAd26 ZEBOV-GP and MVA-BN-Filo | * hAd26 ZEBOV-GP: Replication-defective human adenovirus 26 vector expressing EBOV GP1,2
* MVA-BN-Filo: Replication incompetent modified vaccinia virus Ankara expressing EBOV, SUDV, MARV, TAFV GP1,2
 |  |  |  |  | NCT02509494 |
| Ad26 ZEBOV **[Au: GP?]** , 3 batches |  |  |  |  |  | NCT02543268 |
| Ad26 ZEBOV-GP and MVA-BN-Filo |  |  |  |  |  | NCT02509494 |
|  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |
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|  |  |  |  |
| --- | --- | --- | --- |
| **Candidate vaccine** | **Description** | **Study design and results** | **Disadvantages/Adverse effects** |
| **Recombinant vesicular stomatitis Indiana virus (rVSIV) vectors** |
| rVSVΔG-ZEBOV-GP (BPSC-1001, V920) | Replication-competent rVSIV expressing EBOV GP1,2 in place of VSIV glycoprotein (G) 235,236 | * Studied alone 237 (NCT03161366, NCT02378753)
* Antibody titers increased postvaccination by 28 days and were maintained to 24 months 238
 | Joint pain 236,237,239,240, rash 240,viremia 236,239,241, injection site pain 237,241, headache 240,241, fatigue 240, arthritis with females and history of arthritis as risk factors 238 |
| **Recombinant adenovirus vectors** |
| hAd26 ZEBOV-GP  | Replication-defective human adenovirus 26 vector expressing EBOV GP1,2 | Studied as prime vaccination then boost with MVA-BN-Filo, then a third vaccination with hAd26 ZEBOV-GP (NCT02509494, NCT02543268). |  |
| **Vaccinia virus vectors** |
| MVA-BN-Filo | Replication incompetent modified vaccinia virus Ankara expressing EBOV, SUDV, MARV, TAFV GP1,2 | Studied as boost for hAd26ZEBOV-GP followed by a third vaccination with hAd26ZEBOV-GP (NCT02509494)  |  |

**Table 4. EVD candidate therapeutics evaluated in the PALM study**

 **[Au: title shortened for style, as it’s very unlikely that it’d fit on one line in the final layout]**

 **[Au: as for table 3, the referees had several comments on this table as well (see below). I recommend to reformat the table using a template similar to the one I have suggested for table 3, which would take into account the reviewers’ comments and include both results from previous studies and the current results from PALM]**

 **[Referees’ comments on this table:**

* **The table can be updated with the latest information from PALM.**
* **The PALM RCT (NCT03719586) is a Phase II/III at clinicaltrials.gov, not phase II**
* **The text discusses preliminary data from the PALM randomized control trial (RCT), while the table appears to be based on outdated information obtained from the MEURI review of the investigational agents. The table should be modified to mirror what is discussed in the text.**
* **“Limited data” statements for mAb114 and REGN-EB3 studies implies that safety and preclinical studies were not as complete for REGN-EB3 and mAb114. However, REGN-EB3 and mab114 have published Phase I data in healthy adults and ZMapp did not complete a Phase I study. Similarly, while the PREVAIL IV trial mentions a Phase I for Remdesivir’s in healthy adults as the basis for AST/ALT monitoring, it has not yet been published. Since Phase I studies in healthy subjects are the standard for determining safety, it is not appropriate to single out mAb114 and REGN-EB3 by stating there are limited data for these agents or that they need additional trials to assess benefits and risk, without doing the same for the other two agents. Indeed, the recent PALM trial publication appears to render moot any questions about supportive data for benefits.**
* **Even though it is only preliminary data, the table at minimum should state that remdesivir was found to be equivalent to ZMapp and mAb114/REGN-EB3 were found superior to ZMapp.**
* **Given preliminary results of the PALM RCT showing that ZMapp was inferior to mAb114 and REGN-EB3, the statement saying ZMapp is “Considered standard of care” should be removed.**
* **All of the agent in PALM RCT are available under expanded access in DRC, not just REGN-EB3. REGN-EB3 is the MEURI therapeutic trial registered at clinicaltrials.gov. It is likely that in non-endemic countries each of the agents in the PALM study would be used under an eIND or equivalent mechanism in the country of interest**
* **REGN-EB3: Isolation from humanized mice may not be an advantage and depends on the comparator – it could be argued that it is either neutral or a disadvantage compared to mAbs that originate from a human since the variable domains that have been selected in a mouse might cross-react with human proteins. There are no published data on tissue reactivity for this agent.**
* **Remdesivir & ZMapp: Should add that the treatment regimen is over multiple days and infusions (compared to single infusion for REGN-EB3 and mAb114. The multiday regimen is now thought to partially explain the observed inferiority of Remdesivir and ZMapp in the PALM trial.**
* **ZMapp- check West Africa clinical trial #. Probably should be NCT02363322**
* **ZMapp: Infusion reactions were noted in NCT02363322, and required slowing or discontinuing the treatments. This should be noted as a disadvantage.**
* **Like ZMapp, the other 3 agents were determined by MEURI to have benefits that outweigh their risks, therefore this statement should be added to all of the agents or removed as an advantage for ZMapp]**

**[Au: here is my suggested new template, original table (unedited) after it: please fill and complete this table using the information contained in the original one]**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Agent**  | **Agent design** | **Previous study design** **[Au: protocol and outcomes; if more than one study, please separate them in consecutive rows]** | **Previous study results [Au: data on efficacy, limitations, etc]** | **PALM study design** |  **PALM study results** | **Notes** |
|  |  |  |  |  |  |  |
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|  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Investigational Agent | Drug Class | Efficacy | Advantages | Disadvantages |
| mAb114 | Monoclonal antibody against EBOV GP1,2 | Limited data from phase I study (NCT03478891) 242 sufficient for inclusion in phase II study (NCT03719586) | * Antibody isolated by from a COD survivor 242
* Single rapid infusion (30 min)
 | Additional clinical trials needed to assess benefits and risk |
|  REGN-EB3 **[Au: individual antibodies deleted for consistency with text and the other cocktail]**  | Monoclonal antibody cocktail against EBOV GP1,2 | * Limited safety data from phase I study (NCT002777151) sufficient for inclusion in phase II study (NCT03719586) 243,244
* Available through Expanded access (NCT03576690)
 | * Limited data support further drug development 243,244
* Single intravenous infusion
* Antibodies were isolated from humanized mice
 | Additional clinical trials needed to assess benefits and risk |
| Remdesivir (GS-5734) | Prodrug of adenosine nucleoside analog | Sufficient data from anecdotal report and animal data 243,245 for inclusion in phase II trial in COD (NCT03719586) | Does not require refrigeration 246 | * Limited data in humans to predict efficacy in humans 243
* Requires monitoring of ALT/AST; may not be available in limited resource settings
 |
| ZMapp | Monoclonal antibody cocktail (c2G4, c4G7, c13C6) against EBOV GP1,2 | Trend in efficacy from phase I trial in Western Africa (NCT03719586) sufficient for inclusion in ongoing phase II trial in COD (NCT03719586) 150  | Considered standard of care 247. MEURI-assessed benefits outweigh risks | * Long IV infusion duration under supervision
* Requires cold chain 246
* Antibodies were isolated from immunized mice 242
 |

COD, Democratic Republic of the Congo; mAb, monoclonal antibody; PALM, Pamoja Tulinde Maisha

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