

## **Insights from clinical research conducted during the West Africa Ebola Virus Disease epidemic**

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## **SUMMARY**

The West Africa Ebola virus disease (EVD) epidemic was extraordinary in scale. Now that the epidemic has ended, it is a relevant time to examine published studies with direct relevance to clinical care and, more broadly, to examine the implications of the clinical research response mounted. Clinically relevant research includes literature detailing risk factors for and clinical manifestations of EVD, laboratory and other investigation findings in patients, experimental vaccine and therapeutic clinical trials and analyses of survivor syndrome. In this review, we discuss new insights from patient-oriented research conducted during the West Africa epidemic, identify ongoing knowledge gaps, and suggest priorities for future research.

## **BACKGROUND**

The world's largest ever epidemic of Ebola virus disease (EVD) probably commenced in December 2013, following the infection of the presumed index case, a two year-old child living in rural Guinea.<sup>1</sup> The subsequent outbreak soon crossed into Sierra Leone and Liberia and case numbers escalated rapidly. When the World Health Organization (WHO) acknowledged in August 2014 that the outbreak was a Public Health Emergency of International Concern, there were already 1,711 reported cases and 932 deaths.<sup>2</sup> By the end of the epidemic, 28,646 cases and 11,323 deaths had been reported,<sup>3</sup> but the true numbers are likely to be much higher. The epidemic had far reaching effects in West Africa, including enormous economic costs and significant strain on already stretched healthcare systems.<sup>4,5</sup> A staggering 881 healthcare workers were infected and 513 died.<sup>6</sup>

The focus of global response efforts was, quite rightly, to provide humanitarian assistance and medical care, and to interrupt chains of transmission.<sup>7</sup> But there were also calls from WHO, funding bodies and governments to urgently scale the conduct of scientific research.<sup>8</sup> Prior to 2014, outbreaks were short-lived, occurred in remote locations, and involved relatively small case numbers. Such factors, coupled with limited research interest and funding, meant that our understanding of EVD was limited. The West Africa epidemic provided an important opportunity to improve patient outcomes by conducting clinical studies that would enhance knowledge and allow investigation of potential interventions. There were major hurdles to overcome, however, including logistical challenges,<sup>9,10</sup> and ethical and societal considerations<sup>11,12</sup> that could impact the ability to reach conclusions within the lifetime of the epidemic.

This review summarises published findings from clinical research conducted during the epidemic, and then discusses the implications for countries at risk of EVD outbreaks, ongoing clinical research gaps, and priorities moving forward. A broad range of research was conducted, so we have placed emphasis on patient-centred developments and progress made investigating Ebola virus vaccines.

## Search strategy and selection criteria

We searched PubMed for articles published from the beginning of the West Africa outbreak (January 1, 2014) to November 30, 2016, using search terms ‘Ebola’ or ‘Ebolavirus’ or ‘Ebola Virus Disease’ or ‘Ebola Haemorrhagic Fever’ using British and American spelling variations. Relevant references cited in those articles, and conference and international meeting reports were also reviewed.

Only articles with an abstract available in English were reviewed. All publications that contained original research or patient data and were reviewed for quality and relevance. To identify ongoing unpublished clinical research, we searched clinical trial databases [clinicaltrials.gov](http://clinicaltrials.gov), the Pan African Clinical Trials Registry, and the ISRCTN Registry.

Two authors (AR, JD) categorised all papers according to pre-defined subject area, using publication review software (<http://rayyan.qcri.org/>) (see supplementary figure 1). There were no discrepancies in individual categorization that required mediation from the third author (PWH). Papers were selected for inclusion on the basis of clinical relevance by joint review of two authors (AR, JD). A small number of papers that were published before the outbreak were included where comparison to existing knowledge was considered to be necessary; these were identified from the libraries of the authors.

## FINDINGS

### CLINICAL FEATURES OF EVD

#### *Burden of disease*

In the West Africa epidemic, the greatest burden of EVD was in young adults (median 32, IQR 21-42 years).<sup>13</sup> There is no certainty whether this represents an increased risk in young adults (perhaps due to increased exposure) or a case ascertainment bias (if children or the elderly were less likely to be in the

official count). There was no marked gender difference in disease prevalence (48.8% of probable and confirmed cases were male).<sup>14</sup>

### *Groups at risk of adverse outcomes*

We now know that young age is a predictor of death (odds ratio per year of life = 0.91 (95% CI 0.85-0.97)) and that children tend to deteriorate rapidly (median 3 days from Ebola Treatment Centre (ETC) admission to death, in a cohort of 300 children).<sup>15,16</sup> Likewise, new data demonstrate mortality is higher in patients over the age of 45 and in men.<sup>13,14,17-20</sup> The previously published maternal and neonatal EVD case fatality rates (90% and 100%, respectively) may be an overestimation,<sup>21</sup> as there have been subsequent case reports of maternal<sup>22,23</sup> and, very rarely, neonatal survival.<sup>24</sup> Without systematic data collection, however, the prognosis for pregnant women is uncertain.

### *Principal features of illness*

Although first described as ‘Ebola Haemorrhagic Fever’, due to the frequency of bleeding observed during the initial outbreaks of 1976,<sup>25,26</sup> a spectrum of illness was evident in the West Africa epidemic and haemorrhage, when present, was a late finding associated with fatal disease.<sup>27,28</sup> The hallmark of advanced disease in this epidemic was severe gastrointestinal illness.<sup>13,18,29-33</sup>

The most frequent symptoms at presentation (table 1) were fever, fatigue, anorexia, vomiting, diarrhoea, headache and abdominal pain.<sup>13,18,31-33</sup> Anecdotal reports of large volume, ‘cholera-like’ diarrhoea emerged from ETCs in West Africa, and volumes of up to 10 litres of diarrhoea per day were observed in medically evacuated patients.<sup>29,30</sup> Notably, fever was absent in at least 10% of patients,<sup>13,18,31-33</sup> which has important implications for clinical triage and case definitions that include fever as a prerequisite symptom. Less common clinical manifestations, including confusion, conjunctivitis and hiccups<sup>20,33</sup>, had good discriminatory value in identifying EVD cases amongst all patients presenting to ETCs and therefore remain valuable for presumptive clinical diagnosis in the context of a known outbreak.

A cross-sectional seroepidemiological study conducted in Sierra Leone found that 7.5% (14/187) individuals who had not been diagnosed with Ebola had detectable anti-Ebola glycoprotein antibodies.<sup>34</sup> Twelve of the 14 denied any symptoms compatible with EVD. These results, when considered alongside related data from previous outbreaks,<sup>35,36</sup> suggest that a proportion of Ebola virus infections are sub-clinical, although the contribution of such cases to transmission is unknown and the specificities of serological assays need to be considered.

### *Prognosis*

WHO's estimated case fatality rate (CFR) for the epidemic was 70% (95% CI 69-72%).<sup>32,37</sup> Overall, mortality was lower in hospitalised patients (CFR 60·7% (95%CI 59·2-62·3%)) compared with non-hospitalised patients (88·4% (95%CI 86·3-90·3%)).<sup>32</sup> Small hospital series have reported substantially improved survival (e.g. CFR 31·5%<sup>38</sup>), but these data should be interpreted with caution, since there are many potential explanations for the variability in CFR. For example, while medical intervention may have conferred a survival benefit, the influence of case selection bias (arising from self-presenting patients who are not representative of EVD patients in the community) or a survival bias (where the sickest patients succumbed to disease prior to admission) has not been fully assessed. The 18·5% CFR seen in patients treated in Europe and the USA was much lower than that reported in West Africa;<sup>39</sup> although not confirmed, possible explanations include fewer untreated comorbidities and lower levels of viraemia at admission, and access to advanced physiological support and experimental therapies that were not available routinely in West Africa.

## COMPLICATIONS OF ACUTE ILLNESS

EVD can be a severe and complex multi-system disease, with inflammation, vascular leakage, hypovolaemic shock, electrolyte disturbance, and direct end-organ damage all contributing to illness. Most existing knowledge about the pathogenesis of EVD has come from in vitro studies and animal models (reviewed elsewhere<sup>40-42</sup>), and limited histopathological data from previous human cases of EVD.<sup>42</sup> Improved characterisation of the broad spectrum of organ involvement (table 1) is an important contribution to knowledge about EVD.

### *Gastrointestinal complications*

The mechanism of severe diarrhoea in EVD is unclear. While clinical descriptions of large volume, 'rice water' diarrhoea draws analogy with cholera and implies a secretory process, previous autopsy findings indicate that intestinal wall inflammation also occurs.<sup>42</sup> There have been small gains in explaining why patients experience abdominal pain (including peritonism in a subset of cases), with case reports from resource-rich countries identifying paralytic ileus by ultrasonography.<sup>29,43,44</sup> In one case, marked bowel wall oedema was observed; the treating clinicians speculated that both viral mediated damage and iatrogenic hypoproteinaemia may have contributed to this finding.<sup>29</sup> They also suggested that an inflamed gastrointestinal tract was likely the source of the bacteraemia observed in this patient, but there is a lack of comparable data to suggest whether this was a common phenomenon in West Africa.

### *Renal complications*

Renal dysfunction is more common than previously thought. In one series of 150 patients, acute kidney injury (defined according to RIFLE criteria) occurred in 50% of patients and was an independent predictor of mortality (OR 5·84, 95%CI = 1·15-29·58);<sup>45</sup> a similar pattern has been seen in other cohorts.<sup>19,46,47</sup>

Importantly, these studies suggest that renal dysfunction occurs earlier in the disease trajectory than previously recognised and, at times, prior to the onset of severe vomiting and diarrhoea. For these patients, this indicates a mechanism partially independent of pre-renal hypovolemia due to gastrointestinal losses.<sup>45,48</sup> There are probably various contributors, including renal hypoperfusion from septic shock or, in patients with disseminated intravascular coagulopathy, thrombus formation in the renal microvascular system, or rhabdomyolysis.<sup>45</sup> In particular, the risk of AKI from rhabdomyolysis has yet to be fully elucidated. While approximately half of EVD patients experience myalgia,<sup>32</sup> and suggestive laboratory findings of raised creatine kinase (CK)<sup>39,45,49</sup> and hyperkalemia have been reported, identification of true rhabdomyolysis has been limited by a lack of urine myoglobin measurement. Furthermore, mechanistic studies of ebolavirus-induced muscle damage are lacking.

Hyperkalemia has been reported in 13% of patients in one series of West African patients.<sup>45</sup> This finding is plausible given the prevalence of AKI and the hypothesis of rhabdomyolysis, but hyperkalemia has been reported infrequently in other series, both in West Africa<sup>47</sup> and in medically evacuated patients (albeit confounded by frequent use of renal replacement therapy in this setting). Therefore, there is no certainty and caution is required when interpreting potassium findings obtained under field conditions, since artefactual hyperkalemia due to specimen haemolysis is possible. Hypokalemia is common<sup>45,47,50</sup> and while this is not an unexpected finding, given the severity of gastrointestinal losses in EVD, the variability in reported blood potassium disturbances highlights the necessity of biochemical testing to inform clinical decision making. Although data are limited, other commonly observed metabolic abnormalities include hyponatraemia, hypocalcaemia and hypomagnesemia.<sup>39,45,50</sup> Additionally, severe and frequent hypoglycaemia has been described in children with EVD.<sup>16</sup>

### *Hepatic complications*

There is little new knowledge regarding liver injury in EVD. Normal bilirubin levels were the norm in patients in West Africa cohorts, but transaminitis was common, typically with a high AST:ALT ratio.<sup>19,39,45,50</sup> It is not clear whether the increased ratio represents liver damage, muscle damage, or both.<sup>39,45,49</sup> A high AST level during the first week of illness was shown to be associated with fatal outcome. In the same study, AST correlated with the ebolavirus cycle threshold (Ct) value, suggesting it could be used as a surrogate marker of viral load.<sup>51</sup>

### *Respiratory complications*

Dyspnoea and tachypnoea were observed frequently in West African patients. Difficulty in breathing was reported in 41%<sup>20</sup> to 50% of patients<sup>52</sup>. Tachypnoea was observed in all 35 patients in one cohort.<sup>19</sup> Other groups have reported much lower rates of dyspnoea<sup>10,33,53</sup>, but there is likely to be variability in reporting since the intensity of monitoring varied and dyspnoea is a subjective symptom. Acute lung injury has been

observed in EVD patients who were medically evacuated and had access to more intensive monitoring. In this setting, hypoxaemia was observed in 52% (14/27) patients and noninvasive or invasive mechanical ventilation was required in 33% (9/27).<sup>39</sup> Tachypnoea could occur secondary to acidosis, which is common in EVD,<sup>19,45,47</sup> but pulmonary oedema associated with vascular leakage and/or fluid overload may also contribute.<sup>30,44</sup> Direct viral pneumonitis was suggested as the cause of acute respiratory failure in one case, as evidenced by interstitial pulmonary infiltrates and the detection of ebolavirus in bronchial aspirate fluid.<sup>54</sup>

### *Cardiovascular complications*

Further reports of inappropriate bradycardia in EVD patients surfaced during this epidemic.<sup>55</sup> Because some patients in this report were also encephalopathic, the authors suggest a possible central neurological cause, as opposed to previous hypothesis of toxin-mediated damage.<sup>55</sup> Arrhythmias have been reported in medically evacuated patients<sup>43</sup> and have been the presumed proximal cause of sudden death in some EVD patients who die suddenly during acute illness, or during early recovery<sup>56</sup> in West Africa. Electrolyte disturbances may be possible precipitants, but there is also evidence that viral myocarditis can occur during acute illness and recovery.<sup>57,58</sup> Additionally, a hypercoagulable state has been demonstrated during early recovery,<sup>59</sup> while this raises the possibility of venous thrombosis and pulmonary thromboembolism,<sup>57</sup> evidence of these complications is lacking. In addition, higher haemoglobin concentration and haematocrit were associated with mortality in a West Africa cohort;<sup>45</sup> this may have resulted from haemoconcentration in volume depleted patients,<sup>45</sup> but whether the increase in blood viscosity has clinically important consequences is unknown.

### *Neurological complications*

Neurological complications were common amongst patients in West Africa and included headache (61%), confusion (13%), and coma/unconsciousness (6%).<sup>32</sup> One third of patients treated in Europe and the US were encephalopathic at some point during their illness.<sup>39</sup> Encephalitis during acute illness and early recovery has been described, with detection of ebolavirus RNA in cerebrospinal fluid (CSF).<sup>60,61</sup> This association alone is insufficient to assume an infective mechanism, but is supported by isolation of virus from CSF in a survivor with meningoencephalitis (discussed below).<sup>62</sup> Detailed radiological investigation is challenging even in resource-rich settings, but MRI brain imaging performed at day 33 of illness demonstrated microvascular disease and ischaemia in a patient with meningoencephalitis.<sup>63</sup>

### *Inflammatory response*

The association between high viral load in blood and increased mortality is now well established<sup>18-20,45,52,64-66</sup> with the relationship following a sigmoid (logistic) function.<sup>67</sup> Severe EVD is associated with an intense inflammatory response, characterised by high levels of pro-inflammatory mediators.<sup>42,68,69</sup> The kinetics of soluble immune mediators and biomarkers in serial blood samples obtained from seven patients treated in

the US showed an association between more severe disease and biomarkers suggestive of endothelial or coagulatory dysfunction, and a comparative absence of biomarkers demonstrative of an immune response (compared with those found in patients with less severe EVD).<sup>70</sup> Two case series reported high levels of CRP and lactate, especially in fatal cases.<sup>39,45</sup>

### *Sepsis and co-infections*

There are new and unexpected findings describing how concurrent infections affect EVD prognosis. Analysis of blood samples from 1182 patients infected with ebolavirus found that patients with *Plasmodium* species parasitaemia were 20% more likely to survive, even after accounting for the mortality risk factors of marked ebolavirus viraemia and increasing patient age.<sup>71</sup> In the same study, the survival advantage was independent of treatment with antimalarials, and administration of different antimalarials failed to improve survival in mice infected with EVD. The authors hypothesized that concurrent *Plasmodium sp.* infection may moderate the host immune response, perhaps by reducing the exuberant cytokine response observed in EVD.<sup>71</sup> By contrast, a separate study performed multivariate analysis on data from 1047 cases and found that malaria parasite coinfection was an independent determinant of fatal outcome, but only for children who were 5 to 14 years of age; all patients in this study received antimalarial therapy.<sup>72</sup> The reasons for these discrepant findings are unclear.

A study of 49 EVD patients found that coinfection with GB virus C was associated with improved survival.<sup>73</sup> Similar to the hypothesis for the effect seen with concurrent *Plasmodium sp.* infection, GB virus C might also have beneficial immunomodulatory functions in EVD infection.<sup>71</sup> Studies determining the effect of HIV coinfection on survival in EVD have either not been conducted or have yet to report their findings.

Physiological and biochemical findings that would fulfil the commonly accepted criteria for septic shock have been described for EVD patients treated in Europe and the US. It seems likely that sepsis and septic shock also occurred in many EVD patients treated in West Africa, but data are lacking to confirm this. Sepsis could be caused by Ebola virus infection alone, or by bacterial co-infections (note these have not been investigated systematically).<sup>29</sup>

## **SUPPORTIVE CARE**

Supportive care remains the principal management strategy in patients with EVD. Several authorities advocate focusing efforts on correcting gastrointestinal fluid losses and electrolyte imbalances, and preventing hypovolaemic shock.<sup>74</sup> Recommended components of care often included oral and/or intravenous fluids, analgesia, anti-emetics and anti-diarrhoeal medications alongside empirical antimicrobials and anti-malarials.<sup>75,76</sup>



The lower case fatality rate in patients treated in the US and Europe (18·5%) suggests that intensive supportive care strategies can contribute significantly to improved survival.<sup>39</sup> Trials of supportive care were not completed during the West Africa EVD epidemic, however, and the evidence base for defining optimal supportive care for EVD remains limited.<sup>77</sup> Intensive intravenous fluid resuscitation was shown previously to be harmful in severe paediatric infections in resource limited settings, albeit in a different context to EVD; therefore, a universal fluid resuscitation protocol for ETCs could potentially cause harm in some patients.<sup>78</sup> The complexities of detecting and correcting abnormalities in fluid distribution and organ perfusion have been demonstrated by studies of patients treated in the US and Europe.<sup>39,79</sup> Some have questioned whether EVD-associated sepsis differs significantly from bacterial or fungal sepsis and, accordingly, whether applying general principles of sepsis management (or administering experimental sepsis treatments) to EVD patients could improve survival.<sup>74,80</sup>

Several interventions were used routinely in some ETCs but not in others, with little evidence of their benefit or risks. For example, the role of empirical vitamin K<sup>81</sup> remains unclear, given the limited understanding of the frequency and mechanisms of coagulopathy in EVD. NSAIDs were prescribed in some centres,<sup>38</sup> despite their potential to worsen gastrointestinal and renal complications. Loperamide is known not to confer benefit in cholera patients, but it is uncertain whether a similar, secretory process causes the large volume diarrhoea described in EVD. Additionally, paralytic ileus is a known complication of EVD and a contraindication to loperamide use, but may go unrecognised in a typical ETC setting. The apparent variability of electrolyte disturbances also raises concerns about routine empiric electrolyte supplementation in the absence of blood electrolyte monitoring.

Any future trials of supportive care strategies in EVD will be challenging if new outbreaks are more typical (i.e. smaller and of shorter duration), but high quality supportive care is clearly a major factor influencing survival and it is important that recommended supportive care strategies are evidence based. An expert consensus statement on the optimal package of supportive care for EVD in various settings would be a helpful interim measure, even more so if this identified the most important evidence gaps to guide the design of prospective clinical studies should a situation arise where such trials were possible.

## **SURVIVORS**

The enormity of the West Africa outbreak has led to an unprecedented number of EVD survivors. The most frequently reported post-EVD complications in this epidemic (table 1) are consistent with previous outbreaks.<sup>82,83</sup> These include arthralgia, visual disturbances (including uveitis and loss of visual acuity), hearing impairments, myalgia, fatigue, abdominal pain, and sleep disturbances.<sup>57,84-87</sup> Neurological deficits were reported infrequently before this outbreak, but now appear to be an important contributor to

morbidity.<sup>88</sup> Psychological distress in response to a life threatening illness may also contribute to neuro-cognitive manifestations.<sup>89</sup> Survivors report very poor acceptance by their communities.<sup>84,86</sup> Although this is known to impact survivor confidence and social engagement,<sup>86</sup> long-term psychological needs are unknown.

The pathogenic mechanisms that underlie EVD sequelae remain poorly understood. There is a long-held assumption that autoimmune or post-infectious inflammatory processes play prominent roles, but an association between viral replication in immune privileged sites and late complications in some survivors is newly established.

Ebolavirus was isolated from the aqueous humour of a survivor with panuveitis, fourteen weeks after diagnosis.<sup>90</sup> The total duration of viral sequestration was unknown, but was less than 18 months.<sup>91</sup> Additionally, infectious virus was detected in the CSF of a survivor with meningoencephalitis, nine months following acute illness.<sup>62</sup> At this time, there was also a transient viraemia, thought to represent ‘spillover’ of Ebolavirus from its site of replication in the central nervous system.<sup>24,62</sup> Both of these patients were medically evacuated to settings with advanced care and in addition, they received experimental therapies. Therefore, it is unclear if the nature, timing and severity of these complications are representative of sequelae seen in West Africa. Follow up of 151 survivors in Sierra Leone demonstrated that late recrudescence, defined as illness or death that could not be attributed to a non-EVD related cause after a period of full recovery from confirmed EVD, was rare (maximum estimate of 0.7%).<sup>92</sup>

Persistence of ebolavirus in body fluids had been demonstrated before this outbreak<sup>93</sup> but the long duration of persistence has been an unexpected finding.<sup>94</sup> For example, viral RNA is detectable in semen up to 18 months following discharge from an ETC.<sup>95</sup> There are few data available to estimate the proportion of male survivors affected. In one small convenience sample of survivors who were at varying durations into recovery, the overall prevalence of viral RNA positive semen was 49%.<sup>96</sup> Determinants of viral persistence in semen require further study.

There is also new evidence that women who recover from EVD during pregnancy can harbour persistent virus in the amniotic fluid and placenta and deliver an infected, stillborn foetus.<sup>23,97,98</sup> In addition, there are reports of viral persistence in other body fluids that would not be considered ‘immune privileged’, albeit for a briefer timeframe. Case reports suggest shorter-lived persistence of viable virus (and viral RNA) in urine and viral RNA in sweat<sup>79,99</sup> and contribute to existing knowledge of persistence in vaginal, rectal and conjunctival swab specimens and from breast milk.<sup>93,100,101</sup> Some caution is required when interpreting these small case studies; for example, the method used to collect a positive urine sample from a male patient was not described, raising the possibility of cross contamination by virus present in semen.<sup>79</sup>

Nonetheless, the viral kinetics of persistence in these fluids requires closer examination, particularly when there are implications for guidance on preventing sexual transmission or transmission by breastfeeding.

The phenomenon of viral persistence means that, in limited circumstances, survivors can act as a reservoir for ongoing disease transmission. Convincing evidence now exists to show that men can transmit ebolavirus to women during sexual intercourse.<sup>102,103</sup> The prolonged duration of viral persistence in semen raises the possibility of sexual transmission occurring long after the resolution of acute illness. There is compelling evidence that a flare of EVD in Guinea, which occurred months after the end of the Guinean outbreak, was caused by male-to-female sexual transmission (at approximately 470 days after initial illness in the male partner).<sup>102</sup> There are no population-level data that predict the risk for sexual partners of EVD survivors, but the low incidence of new flares of disease provides some indication that transmission leading to disease is uncommon. There is no published, definitive evidence of female to male sexual transmission having occurred, or of mother-to-child transmission by breastfeeding.

There are several ongoing research priorities for survivors. Long term studies are a priority because the longest survivor follow-up reported to date has been just over two years,<sup>82</sup> with ongoing symptoms reported at that time. Of note, there are no descriptions of the impact of EVD on childhood development and outcomes, and while limited evidence suggests that EVD survivors may be at greater risk of pregnancy-related complications including stillbirth, these data require comparison to age matched controls.<sup>24,104</sup> The risk of EVD recurrence and subsequent transmission by survivors is a key concern and so biological sampling in survivor cohorts is important to direct guidance on prevention strategies.<sup>105</sup> While a biological sampling approach (based on sequential negative samples) seems reasonable, we first need to know the natural history of persistence (i.e. whether detection of ebolavirus in semen can follow non-detection in earlier samples). Clinical trials of experimental drugs to clear persistent virus have commenced (registration numbers NCT2818582 and NCT02739477). To date, many of the available viral persistence studies have relied on RT-PCR to identify viral presence, but future studies should also focus on identifying live virus, which is more indicative of potential transmission risk.

## **THERAPEUTICS**

### **Experimental treatments**

Prior to the West Africa epidemic, experimental therapeutics had not been studied in patients with EVD, although transfusion of blood from convalescent patients had been tried.<sup>106</sup> The sheer scale of the West Africa epidemic demanded that effective, specific treatments should be identified and made available to patients as soon as possible. Accordingly, an expert panel was convened by WHO in September 2014 to prioritise promising candidates for clinical trials.<sup>12</sup>

Disappointingly, no clinical trial of potential therapeutic agent has produced conclusive evidence of a beneficial effect (table 2). None of the trials demonstrated safety concerns for the respective agents, but safety and tolerability will need to be confirmed in subsequent studies. A phase II clinical trial of the antiviral favipiravir demonstrated no survival benefit for EVD patients with a high viral load ( $Ct < 20$ ), but suggested that further efficacy studies in patients with less advanced disease ( $Ct \geq 20$ ) may be warranted.<sup>46</sup> A trial of the antiviral brincidofovir in Liberia was stopped before a conclusion could be reached after the drug company withdrew involvement in Ebola trials, in the setting of falling case numbers.<sup>107</sup> A phase II, single-arm trial of the small interfering RNA lipid nanoparticle compound TKM-130803, conducted in Sierra Leone, showed no survival advantage in patients with severe EVD, compared to survival in historical (untreated) controls.<sup>108</sup> The Ebola-Tx trial demonstrated no survival benefit in patients who received convalescent plasma compared to historical controls.<sup>65</sup> A separate report of the antibody titres in the transfused plasma found that concentrations of neutralizing antibody were generally low and no significant association was found between antibody concentrations in the transfused units and patient survival.<sup>109</sup> A multi-centre, randomised controlled trial of the ZMapp triple monoclonal antibody cocktail found that the CFR in patients receiving ZMapp in addition to standard care (22%) was lower than patients receiving standard of care alone (37%). Although this finding did not meet the pre-specified statistical threshold for efficacy, the posterior probability that the addition of ZMapp improved survival was 91.2%.<sup>110</sup>

Other EVD patients received experimental therapies on a compassionate basis, outside of clinical trials.<sup>30,39,111,112</sup> Many of these patients were treated in resource-rich countries and received a combination of experimental agents alongside intensive care support and nursing care, so it is difficult to assess safety or efficacy. A small number of patients in West Africa received repurposed agents (including lamivudine, amiodarone, atorvastatin, irbesartan, clomiphene, and favipiravir) without enrolment in a registered trial.<sup>113,114</sup> Anecdotal reports of survival benefit have been reported for some of these agents,<sup>113,115</sup> but it is impossible to draw any meaningful conclusions.

A retrospective study of patient outcome data from an ETC in Liberia found a temporal association between the use of antimalarial combination artesunate-amodiaquine and a period of reduced EVD mortality.<sup>116</sup> Patients received this combination when there was a supply failure of the first line agent (artemether-lumefantrine), rather than for hypothesis-driven reasons. This, along with other limitations described by the authors, makes it difficult to interpret the findings from this study, but additional studies are warranted, since in-vitro activity of amodiaquine against ebolavirus provides biological plausibility.<sup>117</sup>

Despite the largely negative outcomes from clinical trials, it must be recognized that the ability of researchers to overcome regulatory and operational barriers to complete trials to internationally accepted standards represents real progress, compared to previous outbreaks caused by high-hazard or emerging

pathogens. Several ongoing challenges remain, however. For some drugs, the 100% survival rates seen in NHP models<sup>118,119</sup> were not replicated in clinical trials. The reasons underlying these discrepancies should be explored, to maximise the utility of the animal model in drug development. Explanations may include inherent biological differences between species, or animal models that do not match human illness,<sup>108,120</sup> differences in exposure route and infectious dose, or that some patients present late in the course of illness with complex end-organ manifestations that cannot be simulated completely in an animal model.

## Vaccines

The epidemic also prompted accelerated efforts to take leading vaccine candidates to clinical trials, and to advance pre-clinical pipelines for less-developed candidates.<sup>121</sup> Overall, four candidate vaccines met WHO criteria for fast-tracked clinical evaluation: initially, the replication competent recombinant vesicular stomatitis virus (rVSV) vaccine expressing Zaire ebolavirus glycoprotein (ZEBOV) and the replication-deficient chimpanzee adenovirus serotype 3 vector vaccine (ChAd3-ZEBOV), followed later by another adenoviral vectored vaccine (Ad26-ZEBOV) with a heterologous boost (modified vaccinia virus Ankara, MVA), and also a nanoparticle vaccine (Novavax).<sup>122</sup> The first clinical trial in a highly affected country commenced in February 2015; with the exception of the nanoparticle vaccine, for which the phase I trial is ongoing, all of the candidates have been investigated in clinical trials in the region (see table 3 for details).

The phase III *Ebola ça suffit* rVSV-ZEBOV trial conducted in Guinea yielded remarkable interim findings.<sup>123</sup> This study used a novel approach of ring vaccination, a method that was first used during smallpox eradication programmes and involves vaccination of high risk contacts (defined geographically or socially) of known EVD cases, with the aim of interrupting transmission. Cluster randomization was utilized and rings of contacts received either immediate or delayed (21 days post-exposure) vaccination. This pragmatic approach aimed to balance the requirement for high-quality efficacy and safety data against ethical concerns about using placebo designs in highly vulnerable populations in the midst of an EVD outbreak.<sup>124</sup> Preliminary results suggest excellent efficacy (100%, 95% CI 74·7 – 100%). There were no new infections after a 6 day period in participants that were immediately vaccinated (n=2014), compared to 16 infections in the delayed vaccination group (n = 1930).<sup>123</sup> In light of these findings, randomisation was stopped and all subsequent participants received immediate vaccination. Concerns have been raised about the reactogenicity of rVSV-ZEBOV following observed, transient fever (up to 30%), arthritis (3-22%), rash, and dermatitis in phase I trials in Africa and Europe.<sup>125</sup> Whether these findings apply to other populations is unknown, as is the impact of potential side-effects on the acceptability of the vaccine among individuals at varying levels of risk of EVD. A significant practical challenge to rolling out this vaccine in an outbreak would be differentiating those with transient vaccine-related fever from those who are developing symptomatic EVD. Additionally, the transient viraemia triggered by vaccination may also result in a false positive PCR result with some tests.<sup>126</sup>

Adenovirus vector vaccines were the second type of vaccine to reach clinical trials in the affected countries. Phase I/IIa trials of ChAd3-ZEBOV demonstrated safety.<sup>127-129</sup> However, a trial with study arms in the US and Mali demonstrated that a single dose of vaccine elicited sufficient immunogenicity likely to be effective in post-exposure prophylaxis scenarios, but that a heterologous prime and boost (with modified vaccinia Ankara expressing Zaire Ebola virus glycoprotein) would be more appropriate when an extended period of protection was required.<sup>127</sup> The superior protective efficacy of a heterologous prime-boost regime has been demonstrated in other phase I trials of ChAd3<sup>130</sup> and Ad26-ZEBOV,<sup>131</sup> and in practical terms, may make it valuable for groups who have prolonged exposure periods e.g. healthcare workers and burial teams.<sup>127</sup> As we learn more about viral sequestration and sexual transmission, more durable vaccine induced immunity may be required to provide longer-term protection of sexual partners or EVD survivors. However, the inclusion of a boosting component will add to the logistical complexity of conducting mass vaccination. The results of field trials of adenovirus vector-based vaccines are awaited.

Other ongoing vaccination trials in the region commenced too late to determine effectiveness. However, they should be able to provide important safety and immunogenicity data, including comparative data for different candidate vaccines. This presents a dilemma, in respect to licensure of these vaccines. While it is possible that promising Ebola vaccines may receive regulatory approval if human safety and immunogenicity data are supported by evidence of efficacy in NHP studies (known as the ‘animal rule’), the limitations of the present animal model and an imprecise understanding of immune correlates of protection, means there is little certainty in this process. The ongoing development and assessment of different vaccines is valuable, because it is unlikely that a single vaccine will meet all of the criteria in the WHO target therapeutic profile.<sup>122</sup>

## CONCLUSIONS

There have been several notable successes in the scientific response to this epidemic, including improved characterisation of EVD complications and the completion of clinical trials of experimental therapeutics (figure 1). Progress was slow in other areas. Despite the very large number of patients, the reporting of clinical manifestations was fragmented and many published studies described small cohorts or single cases. Data collection was frequently *ad hoc* or retrospective, highlighting the need to embed clinically relevant research in outbreak preparedness and response. Knowledge of how EVD affects vulnerable populations, such as pregnant women and children, has not progressed significantly. We do not know the true benefits (or potential harms) of administering specific components of supportive care. Reporting on the outcomes of patients treated in resource-rich countries has been descriptive and repetitive, and only one medically-evacuated patient was recruited to a clinical trial.

An important question is how to apply findings from studies that have generated new information, particularly when the results are inconclusive. For example, despite the lack of incontrovertible evidence of efficacy, it is possible that ZMapp will be included as standard of care in future EVD outbreaks; if this happens, it is likely that trials of any new agents will need to demonstrate superiority of the new agent given alongside ZMapp, compared to ZMapp alone. Such trials will also need to stratify by viral load on admission.<sup>67</sup>

Individual components of supportive care interventions have not been assessed in EVD-specific trials. The rationale of providing IV fluid replacement to patients with significant gastrointestinal fluid losses is clear, but there is scope to compare different, empirical fluid replacement regimens and investigate the optimal timing of fluid replacement. While the observational studies of patients treated in Europe and North America suggest that physiological support does contribute to survival, many of the advanced interventions used will be difficult to translate to the typical ETC environment and so a key component of assessment will be feasibility and practicality.

For pharmaceutical interventions that could alter the course of future outbreaks, the greatest hope comes from the *Ebola ca suffit!* ring vaccination trial. If the final results from this trial confirm the highly promising interim findings, it is likely that ring vaccination strategies will be adopted in future outbreaks caused by Zaire ebolavirus strains.

Additional findings from the West Africa epidemic are expected and it is hoped that new data will contribute to the knowledge base. The degree to which findings from this epidemic can be applied to future outbreaks, including those caused by different species of ebolavirus, is unknown; a comparison of key clinical findings from different outbreaks would be useful, but would rely on high quality, comparable data-sets being available. Future, smaller EVD outbreaks can be expected in at-risk countries and clinical studies will need to be rapid and efficient; greater yields may be obtained if research priorities are agreed in advance, with centralised coordination of studies.

In all of the fields reviewed, we have discussed areas of priority for future investigation. To achieve the most rigorous outcomes from future studies, there must be an improved commitment to producing protocol-directed, hypothesis-driven research whenever possible. When this is infeasible, recommendations should be based on careful, systematic data collection and use of shared platforms that facilitate data collation across different sites. This requires not only a commitment from scientists, but also funding and publishing mechanisms that facilitate and reward collaborative science.

## **Contributors**

AR performed the literature search, according to the stated search strategy. AR, PH and JD reviewed and selected articles to include in the review, based on the stated selection criteria. AR produced the figures and tables. All authors contributed to writing the review.

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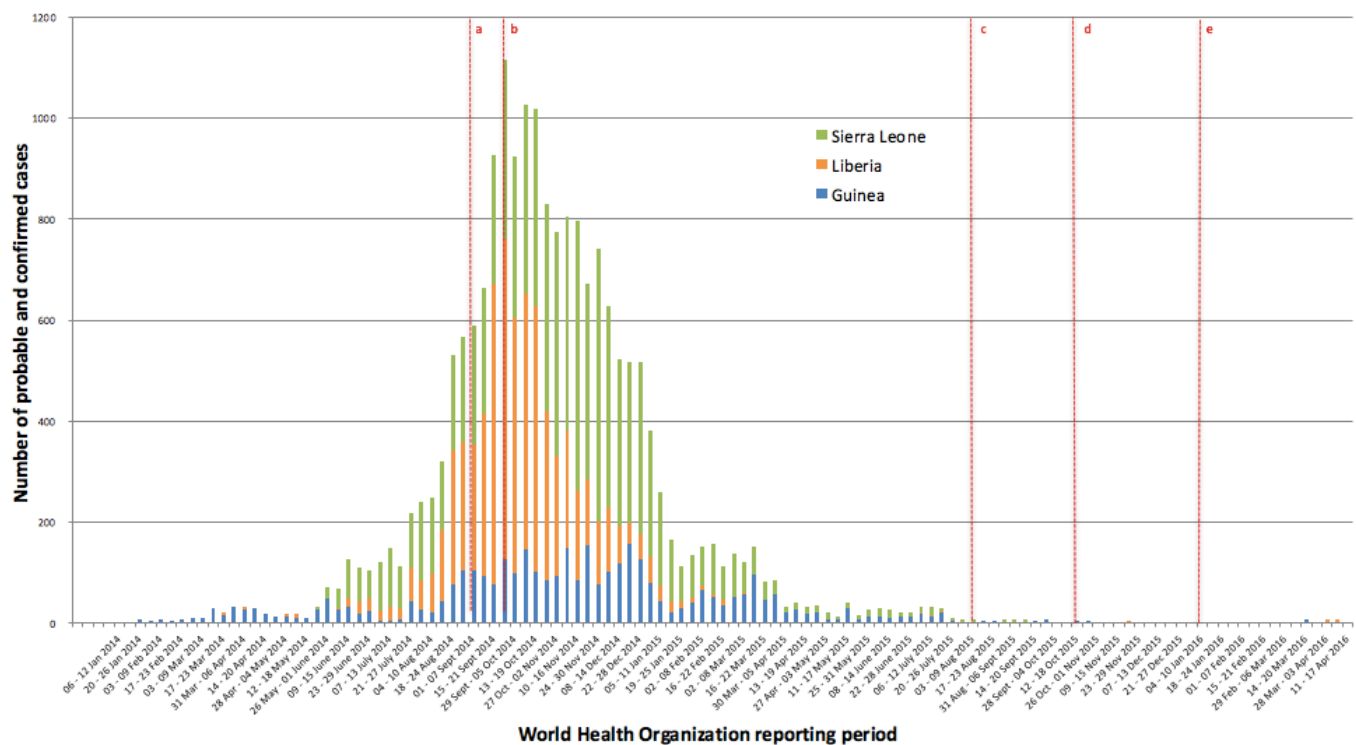
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### **Conflicts of interest**

We declare we have no conflicts of interest. The authors were investigators for two clinical trials described in this review (TKM-130803 and brincidofovir trials).



## Figures and Tables



**Figure 1: Significant research advances during West Africa EVD epidemic.** a) WHO holds consultation on potential Ebola therapeutics and vaccines<sup>132</sup> b) WHO Response Team publishes first large observational patient data set<sup>13</sup> c) interim results of VSV-ZEBOV vaccine trial published<sup>123</sup> d) Molecular evidence for sexual transmission published.<sup>103</sup> e) First clinical trial of experimental treatment (convalescent plasma) published.<sup>65</sup> Adapted from Bausch and Rojek (2016).<sup>133</sup>

	ACUTE ILLNESS		SURVIVOR SYNDROME
System	Signs and symptoms <sup>13</sup>	Investigational findings that have been reported	Signs and symptoms <sup>57, 84-87, 134</sup>
General	Fever, fatigue, hiccups	Raised pro-inflammatory markers, including CRP. Elevated lactate.	Fatigue, depression, anxiety, insomnia
Neurological and visual	Headache, confusion	CSF: detectable EBOV RNA Imaging: diffuse swelling, microvascular occlusions	Difficulty concentrating, mood changes, memory loss, headaches, dizziness, visual disturbances, peripheral paresthesia or dysaesthesia
Cardiovascular	Chest pain, possible sudden death	ECG: bradycardia, arrhythmias. MRI: myocarditis	Chest pain, palpitations
Pulmonary	Cough, dyspnea, sore throat		Dyspnea
Gastrointestinal	Anorexia, vomiting, diarrhea, abdominal pain, odynophagia	USS: Paralytic ileus, bowel wall oedema	Anorexia, abdominal pain, constipation
Hepatobiliary	Jaundice	Transaminitis with high AST/ALT ratio	
Renal, urological, electrolytes		Acute kidney injury, raised creatine kinase, hypo/hyperkalemia, hyponatremia, hypocalcemia, hypoglycaemia	Decreased libido, sexual dysfunction, testicular pain
Haematological	Clinically significant haemorrhage uncommon, likely more frequent in pregnant women.	Leukopenia, thrombocytopenia, raised INR, haemoconcentration.	Anaemia
Skin and musculoskeletal	Myalgia, arthralgia, conjunctivitis		Arthralgia, myalgia, alopecia, skin peeling, pruritis

**Table 1. Clinical manifestations of investigational findings in EVD, reported by studies conducted during the West Africa EVD epidemic.** ALT = alanine transaminase; AST = aspartate transaminase; CRP = C-reactive protein; CSF = cerebrospinal fluid; EBOV = ebolavirus; ECG = electrocardiogram; INR = international normalized ratio; MRI = magnetic resonance imaging; PCR = polymerase chain reaction; USS = ultrasound scan.

Experimental therapy	Trial design	Research question (PICO model)	Declared status (as of November 2016)	Result
ZMapp	Open label RCT with adaptive trial design	<u>Intervention:</u> 50mg/kg ZMapp, i.v, every three days; total of three doses <u>Comparison:</u> optimised care alone (including favipiravir in Guinea) <u>Outcome:</u> Day 28 survival.	Registered as PACTR201503001065306 NCT02363322  Completed	No statistically conclusive benefit <sup>110</sup>
TKM-130803	Open label, single arm. Component of a multi-stage approach	<u>Intervention:</u> 0.3mg/kg of TKM-130803, i.v., once daily. Total of 7 doses. <u>Comparison:</u> historical controls <u>Outcome:</u> Day 14 survival.	Registered as PACTR201501000997429  Completed	No overall survival benefit <sup>108</sup>
Favipiravir	Open label, single arm	<u>Intervention:</u> 6000mg (day 0) and 2400mg (day 1-9), po, daily of favipiravir. Total of 10 doses. <u>Comparison:</u> historical controls <u>Outcome:</u> Day 14 survival.	Registered as NCT02329054  Completed	No overall survival benefit <sup>46</sup>
Convalescent plasma (CP)	Open label, single arm	<u>Intervention:</u> 400-500mL of CP from two donors. Administered as two consecutive (200-250mL) transfusions. One treatment cycle in total. <u>Comparison:</u> historical controls <u>Outcome:</u> Day 14 survival.	Registered as NCT02342171  Completed	No overall survival benefit <sup>65</sup>
Convalescent plasma	Open label, single arm	<u>Intervention:</u> 180-220mL of CP from two donors. Administered as two consecutive (90-110) infusions. Up to 3 treatment cycles, at least 48hrs apart. <u>Comparison:</u> none <u>Outcome:</u> EBOV viral load.	Registered as NCT02333578  Recruiting	N/A
Convalescent plasma	Open label, single arm	<u>Intervention:</u> INTERCEPT plasma, dose not defined. <u>Comparison:</u> not defined. <u>Outcome:</u> 1 year survival.	Registered as NCT02295501  Open to enrolment	N/A
Convalescent plasma	Open label, random allocation	<u>Intervention:</u> <u>Single transfusion of convalescent plasma, dose not defined</u> <u>Comparison:</u> <u>Ringer's Lactate solution</u> <u>Outcome:</u> <u>All-cause mortality at 14 days post treatment</u>	Registered as ISRCTN13990511  Ongoing; no longer recruiting	N/A
Brincidofovir	Open label, single arm trial. Component of a	<u>Brincidofovir:</u> 200 mg, po, initial dose, then 100 mg, po, twice weekly; total of 5 doses <u>Comparison:</u> historical controls	Registered as PACTR201411000939962  Recruitment suspended	No statistical conclusion <sup>107</sup>

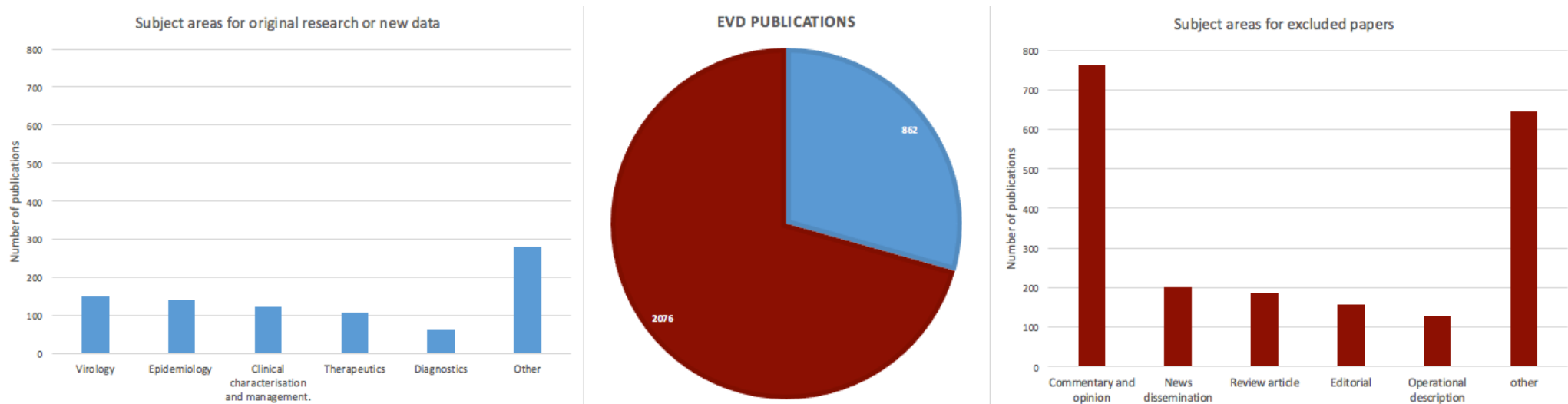
	multistage approach	<u>Outcome:</u> Day 14 survival.		
Azithromycin Sunitinib Erlonitib Atorvastatin Irbesartan	Multi-arm RCT with adaptive trial design	<u>Intervention:</u> Azithromycin (1500 mg, po, daily for 5 days) vs Sunitinib (50 mg, po, daily for 7 days) and Erlonitib (150 mg, po, daily for 7 days) vs Atorvastatin (40 mg, po, daily until discharge) and irbesartan (150 mg, po, daily until discharge)  <u>Comparison:</u> IV fluids and laboratory testing alone  <u>Outcome:</u> Day 14 survival	Registered as NCT02380625  Not yet open to recruitment	N/A
Interferon-beta	Open label, single arm	<u>Intervention:</u> subcutaneous interferon-beta once daily for up to 10 days  <u>Comparison:</u> not defined (safety and effectiveness study)  <u>Outcome:</u> not defined	Registered as ISRCTN17414946  Completed	N/A
Amiodarone	Open label, RCT	<u>Intervention:</u> amiodarone (20 mg/kg, i.v., on day 1,2,3 then 200 mg, po, three times daily, on day 4-10)  <u>Comparison:</u> supportive care alone  <u>Outcome:</u> Day 10 survival	Registered as NCT02307591 and PACTR201501001014425  Withdrawn	N/A

\*Where a dose of an intervention has been stated, it refers to the stated adult dose. Refer to trial protocols for weight adjustment. po= oral administration. i.v. = intravenous administration. N/A = not available.

**Table 2: Patient-based clinical trials of experimental therapeutics registered on clinical trial databases during the West Africa EVD outbreak.**

<b><u>Vaccine</u></b>	<b><u>Trial name</u></b>	<b><u>Trial design</u></b>	<b><u>PICO research question*</u></b>	<b><u>Declared status November 2016)</u></b>
rVSV ZEBOV	Ebola ça Suffit!	Open label, Cluster randomized, ring vaccination	<p>Participants: Contacts of confirmed EVD patients</p> <p>Intervention: immediate vaccination with rVSV ZEBOV</p> <p>Comparison: delayed (day 21) vaccination</p> <p>Outcome: Safety and efficacy</p>	<p>Registered as PACTR201503001057193</p> <p>Interim results available<sup>123</sup></p>
	Ebola ça suffit!	Open label, single arm	<p>Participants: Adult frontline workers</p> <p>Intervention: immediate vaccination with rVSV ZEBOV</p> <p>Comparison: delayed (day 21) vaccination</p> <p>Outcome: Safety and efficacy</p>	<p>Registered as PACTR201503001057193</p> <p>Closed to recruitment, follow up complete<sup>135</sup></p>
	STRIVE	Open label, randomized, with 2 sub studies	<p>Participants: Adult frontline workers</p> <p>Intervention: immediate vaccination with vVSV ΔG ZEBOV</p> <p>Comparison: delayed (18-24 weeks) vaccination</p> <p>Outcomes: Safety, efficacy and immunogenicity</p>	<p>Registered as NCT02378753 PACTR201502001037220</p> <p>Ongoing but not recruiting</p>
Multiple	PREVAC	Double blind RCT	<p>Participants: Children and adults</p> <p>Intervention: immediate vaccination with rVSV-ZEBOV (with or without rVSV boost) or Ad26.ZEBOV + MVA-BN-Filo boost</p> <p>Comparison: Placebo</p> <p>Outcomes: Safety and immunogenicity</p>	<p>Registered as NCT02876328</p> <p>Not yet open for recruitment.</p>
	PREVAIL	Double blind RCT	<p>Participants: Adults</p> <p>Intervention: Immediate vaccination with VSVG-ZEBOV or ChAd3-EBO Z</p> <p>Comparison: Placebo</p> <p>Outcome: safety and immunogenicity</p>	<p>Registered as NCT02344407</p> <p>Ongoing, but not recruiting, no results available</p>
Ad5-EBOV		Double blind RCT	<p>Participants: Adults</p> <p>Intervention: high dose, or low dose immediate vaccination with Ad5-EBOV</p> <p>Comparison: Placebo</p> <p>Outcome: safety and immunogenicity</p>	<p>Registered as NCT02575456 PACTR201509001259869</p> <p>Completed, no results available</p>
Ad26. ZEBOV + MVA-BN-Filo	EBOVAC	Open label, single arm, followed by double blind RCT.	<p>Participants: Adults and children</p> <p>Intervention: immediate vaccination with Ad26-ZEBOV, with MVA-BN-Filo boost</p> <p>Comparison: Placebo (meningococcal vaccine during immediate vaccination) during stage 2</p> <p>Outcome: safety, immunogenicity and efficacy</p>	<p>Registered as NCT02509494 PACTR201506001147964</p> <p>Recruiting</p>

**Table 3: Vaccine trials recruiting in the most affected countries during the EVD outbreak in West Africa.**



**Supplementary Figure 1: EVD publications published from January 1 2014 to November 30, 2016.** Publications were categorized according to subject area and type. 2938 peer-reviewed publications were identified; of these, 862 included original research or new patient data and were eligible for inclusion. 2076 papers were excluded on the basis that they provided commentary, opinion, or editorial information without new data.



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