Ebola virus disease: a review on epidemiology, symptoms, treatment and pathogenesis

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ABSTRACT

Currently, West Africa is facing the largest outbreak of Ebola virus disease (EVD) in history. The virus causing this outbreak, the Zaire Ebolavirus (EBOV), belongs to the genus *Ebolavirus* which together with the genus *Marburgvirus* forms the family of the *Filoviridae*. EBOV is one of the most virulent pathogens among the viral haemorrhagic fevers, and case fatality rates up to 90% have been reported. Mortality is the result of multi-organ failure and severe bleeding complications. By 18 September 2014, the WHO reported of 5335 cases (confirmed, suspected and probable) with 2622 deaths, resulting in a case fatality rate of around 50%. This review aims to provide an overview of EVD for clinicians, with the emphasis on pathogenesis, clinical manifestations, and treatment options.

KEYWORDS

Ebola virus disease, viral haemorrhagic fever, filovirus, pathogenesis, treatment

INTRODUCTION

On 8 August 2014 the World Health Organisation (WHO) declared the Ebola virus disease (EVD) outbreak in West Africa a Public Health Emergency of International Concern (PHEIC),[1] stressing the need for international attention and collaboration to control the outbreak. At this moment (18 September 2014) a total of 5335 cases with 2622 reported deaths have been notified, in Guinea, Liberia, and Sierra Leone. The imported EVD case in Nigeria that resulted in a relatively small outbreak, and similar imported cases in the USA and Spain which at first appeared to have been well contained, but

eventually lead to infection of healthcare workers, show the importance of adequate isolation methods, training of personnel and the adequate use of personal protective equipment (PPE).[2] For the West Africa outbreak the total number of cases is subject to change due to ongoing reclassification, retrospective investigation and the availability of laboratory results. A second, non-related, EVD outbreak has been reported in the Democratic Republic of Congo with currently a total of 62 confirmed and suspected cases.[3,4]

VIROLOGY

The virus causing the outbreak has been characterised as Zaire Ebolavirus (EBOV). EBOV belongs to the genus *Ebolavirus* which together with the genus *Marburgvirus* forms the family of *Filoviridae*. This family belongs to the order of the *Mononegavirales* which further contains members of *Bornaviridae*, *Paramyxoviridae* and *Rhabdoviridae*. *Ebolaviruses* are linear, negative-stranded, RNA viruses with a genome of approximately 19 kilobases. Morphologically, when studied under an electron microscope, the viral particles look like long stretched filaments with some particles tending to curve into an appearance looking like the number 6. At this moment the genus *Ebolavirus* consists of five species: EBOV, Sudan ebolavirus (SUDV), Tai forest ebolavirus (TAFV), Bundibugyo ebolavirus (BDBV) and Reston ebolavirus (RESTV). RESTV is considered to be non-pathogenic to humans.[5] The genus is named after the first recognised outbreak that took place in the village of Yambuku, in Zaire (now Democratic Republic of Congo), close to the Ebola river.[6] Since then there have been multiple EVD outbreaks, mostly with EBOV and SUDV. The EBOV responsible for

the current outbreak was introduced into West Africa from Central Africa in the last few decades.[7]

EPIDEMIOLOGY AND WEST AFRICA OUTBREAK

Figure 1 summarises the current area of the West Africa EVD outbreak. This region is known to be endemic for two additional viral haemorrhagic fever viruses (VHF), namely the rodent-borne Lassa fever virus and the mosquito-borne Yellow fever virus.[8,9] Furthermore, a single case of EVD, caused by TAFV, has been reported from this area concerning a female researcher investigating (autopsy) infected chimpanzees.[10] Historically, EVD outbreaks often occurred in small villages close to or located in tropical rainforests. This partly explains why the first outbreaks of EVD, due to EBOV and SUDV, remained restricted to a limited area.[11] No EVD outbreaks were reported between 1979 and 1994, but after 1994 the number of recognised outbreaks increased, leading to the discovery of two new *Ebolavirus* species (BDBV and TAFV).[12] Multiple causes of this increase in EVD outbreaks have been mentioned in the literature, with the most likely being increased bush meat consumption and transportation to previously inaccessible areas.[5,12] EVD is a zoonotic disease and each EVD outbreak in the human population is initiated by a (single) introduction from an animal reservoir. For the current outbreak this introduction occurred in Guinea

in December 2013, but it is not known with certainty how the index case became infected.[7] The index case of the unrelated outbreak in the Democratic Republic of Congo had consumed bush meat, which is considered the most likely source of infection.[7,13] Species implicated in introduction of EBOV into the human population are chimpanzee, gorillas, duikers and specific species of fruit bats, all found to be infected with EBOV during targeted studies (*figure 2*). Given the lack of overt disease, bats are considered the most likely reservoir host.[14,15] Once introduced into the population EBOV may spread rapidly, due to the rapid uncontrolled rate of high levels of viraemia and virus shedding in body fluids (saliva, urine, faeces and sweat) by EVD patients.[16] When hygiene and personal protective measures are not adequate, the risk for infection of healthcare workers is considerable, as illustrated in the

Figure 1. *Overview of the area of the current EVD outbreak as per 8 September 2014*
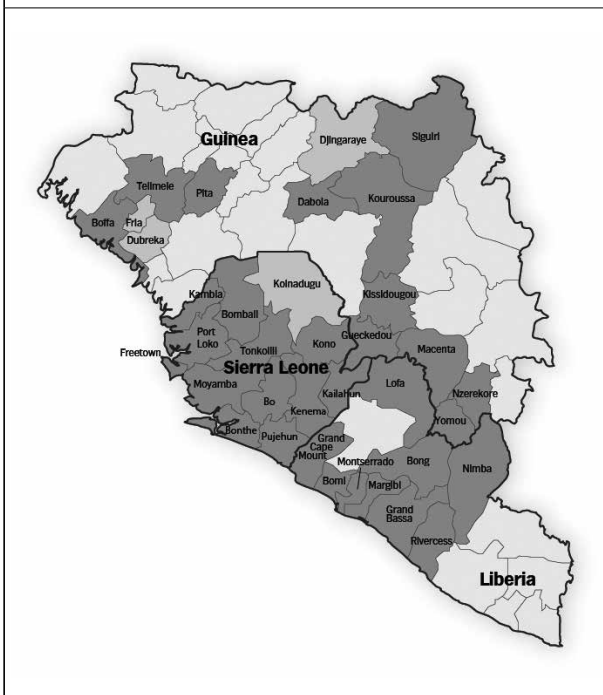

Figure 2. *Transmission of Ebola virus disease (EVD) Ebolaviruses enter the human body via mucosal surfaces, abrasions and injuries in the skin or by direct parental transmission. For each outbreak of EVD a single introduction from the animal kingdom is needed. It is likely that, as for the index case, infection occurs after human contact with primates, e.g. due to hunting or consuming of infected animals, while also other mammals such as antelopes and rodents have been mentioned as potential reservoirs.[61] Another potential cause for human infection was described in 2005 where data from a large study in bats showed three fruit bat species to be a potential reservoir for Ebolaviruses.[14] This was later confirmed by an EVD outbreak that resulted after direct contact with bats.[12,15] Due to the high viral loads seen in the body fluids of EVD patients human to human transmission can easily occur. This transmission seems to take place through body fluid contact and not by airborne transmission (e.g. infective aerosols)*
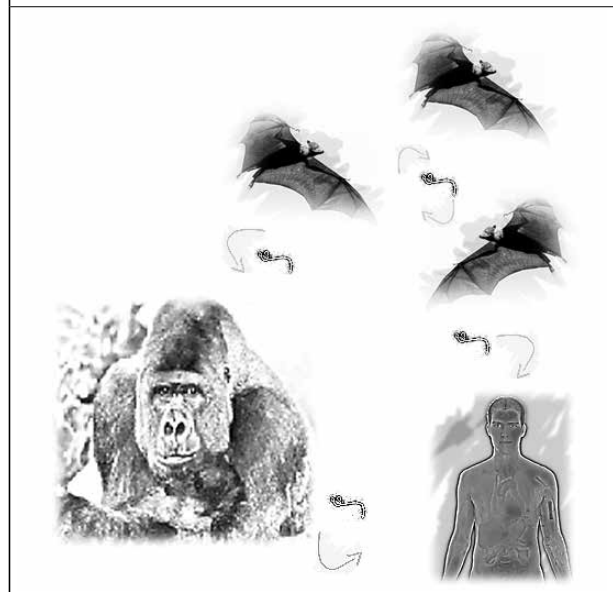
current outbreak.[17] Furthermore cultural aspects, such as local funeral ceremonies with potential contact with body fluids from patients who have died from EVD, contribute to the magnitude of this outbreak.[18]

CLINICAL MANIFESTATIONS

Symptoms in EVD patients normally occur after an incubation period of 4-10 days, with a range of 2-21 days.[19,20] After a sudden onset of 'flu-like' symptoms (fever, myalgia, chills) and vomiting and diarrhoea, the disease can rapidly evolve into a severe state with a rapid clinical decline. This disease phase is characterised by potential haemorrhagic complications and multiple organ failure.[19,21] EVD patients may present with gastrointestinal symptoms (nausea, stomach ache, vomiting and diarrhoea), neurological symptoms (headache, profound weakness and coma), respiratory symptoms (coughing, dyspnoea and rhinorrhoea), and generalised symptoms related to failure of the cardiovascular system resulting in shock and oedema.[5,20,21] The most commonly described symptoms are fever in combination with anorexia, asthenia and a maculopapular rash between day 5 and 7 after the onset of the disease,[5,20,21] but in the current outbreak the primary clinical presentation is gastrointestinal. Clinical symptoms and chemical laboratory tests confirm multi-organ involvement. Most common haematological changes are leucopenia and lymphopenia, with a specific decreased neutrophil count, and an increase in liver enzymes. With progression of the disease, EVD patients develop thrombocytopenia, lengthening of the pro-thrombin time and activated partial thromboplastin time. The lengthening of the clotting times together with the observed increase in fibrin degradation products suggest a consumptive coagulopathy due to disseminated intravascular coagulation, which contributes to multi-organ failure. Lethal EVD cases generally succumb between day 6 and 16 after the onset of symptoms. Patients die due to shock, haemorrhage and multi-organ failure.[5] If patients recover, clinical improvement arises simultaneously with the development of the antibody response. In lethal cases the antibody response sometimes remains absent.[22,23] Long-term complications of EVD have not been studied extensively, but available literature suggests that patients recovered from EVD could develop long-term symptoms and disorders such as recurrent hepatitis, myelitis, prolonged hair loss, psychosis and uveitis.[5,19,21]

DIAGNOSIS

The diagnosis of acute EVD is made by viral genome detection via RT-PCR. The virus is generally detectable 48 hours after infection in both lethal and non-lethal cases. This means that a negative test result within the first 48 hours after exposure does not rule out EBOV infection. Due to the rapidity of the acute disease, serology does not play a role in diagnosis of acute EVD patients but may be of use in epidemiological and surveillance studies. In general, IgM antibodies can be detected starting from two days after the first symptoms appear and disappear after 30-168 days.[24] IgG response is generally considered to start between day 6 and 18 post onset of illness and remains detectable for years. The antibody profile of the sera from patients with lethal disease as compared with those that survive is markedly distinct. This difference can serve as a prognostic marker for the management of the patient since antibody responses strongly differ between lethal and survivor cases and it has been shown that deceased patients show a much lower or even absent antibody response compared with survivors.[25,26]

PATHOGENESIS AND TRANSMISSION

After infection, development of disease is a complex interplay between virus, host and environment. Different case fatality rates (CFR) have been reported between the four human-pathogenic *Ebolaviruses*. For EBOV the CFR ranges from 50-90% of the EVD cases.[27] For the current outbreak, CFR is estimated to be around 50%,[28] although there is some evidence of improved outcomes with intense symptomatic treatment. There is an indication of differences in the CFR for different EBOV species, but these data are hard to interpret as they rely on reporting, which may be suboptimal.[29] *Ebolaviruses* enter the human body via mucosal surfaces, abrasions and injuries in the skin or by direct parental transmission. Infection through intact skin is considered unlikely, although not excluded. The virus has been successfully isolated from skin (biopsy) and body fluids.[30] Several laboratory associated infections have been reported in the past decades, often after needle accidents or direct contact with infectious materials.[31] The route of transmission seems to affect the disease outcome; in the early EBOV outbreak in 1976, CFR after transmission by injection was 100% versus 80% in contact exposure cases.[5] This has been confirmed in a non-human primate model, showing faster disease progression in animals infected via injection versus those that received an aerosol challenge.[32] Due to the high CFR in EVD and the potential use of EBOV as a biodefense weapon, the pathogenesis of EVD has been relatively well studied during the past 15 years.[33] Most studies have been performed in rodent, guinea pig, primate and *in vitro* models. Since the virus needs to adapt to cause disease in rodent and guinea pig experimental study models, the most relevant data representing human disease come

from non-human primate studies.[34] Upon entry, EBOV have proven to be able to infect numerous cell types. Post mortem studies of patients and experimentally infected animals showed infection of immune cells (macrophages, monocytes and dendritic cells), epithelial and endothelial cells, fibroblasts, hepatocytes and adrenal gland tissue.[35] Replication in infected cells is very efficient resulting in a rapid and high peak viraemia.[35] Furthermore, cell death of infected cells has been hypothesised to play an important role in the signs and symptoms seen in EVD patients, for instance the decreased ability of the immune system to respond to the infection due to necrosis of infected lymphocytes or a decreased production of clotting factor due to the loss of hepatocytes.[5] Hallmark characteristics of EVD, as in any VHF, are the bleeding manifestations although these are infrequently observed in the current outbreak.[36] Studies addressing the mechanism behind these coagulation abnormalities first showed that haemorrhage was most likely not a direct effect of endothelial cell infection, followed by cytolysis.[37] A more likely explanation seems to be an overexpression of tissue factor in monocytes/macrophages resulting in (over)activation of the extrinsic pathway of coagulation followed by a consumptive coagulopathy and eventually a disseminated intravascular coagulation.[38] Furthermore antibody enhancement has been hypothesised to play a role in the later phase of the EVD course.[39] Although data on this theory are still limited, antibody-dependent enhancement seems to enhance infectivity of the virus *in vitro* not only for EBOV but also the closely related *Marburgvirus*.[38,40] A similar disease mechanism has been hypothesised for the development of dengue haemorrhagic fever.[41,42] Interesting data about EVD pathogenesis come from asymptomatic cases and EVD patients who survived infection. A cluster of asymptomatic infections have been described after EBOV infection. Of these 24 contacts, 11 were asymptomatically infected and developed an IgM and IgG response plus a mild viraemia between day 7 (first day of sampling) and day 16.[43] The other 13 patients had high levels of plasma viraemia associated with high levels of pro-inflammatory cytokines. These data suggest that a correlation exists between the height of peak viraemia and levels of pro-inflammatory cytokines contributing to disease severity.

CLINICAL MANAGEMENT AND (EXPERIMENTAL) TREATMENT

The first step is to identify patients with symptoms consistent with the case definition as outlined by the WHO and the Centers for Disease Control and Prevention (CDC), Atlanta, Georgia, USA specially for patients in geographical areas where *Ebolavirus* infections have

previously been reported and/or patients in other countries with similar symptoms who have travelled to these countries within the past 21 days. These patients need to be rapidly isolated and the patient contacts identified and appropriate containment and preventive measures instituted. Blood samples need to be immediately obtained and submitted to the nearest clinical laboratory certified to conduct diagnostic evaluation for *Ebolavirus*. Currently, the treatment of EVD includes the administration of 'supportive care' and treatment strategies. EVD patients benefit most from managing the haemodynamics and haemostasis. When started in the early phase of the disease, fluid replacement therapy drastically increases the chance of survival.[44] Ribavirin, the only known antiviral that is effective against certain VHF pathogens such as Lassa fever, is not effective against *Ebolaviruses*.[45,46] Various drugs with a potential effect in EVD are in the experimental phase and have shown beneficial effects against *Ebolaviruses* (mainly EBOV and SUDV) in animal models and have been used in small numbers to treat EVD patients. The WHO declared that, considering the magnitude and severity of the current outbreak, it is ethical to use experimental drugs for treatment and prevention of EVD. *Table 1* shows the most promising experimental compounds with activity against EBOV, and the degree of available information from preclinical and clinical trials published in peer-reviewed journals. ZMapp is a cocktail of monoclonal antibodies and is being used to treat some victims of the current EBOV outbreak. Its role in treatment of EVD still needs to be established since efficacy data in humans have not been published yet. The strongest evidence that ZMapp is indeed effective in EVD comes from experiments in non-human primates in which ZMapp was able to revert advanced EVD when administered up to five days post infection.[47] Unfortunately, there is a limited supply of ZMapp at this moment. Of the non-antibody based antiviral preparations, only the nucleoside analogue favipiravir has been tested extensively in humans. Recently the drug gained approval in Japan for use in humans infected with novel and re-emerging influenza viruses. Besides activity against influenza virus infection, this drug also has documented activity against a wide variety of RNA viruses including *Ebolaviruses*.[48,49] Favipiravir prevented death in mice infected with EBOV when treatment was started six days post infection.[50] These results are promising, but need to be confirmed in a non-human primate model. BCX-4430 is also a nucleoside analogue with broad spectrum activity against RNA viruses and has proven to be effective against the Marburg virus in a non-human primate model and Ebola virus in a mouse model.[51] Finally, TKM-ebola and AVI-6002 are under development for the treatment of EVD and exert their action via gene silencing. Both drugs

Table 1. *Experimental treatments for Ebola viral disease*

Drug	Drug type	Mode of action	*In vitro* data on Ebola	Non-primate animal data on Ebola	Primate data on Ebola	Drug tested in humans	Drug tested in Ebola infected humans	Approval status
Favipiravir (T-705) (Fujifilm Holdings Corp)	Nucleoside analogue – broad spectrum activity against RNA viruses	RNA chain termination and/or lethal mutagenesis	Yes EC_{50} 31-63 mg/l[48] IC_{50} 10 mg/l[50]	Yes 300 mg/kg/d started 1 hour post infection prevented death in 100% of Ebola infected mice[48] 300 mg/kg/d started 6 days post infection prevented death in 100% of Ebola infected mice[50]	Ongoing at USAMRIID [personal communication M. Koopmans and S. Gunther]	Phase-2 completed (influenza) and phase-3 ongoing (influenza)	No	Approved in Japan for novel and re-emerging influenza viruses[49]
TKM-Ebola (Tekmira Pharmaceuticals Corp)	Lipid nano-particle with siRNA – Ebolavirus specific compound	Gene silencing	Yes	Yes TKM-Ebola started 1 hour post infection resulted in survival of 3/5 guinea pigs (2 deaths unrelated to Ebola)[57]	Yes TKM-Ebola started 30 minutes post infection resulted in survival of 6/8 rhesus monkeys (2 Ebola related deaths)[52]	Phase-1 study partially on hold[9]	No	Not approved
BCX-4430 (BioCryst Pharmaceuticals)	Nucleoside analogue – broad spectrum activity against RNA viruses	RNA chain termination	Yes EC_{50} 3,4 – 11,8 microM	Yes	No, but activity against Marburgvirus in cynomolgus macaques[58]	No	No	Not approved
AVI-6002 (Sarepta Therapeutics)	Phosporo-diamidate morpholino oligomer – Ebolavirus specific compound	Gene silencing	Yes[59]	Yes	Yes AVI-6002 started 30-60 minutes post infection resulted in survival of rhesus monkeys in dose dependent manner (5/8 survived using high dose)[53]	No	No	Not approved
ZMapp (Mapp Biopharmaceuticals)	Cocktail of 3 monoclonal antibodies – Ebolavirus specific compound	Most likely virus neu-tralisation	Yes	Yes	Yes started 24-48 hours post infection prevented death in cynomolgus macaques and Zmapp is able to revert advanced EVD when administered up to five days post infection[47;60]	Currently being used to treat small number of victims of the current EBOV outbreak	Yes	Not approved

have proven to be effective in mouse and primate models, and some safety and pharmacokinetic data in humans are available for AVI-6002.[52-54] In earlier outbreaks attention was paid to potential treatment of EVD patients with blood transfusion from EVD survivors. For instance, in the EVD outbreak in Kikwit (Democratic Republic of Congo) in 1995, patients receiving convalescent serum from EVD survivors showed a much lower CFR.[55] However these results were based on a small number of patients with a potential treatment bias. Furthermore, this passive immunotherapy did not seem to be effective in a non-human primate model.[56] Due to the potential for antibodies to enhance viral infections via antibody-enhancement mechanisms,[59] a note of caution is in order

for the use of passive immunotherapeutic strategies. However, there have been studies using such passive immunotherapeutic protocols, especially with monoclonal antibody treatment, which have been shown to be quite effective in non-human primate models of *Ebolavirus* infection and need to be considered.

CONCLUSION

Rapid and wide geographic spread of the current EBOV outbreak are reasons for increased alertness of clinicians dealing with returning travellers from the outbreak areas. Due to the initial non-specific presentation of EVD, the combination of fever (and/or EVD symptoms such as nausea, flu-like illness, headache, diarrhoea, myalgia, conjunctival effusion and redness of the oral and pharyngeal mucosa) in combination with high-risk exposure (contact with EVD patient or body fluids, wild animals, attendance of a funeral, visit to a local healthcare facility or preparing and/or consuming bush meat) is enough to proceed with isolation and management protocols in patients who visited endemic areas in the last 21 days. Currently treatment strategies rely solely on the early start of supportive care, where aggressive fluid replacement therapy is proven to drastically improve the survival rates. Specific antiviral EVD treatment strategies are still in the experimental phase. The current EVD outbreak stresses the already weak healthcare and public health systems in the affected countries, but also triggers increased awareness in countries at risk for EVD import cases. Given the ongoing outbreak, countries and clinical centres should be aware of the potential for admission of an EBOV infected person.

DISCLOSURE

The authors declare no conflicts of interest in the preparation of this manuscript.

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