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Ebola virus disease: a highly fatal infectious disease reemerging in West Africa

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Abstract

Ebolavirus can cause a highly fatal and panic-generating human disease which may jump from bats to other mammals and human. High viral loads in body fluids allow efficient transmission by contact. Lack of effective antivirals, vaccines and public health infrastructures in parts of Africa make it difficult to health workers to contain the outbreak.
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Ebolavirus has been known to cause outbreaks of severe hemorrhagic fever with high fatality in Africa since 1976 [1]. However, *ebolavirus* has been out of the spotlight of the clinical and scientific community because it mainly affects remote villages involving at most few hundred people, and these outbreaks often stopped spontaneously. In 2014, a large *ebolavirus* outbreak occurred in West Africa. This outbreak was first reported from Guinea in March 2014, although epidemiological investigation suggested that the first fatal case had occurred in December 2013 [2]. The outbreak then spread to Liberia, Sierra Leone, Nigeria, Senegal, and Mali in Africa. The first case diagnosed outside Africa was reported from USA on September 30, 2014 [3]. In October 2014, three nurses acquired *ebolavirus* locally in the United States and Spain which has generated huge media attention and public panic. The 2014 West Africa *ebolavirus* outbreak is unprecedented in many ways. Firstly, this is the largest *ebolavirus* outbreak recorded in history, with over 10,000 cases and a mortality rate of 48.5% [4]. Secondly, the outbreak involved major cities, including Conakry in Guinea, Free-town in Sierra Leone, Monrovia in Liberia, and Lagos in Nigeria [5,6]. The involvement of major cities increases the risk of rapid local dissemination, spread to neighboring countries, and transcontinental spread by air travel, and therefore presenting a major health threat to the entire world [7]. Here, we review the basic science, epidemiology and clinical aspects of *ebolavirus* which are relevant for the control of the current outbreak.

1. Taxonomy

Ebolavirus, together with *Marburgvirus* and *Cuevavirus*, are the three genera belonging to the family *Filoviridae* in the order *Mononegavirales* [8]. Four species within the *ebolavirus* genus can cause fatal human disease, including *Sudan*

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ebolavirus, *Zaire ebolavirus*, *Taï Forest ebolavirus* (also known as *Ivory Coast ebolavirus* or *Cote d'Ivoire ebolavirus*), and *Bundibugyo ebolavirus*. *Reston ebolavirus* can cause disease in pigs and monkeys but only asymptomatic infections in humans.

Although *ebolavirus* is the accepted terminology for the virus genus according to both ICTV and NCBI, the World Health Organization (WHO) and the Centers for Disease Control and Prevention have used the term "Ebola virus disease" (EVD) to describe the clinical disease caused by *ebolavirus*. In this review, we will use the term "*ebolavirus*" when referring to the virus, and EVD when referring to the clinical disease.

2. Epidemiology

Ebolavirus was first discovered in 1976 during an outbreak in Ebola River valley in Zaire (now Democratic Republic of Congo [DRC]) in Central Africa. Another simultaneous outbreak occurred in Sudan [9]. It was postulated that two earlier cases of EVD may have occurred in 1972. The evidence came from the investigation of the 1977 outbreak in Zaire [10]. A serum sample obtained from a physician was found to be seropositive. In 1972, that physician developed a febrile illness associated with sore throat, headache, myalgia, vomiting, diarrhea, rash and leukopenia about 2 weeks after he lacerated his finger during an autopsy on a Zairois student who died from hemorrhagic illness.

Since then, there have been numerous outbreaks in Africa, affecting six countries (Table 1). Most of the outbreaks occurred after 1994 in Sudan, Congo, DRC, Uganda, and Gabon, which are located in Central and East Africa. *Zaire* and *Sudan ebolavirus* are responsible for most outbreaks, and these species are associated with highest case-fatality rates, ranging from 44–100% and 41–69%, respectively. *Taï Forest ebolavirus* caused illness in an ethnologist who performed a necropsy on an infected chimpanzee in 1994 in Cote d'Ivoire of West Africa [11]. *Bundibugyo ebolavirus* has only been associated with two outbreaks since 2007, with relatively low case-fatality rate [12]. *Reston ebolavirus* can cause disease in pigs and be fatal in monkeys [13], but has not been definitively associated with any human disease, although asymptomatic infection, diagnosed with serological test, was identified in persons with contacts with infected monkeys and pigs [13,14].

In addition to clinically apparent EVD outbreaks, seroepidemiology studies showed that there is a high prevalence seropositive individuals, suggesting that asymptomatic or mild infection can occur [15]. In a study testing blood samples collected from 4349 individuals from 220 randomly selected village in Gabon between 2005 and 2008, 15.3% of samples were found have *ebolavirus*-specific antibodies using ELISA [16]. *Ebolavirus*-specific antibodies can also be found in individuals from areas without apparent EVD outbreak. For example, *ebolavirus*-specific antibodies, detected using indirect immunofluorescence slide test, were found in 13.4% of healthy individuals from a rainforest area of Liberia in the early 1980s [17]. Though these serological test results have not been confirmed by neutralization antibody study, it is highly likely that asymptomatic and mildly symptomatic infections are much more common than severely symptomatic and fatal illness.

The current West Africa EVD outbreak started in December 2013, when cases first appeared in Meliandou Village, Guéckédou of Guinea (Table 2) [5]. The index patient was a 2-year-old child with fever, black stool, and vomiting, with symptom onset on December 2, 2013, and died 4 days later. The disease then spread to other villages of the Guéckédou district, and also Macenta and Kissidougou district. The first peak occurred in March 2014 when patients were diagnosed with EVD in Liberia. The second peak occurred in May and June 2014, coinciding with the first report of cases from Sierra Leone. Contact tracing found that the initial cases in Sierra Leone attended a funeral of a highly respected "traditional healer", who has treated patients with EVD in Guinea [6,18]. There was a large increase in cases since July 2014. The first case in Nigeria was a traveler from Liberia, who has caused an outbreak involving 19 laboratory-confirmed cases from July to September [19]. Senegal and Mali reported the first imported cases on August 29 and October 22, 2014, respectively [20]. The first case of EVD diagnosed outside Africa was confirmed on September 30, 2014 [3]. The patient, from Liberia, arrived in USA on September 20, and developed symptoms on September 24. A separate EVD outbreak, also caused by *Zaire ebolavirus*, has occurred in DRC since July 2014 [21]. As of October 25, 2014, the DRC outbreak has involved 67 cases with 49 deaths [22].

Table 1
Ebola virus disease outbreaks from 1976 to 2012 [1].

Year	Place	*Ebolavirus* species	Number affected (case-fatality)
1976	Sudan	Sudan	284 (53%)
1976	DRC	Zaire	318 (88%)
1977	DRC	Zaire	1 (100%)
1979	Sudan	Sudan	34 (65%)
1994	Gabon	Zaire	52 (60%)
1994	Cote d'Ivoire	Taï Forest	1 (0%)
1995	DRC	Zaire	315 (81%)
1996 (Jan–Apr, Jul–Dec)	Gabon	Zaire	91 (73%)
1996	South Africa (ex-Gabon)	Zaire	1 (100%)
2000	Uganda	Sudan	425 (53%)
2001–2002	Gabon, Congo	Zaire	124 (78%)
2003 (Jan–Apr, Nov–Dec)	Congo	Zaire	178 (88.2%)
2004	Sudan	Sudan	17 (41%)
2005	Congo	Zaire	12 (83%)
2007	DRC	Zaire	264 (71%)
2007	Uganda	Bundibugyo	149 (25%)
2008	DRC	Zaire	32 (44%)
2011–2012	Uganda	Sudan	32 (69%)
2012	DRC	Bundibugyo	57 (51%)

DRC, Democratic Republic of Congo.

Table 2
Timeline of the 2014 West Africa Ebola virus disease outbreak.[a]

Date	Event
December 2013	The first case of EVD in West Africa, retrospectively identified.
March 21, 2014	The Guinea Ministry of Health reported a rapidly evolving outbreak of EVD in southeastern Guinea
March 30, 2014	First cluster of EVD cases reported from Liberia
May 2014	First EVD diagnosed in Sierra Leone
July 20, 2014	An acutely ill traveler from Liberia arrived in Nigeria. This is the first case of EVD in Nigeria
August 8, 2014	WHO declared that the West Africa EVD outbreak a Public Health Emergency of International Concern (PHEIC)
August 29, 2014	The first laboratory confirmed imported case of EVD in the Senegal.
September 30, 2014	CDC confirmed the first imported case of EVD in the United States.
October 6, 2014	The first laboratory confirmed local case of EVD in Spain
October 10, 2014	CDC confirmed the first local case of EVD in the United States
October 17,2014	WHO declares the end of EVD outbreak in Senegal
October 20, 2014	WHO declares the end of EVD outbreak in Nigeria
October 22, 2014	The first imported case of EVD in Mali

[a] The information is obtained from the references [2,3,5,19,20,132,133].

Together with these epidemiological data, viral genomic data has provided important information on the origin and the transmission dynamics of the 2014 West Africa *ebolavirus* strain. Phylogenetic analysis using the whole genome sequences of 99 *ebolavirus* strains from 78 patients revealed that the 2014 West Africa EVD outbreak is caused by a *Zaire ebolavirus* lineage that is most closely related to the one causing the 2007 EVD outbreak in DRC [2]. Molecular dating suggested that this 2014 West African lineage likely diverged from the Central African lineage in 2004 [23]. Analysis of the *ebolavirus* strains from Guinea and Sierra Leone showed that they are highly similar, which is in agreement with the findings from contact tracing (Fig. 1). However, there are two distinct lineages of *ebolavirus* in Sierra Leone which were estimated to have diverged in April 2014. This finding suggests that the virus has further mutated either in Guinea or in Sierra Leone [23]. On the other hand, the *ebolavirus* strain causing the DRC EVD outbreak in July 2014 is another Zaire strain most closely related to 1995 DRC outbreak Zaire strain [21], confirming that this is a separate outbreak.

Before 2014, the largest EVD outbreak affected less than 500 people. However, in the current outbreak, there are already 10,141 cases and 4922 deaths as of October 25, 2014 [4]. Further studies must be undertaken to understand the viral and environmental factors that contribute to the unprecedented scale of this outbreak. It is still uncertain at this stage whether the virus has become more transmissible in human or has increased environmental stability.

It is unclear why *Zaire ebolavirus* suddenly appear in West Africa. The only human case of *ebolavirus* infection in West Africa before the 2014 outbreak occurred 20 years ago. Epidemiological investigation suggested that the first patient was a 2-year-old child in Meliandou Village, Guéckédou. One postulation is the spread of the virus by fruit bats from Central Africa [24], but this will require confirmation by further field studies.

Humans can acquire the infection from infected animals or infected persons. The index patients of EVD outbreaks are usually persons working in forests, caves or mines. Many of these index patients are bushmeat hunters with direct contact with animals. In the natural setting, transmission from animals usually involves direct contact with the animal or handling of the carcasses. However, most humans acquire the infection through direct person-to-person transmission that can occur via direct contact with body fluids. Many clusters occurred when people attended the funeral of an infected patient [6,18]. A case–control study showed that household contacts with direct physical contact with the ill patient or cadaver and exposure to body fluids were risk factors for acquiring infections [25]. *Ebolavirus* can be transmitted directly through broken skin or mucous membranes from the blood, body fluid, and secretions of the infected person, as the virus could be detected in blood, urine, saliva, seminal fluid, breast milk, tears, stool, skin, and swabs from vagina, rectum and conjunctiva [26]. Virus shedding can be prolonged. Reverse-transcriptase polymerase chain reaction (RT-PCR) remained positive in the blood for up to 21 days, in the vaginal, rectal and conjunctival swab for up to 33 days, and in the seminal fluid for up to 101 days [27]. Live virus could be isolated from a patient's seminal fluid 82 days after symptom onset. Transmission through environmental surfaces is possible. It was shown that live *ebolavirus* can survive on dried glass or plastic surface for up to 50 days [28]. Studies have shown that lower temperature and higher absolute humidity are associated with EVD outbreaks [17]. Animal studies showed that other routes may be possible. *Reston ebolavirus* can be transmitted from pigs to cynomolgus macaques without direct contact, suggesting that *ebolavirus* can be transmitted from animal reservoirs to humans without direct contact [32]. Studies in rhesus macaques showed that *ebolavirus* given orally can cause fatal infections [33]. Aerosol transmission in macaques has been documented [34], but this route of transmission has not been documented in humans. Almost all cases in the current outbreak are related to person-to-person transmission. The effective reproduction numbers for the 2014 outbreak were estimated to be 1.71 for Guinea, 1.83 for Liberia, 1.20 for Nigeria, and 2.02 for Sierra Leone [5].

Hospital-acquired infections are common. During the 1976 EVD outbreak, the index case had transmitted the virus to healthcare workers and hospitalized patients with at least 15 generations of person-to-person transmission [29]. During the current outbreak, at least 318 healthcare workers were infected, with at least 151 deaths [5]. In addition, *ebolavirus* is also transmitted indirectly when the broken skin or mucous membranes come into contact with the contaminated environment or items such as soiled clothing, bed linen, or used needles. Unsterilized syringe was associated with the 1976 outbreak in Zaire [9]. In one study, the virus could also be found on the doctor's blood-stained glove and the bloody intravenous insertion site, but was not found on the patient's surrounding environment [26]. Laboratory-acquired infections from accidental puncture while handling infected materials have been reported [30,31].

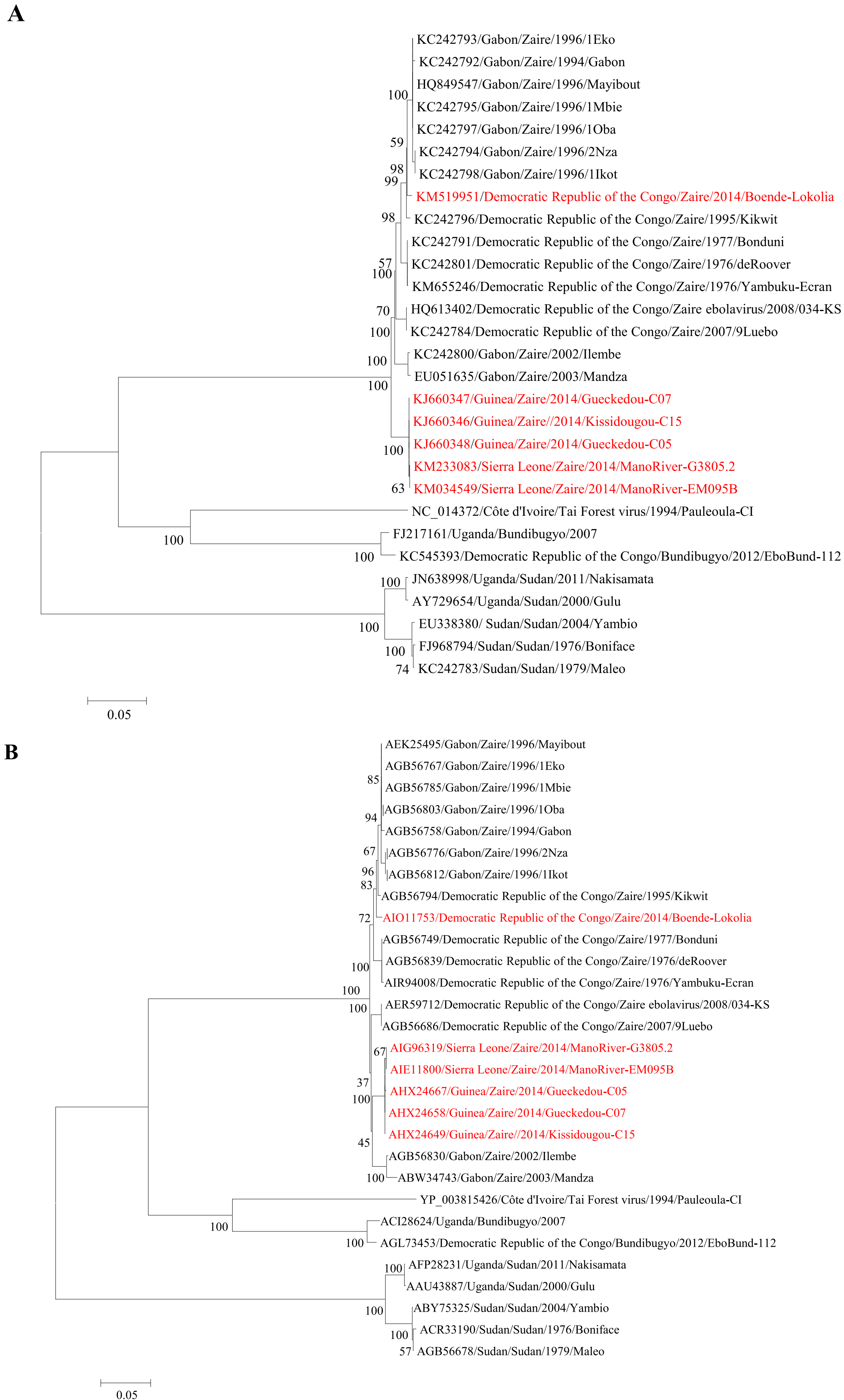

Fig. 1. The phylogenetic tree of *ebolavirus* glycoprotein (GP) nucleotide (A) and protein (B) sequences. The neighbor joining tree of 29 GP nucleotide and protein sequences built using distances inferred by the K2P and JTT models, respectively. Bootstrap values (1000 replications) for key nodes are shown. The 2014 strains are highlighted in red.

In a detailed analysis of 3343 confirmed and 667 probable *ebolavirus* cases in Guinea, Liberia, Nigeria and Sierra Leone from December 30, 2013 to September 14, 2014, the median age was 32 years with an interquartile range from 21 to 44 years. The male to female ratio was 1:1 [5]. The incubation period is usually 4–10 days, but can be as short as 2 days and as long as 21 days [32]. In the current outbreak, the mean incubation period was 11.4 days, with 95% of patients had symptom onset within 21 days.

3. Animal reservoir

Ebolavirus has been found in several animals, including bats, primates (chimpanzee, gorilla), rodents (rats, mice, shrews), duikers (*Cephalophus* species), and pigs [33]. Although *ebolavirus*-specific antibody can be detected in up to 25.2% of serum samples from dogs in *ebolavirus* endemic areas [34], there have not been any reports on the isolation of *ebolavirus* or detection of *ebolavirus* nucleic acid from dog's samples. In some animals, *ebolavirus* causes epidemic fatal disease. It was estimated that after the *ebolavirus* outbreak in the DRC between 2001 and 2003, there were 89%, 56% and 53% reductions in the chimpanzee, gorilla and duiker populations, respectively [35].

Bats have been proposed to be the source of *ebolavirus*. Bats have been known to be the source of several human viruses including *Severe Acute Respiratory Syndrome-related Coronavirus* (SARS-CoV), *Hendra virus*, *Nipah virus*, *Menangle virus*, *Rabies virus* and *Lyssaviruses* [36,37]. *Ebolavirus* was first reported to be found in the fruit bat species *Hypsignathus monstrosus*, *Epomops franqueti* and *Myonycteris torquata*, which were captured during the 2001 and 2003 outbreak in Gabon and the DRC [38]. During the investigation of the 2007 outbreak in DRC, it was found that the affected area has a large palm plantation where migratory fruit bats settled for food between April and May, and that the first human case ate a freshly killed bat which was bought from a hunter in May [39].

Ebolavirus is not limited to Africa. Serological study showed that antibodies against *Zaire* and *Reston ebolavirus* could be detected in fruit bats from Bangladesh [40], while *Reston ebolavirus* could be detected in fruit bats from the Philippines [41]. A study in China showed that up to 3.8% of bats were seropositive for *ebolavirus* [42]. The most common bats species with *ebolavirus* identified in China include *Rousettus leschenaultia*, *Hipposideros Pomona*, *Miniopterus schreibersii*, *Pipistrellus pipistrellus*, *Myotis ricketti*, in which other novel viruses have also been identified [43–45]. *Reston ebolavirus* have been found in domestic pigs in the Philippines and China [33,46]. Antibodies specific against all 5 *ebolavirus* species have been found in apes of Indonesia [47].

4. Virology

Like all filoviruses, *ebolavirus* is a filamentous enveloped virus with a negative-sense, non-segmented single-stranded genome of about 19 kb, measuring 80 nm in diameter and up to 1100 nm in length [48]. The 3′ end of the viral genome consists of a non-coding region, followed by 7 genes (nucleoprotein [NP], virion protein 35 [VP35], virion protein 40 [VP40], glycoprotein [GP], virion protein 30 [VP30], virion protein 24 [VP24], and RNA-dependent RNA polymerase [L]), and then a non-coding region at the 5′ end. Each gene encodes one protein, except for the GP gene, which encodes three glycoproteins. The full-length GP is produced by RNA editing, in which the two reading frames are joined together by slippage of viral polymerase at an editing site, generating an mRNA transcript that allows read-through translation of GP. GP contains GP1 subunit for host cell receptor binding and the GP2 subunit for cell-virion membrane fusion. The soluble GP (sGP) is generated by an unedited transcript, which is much more abundant than GP [49]. A third protein, called small soluble GP (ssGP), is produced via RNA editing [50]. GP, VP24 and VP40 are associated with membrane, while NP, VP35, VP30, and L protein bind to the viral genome, which are required for viral genome replication and transcription. VP24 is also required for the assembly of nucleocapsid.

The viral life cycle starts when GP1 attaches to cell surface receptors [51,52]. Although *ebolavirus* can bind to host cell surface dendritic cell (DC)-specific ICAM3-grabbing non-integrin (SIGN) (DC-SIGN), liver and lymph node SIGN (L-SIGN) and T cell immunoglobulin and mucin domain-containing 1 (TIM1), it is currently unclear which cell surface receptor is most important. After attachment, viral entry occurs via macropinocytosis and clathrin-mediated endocytosis [53]. After entry, the virus is then trafficked into endosome. Inside the endosome, several factors are required for the fusion of the viral and endosomal membrane to occur, including acidification of the endosome, and priming and triggering of the GP. Priming of GP occurs when GP1, which is bound to GP2, is cleaved into the 20 kDa GP1 by cathepsin L and then to 19 kDa by cathepsin B. Triggering occurs when the 19 kDa GP1 undergoes conformational change to expose the fusion loop [52]. Another important event in the fusion step is the binding of the endosomal membrane protein, Niemann-Pick C1 (NPC1), to cleaved GP1 [54]. Viral genome replication and transcription then take place in the cytoplasm, which require the viral polymerase L protein, VP35, VP30 and NP. VP24 is required for nucleocapsid formation and assembly. Viral transcription is also regulated by VP40 [55]. The GP, after modification in the Golgi, is trafficked to the plasma membrane, where it is associated with other proteins. Virus budding and release then occur, and require the matrix protein VP40.

In addition to the viral life cycle, these viral proteins are also involved in the pathogenesis of the infection. GP can cause destruction of endothelial cells [56,57]. Several lines of evidence suggest that the sGP is important in the modulation of host inflammatory response and immune defense. *In vitro*, sGP can inhibit the neutralizing activity of anti-GP antibody [58]. sGP also subverts anti-GP immune response by inducing a host antibody that competes for the binding site of anti-GP antibody [59]. sGP can also interact with neutrophils, although the receptor for this interaction is controversial

[60,61]. On the other hand, sGP may limit the virulence of the virus. Recombinant *ebolavirus* without sGP are less cytotoxic than those with sGP [56]. sGP can protect endothelial cells from TNF-α [62]. sGP can also bind to GP2, but the importance of this finding requires further study [63]. VP35 also inhibits the innate immune RIG-I signaling, interferon(IFN)-α and IFN-β production, and dendritic cell maturation [64]. VP24 is important in the inhibition of IFN signaling [65,66].

It is currently unclear whether the 2014 West Africa *ebolavirus* strain possesses unique characteristics that favor its spread among the human populations. One possible reason is the higher mutation rate. It has been shown that the mutation rate in the current outbreak is about twice as high than that in previous outbreaks [23]. Decontamination methods for *ebolavirus* include heat inactivation at 58–60 °C for 1 h or at 75 °C for 30 min, γ-irradiation, chemicals, including formalin and quaternary ammonium ion, and nanoemulsions, which disrupts the membrane [70].

5. Pathogenesis

In most cases, *ebolavirus* likely enters the body via breaks in the skins or mucous membranes. Infection of monocytes, macrophages and dendritic cells helps to disseminate the virus to the lymph nodes via the lymphatics, and to the liver and spleen via the blood. Notably, *ebolavirus* does not infect lymphocytes, although lymphocyte depletion occurs due to apoptosis [67]. When the infected monocytes, macrophages or dendritic cells move out of the lymph nodes and spleen, the virus can disseminate to other organs [68]. *Ebolavirus* can also infect endothelial cells, fibroblasts, hepatocytes, adrenal cortical cells, and epithelial cells. Since patients with severe disease have higher viral load in blood, uncontrolled viral replication may play an important role in the pathogenesis of severe EVD [69].

Macroscopically, there are hemorrhagic lesions on the skin, mucous membranes, and visceral organs at autopsy. Microscopic examination reveals necrosis in many organs, including the liver, spleen, kidneys and gonads [70]. In the liver, there is also evidence of apoptosis, microvesicular steatosis and kupffer cell hyperplasia. Councilman bodies, which are apoptotic liver cells that have dislodged from adjacent hepatocytes, may be present. Eosinophilic oval or filamentous cytoplasmic inclusions may be present, and they are aggregates of *ebolavirus* NP. Examination of the lung shows hemorrhages and diffuse alveolar damage. Infection of the adrenal gland has been documented in humans [68]. Adrenal necrosis may be one possible pathogenic mechanisms leading to hypotension.

Marked coagulopathy is a hallmark of EVD. Disseminated intravascular coagulation frequently occurs. It is believed that tissue factors secreted from monocytes/macrophages are related to the coagulopathies in macaques [71]. The level of protein C is reduced during coagulopathy [72]. Protein C is important in inflammatory response. A study in which rNAPc2 and rhAPC-treated rhesus macaque had better outcome, had higher levels of genes transcriptionally regulated by CCAAT/enhancer-binding protein alpha, tumor protein 53, and megakaryoblastic leukemia 1 and myocardin-like protein 2 [73]. Although the virus can infect endothelial cells, vascular lesions are not seen in postmortem tissues, and therefore the severe bleeding is unlikely to be related to the direct destruction of the blood vessels by the *ebolavirus*.

Similar to other causes of severe sepsis, cytokine/chemokine dysregulation occurs in patients with severe disease. Fatal cases had high levels of MIP-1β, IL-6, IL-8, and IL-10 [74]. In one study, asymptomatic patients had elevated levels of IL-1β, IL-6, TNFα, MCP-1, MIP-1α and MIP-1β in the plasma [75]. However, in one study, gene expression levels of cytokines in peripheral blood mononuclear cells from infected patients were not different from that of non-infected patients. The level of plasma nitric oxide is higher in fatal than that of non-fatal cases [69]. The high levels of nitric oxide may have contributed to lymphocyte apoptosis, tissue and vascular damage, and may be associated with the hemodynamic instability seen in fatal cases.

6. Immune response

One of the major innate defense mechanisms against viral infection is the IFN pathway. IFN are produced by cells upon viral infection, and induced several proteins, including the IFN-induced transmembrane proteins (IFITMs). It has been shown that the type 1 IFNs and the IFITM1, and to a lesser extent IFITM3, restrict the cell entry of *ebolavirus* [76]. As mentioned above, the viral proteins VP35 and VP24 interfere with the IFN pathway which may in turn dampen the priming effect on the adaptive immune response, thus allowing the virus to replicate to high titers.

The importance of humoral and cell-mediated immune response is illustrated by a study comparing survived and fatal cases [77]. *Ebolavirus*-specific IgM and IgG were detected in all survivors during the early course of illness, with positive titer detected as early as 2 days after symptom onset. In contrast, only one third and none of fatal cases had detectable IgM and IgG response, respectively. Activation of cytotoxic T cells, as indicated by the upregulation of FasL and perforin mRNA expression, was observed at the time of viral clearance for survivors, and the levels of IFNγ, soluble Fas and soluble FasL were low during the recovery phase, suggestive of a regulated cytotoxic T cell response during the recovery phase. For the fatal cases, the levels of IFNγ, soluble Fas and soluble FasL were elevated and increasing before death, suggestive of massive activation of cytotoxic T cells.

After natural infection, neutralizing antibodies are produced in some patients. Persistent serum-neutralizing activity and IgG immunoreactivity for at least 12 years after infection have been found in some survivors [78]. Monoclonal antibodies against GP have been shown to protect non-human primates from lethal infection as both post-exposure prophylaxis [79–81] and treatment [82,83]. The level of anti-GP IgG highly correlated with survival in guinea pigs and cynomolgus macaques which were vaccinated by GP expressed in *adenovirus* or *vesicular stomatitis virus* vectors [84]. Antibodies are

also critical in conferring protection for cynomolgus macaques after vaccination with recombinant vesicular stomatitis virus expressing GP [85].

Vaccine studies in animals have provided clues to the contribution of cell mediated immunity in conferring protection. Mice study showed that virus-like particles induced protective immunity only in wild type mice but not in NK-cell depleted mice, therefore suggesting that NK cells are important in protective immunity [86]. The role of T cells is controversial. While one study showed that CD8+ T cells are required for conferring protection [87], another study showed that it is not required [85].

Despite a high case-fatality rate, there are many individuals with asymptomatic infections, as evidenced by a high percentage of seropositive individuals. Although many factors may determine whether a patient develops symptomatic disease, host genetic differences likely play an important role, as in other infectious diseases [88,89]. Mice with different genetic backgrounds had different susceptibility to *ebolavirus* infection, and possibly related to the variations in the *Tek* gene responsible for coagulation [90].

7. Clinical features

EVD typically progresses rapidly with multisystem involvements, and in particular coagulopathy leading to severe hemorrhage. During the early stage of illness, the patients usually exhibit an acute onset of non-specific flu-like symptoms, including fever, chills, myalgia, and headache, followed by gastrointestinal symptoms including abdominal pain, nausea, vomiting and diarrhea [68]. Respiratory symptoms, such as cough and sore throat may also occur. A maculopapular rash typically occurs on day 5–7 after symptom onset, and is associated with erythema and desquamation. Hemorrhagic phenomenon then appears, which can include petechiae or ecchymoses, uncontrolled oozing from venipuncture sites, and mucosal hemorrhages. However, it should be noted that massive hemorrhage occurs in fewer than half of patients and is seldom the cause of death. In the 2014 outbreak, unexplained bleeding was reported in only 18% of patients [5]. Hypovolemia can develop rapidly. As in other causes of severe sepsis, complications including disseminated intravascular coagulopathy and multi-organ failure can occur. Death usually occurs between days 7 and 16 after symptom onset. Survivors usually improved on day 6–11, when neutralizing antibodies start to develop. In the convalescent phase, myelitis, recurrent hepatitis, psychosis and uveitis may develop [91].

For pregnant women, there may be an increased risk of severe illness and death. There is also an increased risk of spontaneous abortion and pregnancy-related hemorrhage. In the 1996 outbreak in DRC, fetal or neonatal loss occurred in all 5 third trimester pregnancies [92]. In fact, the first case of EVD in Sierra Leone was a pregnant woman with miscarriage [18].

Blood test may show thrombocytopenia, leukopenia, hepatic dysfunction with elevated levels of aspartate aminotransferase more than that of alanine aminotransferase, amylase and d-dimer. Hemolysis is severe especially in the acute stage [93]. Blood film may also show atypical lymphocytes [69]. Renal impairment usually appears by the end of the first week. Fatal cases have higher viral load in the blood [6,77].

Despite a fatal disease in over 50% of infected patients, some individuals did not develop symptoms. During the 2 EVD outbreaks in Gabon in 1996, 24 asymptomatic individuals with direct exposure to infected materials were identified [75]. Eleven of these patients developed specific IgM and IgG response to *ebolavirus*. Furthermore, RT-PCR for *ebolavirus* was positive in the peripheral blood mononuclear cell samples from 7 of these 11 seropositive individuals. Positive-strand RNA, the presence of which suggests active replication, was detected in 4 individuals.

8. Diagnosis

Since high grade viremia occurs in the acute period, the preferred diagnostic test is RT-PCR of the blood. RT-PCR targeting the NP can be performed in the serum, plasma, whole blood, or oral fluid [93,94]. RNAemia can be detected on the day of symptom onset with viral loads of about 4–5 logs copies per ml. The viral load increases rapidly and reaches 7 to 8 logs on day 2 of symptom onset. The level of RNAemia peaks on about day 5 after symptom onset, and the level of RNAemia is higher in fatal cases than that in survivors [93]. Antigen-capture ELISA can also be used on blood samples, but is less sensitive than RT-PCR [93]. A rapid immunochromatographic assay for the detection of *ebolavirus* antigen, which claimed to provide result in 15 min, was recently announced by the France's Atomic Energy Commission [95]. Viral culture from the blood using Vero E6 is usually positive in the acute stage, but should not be performed except in biosafety level 4 facilities. Viral particles may be seen in the serum under electron microscope, which was used in the confirmation of the first cases in the current outbreak [2]. Other than blood samples and oral fluids, the virus can also be detected in other body fluids, but these are not usually used for diagnosis. Serum IgM is useful during the convalescent phase, but is not useful in the acute setting. Serum IgG is not reliable, as one study showed that 4 out of 18 survivors did not have detectable IgG levels at the time when viral antigen was no longer detected [93].

Several biomarkers have been proposed to be associated with adverse outcomes. In addition to elevated cytokine/chemokine levels, levels of thrombomodulin and ferritin are also elevated in patients with poor outcome, while the sCD40L, a protein produced by platelet responsible for repairing damaged endothelium, is higher in survivors [74].

9. Treatment

Currently, the cornerstone in the management of patients with EVD is supportive care. Although this is taken for

granted in developed countries, these supportive measures are usually lacking in the most affected areas with poor healthcare infrastructures. Aggressive volume and electrolyte management, oral and intravenous nutrition, medications to control fever and gastrointestinal distress, and medications to treat pain, anxiety and agitation are important measures [68]. Co-infections should be actively sought and treated appropriately [96].

There are currently no licensed antiviral drugs to treat EVD. Before the 2014 outbreak, specific therapy has been used in humans with some success. During the 1976 EVD outbreak, a male investigator pricked himself while transferring homogenized liver from an infected guinea pig [30]. Six days after the injury, he developed fever, central abdominal pain and nausea. On the same day, he started to receive a 14-day course of human IFN 3 million units every 12 h administered intramuscularly. The human IFN was prepared by stimulating peripheral lymphocytes with Sendai virus *in vitro*. On day 8 after the injury, he received 450 ml of convalescent sera which was obtained from infected people from Zaire, and the viral load was reduced from $10^{4.5}$ guinea-pig infective units per ml to 3-10 guinea-pig infective units per ml. On day 11 after the injury, he received the 2nd infusion of convalescent sera. He eventually recovered. Subsequently in the 1995 *ebolavirus* outbreak in Kikwit, 7 of 8 patients who received blood donated from 5 convalescent patients survived [97]. Both convalescent plasma and IFN-β were later tested in rhesus macaques, but only convalescent plasma was found to improve survival [98,99]. In the 2014 EVD outbreak, convalescent plasma has been given to several patients, but the efficacy of convalescent plasma remains to be determined.

In addition to convalescent plasma and IFN-β, several experimental treatments have been shown to improve survival in non-human primates (Table 3). The first strategy employs the antiviral effect of neutralizing antibodies, either through direct administration of the antibodies or through active immunization. Monoclonal antibody cocktails targeting different sites of the *ebolavirus* were shown to protect primates [68,82,83]. ZMapp, a cocktail of monoclonal antibodies that are originally contained in the preparation MB-003 (consisting of human or human-mouse chimeric mAbs c13C6, h13F6 and c6D8), and ZMab (consisting of murine mAbs m1H3, m2G4 and m4G7 targeting GP) have been shown to protect rhesus macaques from lethal challenge when given up to 5 days post infection [82]. Post-exposure vaccine, such as the vesicular stomatitis virus-based vaccine, can elicit anti-GP antibodies and improve the survival of rhesus macaques when given 20–30 min post infection [100]. The second strategy is to inhibit the activity of virus proteins. Antisense oligonucleotides target the viral L protein and VP proteins, can also improve survival of infected rhesus macaques [101,102]. The third strategy aims to ameliorate the deleterious host immune response. Recombinant nematode anticoagulant protein c2 and recombinant human activated protein C could alleviate the coagulopathy and improve survival in animal models [103,104]. However, the clinical efficacy of recombinant human activated protein C is questioned because a randomized double-blind placebo-controlled study did not show survival benefit in patients with septic shock [105]. Among these experimental treatments, monoclonal antibody cocktail (ZMapp) and small interfering RNA (Tkm-Ebola) have been used in patients during the current 2014 outbreak [106,107]. Both antibody and RNA-based therapy might be limited to a particular species, and may become ineffective if there are mutations affecting the related antigenic epitopes or gene targets. The efficacy of these experimental treatments in humans remains to be determined.

Several drugs currently undergoing clinical trials have antiviral activity against *ebolavirus*. One of the most promising is nucleotide analog brincidofovir (CMX-001), which is a lipid-conjugated prodrug of cidofovir that is converted intracellularly to cidofovir. Brincidofovir is currently undergoing phase III clinical trials for *adenovirus* and *cytomegalovirus* infection. This drug has *in vitro* activity against *ebolavirus* [108], and has been used as an experimental treatment in the current outbreak [107].

Repurposing of licensed drugs has been considered for the treatment of EVD as in other emerging infectious diseases which have no specific antiviral treatment [109–113] (Table 4). The IC_{50} of clomiphene, chloroquine and imatinib

Table 3
Experimental post-exposure prophylaxis/treatment after lethal *ebolavirus* challenge in non-human primates.

Class	Drug	Animal model	Timing of the first dose of treatment p.i.	Survival
Monoclonal antibody	ZMapp	Rhesus macaques	5 days	100% [82]
Vaccine targeting GP	Vesicular stomatitis virus-based vaccine	Rhesus macaques	20-30 min	50% [100]
Passive immunotherapy	Convalescent whole blood	Rhesus macaques	Immediately after infection	0% [98]
RNA interference	siRNAs targeting RNA polymerase L protein, VP-24 and VP-35	Rhesus macaques	30 min	100% (7 doses) [101]
	PMO*plus* (targeting VP24)	Rhesus macaques	30-60 min	62.5% [102]
Modulators of blood coagulation	Recombinant nematode anticoagulant protein c2	Rhesus macaques	10 min	66% [103]
	Recombinant nematode anticoagulant protein c2	Rhesus macaques	24 h	33% [103]
	Recombinant human activated protein C	Rhesus macaques	30-60 min	18% [104]
Modulation of host innate immune response	Interferon-β	Rhesus macaques	18 h or 2 days	Prolonged survival, but all died [99]

N. A., not available; p. i., post infection; PMO*plus*, positively-charged phosphorodiamidate morpholino oligomers.

Table 4
Licensed drugs with antiviral activity against *ebolavirus*.

Drug	Class	IC50 (μM)	Peak serum level	Animal models
Clomiphene	Selective estrogen receptor modulators	3.83-11.1 [109]	Single dose 50 mg orally: 0.025 μM [134]	Mouse model: when given 1 h p.i., 90% survival [109]
Toremifene	Selective estrogen receptor modulators	0.937-6.17 [109]	Single dose 600 mg orally: 13.7 μM [135]	Mouse model: when given 1 h p.i., 50% survival [109]
Favipiravir[a]	Viral RNA polymerase inhibitor	67 [110]	Single dose 2400 mg orally: 587 μM (92.17 μg/ml) [136]	Mouse model: when given on 1 h or day 6 p.i., 100% survival [110,137]
Chloroquine	Anti-malarial	16 [111]	Single dose, 300 mg orally: 0.15 μM [138]	N.A
Imatinib	Tyrosine kinase inhibitor	20 μM inhibited viral replication by 95% [112]	Single dose, 200 mg orally: 1.56 μM [139]	N.A

N.A, not available.
[a] Only licensed in Japan.

are above the peak serum level in humans, and therefore these are unlikely useful clinically. Both toremifene and favipiravir had peak serum levels above the IC_{50}, and both of these drugs have shown to improve survival in mouse models [109,110]. Toremifene can achieve plasma concentrations of about 10 μM at a high dose of 600 mg/day that are inhibitory in cell culture (IC_{50} 1−4 μM in Vero cells). However the murine model utilizing a high dose of 60 mg/kg initiated at 1 h post infection can only achieve a 50% survival [109]. Moreover prolonged QTc changes have been reported at a dose of 300 mg once daily [114]. Thus clinical trials should consider dosage adjustment, cardiac and electrolyte monitoring. Favipiravir also appears effective in mice model, and has been administered to a patient in France [115]. Although the efficacy of these drugs in humans is uncertain especially when non-human primate treatment data are not available, they may be considered when better options are not available and the benefit-risk ratio is favorable.

Since fusion of the viral and cellular membrane in the endosome plays an important role in the viral life cycle, several studies have used chemical libraries to screen for molecules which can inhibit processes in the fusion step. Using this strategy, molecules that can inhibit cathepsin-L mediated cleavage of GP [116] and binding of GP1 and NPC1 [54] have been identified.

10. Post-exposure prophylaxis in humans

The experience of post-exposure prophylaxis in humans mainly comes from laboratory accidents. In 2004, a scientist from the US Army Medical Research Institute of Infectious Diseases (USAMRIID) suffered from a needlestick injury while working with mouse-adapted variant of *Zaire ebolavirus* [117]. As post-exposure prophylaxis, he received a live-attenuated recombinant vesicular stomatitis virus expressing GP of *Zaire ebolavirus* 48 h after the accident. The scientist had fever 12 h after receiving the vaccine, but otherwise remained asymptomatic.

11. Pre-exposure vaccination

Three phase-1 clinical trials have been conducted. The first trial used an adenovirus-based vaccine expressing GP, involving 23 subjects in the vaccine group and 8 subjects in the placebo group [118]. The vaccine group was further divided into a high dose and a low dose group. Specific antibody response developed in 100% for recipients of high dose vaccine. The vaccine was well-tolerated. Two vaccine recipients were complicated by the development of antiphospholipid antibody, and one vaccine recipient developed fever >40 °C. In the second trial, two GP (Zaire and Sudan) DNA vaccines were expressed in a VR-1012 expression vector and produced in *Escherichia coli* [119]. There were 20 subjects in the vaccine group and 6 subjects in the placebo group. Specific antibody responses to at least one of the vaccine antigens developed in all vaccine recipients. This second vaccine is also well-tolerated. In the vaccine group, 1 subject developed raised creatine phosphokinase (associated with vigorous exercise) and 1 subject developed herpes zoster. The third trial (VRC206 study) evaluated a DNA vaccine encoding the wild type GP antigens from *Zaire* and *Sudan ebolavirus* which is produced in *E. coli* [120]. Ten subjects were enrolled. There were no serious adverse events. The vaccine elicited specific antibodies against both GP antigens. At the time of writing, two other vaccines are undergoing phase I clinical trials [121].

Vaccines against *ebolavirus* consisting of virus-vectors such as adenovirus type 5, human parainfluenza virus type 3, vesicular stomatitis virus; virus-like particles with VP40, NP and GP, and recombinant *ebolavirus* have been tested in animal models [122]. The efficacy of these vaccines in humans awaits further studies.

12. Infection control in hospitals

Current evidence suggests that *ebolavirus* is transmitted via contact with contaminated body fluid or the contaminated environment, and therefore the practice of contact precautions with appropriate personal protection equipment (PPE) is of utmost importance when handling suspected or confirmed cases of EVD. Healthcare workers should preferably work in pairs so as to mutually guard against breaks in infection control measures. They are required to put on the PPE in the following sequence, from N95 respirator, water repellent cap or hood, full length shoe cover or boot, water resistant gown, face shield, and long nitrile gloves. If the patient has hemorrhagic symptoms, double nitrile gloves should be worn. In

view of the high virulence and mortality, patients suspected to have EVD should be isolated in airborne isolation room in the developed countries, although WHO allows cohorted nursing in the designated areas with dedicated instruments, where access should be restricted in the developing countries with limited isolation facilities [123].

Degowning remains the most critical procedure for the healthcare workers. The most contaminated PPE should be removed first, from long nitrite gloves, water resistant gown, full length shoe cover or boot, face shield, water repellent cap or hood, and finally N95 respirator. Hand hygiene with alcohol-based hand rub should be performed in each step of degowning. When the hand is visibly soiled, it should be washed with soap and water. Healthcare workers must be well trained and audited for the proper procedure of gowning and degowning.

When the suspected or confirmed case of EVD dies, the healthcare and mortuary workers are required to wear PPE as described above. The dead body is placed in double bags with leak-proof characteristic of no less than 150 μm thick. Absorbent material should be put under the body and placed in the first bag. The surface of each body bag is wiped with 10,000 ppm sodium hypochlorite solution. The bags are sealed and labeled with the indication of highly-infectious material (category 3) and moved to the mortuary immediately. Viewing in funeral parlor, embalming and hygienic preparation are not allowed. The dead body should not be removed from the body bag and should be sent to cremation as soon as possible.

13. Public health response

Since the outbreak was first reported to WHO on March 23, 2014, the situation continues to deteriorate, and the consequences can be catastrophic in terms of lost lives but also severe socioeconomic disruption and a high risk of spread to other countries. On August 8, 2014, WHO declares the EVD outbreak in West Africa a "Public Health Emergency of International Concern". Preparedness and response plan were made available in most of the health authorities all over the world. The aim is to detect the first imported case for early isolation in order to prevent local transmission in the community and healthcare setting. Risk assessment in the port health, emergency room, and outpatient clinics for any patient fulfilling both clinical and epidemiological criteria for EVD is important. For the clinical definition, patient suffering from a sudden onset of fever with over 38 °C, or having at least one of the following signs including inexplicable bleeding, bloody diarrhea, bleeding from gums, bleeding into skin or eye, or hematuria should be alerted, while the epidemiological definition includes close contact with a confirmed or probable case of EVD or resided in or history of travel to an affected area or countries within 21 days before onset of symptoms. For healthcare workers working in volunteer medical services or non-government organizations, who have direct contact with patients in the affected areas or countries, should also perform medical surveillance for at least 21 days after leaving the affected areas or countries. They are required to seek medical advice promptly if there are any symptoms of fever, diarrhea, vomiting, rash or bleeding during medical surveillance.

One of the major problems with the current outbreak is the panic associated with the disease. Many patients with symptom did not seek medical care because of the fear of contracting the disease from the hospital [124]. Therefore, the local governments and health authorities should focus on the health education and give a clear instruction to the person for seeking early medical attention in the unaffected areas of Africa. However, when the community transmission of *ebolavirus* is uncontrolled, implementation of home quarantine for up to 21 days (one incubation period) can be considered. Home quarantine measure had been used to control the community spread of SARS in Beijing, Taiwan, Singapore, and Toronto [125]. However, the public health staff is expected to face unprecedented challenges in implementing an extensive quarantine policy, as they have a dual role of monitoring compliance and providing support of daily necessities to people in quarantine. The countries next to the affected areas require implementing border control measures to screen for any suspected case of *ebolavirus*. Although these measures may adversely affect the international travel and economy, it may be worthwhile to implement such a strict measure to control this re-emerging infectious disease with high mortality and psychological fear in a timely manner.

14. Conclusion

Humans are constantly under the threat of infectious diseases. Some emerging infectious diseases have been especially important in human history with significant loss of population, economic disruption and political instability. *Yersinia pestis* caused the black death in Europe, killing up to one-third of the population [126]. The 2010–2013 cholera outbreak after the major earthquake in Haiti reminded us that a seemingly easy-to-treat and control infection can cause large outbreaks when the infrastructure is damaged [127]. SARS coronavirus, MERS coronavirus, and the avian influenza viruses have caused epidemics with major health and economic effects [128–131].

The current 2014 West Africa EVD outbreak is unprecedented in that this is the largest EVD outbreak with local transmission outside Africa. One of the major differences from previous outbreaks is that it has affected crowded major cities in West Africa where the infrastructure has been heavily damaged due to civil wars. The rapid spread is facilitated by the efficient person-to-person transmission due to high viral loads in blood, bodily secretions and the contaminated environment. The large amount of virus particles shed in body fluid makes this virus very contagious, even among healthcare workers who are already equipped with PPE. Whole genome study showed that the current West Africa *ebolavirus* strain is phylogenetically distinct from previous outbreak strains, and this current *ebolavirus* strain has higher mutation rate than previous strains. However, it is currently not known whether this strain is particularly virulent or transmissible.

Currently, there are major gaps in our understanding of the disease due to the lack of systematic epidemiological, pathological, clinical and virological studies that are taken for granted in developed countries. For example in the pandemics and epidemics caused by coronaviruses and influenza viruses, many studies were conducted quickly within few months of the outbreak, and the results allowed early control of the outbreak and the implementation of scientifically sound clinical management of the patients [128,130,131]. A coordinated effort involving virologists, clinicians, epidemiologists, governments and international organizations is necessary to prevent this EVD outbreak to become a pandemic.

Conflict of interest

All authors declare no conflict of interest.

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