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A review on the Ebola virus, outbreak history and the current research tools to control the disease

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ABSTRACT

The Ebola virus is a zoonotic pathogen causing hemorrhagic fever disease with a high mortality rate. The distribution of this pathogen has been limited to woodlands from Central and West Africa and the forest-savannah ecotone in East Africa. The likely reservoir species are frugivorous bats living in these areas. This pathogen is becoming an increasing threat to human populations since its distribution range is expanding faster than expected. The current Ebola outbreaks in Western Africa and in the Democratic Republic of Congo have rapidly spread infecting high numbers of individuals in five African countries. The disease has reached the United States and Spain. This expansion is due partly to increasing global connectivity. This situation represents a new challenge to control the spread of the disease. Experimental drugs have been used to treat a few infected people with promising results. This gives hope for an effective treatment against Ebola hemorrhagic fever in the near future, though thousands of people remain at risk of infection. The present review aims to give an update of the knowledge on the disease, including features of the Ebola virus, the history of disease outbreaks in Africa and the tools that are being developed in order to control this re-emergent disease.

1. Introduction

Zoonotic diseases are increasingly affecting human health. Causative agents include viruses, bacteria, parasites and fungi[1]. In recent years, zoonotic diseases originated in wildlife such as birds, swine and bats, have hit several countries causing infection and mortalities to large numbers of human individuals.

Viruses are responsible for the most lethal and widespread zoonotic outbreaks in human populations. Examples of these epidemics are those of influenza A virus, serotype H5N1, originated in China in 2003. This epidemic affected 15 countries with 650 cases and a mortality rate of 60%. In 2011, five countries: Bangladesh, Cambodia, China, Indonesia and Egypt, reported 62 cases of human infections of which 34 died[2]. An outbreak of the influenza virus serotype H1N1 "swine flu" was first reported in Mexico in early 2009 and soon after it spread worldwide[3]. An estimate of the global mortalities caused by this outbreak is 201 200 deaths by respiratory failure (range 105 700-395 600) and 83 300 deaths by cardiovascular arrest (46 000-179 900). Most of the people who died (80%) were younger than 65 years and most of them (51%) from Asia and Africa[4].

Another epidemic occurred in 2003, when the severe acute respiratory syndrome (SARS) was first reported in Asia. The agent is a coronavirus. This pathogen spread to more than 24 countries in North and South America, Europe and Asia before the global outbreak was contained. The World Health Organization estimated that during the 2003 outbreak, a total of 8 098 people worldwide became sick with SARS and 774 died[5].

A recent outbreak caused by another coronavirus has been responsible for a respiratory syndrome in the Middle East. It is called Middle East Respiratory syndrome (MERS)-CoV[6]. This disease originated in Saudi Arabia in 2012 and it has spread to virtually all the Arabic Peninsula, and it has reached countries in Europe, Asia, Northern Africa, as well as the United States[7]. Until mid-2013, 90 cases of MERS-CoV have been reported with 50% mortality rate[8].

The most recent and expanded outbreak of a lethal viral disease has occurred since early 2014 in West African countries (Guinea, Liberia, Sierra Leone, Nigeria). This epidemic further expanded to Senegal and Mali. Later this year, another outbreak independent from that of West Africa occurred in the Democratic Republic of Congo[7]. The pathogen responsible for these two outbreaks is the Ebola virus. Until October 1st, 2014, 3 431 out of 7 470 infected persons have died (46% mortality rate)[9]. Most infected people are

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located in Guinea, Liberia and Sierra Leone, whereas the outbreaks in Senegal and Nigeria seem to be contained. The Ebola outbreak from The Democratic Republic of Congo has recorded 70 cases and 43 deaths (61%) by October 1st, 2014[9]. The concern that this epidemic may extend beyond West Africa is supported by the fact that people who may be in contact with Ebola-infected people may travel abroad. The first confirmed case of *Ebolavirus* infection in the United States was announced on September 30th[10], and became the first Ebola casualty in America. Also, foreign personnel doing humanitarian work in Africa have become infected with the virus. Two American physicians and two missionaries from Spain became infected with the virus, and were taken back to their countries to receive an experimental treatment. The two Americans were cured whereas the two Spanish priests died. Another person travelling from Liberia to the United States was infected with the Ebola virus and died. Because of breaches in safety protocols, health workers in contact with this patient became exposed to infection and at least two nurses have tested positive to Ebola infection. The two American physicians and other patients in the United States, Germany, Spain and Norway have been treated with experimental drugs against the infection. In the case of the two American medics, the experimental treatment was successful. Although such results against Ebola infection in humans is encouraging, still no effective or safe treatment exist for Ebola virus infection. A number of different treatments have been experimentally tested in animal models, including non-human primates. Some results have shown great rates of virus inhibition and reduction of clinical signs. Yet, large numbers of persons are at risk of Ebola infection not only in Africa, but also in Asia, Europe and America.

Because of the sanitary importance of this pathogen and the high mortality rates in infected people, the aim of this paper is to present data on the features of the Ebola virus, the history of disease outbreaks in Africa and the tools that are being developed in order to control this re-emergent disease.

2. The Ebola virus

2.1. Classification

The Ebola virus belongs to the Order Mononegavirales. This group includes enveloped viruses with a negative, single-stranded RNA genome and a RNA polymerase associated to the virion[11,12]. Filoviridae which is the family of Ebola virus[13], was first discovered in 1967, when laboratory workers in Germany and Yugoslavia got a severe hemorrhagic fever as result of exposure to tissues of African green monkeys (*Cercopithecus aethiops*) imported from Uganda[14-16]. At present, this family has three genera: *Marburgvirus*, *Ebolavirus* and *Cuevavirus*[17-22]. The genus *Ebolavirus* consists of five species: *Bundibugyo ebolavirus* (BDBV), *Zaire ebolavirus* (EBOV), *Reston ebolavirus* (RESTV), *Sudan ebolavirus* (SUDV) and *Taï Forest ebolavirus* (TAFV)[18-20,22,23]. The species of *Marburgvirus* is *Marburg marburgvirus* with two kinds of virus, Marburg virus (MARV) and Ravn virus (RAVV). *Lloviu cuevavirus* is the species of *Cuevavirus*, with one kind virus, Lloviu virus.

A difference in pathogenicity was suggested to exist among ebolaviruses. *Zaire ebolavirus* is thought to be the most pathogenic, causing up to 90% mortality in infected people. In contrast, *Reston ebolavirus*, (which is thought to be an Asian species) has never caused a lethal infection in humans and is weak pathogenic virus in experimentally infected nonhuman primates compared with *Zaire ebolavirus*[21,23]. Three *Ebolavirus* species (Zaire, Sudan and Bundibugyo) are the main responsible for the Ebola outbreaks in Africa[24-26]. The 2014 Ebola outbreak from West Africa is caused by a *Zaire ebolavirus* species with a mortality rate of 47%[27].

Since *Ebolavirus* is a highly contagious and lethal pathogen, it is classified as a biosafety level 4 pathogen[28], based on its high mortality rate, ease to transmission among persons, with potential infectivity by aerosol and lacking of treatment such as vaccines or chemotherapy. Maximum containment is required for all laboratory work with infectious material[14].

2.2. Virion morphology and ultrastructure

Ebola virions are filamentous or pleomorphic and flexible. Different types of virions may exist. Single virions have one nucleocapsid and may have linear, comma or toroid shapes[29]. In addition, polyploid virions may be continuous or linked. These virions may contain two or more nucleocapsids bound together by the envelope. Continuous virions may have two or more nucleocapsids covered by the envelope and most of them are rod-shaped. Linked virions are also composed by two or more nucleocapsids, connected by a short empty section of envelope between or among nucleocapsids. The latter organization allows them to bend without breaking[29,30]. Under the transmission electron microscope linked virions may appear as U-, 6-, or circle-shaped (Figure 1)[13,14,31]. Ebola virion size is about 80 nm in diameter but its length is variable. Single virions are between 790 to 982 nm long, but polyploid virions can reach lengths from 1.9 up to 14 μm[13,30].

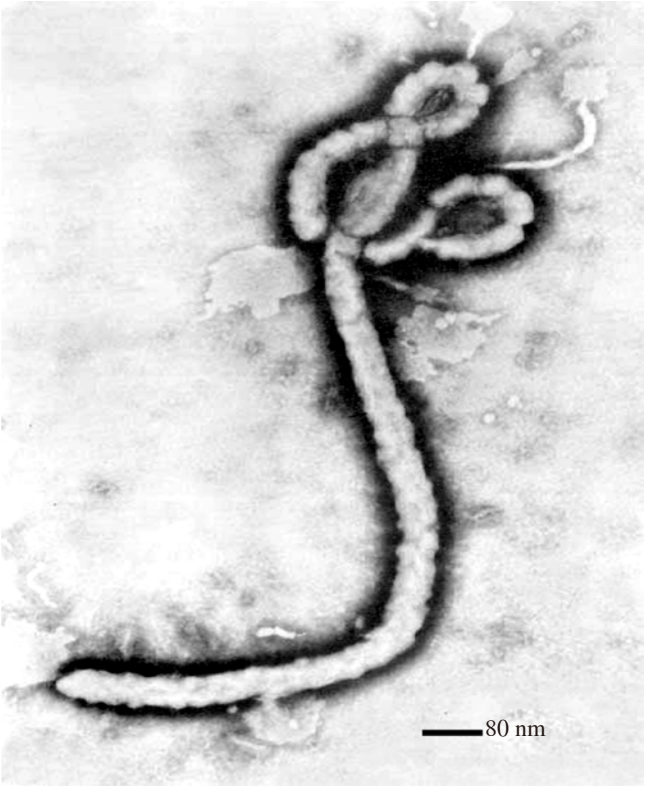

Figure 1. Transmission electron microscope view of an *Ebolavirus* virion. The bar shows an approximate size of 80 nm (picture modified from F. A. Murphy, School of Veterinary Medicine, University of California, Davis).

Virion ultrastructure consists of the following components (Figure 2): an outer envelope or membrane, which contains a surface glycoprotein (GP). This GP is a knob-shaped projection, evenly distributed over the envelope. These peplomers are 10 nm long and spaced 10 nm apart[13,30]. The lipidic envelope is obtained from the cell membrane during budding. The inner surface of the envelope is coated with a protein matrix, mainly composed by VP40, and in lesser extent by VP24[32], which gives stability to the virion[30,33-35], and has an important role in virus assembly and budding[31]. The matrix protein also provides a link to the ribonucleoprotein complex[36]. Between the envelope and nucleocapsid there is a gap separating them. The nucleocapsid is a right-handed, cross-striated double-layered helix. It has 41 nm in outer diameter and 16 nm wide in a hollow inner channel[30]. The nucleocapsid consists of units of the nucleoprotein (NP) associated to the genomic RNA. The inner nucleocapsid is formed by large subunits linked with vertical contacts, which contains a complex of RNA and NP[30,37]. The NP-RNA complex works as the template for genome replication[12,30,38]. Further, the outer nucleocapsid contains NP subunits linked by an outer horizontal layer. This layer consists of a ring of bridges between adjacent large subunits. These bridges are composed of two lobes, one bigger than the other[30]. Experimental results showed that recombinant nucleocapsid-like structures expressed VP24 and VP35. Each of these two proteins independently associates with NP, but all three proteins are required to be together to produce 50 nm diameter helical nucleocapsid-like structures[30,38-40]. The ribonucleoprotein complex is formed by NP, VP30 (transcription factor), VP35 (polymerase cofactor) and the RNA-dependent RNA polymerase[28,31,41,42].

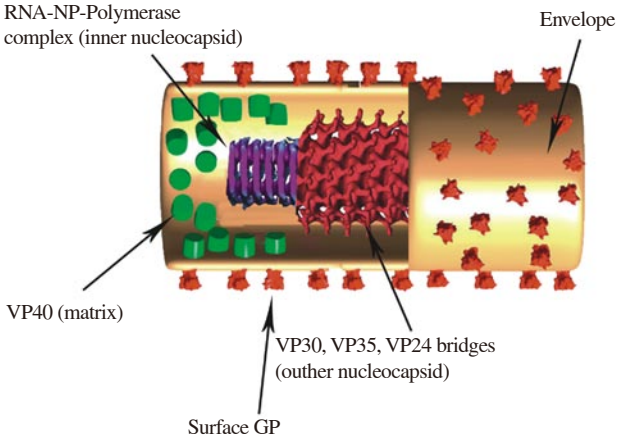

Figure 2. Virion structure of the Ebola virus.
Scheme shows the GP spikes, envelope, matrix, and ribonucleocapsid complex[12,30].

2.3. Genome organization

The genome of *Ebolavirus* is a nonsegmented, single-stranded, negative-sense RNA with size varying from 18 875 to 18 959 bases[13,14,29,43,44]. The Ebola virus genome consists of seven linearly arranged genes[11,12,28,43]. These genes have the following order from the 3´to the 5´ terminus: np, vp35, vp40, gp, vp30, vp24 and l (Figure 3)[11,12,28,43]. Nucleotide sequences at the 3'-terminus are complementary to similar regions on the 5' end[34]. The viral ARN alone is not infectious and it is not polyadenylated[28].

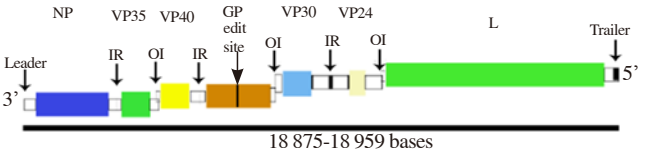

Figure 3. General genome organization of *Ebolavirus*.
Genome size ranges from 18 875 to 19 111 bases, depending on the species. Open reading frames encoding each of the seven genes are represented as color boxes. L: large protein (RNA-dependent RNA polymerase); Ol: overlapping sequence; IR: intergenic gene overlap[11].

2.4. Ebolavirus proteins

The gene *np* is 2953 nucleotides long, encoding a RNA-binding protein. The NP protein is 738 aminoacids long with a predicted size of 80 KDa. The apparent weight of NP in polyacrylamide gel electrophoresis is 105 KDa due to phosphorylation[14]. Protein NP is coupled to RNA synthesis and all replicative products are resistant to nucleases[45].

The gene *vp35* has 1 371 nucleotides, encoding for a protein of 329 to 340 amino acids, depending on the species[46]. The protein VP35 is the functional equivalent of the phosphoprotein P of other Mononegavirales[42]. Protein VP35 also acts as a viral polymerase cofactor and an inhibitory protein of IFN-α/β production by blocking the activation of interferon regulatory factors-3 and -7[47,48]. Further, VP35 also inhibits the activation of protein kinase R, an interferon-induced, double-stranded RNA-activated kinase with antiviral activity, preventing RNA silencing[49].

The gene *vp40* is 980 nucleotides long and encodes for a protein of 326 amino acids with a predicted molecular mass of 35.5 KDa[50-52]. The apparent size of VP40 in polyacrylamide gel electrophoresis is 40 KDa, probably due to post-translational modifications[52]. Protein VP40 is the most abundant protein in the virion and it alone can assemble virus-like particles (VLPs) when expressed in human cells[36,53,54]. This protein has been related to the assembly and budding of viral particles from infected cells[34,36,53,55]. The crystal structure of VP40 showed two domains: an N-terminal oligomerization and a C-terminal membrane-binding domain, connected by a flexible linker[16]. The conformation of VP40 is metastable, allowing the transition into hexameric or octameric ring-like structures *in vitro*[16]. It was also found that when VP40 oligomerizes into octamers, this new structure binds a RNA tri-ribonucleotide containing the sequence 5´ UGA 3´. This novel function suggests another role of VP40 in the virus life cycle[36,56]. This other function of VP40 was found to be as a regulator of viral transcription within infected cells[57]. Additional functions that matrix protein VP40 might have include targeting late endosomes and interactions with cytoskeleton[16].

The *Ebolavirus gp* gene consists of 2029 nucleotides encoding a protein of 676 amino acids with a predicted mass of 74.6 KDa[58-60]. The apparent size of this protein in polyacrylamide electrophoresis gel is 125-140 kDa due to heavy glycosylation[61,62]. The *gp* gene undergoes transcription by the L protein, involving furin cleavage and disulfide-bond formation between the N-terminus and the membrane proximal portion of GP[62-64]. This processing yields two peptide subunits required to synthesize the surface GP[60,65]. The membrane-anchored GP2 is covalently linked via disulfide bond to the N-terminus of GP1, which has a highly O-glycosylated mucin-like domain[63]. Upon infection, high amounts of GP1 is secreted

from the cells after release from the GP2 subunit. Moreover, an additional nonstructural soluble GP deriving from GP1 (identical sequence of 295 amino acids with N-terminal GP1), is produced by EBOV-, but not Marburg virus- infected cells[63,66]. The GP (class 1 membrane GP) is arranged in trimers that forms spikes at the surface of virions[67], and it is the only surface protein in the virion, which indicates that this protein mediates virus entry through receptor binding and fusion[34,54,62,65]. *In vitro* expression of full-length Ebola virus GP has been reported to cause cytotoxicity such as massive rounding and cell detachment in several cell lines[56]. It is possible that GP cytoxicity is related to disruption of blood vessel walls and may contribute to the haemorrhagic symptoms characteristic of the disease[68,69]. Nonetheless, it was experimentally shown that early expression of normally-processed GP in infected cells did not cause cytotoxic effects[67].

The gene *vp30* consists of 886 nucleotides encoding for a protein of 288 amino acids with a predicted size of 32 KDa[34,70]. VP30 is a minor virion protein and it is a strong specific transcriptional activator. It was shown that VP30 alone is not required for replication[71,72]. *In vitro* assays using an *Ebolavirus*-specific minigenome system, VP30 led to a 160-fold increase of transcription activity. Moreover, the presence of VP30 was necessary to rescue full-length *Ebolavirus* clones. These experiments showed the importance of VP30 for the viral replication cycle[34,71]. This phosphoprotein is activated and regulated via phosphorylation. The phosphorylated form of VP30 impacts viral transcription and replication by modulating interaction with the nucleocapsid proteins VP35 and NP[73]. Co-expression studies showed an interaction between VP30 and NP, inducing a re-localization of these proteins into cytoplasmic inclusions. Likewise, it was observed that VP30 serves as bridge between NP and L[72]. From these results, a proposed arrangement of the *Ebolavirus* ribonucleoprotein complex includes linking NP to L either by VP35 or VP30 bridges[72].

The gene *vp24* has 755 nucleotides, encoding a protein of 251 amino acids and a predicted weight of 28 KDa[74,75]. Protein VP24 is a minor constituent of the matrix[32,76,77], and is associated with the envelope[66]. It is known that VP24 is involved in production of VLPs when co-expressed with NP and VP35[32,39]. Recent studies on VP24 have shown that VP24 is involved in the structural rearrangement of the ribonucleoprotein complex, from a relaxed to a condensed state (nucleocapsid formation)[39,78]. According to a proposed model, when VP24 condenses the ribonucleoprotein complex, it prevents the polymerase complex from acting on the genome, locking the polymerase complex into the 3´ end of the genome. This model can explain why VP24 is required for the formation of nucleocapsids and nucleocapsid-like structures, and why it is not needed for genome replication and transcription[78]. Similar functions were found for VP24 when the *vp24* gene was silenced by RNAi[40]. Another novel function of VP24 in *Ebolavirus* pathogenesis is that it can inhibit innate immune responses[79]. This effect occurs when *Ebolavirus* VP24 binds karyopherin alpha nuclear transporters (KPNA) to inhibit tyrosine 701-phosphorylated activator of transcription (PY-STAT1) nuclear transport and renders cells refractory to interferons[79].

The RNA-dependent RNA polymerase gene has 6781 nucleotides, encoding a protein of 2212 amino acids in length and a calculated weight of 253 KDa[42,80]. The catalytic functions of L protein include transcription and replication, RNA-dependent RNA polymerization, capping, and methyl transferase activities[34,42]. The L protein, together with ribonucleoprotein complex and VP35 is sufficient for *Ebolavirus* transcription and replication[42,72]. The first 380 amino acids of *Ebolavirus* L protein were shown to be sufficient to bind to VP35[42,49].

2.5. Ebolavirus replication cycle

2.5.1. Entry

Protein GP on the surface of *Ebolavirus* virions is involved in virus attachment to cells via receptor binding and membrane fusion[81-83]. Cellular molecules such as β1 integrins, dendritic cell-specific intercellular adhesion molecule-3 grabbing nonintegrin (DC-SIGN), its homologue DC-SIGNR (DC-SIGN-related molecule), axl receptor tyrosine kinase, asialoglycoprotein receptor and folate receptor α, are some of the molecules that have been identified as potential *Ebolavirus* receptors[82-84]. Recently, a T-cell immunoglobulin and T-cell Ig and mucin domain 1, was determined as a candidate cell-surface receptor for *Ebolavirus*[84]. However, none of these potential cell receptors are essential for virus entry. Instead, an active cellular mechanism seems to be involved in *Ebolavirus* uptake and internalization[82]. Upon virus attachment to the cell membrane, internalization of the virion takes place. The *Ebolavirus* internalization mainly occurs through a process called macropinocytosis[85]. This process involves the formation of actin-based plasma membrane ruffles and blebs that can enclose large extracellular materials[84,85]. Macropinocytosis internalize large virions that do not fit into smaller endocytic vesicles. This uptake route is strictly dependent on GP but not on virus particle size or morphology. This indicates that one or more interactions of GP with the cell surface (one or more of the putative cell receptors) provide an initiating signal[84]. Internalized pseudotyped GP-bearing virions colocalized with early endosomal antigen-1. Later, the virions were found in Rab5-positive early endosomes and colocalized with perinuclear Rab7/Lamp-1-positive late endosomes. Delivery to these compartments appears to be important for entry, since a dominant-negative inhibitor of Rab7 reduced infection[84]. Studies done with HIV particles pseudotyped with *Ebolavirus* GP showed that virions colocalized with caveolin-1, and their entry was dependent on clathrin (typical for particle sizes< 200 nm) and membrane lipid composition. Only a small fraction of viral particles entered via clathrin-mediated endocytosis[81,82].

2.5.2. Replication

Replication and transcription of viral genes take place in the cytoplasm of infected cells[31]. *In vitro* infection assays with *Zaire ebolavirus* in Vero E6 cells showed that a single replication cycle occurs in 12 h, whereas in the same *in vitro* system using *Marburgvirus*, one replication cycle lasts 21 h[34,86]. *Ebolavirus*-specific mRNA was first detectable at 6-7 h post-inoculation with a synthesis peak at 18 h[86].

The viral RNA-dependent RNA polymerase transcribes the negative-strand RNA genome into mRNAs to produce virus-specific proteins[34]. Replication of the RNA genome requires the creation of a replicative intermediate, the antigenome that is a complete copy of the negative-strand RNA genome, which is complementary to the genome[71,87]. The antigenome in turn serves as a template for generation of new genomes.

The negative-strand RNA genome is transcribed into seven monocistronic mRNAs[43,86,88], which are capped[34,88,89]. It may exist a single binding site for the transcription complex within the leader region of each genome[34]. Each gene has a conserved transcriptional signal. Transcription of each gene begins with a start site at the 3' end and terminates with a stop (polyadenylation) site[34,90]. The transcription of each downstream gene reduces expression of subsequent mRNAs. Therefore, the amount of transcripts is high for the first gene (NP) and very low for the last gene (L). This explains why mRNA from NP is the most

abundant, whereas the L mRNA is not detectable by Northern hybridization[34,86,88].

2.5.3. Morphogenesis, assembly and budding

In vitro expression of viral proteins (NP, VP24, VP30, VP35, and L) in mammalian cells showed that NP alone formed helical tubes resembling the core of the nucleocapsid[38]. Under normal conditions, NP encapsidates the viral RNA genome[91]. Co-expression of NP, VP24 and VP35 was indispensable for the formation of nucleocapsids that were almost identical to those seen in *Ebolavirus* infected cells[12,38,39]. Post-translational modifications of NP (*e.g.* glycosylation) may allow its interaction with VP35 and VP24 in order to produce viral particles[39]. Proteins VP24 and VP35 form an outer ring of bridges that link to NP and are required to produce 50 nm diameter helical nucleocapsid-like structures[12,30]. Further, NP interacts with the matrix protein VP40, which on its own, is sufficient for virus assembly and budding from the plasma membrane[53,76]. Such an interaction between NP and VP40 is essential for transport of nucleocapsids to the cell surface, to incorporate them into virions and therefore, to produce mature virus particles[12,53,91]. Experiments show that VP40 penetrate 8.1 Å into the hydrocarbon core of the plasma membrane bilayer, inducing important changes to the membrane, forming giant unilamellar vesicles. This is a critical step in viral egress since deep penetration of hydrophobic peptides into the plasma membrane are essential for plasma membrane localization, particle formation and virus release from cells[54]. *Ebolavirus* virions containing nucleocapsids mainly emerge horizontally from the cell surface, whereas virions lacking nucleocapsids bud vertically[38].

3. Ebola virus ecology and hosts

Since the first record of *Ebolavirus* in Africa (1976), 23 outbreaks have occurred in a well-delimited region between the 10° latitude parallels[25,92-94](Table 1, Figure 4). It is possible that *Ebolavirus* ecology is closely related to the rain forest ecosystems in Central and West Africa and to the forest-savanna ecotone in Sudan and Uganda, since most outbreaks have occurred in these areas[95,96]. The *Ebolavirus* prevalence might be in function of one or more unidentified factors[25].

The reservoir species for *Ebolavirus* has been sought ever since the first outbreak. In 1998, detection of small *Ebolavirus*-specific RNA sequences in mice (*Mus setulosus* and *Praomys* sp.) and a shrew (*Sylvisorex ollula*) collected in the Central African Republic led to suggest that rodents could be reservoir species. Nonetheless, these findings have not been confirmed by other diagnostic methods such as serology, antigen detection or virus isolation[25,92]. Bats are most likely the reservoir species. Since 1976, bats were related to the occurrence of the disease[23,25,92]. A link between presence of bats and disease outbreaks was recorded as a hemorrhagic fever case occurred in a cave at Mount Elgon, Kenya (1987) and again in a gold mine in Durba, Democratic Republic of Congo (2000)[95,97].

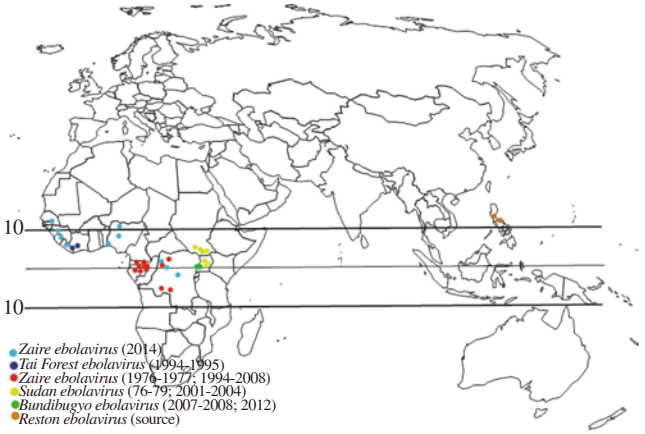

Figure 4. Geographic distribution of the *Ebolavirus* species in Africa and the Philippines[23,25,96].

Table 1

Chronology of outbreaks of Ebola hemorrhagic fever in Africa (from CDC website: http://www.cdc.gov/vhf/ebola/outbreaks/history/chronology.html).

Year	Country	Ebola species	Number of human cases	Number (%) of deaths
1976	Zaire (Democratic Republic of the Congo)	Zaire ebolavirus	318	280 (88%)
1976	Sudan (South Sudan)	Sudan ebolavirus	284	151 (53%)
1977	Zaire (Democratic Republic of the Congo)	Zaire ebolavirus	1	1 (100%)
1979	Sudan (South Sudan)	Sudan ebolavirus	34	22 (65%)
1994	Gabon	Zaire ebolavirus	52	31 (60%)
1994	Ivory Coast	Taï Forest ebolavirus	1	0
1995	Democratic Republic of the Congo (Zaire)	Zaire ebolavirus	315	250 (81%)
1996	(Jan-April) Gabon	Zaire ebolavirus	37	21 (57%)
1996-1997	(July-Jan) Gabon	Zaire ebolavirus	60	45 (74%)
1996	South Africa (imported from Gabon)	Zaire ebolavirus	2	1 (50%)
2000-2001	Uganda	Sudan ebolavirus	425	224 (53%)
2001-2002	(Oct-March) Gabon	Zaire ebolavirus	65	53 (82%)
2001-2002	(Oct-March) Republic of Congo	Zaire ebolavirus	57	43 (75%)
2002-2003	(Dec-April) Republic of Congo	Zaire ebolavirus	143	128 (89%)
2003	(Nov-Dec) Republic of Congo	Zaire ebolavirus	35	29 (83%)
2004	Sudan (South Sudan)	Sudan ebolavirus	17	7 (41%)
2007	Democratic Republic of Congo	Zaire ebolavirus	264	187 (71%)
2007-2008	(Dec-Jan) Uganda	Bundibugyo ebolavirus	149	37 (25%)
2008-2009	(Dec-Feb) Republic of Congo	Zaire ebolavirus	32	15 (47%)
2011	(May) Uganda	Sudan ebolavirus	1	1 (100%)
2012	(June-Oct) Uganda	Sudan ebolavirus	11*	4* (36.4%)
2012	(June-Nov) Republic of Congo	Bundibugyo ebolavirus	36*	13* (36.1%)
2012-2013	(Nov-Jan) Uganda	Sudan ebolavirus	6*	3* (50%)
2014	(March-Present) Guinea, Liberia,Sierra Leone, Nigeria	Zaire ebolavirus	7470*	3431* (45.9%)

*: Confirmed infection.

Evidence of asymptomatic infections in fruit bats was documented during the 2001-2003 Ebola outbreaks in Gabon and Republic of Congo. Viral RNA and antibodies were detected in the tree-roosting bats *Hypsignathus monstrosus*, *Epomops franqueti*, and *Myonycteris torquata*. These species had a 5% prevalence of *Ebolavirus*-specific IgG antibodies[98]. This finding suggests that *Ebolavirus* is rather common in epidemic and non-epidemic regions throughout the woodland countries of Central Africa[25]. Other bat species such as *Epomophorus gambianus*, *Eidolon helvum* and *Nanonycteris veldkampii* from Africa and Asia have tested positive to antibodies against *Ebolavirus* proteins[23]. Recently in the Philippines serum samples from 16 bats of the species *Rousettus amplexicaudatus* collected at Diliman forest and Quezon forest, resulted in 5 samples (31%) being positive by IgG ELISA to *Reston ebolavirus* NP and GP antibodies[99]. Likewise in China, serological evidence of *Ebolavirus* infection in several bat species was reported. Serum reactions and relatively high titers against the *Ebolavirus* protein NP was found in samples of the bat species *Rousettus leschenaulti*, *Pipistrellus pipistrellus* and *Myotis* sp.[100].

Experimental data showed that frugivorous and insectivorous African bats became infected with *Ebolavirus* without signs of disease[25,93]. Although under experimental conditions, high virus titers have been recorded in infected bats and replication-competent virus can easily be isolated, this has not been possible in naturally infected animals[93]. One possible explanation is that in naturally infected bats the virus titer might be very low, along with the possibility that specific physiological or environmental stimuli are needed to trigger virus replication[25]. *In vivo* and *in vitro* experiments have suggested that such stimuli might be stress, co-infection, shift in food resources and pregnancy. These factors would determine the occasional emergence of Ebola haemorrhagic fever in Africa[25,93]. In contrast, *Marburgvirus* was identified and isolated from the Egyptian frugivorous bat *Rousettus aegyptiacus*[97,101].

New data on the number of bat species that may serve as natural reservoirs of *Ebolavirus* indicate that its geographic range has increased. Serologic data indicate that the geographic distribution of *Ebolavirus* in Africa may extend to the east until Madagascar[95]. Bats in the Asian continent (Philippines, China and Bangladesh) have also tested positive to antibodies against *Ebolavirus*[23,99,100]. Moreover, a new member of the Filoviridae (*Cuevavirus*) has recently been described in the bat species *Miniopterus schreibersii* from southern Europe[18]. The bat genus *Rousettus* has at least three species that have tested positive to *Ebolavirus* in Africa and Asia. The geographic range of this genus includes countries in West and East Africa, Egypt, the middle East (Syria, Jordan, Israel), and Asia (Iran, India, Bangladesh, Thailand, Vietnam, New Guinea, Malaysia, the Philippines and Indonesia)[102]. These data show that at least the species *Reston ebolavirus* and *Zaire ebolavirus* are present in bat populations in mainland Asia[23].

Apes, man and other mammals that may be susceptible to *Ebolavirus* infection are regarded as accidental hosts and not as reservoir species[23,25,93].

4. History of Ebola virus outbreaks in Africa

Ebola hemorrhagic fever was first reported in northern Zaire (Yambuku and surrounding areas) and southern Sudan (Nzara, Maridi and surrounding areas) in 1976. Two distinct types of the virus were isolated from these simultaneous outbreaks: *Zaire ebolavirus* and *Sudan ebolavirus*[92,96]. These two species re-emerged in outbreaks in 1977 and 1979 at the same locations, causing a smaller epidemic[93]. In 1994, outbreaks of hemorrhagic fever was caused by a new Ebola species: *Côte d'Ivoire ebolavirus*. Later, this species was renamed *Täi Forest ebolavirus*[14,92,94,96]. The virus was isolated from a nonfatal case, in which a worker was infected during the autopsy of a wild chimpanzee[14,93]. The outbreak from Gabon was caused by the *Zaire ebolavirus* species[25]. In 1995, another Ebola outbreak appeared in the southwestern city of Kikwit and the surrounding villages in Bandundu Province, in the Democratic Republic of Congo (former Zaire). The causative virus was closely related to the 1976 Zaire isolate[14]. In 1996-1997, outbreaks of *Zaire ebolavirus* occurred in Gabon during January-April (Mayibout area) and July-January (Booué area). In both occasions, contact with non human primates (chimpanzees) for food are thought to be the onset of the outbreaks[25,96]. A physician who treated some of the infected patients in Gabon traveled to South Africa. He became infected and was hospitalized. The nurse taking care of him became infected and died[94].

In the period 2000-2003, five outbreaks occurred in Uganda (Gulu, Masindi, and Mbarara districts), Republic of Congo (Mbomo, Mbandza villages and Kéllé in Cuvette located in Mbomo distric, Ouest Département) (2001-2003) and Gabon (2001-2002). The Uganda outbreak was caused by *Sudan ebolavirus*, whereas the outbreaks from Gabon and Congo were caused by *Zaire ebolavirus* species[25,92,94,96].

In the period 2004-2009, four outbreaks occurred in Africa. In 2004, southern Sudan (Yambio county) was affected by *Sudan ebolavirus*. In 2007-2009, three outbreaks were recorded[96]. Two in Congo in 2007 and 2008 (Kasai occidental province) caused by *Zaire ebolavirus*, and one in 2007-2008 in Uganda. Here, a new species of *Ebolavirus* was reported: *Bundibugyo ebolavirus*[97].

In the period 2011-2013, four outbreaks were recorded[94]. Three of them occurred in Uganda and were caused by Sudan virus. In 2011, *Sudan ebolavirus* killed one patient in Luwero district, Uganda. In 2012, 11 persons became infected with *Sudan ebolavirus* species and four died in the Kibaale district of Uganda. Again in 2012-2013, another outbreak by *Sudan ebolavirus* species happened at the Luwero district in Uganda. The fourth outbreak caused by *Bundibugyo ebolavirus* species occurred in late 2012 in the oriental province of Congo and killed 13 out of 36 infected patients.

Since early 2014, an outbreak of *Zaire ebolavirus* was reported in Guinea, Liberia and Sierra Leone. These countries account for more than 98% of the Ebola cases in western Africa (as October 1st report, there were 7470 cases and 3431 deaths). Senegal and Nigeria have reported 1 and 20 imported cases, respectively. In these countries, Ebola outbreak seems to have been contained. In August 2014, an Ebola outbreak unrelated to that of West Africa appeared in the Democratic Republic of Congo. Until October 1st, 2014, 70 cases and 43 deaths have been recorded[103].

In the United States, the first Ebola case in a person who traveled from Liberia has been reported. This patient died, but he had contact with 48 people who are at risk of developing Ebola symptoms[104]. There is the risk of Ebola crossing the Mexican border. In Spain, a nurse cared for a missionary priest for Ebola disease, has also tested positive to Ebola[105]. The nurse also had contact with various family members and colleagues who are at risk

of contracting the virus. In France, the risk of Ebola entering the country is as high as 75% by the end of October and 50% for the United Kingdom[106].

5. Ebola pathogenesis

Pathogenesis studies on this virus have been done using animal models. Successful infections have been obtained using guinea pigs, mice and non-human primates[93]. Since wild-type Ebola viruses are not lethal to rodent models, lethal host-adapted strains have been developed in the laboratory through serial passage of the virus into sucklings[107,108]. In rodent models using guinea pigs and mice, the enhanced virulence has been related to mutations in the NP and VP24 viral proteins[109]. Nonetheless, these animal models lack various pathogenic features that occur during a human infection, such as lymphocyte apoptosis or hemorrhage[108]. Hence, features of the human infection can be replicated in non-human primate models with some variations, such as a shorter time to death after infection (6-9 d versus 6-16 d after exposure), or lacking the production of antigen-specific antibodies[108]. It is possible that the latter feature has to do with the high infectious doses. Further, *in vitro* studies have been very useful to understand *Ebolavirus* pathogenesis. The development of minigenomes which are able to produce VLPs has demonstrated the function of most viral proteins during the infection process[2,41,53,78,87].

5.1. Route of infection

Ebola virus probably enters the host through mucosal surfaces, minute lesions in skin or by parenteral route. In most human infections, virus transmission occurs through direct contact with infected patients or corpses or contaminated materials[93,108,110]. Among infected patients, organs and tissues where infectious virus particles or viral RNA appeared include skin, semen and genital secretions, whereas in experimentally infected non-human primates, they have been collected from skin, body fluids, and nasal secretions[93]. Sexual transmission of Ebola virus is possible, since it can be found in semen of men who have recovered from the disease for up to 7 weeks after remission from illness[22]. Other routes of infection in the laboratory have occurred through exposure to tainted needlesticks and blood. In the 1976 outbreak, reuse of contaminated needles was a significant path of virus spread in Sudan and Zaire[93]. In natural conditions, transmission of *Zaire ebolavirus* by contact exposure has been recorded by handling and eating chimpanzee bushmeat in Gabon or freshly killed bats in the Democratic Republic of Congo. Though infectious virus should be destroyed by cooking, handling of contaminated blood and/or ingesting tainted meat are risks of exposure to Ebola virus[93].

In humans, the course of disease and outcome of infection seems to depend on the route of infection. By injection exposure to *Zaire ebolavirus*, the mean incubation period is 6.3 d *vs.* 9.5 d for contact exposures[93]. Data analysis from the 1995 outbreak in Kikwit, Democratic Republic of Congo, determined the mean incubation period only by contact exposure with *Zaire ebolavirus* to be (12.70±4.31) d. This incubation period is longer than previous records (6-10 d). It is possible that such earlier records included data from infections by needle prick[111]. Also, mortality rate seems to be influenced by the exposure route. In 1976, mortality caused by Ebola infection through needle prick was 100% (85/85 cases), whereas the rate of deaths caused by contact exposure was 80% (119/149 cases)[93]. In the case of non-human primates, infection with *Zaire ebolavirus* by intramuscular or intraperitoneal injection, leads to a faster progression of the disease than in animals exposed by aerosol droplets[93]. In gorillas infected with *Zaire ebolavirus* by contact exposure, an incubation period of 11.7 d was determined[111].

5.2. Target cells

Target cells of *Ebolavirus* include several cell types such as monocytes, macrophages, dendritic cells, endothelial cells, fibroblasts, hepatocytes, adrenal cortical cells, and epithelial cells[93,110,112]. Early during infection, macrophages seem to be the first cell type to allow *Ebolavirus* replication. This cell type has been found to harbor the virus in deceased patients and they can become infected in *in vitro* models[77,110]. In non-human primates, macrophages and dendritic cells have become *Ebolavirus*-positive early during infection [2 d post inoculation (dpi)][93]. In rodent models (guinea pigs and mice), macrophages are the primary cell target[108]. After initial replication in macrophages, *Ebolavirus* subsequently spreads to lymph nodes, and then it reaches organs such as liver, spleen and thymus. In these organs, *Ebolavirus* infects other cell types such as monocytes, macrophages, and dendritic cells, which leave these organs spreading the virus[93]. As the course of infection advances, hepatocytes, adrenal cortical cells, fibroblasts, and many other cell types also become infected, resulting in extensive necrosis[110].

The main target organs in *Ebolavirus* infection are lymphoid tissues, liver and adrenal gland. This tropism has a paramount role in the pathogenesis of the disease. The adrenal cortex has an important role in blood pressure homeostasis. This organ is affected by *Ebolavirus* infection, and this could reduce the synthesis of enzymes and steroids that control blood pressure, leading to hypotension and sodium loss with hypovolemia. These traits have been recorded in many cases of Ebola hemorrhagic fever[93]. Hepatocellular necrosis in different grades have been recorded in infected humans and non-human primates. These damages generally are not serious enough to explain the cause of death. Nonetheless, these hepatocellular injuries may reduce the synthesis of coagulation factors and other plasma proteins, thus increasing the chance of hemorrhages[93]. Damage to the endothelium as consequence of *Ebolavirus* infection has only been recorded in a few cases with advanced stage of infection[93].

Lymphopenia and necrosis has been reported in spleen, thymus and lymph nodes of deceased patients and experimentally-infected non-human primates. Lymphocytes do not become infected but they undergo apoptosis in humans and non-human primates. The main affected cell subpopulations in experimentally-challenged macaques with *Zaire ebolavirus* were T cells (CD4 and CD8) and natural killer cells[93,108].

5.3. Impairment of adaptive immunity

Limited data exist in adaptive immune responses in *Ebolavirus*-infected humans. Nonetheless, sharp differences in the response of adaptive immunity have been recorded between fatal and non-fatal cases, showing its importance in the outcome of Ebola infection[108,110]. In survivors, production of Ebola-specific antibodies (IgM) have been reported as soon as 2 d after onset of symptoms, and IgG antibodies have been detected 5-8 d after onset

of symptoms. Conversely, a low proportion of deceased patients (30%) only had low concentrations of specific IgM and none of them had specific IgG antibodies[108]. *In vitro* studies showed that infected dendritic cells do not become mature, thus unable to present antigens to naive lymphocytes. This may explain the lack of virus-specific antibodies in deceased patients[110]. A marked depletion of T-cells occurred in deceased patients prior to death due to apoptosis[108,110]. These observations indicate that early inflammatory responses correlate with survival[108].

5.4. Impairment of innate immunity

In humans and non-human primates, *Ebolavirus* infection produces inflammatory responses and increases cytokine production such as interferons, interleukins 2, 6, 8, and 10, interferon-inducible protein 10, monocyte chemo-attractant protein 1, chemokine, tumor necrosis factor (TNF)-α and reactive oxygen and nitrogen species[93,108,110]. The virus-induced expression of these molecules appears to produce a defective immune response, which contributes to the progression of disease. In fatal cases, pro-inflammatory responses were deregulated. In contrast, early inflammatory responses have been related to remission[93,108].

In early studies done in endothelial cells from people infected with *Zaire ebolavirus*, inhibition of Type I interferon response, appears to be a key feature of filovirus pathogenesis, since suppression of interferon speeds up systemic spread of the virus[93,108,110]. *In vitro* studies have shown that *Ebolavirus* selectively suppresses responses to IFN-α and IFN-γ and the production of IFN-α in response to double-stranded RNA[108,112]. Upon *in vitro Ebolavirus* infection, dendritic cells do not perform their functions, such as production of cytokines or expression of co-stimulatory molecules (CD80, CD86) and fail to promote T-cell proliferation due to maturation arrest. The lack of production of CD80-CD86 has also been observed in a non-human primate experimental infection[108]. Two Ebolavirus proteins block the interferon response by inhibiting phosphorylation of the interferon regulatory factor 3 and probably preventing transcription of interferon-b (VP35), or interfering with interferon signaling (VP24)[93,108,112].

Upon *Ebolavirus* infection, pro-apoptotic tumor necrosis factor-related apoptosis inducing ligand can be induced *in vivo* and *in vitro*. Apoptosis does not occur in infected dendritic cells *in vivo*, but other cell types such as lymphocytes and natural killer cells sharply decrease during the course of infection. Presence of caspase activity and DNA fragmentation in these cells indicate apoptosis[93,108,110]. Reactive oxygen and nitrogen species have an important role in *Ebolavirus* pathogenesis. High concentrations of nitric oxide in blood were found in infected patients with *Zaire ebolavirus* and *Sudan ebolavirus* as well as in experimentally infected non-human primates. Increased blood concentrations of nitric oxide were associated with mortality[93]. High concentrations of nitric oxide have been related to apoptosis of lymphocytes, tissue damage and loss of vascular integrity, which might contribute to virus-induced shock[93].

5.5. Impairment of coagulation

Coagulation defects observed in Ebola hemorrhagic fever are a side effect of the infection[93,108,110]. Different treatments showed a permeability increase of endothelial cells *in vitro*. Human endothelial cell monolayers exposed to supernatants of monocyte/macrophages infected with *Marburgvirus* increased permeability[108]. In experimental conditions, GP from *Ebolavirus*-like particles was able to activate endothelial cells and interfere with their barrier function[16,108]. Also, nitric oxide is a potent endogenous vasodilator. In non-human primates, *Ebolavirus* infection induced high levels of nitric oxide at 3 dpi[108,112].

Virus-infected monocytes and macrophages express or release cell-surface tissue factor (TF)[93,108,110]. This response is important to induce the development of coagulation anomalies reported in Ebola hemorrhagic fever. High levels of pro-inflammatory cytokines such as TNF-α, also trigger production of TF by macrophages. Further, TNF-α can induce the expression of TF in endothelial cells[108]. The synergy of two TF activation sources helps to explain the early appearance, rapid development and severity of the coagulopathy in *Ebolavirus* infection[110].

In the late stages of Ebola hemorrhagic fever, coagulopathy could be triggered by several factors. Cytokine TNF-α can impair the function of the anticoagulant-protein-C pathway by downregulating thrombomodulin[108]. In non-human primates, *Ebolavirus* infection induced a sharp drop of protein C in plasma as soon as 2 dpi, and increased TF mRNA levels by 3 dpi. In this animal model, TF was detected on infected macrophages, endothelial cells and on the surface of the abundant membrane microparticles present in blood[93,108]. Nonetheless, the platelet count only fell until 3-4 dpi, suggesting that activated platelets were attached to endothelial cells. As the disease advanced, hepatic injury could cause a decline in plasma levels of certain coagulation factors[110].

In vitro experiments showed that *Ebolavirus* infection can induce the expression of TF on the surface of macrophages. High levels of TF, either induced by TNF-α, or through macrophage infection, might lead to a condition known as disseminated intravascular coagulation (DIC), an outstanding characteristic of Ebola hemorrhagic fever[93,108]. Such a coagulopathy consists of a host of reactions such as thrombocytopenia, depletion of clotting factors and increased concentrations of fibrin degradation[93,108]. When TF production was inhibited, survival time of *Ebolavirus* infected non-human primates increased. This result indicates the importance of TF in the pathogenesis of *Ebolavirus* infection[108]. High concentrations of virus and pro-inflammatory molecules during the infection trigger a defective immune response that contributes to *Ebolavirus* pathogenesis including hemorrhage and shock. Impairment of function in the vascular and coagulation system lead to multiple organ failure and a syndrome that resembles septic shock[93].

6. Ebola symptoms, pathology, diagnosis

The incubation period of *Ebolavirus* infection (the time interval from virus infection to onset of symptoms) is 2 to 21 d, with a mean of 8-10 d[22,110]. Humans are not contagious until they develop symptoms. The initial diagnosis of Ebola hemorrhagic fever is based on clinical assessment. Because of its high virulence and high mortality rate, proper contingency plans should be developed when a suspected case is documented[93]. Ebola hemorrhagic fever can be suspected in patients presenting with acute fever (>38.6 °C) and with a history of travel to an endemic area. Identification of the causative agent might be difficult since

other acute febrile diseases (malaria, typhoid fever, shigellosis, meningococcal septicaemia, plague, leptospirosis, anthrax, relapsing fever, typhus, murine typhus, yellow fever, Chikungunya fever and fulminant viral hepatitis) exist in the regions where Ebola is endemic[93,110].

The early *Ebolavirus* infection symptoms are non-specific and include sudden onset of fever, fatigue, muscle pain, headache and sore throat. Later during infection, other symptoms appear such as vomiting, diarrhea, rash, impaired kidney and liver function, and in some cases, both internal and external bleeding (*i.e.* bleeding gums, blood in stools)[93,108,110,113]. Other laboratory tests reported to be done in infected patients include hemoglobin and hematocrit, white blood cells and platelets counts, bilirubin, serum electrolyte and glucose, coagulation parameters (prothrombin time, partial thromboplastin time, bleeding time and plasma levels of D-dimers), liver-associated enzymes, pancreatic enzymes and renal function[114]. During *Ebolavirus* infection, impairments in blood coagulation and fibrinolysis are revealed as petechiae, ecchymoses, mucosal hemorrhages, congestion and uncontrolled bleeding at venipuncture sites. Nonetheless, massive blood loss is rare and if present, it is mainly limited to the gastrointestinal tract. Even here, the volume of lost blood is not high enough to cause death[93,114].

Diagnostic methods to determine presence of *Ebolavirus* are done only in specialized national and international reference centers. These institutions should be readily contacted when presence of the pathogen is suspected. Such laboratories should give advice on sampling, sample preparation and sample transport. Diagnosis of *Ebolavirus* infection can be done through two different approaches: (1) detection of host-specific immune response to the virus (antibodies) and (2) detection of viral antigen and/or genomic RNA in the host[14,93,113,114]. Diagnostic methods include immunofluorescence assay, immunoblot, and ELISA (direct IgG and IgM ELISA, and IgM capture assay). Other diagnostic methods can find viral particles (electron microscopy), viral antigen (immunohistochemistry, immunofluorescence on impression smears of tissues) and genomic RNA (RT-PCR) (Table 2)[14,110]. Blood samples usually give positive to *Ebolavirus* by RT-PCR one day before symptoms appear[110]. Antigen detection may be used as a confirmatory test for immediate diagnosis. Data from outbreaks and from laboratory animal experiments show that all individuals becoming ill will test positive using any of the above-mentioned assays[110]. As it is unkown whether infected patients within the incubation period could be accurately diagnosed through the available diagnostic methods, it is suggested that testing for IgM or IgG antibodies to *Ebolavirus* may be useful[110].

Rapid diagnostic assays are RT-PCR and antigen detection ELISA[93,110]. Viral antigen and nucleic acid can be detected in blood as soon as 3 d after onset of symptoms and they can be detected up to 7-16 d. Antibodies (IgM) against *Ebolavirus* can appear as early as 2 d after onset of symptoms and disappear between 30 and 168 d after infection. In contrast, IgG-specific antibodies can develop at 6-18 days after onset of symptoms and persist for many years[110]. Presence of virus-specific IgM or increasing IgG titers are strong indicators of infection. Decreasing titers of IgM or increasing titers of IgG (four-fold), or both, in successive paired serum samples are highly suggestive of a recent infection[93].

Diagnostic assays must be done on materials that have to be rendered non-infectious. Effective virus inactivation procedures include the use of gamma irradiation from a cobalt-60 source or heat inactivation. In the case of nucleic acids, virus RNA purification can be done using guanidinium isothiocyanate. This is a chemical chaotrope that denatures the proteins of the virus and renders the sample non-infectious[93].

Management of people with *Ebolavirus* infection is based on patient isolation and application of strict barrier nursing procedures, such as protective clothing and respirators. These procedures have been sufficient to arrest virus transmission in hospital settings in rural Africa. Other methods to control spread of disease include provision and use of sterile equipment for injections, personal protective equipment to doctors, nurses, and caretakers. In developing countries with minimum health-care provision, basic management of patients should include isolation, malaria treatment, broad spectrum antibiotics and antipyretics before diagnosis[93].

In contrast, many developed countries have devised contingency plans which include the installation of proper isolation facilities and intensive care units to deal with imported cases. Health-care systems should have suitable isolation units where intensive care treatment should aim to manage Ebola-related complications (reduce hemorrhage, coagulation defects and electrolyte balance). Treatment against bacterial infections and organ failure should also be done to increase chances of survival[93,110].

7. Experimental strategies to control Ebola infections

So far there is no clinically proven treatment against Ebola hemorrhagic fever. Only supportive treatment is available and it includes isolation of patients, barrier protection and a combination of intravenous-fluid replacement, administration of analgesics and standard nursing measures[93,108,115,116]. The Ebola virus is highly contagious and some strains (Reston) have also been detected in farmed animals (swine). This finding indicates that *Ebolavirus* infection might also occur from livestock to humans. Therefore, effective treatments are urgently needed[63,116,117].

Different treatment approaches against *Ebolavirus* infections have been developed under experimental conditions. They can be grouped into prophylactic and therapeutic. Treatments assayed include antibodies (poly- and monoclonal), vaccines (live-attenuated, recombinant viral vectors, subunit, DNA, virus-like particles), antivirals, inflammation and coagulation modulators and tissue factor pathway inhibitors (Table 3)[93,116,118,119].

Table 2

Diagnostic methods used for confirmation of Ebolavirus infection[14].

Target molecules	Source	Assay(s)
Antiviral antibodies	Serum	Indirect immunofluorescence (IFA); Enzyme-linked immunosorbent assay (ELISA); Immune blot
Virus antigen	Blood, serum, tissues	Antigen ELISA; immunohistochemistry, fluorescence assay
Virus nucleic acid	Blood, serum, tissues	Reverse-transcription polymerase chain-reaction
Virus particles	Blood, tissues	Virus isolation; Transmission electron microscopy

7.1. Antibody-based treatments

These include passive immunization (transfer of immune serum from animals and/or humans to other animals or humans) and administration of polyclonal or monoclonal antibodies raised against *Ebolavirus* proteins[108,112]. The usefulness of passive immunization is still unclear since results have not been consistent in different animal models. In rodents, serum transfer from Balb/c mice vaccinated with an attenuated replicating virus vector expressing *Ebolavirus* proteins NP and GP did not protect guinea pigs from infection[108,112,120]. Likewise, passive transfer of serum from mice immunized with *Ebolavirus* VP24, VP30, VP35 and VP40 did not protect recipient mice from a lethal challenge[121]. In contrast, passive transfer of IgG polyclonal immune serum in mice before a lethal *Ebolavirus* challenge protected them up to 100%[112,122].

Passive transfer of hyper-immune serum from horse into non-human primates have also yielded inconsistent results. While in hamadryas baboons (*Papio hamadryas*) such a treatment protected monkeys from *Ebolavirus* challenge (110 LD_{50}), in other species such as cynomolgus macaques (*Macaca fascicularis*), treatment did not prevent mortality but only delayed death[108,112]. In humans, during the outbreak in Kikwit in 1995 (Democratic Republic of Congo), whole blood transfusion from convalescent *Ebolavirus* patients were transferred into eight patients showing hemorrhagic manifestations. After treatment, only one patient survived. Nonetheless, it is unknown whether the late stage of infection and the supportive care they received influenced survival in those patients[108,112].

Neutralizing monoclonal antibodies raised against *Ebolavirus* GP protected mice challenged with a lethal dose, even after virus inoculation[112]. In contrast, concerns about vaccine development using GP antigen have been raised when antisera against *Ebolavirus* GP protein produce an infectivity-enhancing response[112]. Antisera produced by DNA immunization with a plasmid encoding the *Zaire ebolavirus* GP, enhanced the infectivity of vesicular stomatitis virus (VSV) pseudotyped with *Ebolavirus* GP. Monoclonal antibodies that specifically enhance *Ebolavirus* infectivity verify the existence of infectivity-enhancing epitopes on the *Zaire ebolavirus* GP molecule. It is possible that the reduced virulence of *Reston ebolavirus* depends on the absence to produce this antibody-dependent infectivity enhancement. This finding raised questions about the effect on the application of passive transfer using anti-Ebola GP antibodies as prophylaxis or treatment. Instead, it is suggested that neutralizing antibodies against Ebola GP should be developed[112].

Recently, combinations of three monoclonal antibodies against different epitopes of *Ebolavirus* GP protein (MB-003 and ZMapp) successfully protected three out of seven (43%) infected macaques administered after onset of symptoms[110,116,123]. In the current West African outbreak, the ZMapp treatment was administered to two United States healthcare workers and one Spanish priest who developed Ebola infection. The two Americans survived the infection and the Spanish died. Before the current outbreak, this treatment was never used in humans. Nonetheless, the role of this treatment in the course of disease in these patients cannot be evaluated since their survival might have been influenced by the high quality care received[110,123]. At present, monoclonal antibodies are the most promising of the experimental post-exposure strategies against filovirus infections. These reagents have consistently produced higher survival rates in non-human primates upon lethal *Ebolavirus* challenges[116].

7.2. Vaccines

Vaccination constitutes one of the main control strategies against infectious diseases[118]. Early vaccine candidates against *Ebolavirus* were based on inactivated virus. These vaccines failed to induce protection[108]. Current standards indicate that effective vaccine candidates should elicit protection in at least two animal models of the disease including non-human primates[93], which are the gold standard model for Ebola hemorrhagic fever[93,118]. Only a few vaccine platforms have met these conditions to continue in the pipeline of vaccine development. These vaccine candidates are based on recombinant technologies such as viral-vector-vaccines, DNA vaccines, recombinant virus proteins, VLPs

Table 3
Treatment approaches against Ebolavirus infection[93,116,118,119].

Treatment	Type	Example	Model evaluated
Antibodies	Passive transfer	Whole-blood / serum from surviving patients	Rodents, NHP and humans
	Neutralizing monoclonal	ZMapp	NHP and humans
Vaccines	Viral vector vaccines (Live-attenuated virus)	Replication competent vaccines (vesicular stomatitis virus expressing GP)	Rodents and NHP
		Human parainfluenza 3 virus expressing Ebola GP	Rodents
		Kunjin replicating VLPs	Rodents
	DNA prime-virus boost	DNA-recombinant Ebola-GP-adenovirus	rodents
	Recombinant virus	Recombinant adenovirus expressing NP, GP; Recombinant human adenovirus type 5 expressing GP	Rodents rodents and NHP
		Recombinant vesicular stomatitis virus expressing GP	NHP
	Inactivated virus	Ebolavirus △VP30 gene	Rodents
	Virus-like particles (VLPs)	NP, VP40, GP	Rodents and NHP
	Recombinant antigen	Ebola GP-human IgG1 Fc	
Inhibitors of TF and modulators of coagulation	Recombinant human activated protein C	Against severe sepsis	NHP
	Recombinant nematode anticoagulant protein c2	Inhibitor of TF-FVIIa complex	NHP
Antivirals	3-deazaneplanocin A (3-DNP)	Inhibits 5' cap methylation of viral messenger RNA	Rodents
	FGI-106 (Quino[8,7-h]quinoline-1,7diamine,N,N'-bis[3-(dimethylamino)propyl]-3,9-dimethyl-tetrahydrochloride)	Not described	Rodents
	BCX4430	synthetic adenosine analog	Rodents and NHP
	Brincidofovir (CMX001)	Acyclic nucleoside phosphonate	In vitro and humans
	Phosphorodiamidate morpholino oligomers (PMOs)	block translation of viral RNA inhibiting viral replication	Rodents and NHP
	siRNA	Block transcription of viral RNA inhibiting replication	Rodents and NHP

and live-attenuated viruses. These vaccines intend to induce a more robust activation of both innate (humoral) and adaptive (cellular) immune responses, maximizing vaccine efficacy[93,118]. Examples of vaccines include a DNA-prime and adenovirus-boost. This strategy required six months to induce protective immunity[108].

A single dose of recombinant adenovirus expressing NP and GP protected non-human primates against a lethal dose (1 500 LD_{50}) of Ebola at 28 d after vaccination[93,108]. An adenovirus serotype 5 expressing the *Zaire ebolavirus* GP protected mice against an adapted *Zaire ebolavirus*[124]. This recombinant virus protected guinea pigs against a lethal guinea pig-adapted *Ebolavirus* challenge when delivered by intramuscular or intranasal routes. Animals with pre-existing immunity to adenovirus had lower survival[124]. A recombinant human adenovirus type 5 viral vector expressing full or truncated *Ebolavirus* GP forms was tested in the interferon α/β receptor knock-out mouse model. The recombinant vaccine induced strong protection and antibody response in this mouse model and animals intranasally inoculated with the recombinant virus vaccine showed 40%-100% survival upon a lethal intraperitoneal challenge[125].

Another approach used recombinant VSV encoding the Ebola GP surface protein. This vaccine showed protection efficacy in 50% of non-human primates challenged with *Zaire ebolavirus* when administered soon after challenge (30 min)[110]. A new-generation of inactivated Ebola virus vaccine has been recently done by deleting the VP35 gene, essential for virus replication[93]. Recombinant vaccines based on human parainfluenza virus protected guinea pigs from Ebola infection after a single intranasal inoculation[108]. A new live-attenuated *Ebolavirus* vaccine using a chimeric human parainfluenza virus type 3 expressing *Ebolavirus* GP as the only transmembrane envelope protein, induced a strong immune response against GP. A single dose protected animals against a lethal intraperitoneal challenge with a guinea pig-adapted *Ebolavirus*[126]. Live attenuated viruses are better than replication-deficient vaccines in terms of production scale and induction of strong innate and adaptive (humoral and cellular) immune responses, though the safety concerns on this type of vaccines may limit its application in humans[93].

A vaccine candidate using *Ebolavirus* VLPs have been synthesized in mammalian or insect cell expression systems[93,119,127]. The VLPs were generated by coexpression of the viral proteins VP40, NP and GP[93,119], or baculovirus pseudotyped with the *Ebolavirus* proteins GP or VP40[127]. These VLPs have successfully protected mice, guinea pigs and non-human primates[108,119,127]. Another VLP-producing approach is to use a replicon-recombinant kunjin virus, expressing VLPs with full length *Ebolavirus* GP or a mutated (D637L) GP. These replicon VLPs induced dose-dependent protection in guinea pigs against a lethal challenge of an adapted *Ebolavirus* strain[128].

An *Ebolavirus* GP-human IgG1 Fc fusion protein was expressed in mammalian cells. The fusion protein was cleaved with enterokinase and purified. Mice immunized with the fusion protein GP-Fc developed T-cell immunity against *Ebolavirus* GP and neutralizing antibodies against an engineered, replication-competent VSV recombinant virus containing *Ebolavirus* GP. Also, mice vaccinated with the GP-Fc were protected against a lethal *Ebolavirus* challenge[63].

Cross protection among different Ebola species in experimental animal systems has been reported indicating the potential value of a multivalent vaccine against different filovirus species[93,118,129].

7.3. Inhibitors of TF pathway and modulators of inflammation and coagulation

Overexpression of TF influences the pathophysiology of *Ebolavirus* infection, including strong inflammatory responses similar to those seen in sepsis and septic shock. These pathological features lead to the appearance of disseminated intravascular coagulation[93,108,116]. These similarities induced to evaluate the effect of inhibiting TF pathway to control the pathology of Ebola hemorrhagic fever[93,108,115,116]. Recombinant human activated protein C (rhAPC), a licensed therapy for severe sepsis in humans, showed limited success in non-human primates as post-exposure prophylaxis for Ebola hemorrhagic fever[93,115,118]. Another molecule involved in inhibiting TF is the recombinant nematode anticoagulant protein c2 (rNAPc2). This is a clotting inhibitor that blocks the action of the TF-factor VIIa complex, which has been used to treat clinical symptoms of coagulopathy so as to reduce DIC and post-inflammatory responses in non-human primates[93,115,116,118]. Post-exposure administration (24 h) of rNAPc2 in non-human primates showed 33% survival rate and a significant extended survival time upon a lethal *Zaire ebolavirus* challenge[93,108,116]. It is possible that rNAPc2 treatment along with other modulators of coagulation increase the success rate to control Ebola hemorrhagic fever and other hemorrhagic diseases[93,108]. These two drugs have been approved for different applications in humans and could be used in emergencies[93].

7.4. Antivirals

Antivirals have emerged since the mid 1980s with the need to treat deadly viral infections such as HIV. Since then, several molecules with antiviral activity have been developed and assayed *in vitro* and *in vivo*.

In *Ebolavirus* infection, various antiviral strategies have been tested. Some approaches include inhibitors of a cellular enzyme, S-adenosyl-L-homocysteine (SAH) hydrolase, which is involved in 5' cap methylation of viral messenger RNA, inducing the intracellular accumulation of SAH and triggering feedback inhibition of methylation[107,130,131]. These type of compounds have broad-spectrum antiviral activity attributed to decreased methylation of the 5' cap of viral messenger RNA, resulting in inefficient translation[107,131]. The molecule 3-deazaneplanocin A (3-DNP) is one of such SAH inhibitors and has showed a potent antiviral effect against *Ebolavirus* infection in a mouse model. Upon treatment, 3-DNP induces an excess of IFN-α production in the tissues of infected animals but not in uninfected ones. A direct relationship between IFN serum concentration and the number of infected cells was observed, indicating that IFN induction by 3-DNP requires presence of virus within cells[107]. Under experimental conditions, 3-DNP given for a week three times daily after an *Ebolavirus* challenge, prevented infected mice from disease and death[130]. This same molecule showed a similar efficacy when given once-daily on Day 0 or 1 at a larger dose[107,131]. The molecular mechanism of this novel form of antiviral therapy has not been determined[107].

Other antiviral molecules termed FGI-103, FGI-104 and FGI-

106 were effective against an *Ebolavirus* challenge in a mouse model[118]. A single administration of molecule FGI-106 (Quino[8,7-h]quinoline-1,7diamine,N,N'-bis[3-(dimethylamino) propyl]-3,9-dimethyltetrahydrochloride) provided high protection to challenged mice when administered 24 h after challenge in a dose-dependent manner (0.5-5.0 mg/kg). Survival ranged from 60% to 90%[118,132].

Recently, a novel synthetic adenosine analog, called BCX4430, showed broad antiviral activity (bunyaviruses, arenaviruses, paramyxoviruses, coronaviruses and flaviviruses), which included filovirus infections in human cells[133]. By using different analyses (biochemical, reporter-based and primer-extension assays) it was shown that BCX4430 inhibited viral RNA polymerase function, acting as a non-obligate RNA chain terminator[110,133]. In a post-exposure challenge model in mice, intramuscular administration of BCX4430 conferred protection against Ebola virus and Marburg virus. Further, BCX4430 post-exposure (48 h) therapy gave a complete protection from a Marburg virus challenge in a non-human primate model[116,133]. Brincidofovir (CMX001) is another broad-spectrum antiviral that has been tested *in vitro* against *Ebolavirus* infection in 2014. Results showed that this nucleotide analog under oral administration had a potent antiviral response[134]. This antiviral was administered as treatment to the imported Ebola patient in Dallas Texas. Nonetheless this patient died. In contrast, another Ebola patient who became infected while working in Liberia, was brought to a Nebraska hospital and he was also treated with this antiviral. The patient survived[135].

Molecules that have shown a strong antiviral activity by blocking virus translation or transcription include antisense oligonucleotides and RNA interference (RNAi), respectively[93,136,137].

Phosphorodiamidate morpholino oligomers (PMOs) are a subclass of antisense molecules, adapted with a phosphorodiamidate linkage and a morpholine ring. These molecules show limited off-target effects, favorable base stacking, high duplex stability, high solubility, cell permeability, and no hybridization problems. These molecules form a PMO/mRNA duplex that effectively block translation of viral RNA inhibiting viral replication. Antisense PMO have shown antiviral effect against vesiviruses, flaviviruses, and the SARS coronavirus[137].

A study developed a set of *Ebolavirus*-specific PMOs against mRNAs from viral proteins VP24, VP35 and L. The combination of these PMOs protected mice in both pre- and post-exposure models. Also, these PMOs showed high antiviral efficacy as a prophylactic treatment in a non-human primate assay, where 75% of the animals were protected from a lethal *Ebolavirus* infection[137]. A similar study using PMOs by intraperitoneal, subcutaneous and intravenous administration, protected 60% of the challenged non-human primates against a lethal *Ebolavirus* challenge. This experiment was done in a post-exposure scenario, where treatments were given between 30-60 min after an *Ebolavirus* challenge[118].

Small-interfering (si) RNA is another antiviral method used against *Ebolavirus* infection. One study developed four siRNAs against the L gene of *Zaire ebolavirus* coupled to polyethylenimine or included into stable nucleic acid-lipid particles (SNALPs). Then, groups of guinea pigs were treated with one of these preparations. Results showed that administration of the siRNA-polyethylenimine complex just before the Ebola challenge reduced plasma viremia and partially protected animals. In contrast, administration of siRNA-SNALP complex 1 h after the Ebola challenge showed a complete protection against viremia and mortality. Other experiments showed that one (EK1) of the four siRNAs alone could completely protect animals from Ebola infection[130]. The same siRNA approach against *Ebolavirus* was assayed in a non-human primate model. A combination of siRNA-SNALPs including the EK1 against L gene, VP24 and VP35 of *Zaire ebolavirus* were evaluated in a post-exposure design. One group of macaques (n=3) were treated with a pool of these molecules (2 mg/kg per dose, bolus intravenous infusion) at 30 min, and on 1, 3, and 5 d after an Ebola challenge. Another set of macaques (n=4) were treated with the same molecule pool at 30 min, and on 1, 2, 3, 4, 5, and 6 d after an Ebola challenge. Protection against Ebola challenge was observed in 2 out of 3 (66%) and 7 out of 7 (100%) monkeys in the first and second experiments, respectively. These results indicate the efficacy of siRNA-SNALPs as a post-exposure treatment against Ebola infection, with potential application in humans[93,110,118,138]. A similar siRNA approach is already in the pipeline. A phase I clinical trial has started. The experimental treatment is termed TKM-Ebola and is based on the EK1-4 molecules silencing the L gene. This treatment has been cleared by the US food and drug administration for use in infected patients[123].

8. Concluding remarks

The Ebola virus is one of the most infectious and lethal zoonotic viruses that have hit human populations in the last four decades. This virus causes a serious disease (Ebola hemorrhagic fever) which often results in death ($\geqslant$50%). Outbreaks leading to considerable mortalities have occurred exclusively in African countries. The current *Ebolavirus* outbreak is still evolving and it is expected that by the end of 2014, as many as 5000 persons may become infected every week unless effective containment protocols and treatments are enforced. The World Health Organization has set a goal to contain the outbreak by applying these three rules: 70% safe burials, 70% of infected people getting proper isolation and treatment and these have to be in place within 60 d[139]. In addition, World Health Organization has recommended the evaluation of a number of treatments that may be applied in the worst-hit areas. These include whole blood transfusion from surviving patients. This tries to apply passive serum transfer as a first-line of treatment. Other efforts should be done to evaluate in fast-track clinical trials some successful experimental treatments, such as recombinant VSV-GP vaccines developed in Canada, siRNA-SNALPs and small broad-spectrum antiviral molecules. The current Ebola virus epidemic clearly demands a fast response of scientific community to develop a safe and effective vaccine against so-far largely ignored, re-emergent disease.

Conflict of interest statement

We declare that we have no conflict of interest.

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