

# Ebola haemorrhagic fever

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Ebola viruses are the causative agents of a severe form of viral haemorrhagic fever in man, designated Ebola haemorrhagic fever, and are endemic in regions of central Africa. The exception is the species Reston Ebola virus, which has not been associated with human disease and is found in the Philippines. Ebola virus constitutes an important local public health threat in Africa, with a worldwide effect through imported infections and through the fear of misuse for biological terrorism. Ebola virus is thought to also have a detrimental effect on the great ape population in Africa. Case-fatality rates of the African species in man are as high as 90%, with no prophylaxis or treatment available. Ebola virus infections are characterised by immune suppression and a systemic inflammatory response that causes impairment of the vascular, coagulation, and immune systems, leading to multiorgan failure and shock, and thus, in some ways, resembling septic shock.

## Introduction

Ebola virus is regarded as the prototype pathogen of viral haemorrhagic fever, causing severe disease and high case-fatality rates.<sup>1</sup> This high fatality, combined with the absence of treatment and vaccination options, makes Ebola virus an important public health pathogen and biothreat pathogen of category A.<sup>2</sup>

Ebola virus and Marburg virus constitute the family Filoviridae in the order of Mononegavirales.<sup>3</sup> Filoviruses are enveloped, non-segmented, negative-stranded RNA viruses of varying morphology. These viruses have characteristic filamentous particles that give the virus family its name.<sup>4</sup> Ebola virus particles have a uniform diameter of 80 nm but can greatly vary in length, with lengths up to 14000 nm.<sup>1,3</sup> The genome consists of seven genes in the order 3' leader, nucleoprotein, virion protein (VP) 35, VP40, glycoprotein, VP30, VP24, RNA-dependent RNA polymerase (L)—5' trailer.<sup>1,3</sup> With the exception of the glycoprotein gene, all genes are monocistronic, encoding for one structural protein. The inner ribonucleoprotein complex of virion particles consists of the RNA genome encapsulated by the nucleoprotein, which associates with VP35, VP30, and RNA-dependent RNA polymerase to the functional transcriptase–replicase complex.<sup>5</sup> The proteins of the ribonucleoprotein complex have additional functions such as the role of VP35, which is an interferon antagonist.<sup>6</sup> VP40 serves as the matrix protein and mediates particle formation.<sup>7</sup> VP24, another structural protein associated with the membrane, interferes with interferon signalling.<sup>8</sup> The glycoprotein is the only transmembrane surface protein of the virus and forms trimeric spikes consisting of glycoprotein 1 and glycoprotein 2—two disulphide-linked furin-cleavage fragments.<sup>1</sup> An important distinction of Ebola virus from other Mononegavirales is the production of a soluble glycoprotein, which is the primary product of the GP gene, and gets secreted to large quantities from infected cells.<sup>9,10</sup>

Despite important achievements during the past two decades to unravel the molecular biology and pathogenesis of Ebola virus, we are still unclear about virulence factors and host responses, which seem to be partly detrimental to the host. The scarce knowledge has long hampered the

development of proper treatment methods and vaccines, although some vaccines have now shown promise in experimental studies.<sup>11</sup> This Seminar reviews the present knowledge about the epidemiology, ecology, disease manifestation, pathogenesis, and case management of Ebola haemorrhagic fever.

## Epidemiology

The first cases of filovirus haemorrhagic fever were reported in 1967 in Germany and the former Yugoslavia, and the causative agent was identified as Marburg virus.<sup>12</sup> Similar cases of haemorrhagic fever were described in 1976 from outbreaks in two neighbouring locations: first in southern Sudan and subsequently in northern Zaire, now Democratic Republic of the Congo (DRC).<sup>13,14</sup> An unknown causative agent was isolated from patients in both outbreaks and named Ebola virus after a small river in northwestern DRC. These two epidemics were caused by two distinct species of Ebola virus, Sudan Ebola virus and Zaire Ebola virus, a fact not recognised until years later (figure 1).<sup>15</sup> The third African Ebola virus species, Côte d'Ivoire Ebola virus was discovered in 1994. The virus was isolated from an infected ethnologist who had worked in the Tai Forest reserve in Côte d'Ivoire and had done a necropsy on a chimpanzee. The animal came from a troop that had lost several members to an illness later identified as Ebola haemorrhagic fever (figure 1).<sup>16</sup> The latest discovery is Bundibugyo Ebola virus, the fourth African species of human-pathogenic Ebola virus found in equatorial Africa (approximate distribution 10° north and south of the equator, figure 1).<sup>17</sup> An additional Ebola

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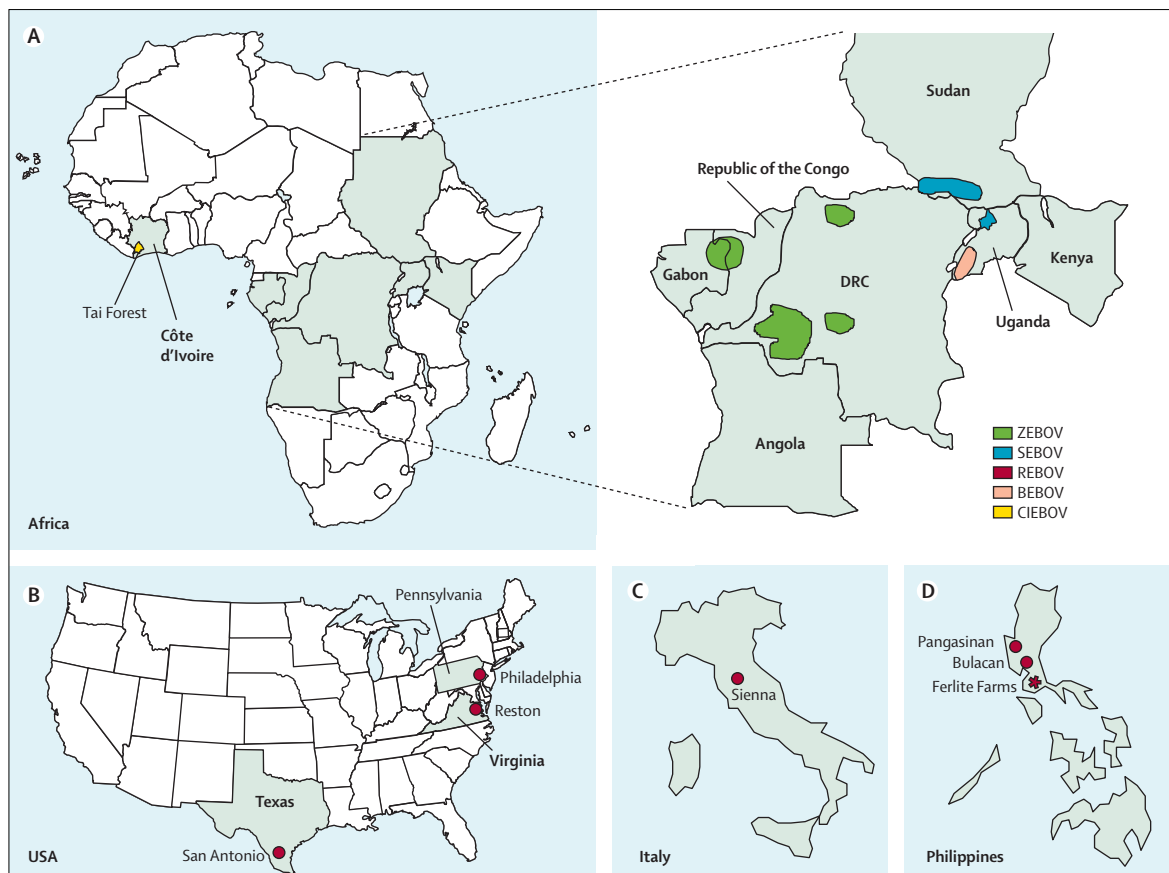
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### Search strategy and selection criteria

We searched PubMed with the terms “viral haemorrhagic fever”, “Ebola virus”, “Marburg virus”, “pathogenesis”, “diagnosis”, “treatment”, and “vaccines” in various combinations without any language restrictions. The search covered the period from 1967, the year of the discovery of filoviruses, until present. Review articles were cited when appropriate. We also included references that were recommended by peer reviewers.



**Figure 1: Locations of Ebolavirus infections and outbreaks**

(A) Regions in Africa (approximate distribution  $10^{\circ}$  north and south of the equator) with reported outbreaks of Ebola haemorrhagic fever caused by the three central African species of Ebola virus, Zaire Ebola virus (ZEBOV), Sudan Ebola virus (SEBOV), and Bundibugyo Ebola virus (BEBOV). The Tai Forest region in Côte d'Ivoire reported the only case so far of Ebola virus in western Africa caused by the species Côte d'Ivoire Ebola virus (CIEBOV). (B) Reston ebolavirus REBOV has been introduced several times through imported macaques into USA from 1989 to 1996 (Philadelphia, PA; Reston, VA; San Antonio, TX) and into Italy (Sienna) in 1992 (C). The source of the introduction in all cases of REBOV has been a primate export facility in the Philippines (Ferlite farm) (D). Animals of this farm have been diagnosed with REBOV infection several times in the 1990s. REBOV has been detected in pigs on two farms in the Philippines (Pangasinan, Bulacan). DRC=Democratic Republic of the Congo.

virus species, Reston Ebola virus, is found in the Philippines. It was first described in 1989 and isolated from *Cynomolgus* monkeys (*Macaca fascicularis*) housed at a quarantine facility in Reston, VA, USA. These monkeys were imported from the Philippines; an unusually high mortality was noted in infected animals during quarantine, but simian haemorrhagic fever virus co-circulated in the animals (figure 1).<sup>18,19</sup> Subsequently, Reston Ebola virus has been found in the Philippines on several occasions,<sup>20</sup> with surprising reports documenting infections in pigs (figure 1).<sup>21</sup>

Ebola haemorrhagic fever remains a plague for the population of equatorial Africa, with an increase in the numbers of outbreaks and cases since 2000 (figure 1). Almost all human cases are due to the emergence or re-emergence of Zaire Ebola virus in regions of Gabon, Republic of the Congo, and DRC, and of Sudan Ebola virus in Sudan and Uganda.<sup>1</sup> These two species together with the single species of Marburg virus, Lake Victoria Marburg virus, are major public health concerns in these

regions. The role of Bundibugyo Ebola virus and Côte d'Ivoire Ebola virus in the occurrence of filoviral haemorrhagic fever in equatorial Africa is not clear since only one outbreak of Bundibugyo Ebola virus has occurred,<sup>17</sup> and the Côte d'Ivoire virus has not yet re-emerged since the original episode in 1994. The presence of Ebola virus in equatorial Africa has been supported by various serosurveys of selected populations in the region, done during the past three to four decades, indicating that the virus, or unknown pathogens that are serologically cross-reactive, are endemic in the region.<sup>1,22,23</sup> Additionally, the emergence of Reston Ebola virus in pigs<sup>21</sup> raises important concerns for public health, agriculture, and food safety in the Philippines and could turn into a serious issue for parts of Asia.

### Ecology

Ebola haemorrhagic fever is thought to be a classic zoonosis with persistence of the Ebola virus in a reservoir species generally found in endemic areas. Apes, man,

and perhaps other mammalian species that are susceptible to Ebola virus infection are regarded as end hosts and not as reservoir species.<sup>22</sup> Although much effort has been made to identify the natural reservoirs with every large outbreak of Ebola haemorrhagic fever, neither potential hosts nor arthropod vectors have been identified.<sup>23–26</sup> Rodents<sup>27</sup> and bats<sup>28</sup> have long been thought to be potential reservoir species. This idea was supported by experimental studies in African plants and animals that resulted in productive infection of African fruit and insectivorous bats with Zaire Ebola virus, but a firm link could not be established.<sup>29</sup> The first evidence for the presence of Zaire Ebola virus in naturally infected fruit bats was documented by detection of viral RNA and antibodies in three tree-roosting species: *Hypsignathus monstrosus*, *Epomops franqueti*, and *Myonycteris torquata*.<sup>30,31</sup> However, despite efforts, Zaire Ebola virus has not been successfully isolated from naturally infected animals. The identification and successful isolation of Marburg virus from the cave-dwelling fruit bat *Rousettus aegyptiacus* further lends support to the idea of bats as a reservoir species for filoviruses.<sup>32</sup> This finding is reassuring since several of the Marburg virus outbreaks have been associated with caves or mines that are usually heavily infested by bats.<sup>33</sup> Data for potential reservoirs for any of the other four Ebola virus species do not exist.

Infections with Ebola virus are rare in equatorial Africa, although probably under-reported. Transmission from the reservoir species to man or other end hosts might therefore be an infrequent event, given the restricted distribution of or restricted contact with the reservoir species. However, bats are frequently encountered in equatorial Africa and hunted for food in many places.<sup>34</sup> Therefore, Ebola virus might persist as an asymptomatic or subclinical infection in the reservoir species, with little or no transmission, and might be sporadically activated through an appropriate stimulus. The stimulus might be stress, co-infection, change in food sources, and pregnancy, as shown experimentally in vivo and in vitro.<sup>35,36</sup> This hypothesis would explain the sporadic nature and periodicity of outbreaks of Ebola haemorrhagic fever in Africa.

Future studies need to address the extent of infections of Ebola viruses in fruit or insectivorous bats in areas endemic for these viruses. Issues such as virus pathology and persistence in bats, potential activation mechanisms of persistent virus, and potential transmission routes need to be addressed by field and experimental studies. However, one should keep an open mind for the existence of other reservoir species and a role for potential amplifying hosts, especially after the discovery of Reston Ebola virus in pigs in the Philippines.<sup>21</sup>

### Clinical manifestations

The different species of Ebola virus seem to cause somewhat different clinical syndromes, but opportunities for close observation of the diseases under good

conditions have been rare. Generally, the abrupt onset of Ebola haemorrhagic fever follows an incubation period of 2–21 days (mean 4–10) and is characterised by fever, chills, malaise, and myalgia. The subsequent signs and symptoms indicate multisystem involvement and include systemic (prostration), gastrointestinal (anorexia, nausea, vomiting, abdominal pain, diarrhoea), respiratory (chest pain, shortness of breath, cough, nasal discharge), vascular (conjunctival injection, postural hypotension, oedema), and neurological (headache, confusion, coma) manifestations. Haemorrhagic manifestations arise during the peak of the illness and include petechiae, ecchymoses, uncontrolled oozing from venepuncture sites, mucosal haemorrhages, and post-mortem evidence of visceral haemorrhagic effusions. A macropapular rash associated with varying severity of erythema and desquamate can often be noted by day 5–7 of the illness; this symptom is a valuable differential diagnostic feature and is usually followed by desquamation in survivors. Abdominal pain is sometimes associated with hyperamylasaemia and true pancreatitis. In later stages, shock, convulsions, severe metabolic disturbances, and, in more than half the cases, diffuse coagulopathy supervene.<sup>1,37–39</sup>

Laboratory variables are less characteristic but the following findings are often associated with Ebola haemorrhagic fever: early leucopenia (as low as 1000 cells per  $\mu\text{L}$ ) with lymphopenia and subsequent neutrophilia, left shift with atypical lymphocytes, thrombocytopenia (50 000–100 000 cells per  $\mu\text{L}$ ), highly raised serum aminotransferase concentrations (aspartate aminotransferase typically exceeding alanine aminotransferase), hyperproteinaemia, and proteinuria. Prothrombin and partial thromboplastin times are extended and fibrin split products are detectable, indicating diffuse intravascular coagulopathy. In a later stage, secondary bacterial infection might lead to raised counts of white blood cells.<sup>1,37–39</sup>

Patients with fatal disease develop clinical signs early during infection and die typically between day 6 and 16 with hypovolaemic shock and multiorgan failure. Haemorrhages can be severe but are only present in fewer than half of patients. In non-fatal cases, patients have fever for several days and improve typically around day 6–11, about the time that the humoral antibody response is noted.<sup>1,40</sup> Patients with non-fatal or asymptomatic disease mount specific IgM and IgG responses that seem to be associated with a temporary early and strong inflammatory response, including interleukin  $\beta$ , interleukin 6, and tumour necrosis factor  $\alpha$  (TNF $\alpha$ ). However, whether this is the mechanism for protection from fatal disease remains to be proven.<sup>1</sup> Convalescence is extended and often associated with sequelae such as myelitis, recurrent hepatitis, psychosis, or uveitis.<sup>1,41</sup> Pregnant women have an increased risk of miscarriage, and clinical findings suggest a high death rate for children of infected mothers. This high death rate could be due to transmission from the infected

mother to the child during breastfeeding, either through milk or close contact.

The virulence of Ebola virus in man is variable and is dependent on the species or strain; a similar variability seems to recapitulate well in non-human primates. Within the genus Ebola virus, infections with the Zaire Ebola virus species have the highest case-fatality rates (60–90%) followed by those for the Sudan Ebola virus species (40–60%). On the basis of one outbreak, case-fatality rates for Bundibugyo strain infections are estimated to be only 25%. The only reported person infected with Côte d'Ivoire Ebola virus became ill but survived.<sup>16</sup> By comparison, case-fatality rates for Marburg virus infection in Africa are 70–85% but were much lower in the outbreak in Europe in 1967, with a case-fatality rate of only 22%. This low rate has led to speculation that proper intensive care with supportive therapy would increase the survival rate of infected patients. This hypothesis is hard to test because of austere field conditions and ethical dilemmas about not providing care to some patients. Reston Ebola virus is deemed non-pathogenic for man, but laboratory tests have documented the occurrence of infection.<sup>1</sup>

### Pathogenesis

Information about the pathology and pathogenesis of Ebola virus infections in man is sparse. This shortcoming is partly attributable to the inaccessibility of the geographical regions in which these natural infections arise. However, comprehensive studies have been done in animals. Rodents such as guineapigs and mice have been used to study Ebola haemorrhagic fever.<sup>42–44</sup> Because isolates of Ebola virus obtained from primates do not typically produce severe disease in rodents on initial exposure, serial adaptation is needed to produce a uniformly lethal infection. Mice and guineapigs have served well as early screens for assessment of antiviral drugs and candidate vaccines, and genetically engineered mice are clearly useful for the dissection of specific host–pathogen interactions. However, the disease pathogenesis recorded in rodents is less accurate in representation of the human disorder than is the disease recorded in non-human primates.<sup>45,46</sup>

### Route of infection

Ebola virus seems to enter the host through mucosal surfaces, breaks, and abrasions in the skin, or by parenteral introduction. Most human infections in outbreaks seem to occur by direct contact with infected patients or cadavers.<sup>13,14,47,48</sup> Infectious virus particles or viral RNA have been detected in semen, genital secretions,<sup>40,49</sup> and in skin of infected patients;<sup>50</sup> they have also been isolated from skin, body fluids, and nasal secretions of experimentally infected non-human primates.<sup>51,52</sup>

Laboratory exposure through needlestick and blood has been reported.<sup>53–55</sup> Reuse of contaminated needles played an important part in the 1976 outbreaks of Ebola virus in

Sudan and Zaire.<sup>13,14</sup> Butchering of a chimpanzee for food was linked to outbreaks of Zaire Ebola virus in Gabon,<sup>56</sup> and contact exposure was the probable route of transmission. Although proper cooking of foods should inactivate infectious Ebola virus, ingestion of contaminated food cannot wholly be ruled out as a possible route of exposure in natural infections. Notably, handling and consumption of freshly killed bats was associated with an outbreak of Zaire Ebola virus in DRC.<sup>34</sup> Organ infectivity titres in non-human primates infected with Ebola virus are frequently in the range of  $10^7$  to  $10^8$  pfu/g;<sup>51</sup> thus, exposure through the oral route could invariably be associated with very high infectious doses. In fact, Zaire Ebola virus is highly lethal when given orally to rhesus macaques.<sup>57</sup> The role of aerosol transmission in outbreaks is unknown, but is thought to be rare.

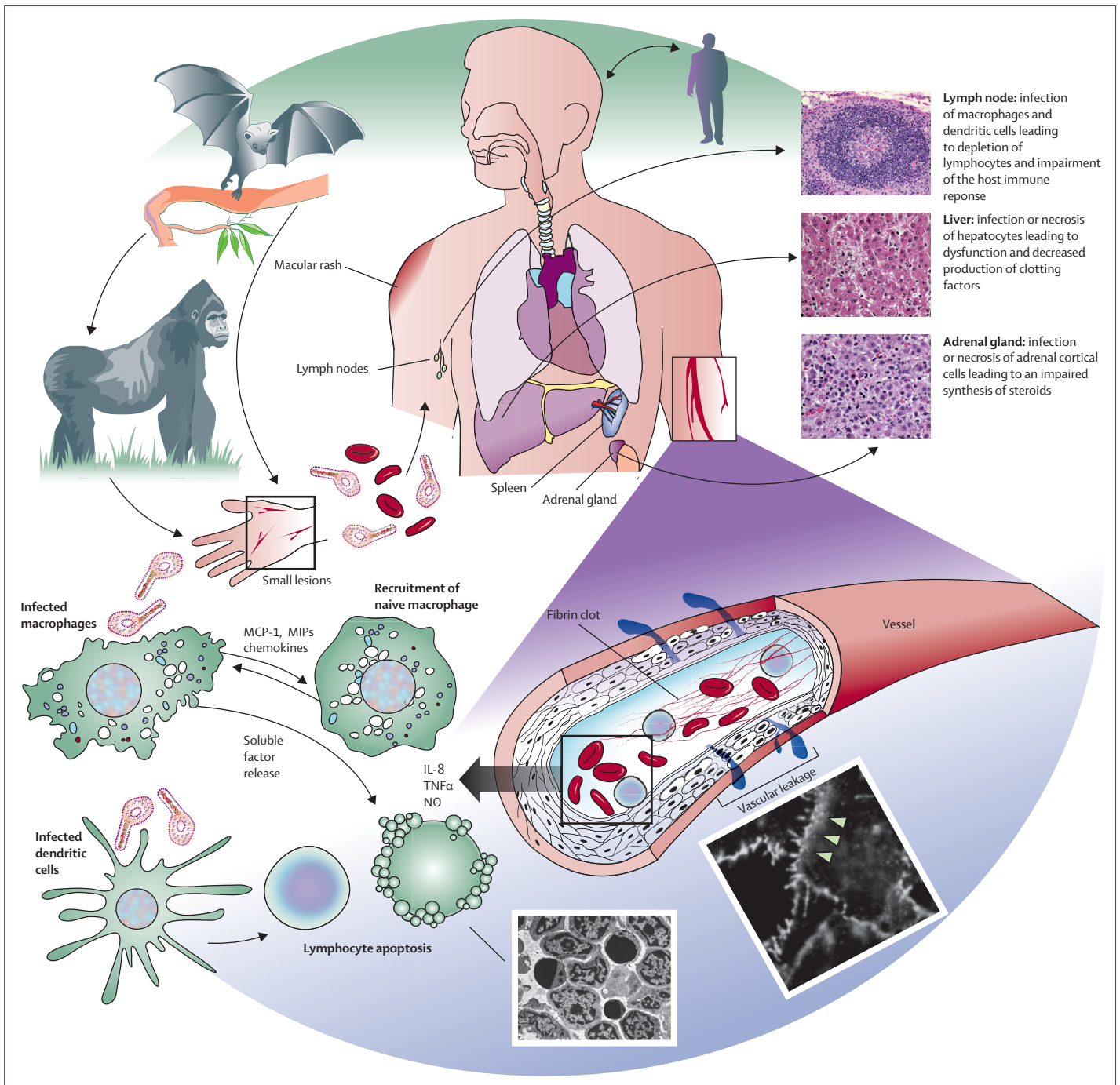
In human beings, the route of infection seems to affect the disease course and outcome. The mean incubation period for cases of Zaire Ebola virus infection known to be due to injection is 6.3 days, versus 9.5 days for contact exposures.<sup>58</sup> Moreover, the case-fatality rate in the 1976 outbreak of Zaire Ebola virus was 100% (85 of 85) in cases associated with injection compared with about 80% (119 of 149) in cases of known contact exposure.<sup>58</sup> For non-human primates infected with Zaire Ebola virus, the disease course seems to progress faster in animals exposed by intramuscular or intraperitoneal injection than in animals exposed by aerosol droplets.<sup>59</sup>

### Target cells and tissues

Ebola virus has a broad cell tropism, infecting a wide range of cell types. In-situ hybridisation and electron microscopic analyses of tissues from patients with fatal disease or from experimentally infected non-human primates show that monocytes, macrophages, dendritic cells, endothelial cells, fibroblasts, hepatocytes, adrenal cortical cells, and several types of epithelial cells all lend support to replication of these viruses.<sup>50,51,57,60–63</sup> Temporal studies in non-human primates experimentally infected with Zaire Ebola virus suggest that monocytes, macrophages, and dendritic cells are early and preferred replication sites of these viruses (figure 2).<sup>62</sup> These cells seem to have pivotal roles in dissemination of the virus as it spreads from the initial infection site via monocytes, macrophages, and dendritic cells to regional lymph nodes, probably through the lymphatic system, and to the liver and spleen through the blood.<sup>52,64</sup> Monocytes, macrophages, and dendritic cells infected with Ebola virus migrate out of the spleen and lymph nodes to other tissues, thus disseminating the infection (figure 2).

Although the endothelium is thought to play an important part in the pathogenesis of Ebola virus (figure 2), studies defining the molecular mechanisms of endothelial impairment are incomplete. Researchers thought that the virus' glycoprotein is the primary determinant of vascular-cell injury and that Ebola virus infection of endothelial cells induces structural damage,<sup>65</sup>





**Figure 2: Model of Ebola virus pathogenesis**

Virus spreads from the initial infection site (small lesions) to regional lymph nodes, liver, and spleen. Although Ebola virus does not infect lymphocytes, their rapid loss by apoptosis is a prominent feature of disease. The direct interaction of lymphocytes with viral proteins cannot be discounted as having a role in their destruction, but the substantial loss of lymphocytes probably results from a combination of factors including infection-mediated impairment of dendritic cells and release of soluble factors from monocytes and macrophages. Soluble factors released from target cells also contribute to the impairment of the vascular system leading to vascular leakage as demonstrated here in cultures of endothelial cells (white arrowheads). The systemic virus spread and replication, the general dysregulation of the host immune response, the coagulation abnormalities, the impairment of the vascular system, and hypotension all together finally result in shock and multiorgan failure. IL=interleukin. MCP-1=monocyte chemoattractant protein-1. MIPs=macrophage inflammatory proteins. NO=nitric oxide. TNF $\alpha$ =tumour necrosis factor  $\alpha$ .

which could contribute to the haemorrhagic diathesis. However, histological analysis of autopsy tissues from several of the early outbreaks did not identify vascular

lesions,<sup>66</sup> and no vascular lesions in any subsequent studies have been reported so far. Similarly, no evidence of substantial vascular lesions in non-human primates

infected with Ebola virus exists.<sup>57,60–62</sup> In one temporal study in cynomolgus macaques, infection of endothelial cells by Zaire Ebola virus was infrequent and was mainly restricted to the terminal stages of disease.<sup>62</sup>

Together with the macrophage-rich lymphoid tissues, the liver and the adrenal gland seem to be important targets for filoviruses (figure 2), and this tropism probably has an equally important role in the disease pathogenesis. Various degrees of hepatocellular necrosis have been reported in infected people and non-human primates;<sup>1,13,51,57,66</sup> however, the hepatocellular lesions are generally not serious enough to explain the cause of death. Importantly, haemorrhagic tendencies could be related to decreased synthesis of coagulation and other plasma proteins because of severe hepatocellular necrosis. Adrenocortical infection and necrosis have also been reported in humans and non-human primates infected with Ebola virus.<sup>1,51</sup> The adrenal cortex plays an important part in control of blood pressure homeostasis. Impaired secretion of enzymes that synthesise steroids leads to hypotension and sodium loss with hypovolaemia, which are important elements that have been reported in nearly all cases of Ebola haemorrhagic fever.<sup>1</sup> Impairment of adrenocortical function by Ebola virus infection could therefore have an especially important role in the evolution of shock that typifies late stages of Ebola haemorrhagic fever (figure 2).

During infection with Ebola virus, lymphoid depletion and necrosis are often noted in spleen, thymus, and lymph nodes of patients with fatal disease and in non-human primates that are experimentally infected (figure 2).<sup>1,13,51,61,63</sup> Although lymphoid tissues are primary sites of Ebola virus infection, there is usually little inflammatory cellular response in these or other infected tissues. Despite the large die-off and loss of lymphocytes during infection, the lymphocytes themselves are not infected. Large numbers of lymphocytes undergo apoptosis in man as well as in non-human primates experimentally infected with Ebola virus,<sup>51,67–69</sup> partly explaining the progressive lymphopenia and lymphoid depletion at death (figure 2). In the 2000 outbreak of Sudan Ebola virus in Uganda, a decrease in the number of circulating T lymphocytes was noted in people with fatal disease whereas cell count did not fall significantly in patients who survived the disease.<sup>70</sup> In macaques infected with Zaire Ebola virus, the lymphocyte loss seemed to be greatest in the T-lymphocyte and natural-killer cell populations.<sup>51</sup>

The mechanism for the underlying apoptosis and loss of bystander lymphocytes during the course of Ebola haemorrhagic fever are unknown but are thought to be provoked through several different agonists or pathways. These pathways or processes might include the TNF-related apoptosis-inducing ligand (TRAIL) and Fas death receptor pathways,<sup>51,71</sup> impairment of dendritic cell function induced by Ebola virus infection,<sup>51,72,73</sup> abnormal production of soluble mediators such as nitric oxide that

have proapoptotic properties,<sup>1,51,71,74</sup> or possibly by direct interactions between lymphocytes and Ebola virus proteins (figure 2). The recognition of an immunosuppressive motif in the carboxyl-terminal region of the virus' glycoproteins lends support to the notion that virus particles or proteins might partly contribute to the dysfunction or the loss of lymphocytes, or both.<sup>75–77</sup>

### Host immune response

Ebola virus infection triggers the expression of several inflammatory mediators including interferons; interleukins 2, 6, 8, and 10; interferon-inducible protein 10; monocyte chemoattractant protein 1; regulated upon activation normal T cell expressed and secreted (RANTES); TNF $\alpha$ ; and reactive oxygen and nitrogen species (figure 2).<sup>1,51,67,71,74,78</sup> Results from studies of various primary human cells in vitro also show that infection of Ebola virus can trigger the production of many of these same inflammatory mediators.<sup>62,71,79</sup> Although monocytes or macrophages seem to produce many of these mediators, as shown in vitro, other cell types could produce inflammatory mediators in the intact animal. Overall, virus-induced expression of these mediators seems to result in an immunological imbalance that partly contributes to the progression of disease. Proinflammatory responses recorded in fatal cases of Ebola haemorrhagic fever are dysregulated, whereas early and well regulated inflammatory responses have been associated with recovery.<sup>80</sup>

Inhibition of the type I interferon response, initially noted by studies of endothelial cells infected with Zaire Ebola virus,<sup>81,82</sup> seems to be a key feature of filovirus pathogenesis. The Ebola virus VP35 functioned as a type I interferon antagonist<sup>6,83,84</sup> by blocking activation of interferon regulatory factor 3 and possibly by preventing transcription of interferon  $\beta$ .<sup>83</sup> Additionally, other studies suggest that expression of VP24 of the Ebola virus interferes with type I interferon signalling;<sup>8,84</sup> mutations in VP24 have been linked to adaptation of Zaire Ebola virus to produce lethal disease in mice<sup>85</sup> and guinea pigs.<sup>86</sup>

Results from several studies show an important role for reactive oxygen and nitrogen species in pathogenesis of Ebola haemorrhagic fever (figure 2). Increased concentrations of nitric oxide in blood were reported in non-human primates experimentally infected with Zaire Ebola virus<sup>51,71</sup> and were noted in patients infected with Zaire Ebola virus and Sudan Ebola virus.<sup>70,74</sup> Increased blood concentrations of nitric oxide in patients were associated with mortality.<sup>70</sup> Abnormal production of nitric oxide has been associated with several pathological disorders including apoptosis of bystander lymphocytes, tissue damage, and loss of vascular integrity, which might contribute to virus-induced shock. Nitric oxide is an important mediator of hypotension, and hypotension is a prominent finding in most of the viral haemorrhagic fevers including those caused by Ebola virus (figure 2).

### Impairment of coagulation

Defects in blood coagulation and fibrinolysis during Ebola virus infections are manifested as petechiae, ecchymoses, mucosal haemorrhages, congestion, and uncontrolled bleeding at venepuncture sites (figures 2 and 3). However, massive loss of blood is infrequent and, when present, is mainly limited to the gastrointestinal tract (figure 3). Even in these cases, the amount of blood that is lost is not substantial enough to cause death. Thrombocytopenia, consumption of clotting factors, and increased concentrations of fibrin degradation products are other indicators of the coagulopathy that characterises Ebola virus infections. Results from clinical laboratory data strongly suggest that the coagulation abnormalities that occur during human Ebola haemorrhagic fever<sup>14,87</sup> are generally consistent with disseminated intravascular coagulation.<sup>88</sup> Furthermore, results from many studies have shown histological and biochemical evidence of disseminated intravascular coagulation during Ebola virus infection in several non-human primate species (figure 3).<sup>45,46,51,57,61,89–91</sup>

The mechanism responsible for triggering the coagulation disorders that typify Ebola haemorrhagic fever are not wholly understood. Results from several studies strongly suggest that expression or release of tissue factor from monocytes and macrophages infected with Ebola virus are key factors that induce the development of coagulation irregularities reported in Ebola haemorrhagic fever.<sup>91</sup> However, coagulopathy noted during Ebola haemorrhagic fever could be caused by several factors, especially during the later stages of disease. For example, rapid reductions in plasma concentrations of the natural anticoagulant protein C were recorded during the course of Zaire Ebola virus infection of cynomolgus monkeys.<sup>91</sup>

Together, the data so far suggest that an impaired and ineffective host response leads to high concentrations of virus and proinflammatory mediators in the late stages of disease, which is important in the pathogenesis of haemorrhage and shock. The prevailing hypothesis at this time is that infection and activation of antigen-presenting cells is fundamental to the development of Ebola haemorrhagic fever. The release of proinflammatory cytokines, chemokines, and other mediators from antigen presenting cells, and perhaps other cells, causes impairment of the vascular and coagulation systems leading to multiorgan failure and a syndrome that in some ways resembles septic shock (figure 2).

### Diagnosis

Ebola haemorrhagic fever presents as a viral prodrome with a high potential for differential diagnosis, especially early in outbreaks. The initial diagnosis of this syndrome is based on clinical assessment. Therefore, proper contingency plans should be developed. Several imported cases of the closely related Marburg virus have been



**Figure 3: Haemorrhagic manifestations noted in non-human primates infected with Ebola virus** Petechiae on the arm and axillary region of a Cynomolgus monkey infected with Sudan Ebola virus (A). Also shown are haemorrhages in the ileum (B) and a gastroduodenal lesion (C) from a Cynomolgus monkey infected with Sudan Ebola virus and fibrin thrombi (arrows) in sinusoids of a rhesus monkey infected with Zaire Ebola virus (D).

reported in Europe and the USA.<sup>92,93</sup> Ebola haemorrhagic fever can be suspected in acute febrile patients with the symptoms described and with a history of travel to an endemic area, if they present with fever and constitutional symptoms. Identification might be difficult because severe and acute febrile diseases can have a wide range of causes in areas endemic for Ebola virus, with the most prominent being malaria and typhoid fever followed by others such as shigellosis, meningococcal septicaemia, plague, leptospirosis, anthrax, relapsing fever, typhus, murine typhus, yellow fever, Chikungunya fever, and fulminant viral hepatitis.<sup>1</sup>

Laboratory diagnosis for viral haemorrhagic fevers is generally done in national and international reference centres, which should be contacted immediately on suspicion for advice on sampling, sample preparation, and sample transport. Laboratory diagnosis of Ebola virus is achieved in two ways: measurement of host-specific immune responses to infection and detection of viral particles, or particle components in infected individuals. Nowadays, RT-PCR<sup>1,94</sup> and antigen detection ELISA<sup>1,94</sup> are the primary assays to diagnose an acute infection. Viral antigen and nucleic acid can be detected in blood from day 3 up to 7–16 days after onset of symptoms.<sup>41</sup> For antibody detection the most generally used assays are direct IgG and IgM ELISAs and IgM capture ELISA.<sup>1,94</sup> IgM antibodies can appear as early as 2 days post onset of symptoms and disappear between 30 and 168 days after infection. IgG-specific antibodies develop between day 6 and 18 after onset and persist for many years.<sup>41</sup> A IgM or rising IgG titre constitutes a strong presumptive diagnosis. Decreasing IgM, or increasing IgG titres (four-fold), or both, in successive



paired serum samples are highly suggestive of a recent infection.<sup>194</sup> All these assays can be done on materials that have been rendered non-infectious. An efficient way to inactivate the virus for antigen and antibody detection is the use of gamma irradiation from a cobalt-60 source or heat inactivation.<sup>95</sup> Similarly, the nucleic acid can be amplified by purification of the virus RNA from materials treated with guanidinium isothiocyanate—a chemical chaotrope that denatures the proteins of the virus and renders the sample non-infectious.<sup>96</sup>

Repeated Ebola virus outbreaks in several countries of equatorial Africa have occurred in recent years.<sup>97</sup> Often these outbreaks occur in remote sites where advanced medical support systems are scarce and timely diagnostic services are very difficult to provide. Provision of basic on-site diagnostics, including confounding differential diagnosis, could help with the management of patients specifically and with the outbreak in general. The development of truly portable real-time thermocyclers and simple serological assays appropriate for field use has made the provision of a field diagnostic laboratory a reasonable undertaking.<sup>194,98,99</sup> However, the launch of diagnostic support in remote areas of equatorial Africa can be logistically and technically difficult since these regions are austere environments with cultural differences and sometimes hostile behaviour.

### Management

Case management is based on isolation of patients and use of strict barrier nursing procedures, such as protective clothing and respirators. These procedures have been sufficient to rapidly interrupt transmission in hospital settings in rural Africa. For members of rural African communities, cadavers are residual risks and should be handled accordingly. Traditional funeral and caretaking methods contribute to the spread of the virus and potentiate outbreaks. Methods to achieve barrier nursing, waste disposal, and other key elements inexpensively and practically in Africa have been devised, and field-tested manuals are available.<sup>47,100,101</sup> Important elements for outbreak prevention are provision of sterile equipment for injections, which is remarkably and tragically missing in Africa, and personal protective equipment to doctors, nurses, and caretakers, who are at high risk of contraction of infections in hospitals.

As a part of their contingency plans, many developed countries have established proper isolation and intensive care units to deal with imported cases.<sup>102,103</sup> Whether patients with viral haemorrhagic fever should be transported at later stages of disease is a persistent debate. Nevertheless, any hospital should be safely capable of minimum management of Ebola and other viral haemorrhagic fevers, and should prioritise an initial crucial assessment and an early rapid diagnosis.

Present treatment strategies are mainly symptomatic and supportive. In developing countries with minimum health-care provision, these strategies should include

isolation, malaria treatment, broad spectrum antibiotics, and antipyretics before diagnosis. Fluid substitution, preferentially intravenous administration, and analgesics should be provided as needed. In developed health-care systems with appropriate isolation units, proper intensive care treatment might be advised and should be directed towards maintenance of effective blood volume and electrolyte balance. Shock, cerebral oedema, renal failure, coagulation disorders, and secondary bacterial infection have to be managed and can be life-saving. Organ failure should be addressed appropriately—eg, dialysis for kidney failure and extracorporeal membrane oxygenation for lung failure. At present, no strategy has proved successful in specific pre-exposure and postexposure treatment of Ebola virus infections in man (table).

### Investigational treatments

Ribavirin, a drug that is believed to interfere with capping of viral mRNAs and has been used to treat viral haemorrhagic fevers caused by arenaviruses and bunyaviruses, has no in-vitro or in-vivo effect on filoviruses.<sup>104,105</sup> Therefore, and because of potential severe adverse effects associated with the drug, ribavirin is not recommended for Ebola virus infections.

With regard to RNA-based treatments, strategies to interfere with transcription and replication include the use of antisense oligonucleotides or RNA interference.<sup>106,107</sup> The approaches are promising on the basis of efficacy in rodents and non-human primates infected with Zaire Ebola virus (table).<sup>108</sup> RNA interference and antisense oligonucleotide-based approaches might be limited by the sequences for a particular Ebola virus species, which might not be known at the early stages of an outbreak. Additionally, these therapies are currently delivered intravenously, which might present logistical challenges in remote outbreak settings.

Treatment of the coagulation abnormalities recorded in Ebola virus infections should be considered (table). The nematode-derived anticoagulation protein rNAPc2 has shown 33% efficacy in the treatment of non-human primates infected with Zaire Ebola virus.<sup>109</sup> D-dimer formation has been identified as an early event during Ebola virus infection in non-human primates and could be used as a marker for treatment.<sup>91</sup> Because rNAPc2 targets signalling mainly through the extrinsic blood coagulation pathway, additional benefits might be gained with inhibitors of factor X, thus targeting the most common pathway of the extrinsic and intrinsic blood coagulation pathways (table). Additional substitution of protein C might be beneficial by activation of one of the crucial anticoagulant mechanisms in blood.<sup>91</sup> Results from a study showed that treatment of rhesus monkeys infected with Zaire Ebola virus with recombinant human activated protein C resulted in some protection of the animals, which is consistent with survival recorded with rNAPc2 (table).<sup>110</sup> All these drugs have been approved for



different applications in man and could be easily and safely used in emergencies.

Recombinant vaccines against Ebola virus based on vesicular stomatitis virus<sup>111</sup> have shown remarkable usefulness when given as a postexposure treatment against Ebola haemorrhagic fever in non-human primates infected with Zaire Ebola virus and Sudan Ebola virus.<sup>112,113</sup> In a laboratory event, a recombinant vesicular stomatitis virus expressing the Zaire Ebola virus glycoprotein was given to a woman shortly after exposure with Zaire Ebola virus.<sup>114</sup> The patient developed fever, headache, and myalgia hours after injection, which was successfully controlled with analgesics and antipyretics. Other adverse effects were not reported, but whether the treatment was effective or the patient never got infected with the virus remains uncertain. As with RNA-based treatments, postexposure vaccination with vaccines based on vesicular stomatitis virus will need some knowledge of the species involved since little cross-protection seems to exist between the various Ebola virus species.

Human convalescent blood or serum has been used for passive immunisation to treat patients naturally infected or non-human primates experimentally infected with Ebola virus,<sup>115,116</sup> but the success is controversial. In vitro, neutralising monoclonal antibodies specific for the glycoprotein of Ebola virus generated from different species, including man, showed protective and therapeutic properties in rodents.<sup>117–119</sup> However, antibody treatment with equine immunoglobulin against Ebola virus,<sup>120,121</sup> with polyclonal whole blood from rhesus monkeys immune against Ebola virus,<sup>122</sup> or with a recombinant human monoclonal antibody<sup>123</sup> did not protect non-human primates from lethal infection with Ebola virus. Although no definite therapeutic conclusion can be drawn from the studies done so far, data suggest the value, in principle, of passively acquired antibodies in reduction of the viral burden during infection. Thus, antibody therapy, perhaps in combination with other pharmaceutical agents, might be beneficial (table).

In view of the severe and rapid progression of Ebola haemorrhagic fever, no one therapy is likely to be sufficiently potent, which strongly favours combination therapy as the best choice. A suitable strategy might be to slow down virus replication and disease progression and to allow innate and adaptive immune responses to overcome infection.<sup>115,124</sup> This idea is supported by data showing that viraemia lower than  $1 \times 10^4$ – $5$  pfu/mL of blood is strongly associated with survival of patients and non-human primates infected experimentally.<sup>1,51,110,112</sup>

## Prevention

Previously, the usefulness of an Ebola virus vaccine was disputed, because of the disease's rarity, little interest by industry, and the potential cost. Frequent outbreaks in the past decade, several imported cases of viral haemorrhagic fever and laboratory exposures, and the potential misuse

of Ebola virus as a biothreat agent has changed that view. Vaccine development is part of many nations' efforts in response to the public health threat posed by emerging or re-emerging biothreat pathogens such as Ebola virus. A protective vaccine would be very valuable not only for at-risk medical personnel, first responders, military personnel, and researchers, but also for targeted vaccination in affected populations, especially during outbreaks, for use in a so-called ring vaccination strategy.

At present, vaccine candidates to be considered should show efficacy in at least two animal models of the disease including non-human primates, the gold standard animal model for viral haemorrhagic fever caused by several pathogens such as Ebola virus.<sup>46</sup> Only a few vaccine platforms have passed these requirements and are considered for further investigation and

	Success in animals	Issues and concerns
<b>Treatment approach</b>		
Antibody therapy	Efficacy in rodents but not in non-human primates	Escape mutants; genetic variability; antibody-dependent enhancement of infection
Antisense oligonucleotides		
Phosphorodiamidate morpholino oligonucleotides	Efficacy in rodents and non-human primates (latter prophylactic only)	Genetic variation; delivery
Small interfering RNAs	Efficacy in rodents and non-human primates	Genetic variation; delivery
Inflammatory modulators		
Type I interferons	Efficacy in rodents but not in non-human primates	Manipulation of immune system
S-adenosylhomocysteine hydrolase inhibitors	Efficacy in rodents but not in non-human primates	Manipulation of immune system
Coagulation modulators		
Heparin sulfate	Efficacy in humans questionable; not tested in animals	Manipulation of coagulation
Tissue factor pathway inhibitors	Not tested in rodents; partial protection in non-human primates	Manipulation of coagulation
Activated protein C	Not tested in rodents; partial protection in non-human primates	Manipulation of coagulation
<b>Vaccination approach</b>		
Postexposure vaccination		
Vesicular stomatitis virus	Efficacy in rodents and non-human primates	Efficacy dependent on filovirus species and time of treatment start
Pre-exposure vaccination		
Adenovirus type 5	Efficacy in rodents and non-human primates; one dose; clinical trials	Pre-existing immunity; high dose
Human parainfluenza virus type 3	Efficacy in rodents and non-human primates; two doses needed for non-human primates	Pre-existing immunity; safety (replication-competent)
Vesicular stomatitis virus	Efficacy in rodents and non-human primates; one dose	Safety (replication-competent)
Virus-like particles	Efficacy in rodents and non-human primates; three doses needed for non-human primates	Boost immunisation needed; production
Recombinant Ebola virus without VP35	Efficacy in rodents	Safety
Only approaches that have shown in-vivo efficacy have been listed.		
<b>Table: Treatment and prophylaxis of Ebola haemorrhagic fever</b>		

perhaps for clinical trials. These vaccine candidates are based on recombinant technologies that use either generated replication-deficient or attenuated replication-competent platforms.

Among the replication-deficient platforms, human-adenovirus-type-5 vectors have been the first successful strategies to protect non-human primates from lethal Ebola virus challenge (table). Originally a DNA prime (glycoprotein and nucleoprotein) adenovirus boost (glycoprotein) approach was used,<sup>125</sup> which was subsequently replaced with an accelerated approach of one immunisation with a recombinant adenovirus expressing the Zaire Ebola virus glycoprotein 28 days before challenge.<sup>126</sup> The approach has been further developed by others by use of a multivalent adenovirus technology for the development of a panfilovirus vaccine that provides protection against several filovirus species.<sup>127</sup> The adenovirus platform seems safe and robust but is weakened by pre-existing immunity<sup>128</sup> in the world population and its failure in an HIV/AIDS trial.<sup>129</sup> The second successful approach with replication-deficient platforms is based on Ebola virus-like particles generated by coexpression of the viral matrix protein (VP40), nucleoprotein, and glycoprotein (table).<sup>130</sup> This approach seems to best address safety issues but might need adjuvant and still needs booster immunisation for efficacy in non-human primates, which is not favourable for emergency use. Other issues are associated with the costs and production of the virus-like particle (VLP) vaccines compared with viral vector-based platforms. Reverse genetics has generated the first new generation inactivated Ebola virus vaccine by deletion of an essential gene rendering the resulting virus replication deficient.<sup>131,132</sup> This technology allows large-scale production, but remaining safety issues still need to be addressed for potential future use of this technology in generation of promising vaccine candidates.

Generally, live attenuated viruses are more advantageous than are non-replicating vaccines because of ease of production and their potent stimulation of innate and adaptive (humoural and cellular) immune responses. However, this idea does not seem feasible for Ebola virus because of difficulties in ensuring the safety of live attenuated Ebola virus strains. However, live attenuated recombinant Ebola virus vaccine vectors have been developed on the basis of the background of less virulent viral systems such as vesicular stomatitis virus<sup>111</sup> and human parainfluenza virus (table).<sup>133,134</sup> The system based on vesicular stomatitis virus has shown tremendous efficacy in non-human primates including both prophylactic and postexposure treatment situations.<sup>112,113,135</sup> These potent vaccine platforms are associated with safety issues despite having a clean record in laboratory animals including immune-deficient animals.<sup>136</sup> As with adenovirus vectors, pre-existing immunity might be an issue with the human parainfluenza virus<sup>137</sup> platform but is negligible for vesicular stomatitis virus. Vaccine platforms of human

parainfluenza and vesicular stomatitis viruses might have potential for delivery without use of needles.<sup>134,138</sup>

Despite good to excellent protective efficacy in animals, correlates and mechanisms of protection have not been well defined for most of the vaccine candidates mentioned in this Seminar. On the basis of present data, antibody responses, T-cell proliferation, and cytotoxic-T-lymphocyte responses show that antibody and T-helper cell memory are essential for protection, and that cell-mediated immunity, although possibly important, is not an absolute requirement. Total antibody response is thought to be a correlate for protection for Ebola virus vaccines.<sup>139</sup> Finally, a multivalent preventive vaccine is clearly needed to provide protection against all species of Ebola viruses and Marburg viruses, and such a vaccine will possibly need at least three components.<sup>140</sup>

## Conclusions

Substantial progress has been made during past decades in the understanding of the biology and pathogenesis of Ebola virus infections in vitro and in vivo. The identification of bats as potential reservoir species is a milestone, with implications for public health. Substantial progress has also been achieved in the development of countermeasures, with rapid diagnostics being implemented in developed settings and with some promising therapeutics and vaccine candidates having entered or being close to entering clinical trials. However, most of our knowledge is based on infections with Zaire Ebola virus, the most pathogenic species within the genus *Ebolavirus*, and on studies done in non-human primates. The other species of Ebola virus are genetically and serologically distinct, might differ in their ecology, and possess biological characteristics that make them less virulent in man.

Future efforts need to focus on the knowledge gaps about other species of Ebola virus. To prevent primary transmission from bats to man, we need more field studies into the ecology of reservoir species and their infection status and shedding mechanisms. More detailed investigations into the pathophysiology of Ebola virus infections with laboratory animals should provide us with new targets for intervention strategies. Promising therapeutics and vaccines need to be moved forward into clinical trials, and provision needs to be made for emergencies such as laboratory exposures. Finally, we urgently need strategies, financial support, and political will to bring these developments to the populations of endemic areas in equatorial Africa who are in primary need for intervention and for whom financial resources are scarce.

### Contributors

Both authors contributed equally to concept, literature search, writing, and figure design of this Seminar.

### Conflicts of interest

HF claims intellectual property for VSV-based filovirus vaccines. TWG claims intellectual property for VSV-based filovirus vaccines, adenovirus-based filovirus vaccines, and RNA interference for the treatment of filoviral infections.

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