Updates in diagnosis and management of Ebola hemorrhagic fever

Salah Mohamed El Sayed, Ali A. Abdelrahman, Hani Adnan Ozbak, Hassan Abdullah Hemeg, Ali Mohammed Kheyami, Nasser Rezk, Mohamed Baioumy El-Ghoul, Manal Mohamed Helmy Nabo, Yasser Mohamed Fathy

INTRODUCTION

Ebola virus infection constitutes a highly infectious lethal zoonosis affecting both human and nonhuman primates that usually occurs in sporadic epidemics every few years (average every 1.5 years).[1] Central Africa and sub-Saharan Africa are the most badly affected localities and constitute the source of world epidemics possibly due to human infections from the forest bats Zaire Ebola virus caused the largest reported outbreak of Ebola in 2014 in the West Africa. This virus was transmitted to human through infected fruit bats, monkeys, apes, and pigs. Animals got the infection mostly through contact with bat saliva or feces.[1-4]

Due to urgency, the WHO allowed the use of experimental treatments.[5] This review aims to elucidate the health efforts exerted to combat Ebola virus and update the diagnostic and treatment lines to fight this lethal health problem. In this article, we review the

Key words: Ebola hemorrhagic fever, filoviruses, fruit bats, pathogenesis, reverse transcription-polymerase chain reaction

Access this article online
Quick Response Code: Website: www.jmsjournal.net DOI: 10.4103/1735-1995.192500

How to cite this article: El Sayed SM, Abdelrahman AA, Ozbak HA, Hemeg HA, Kheyami AM, Rezk N, El-Ghoul MB, Nabo MMH, Fathy YM. Updates in diagnosis and management of Ebola hemorrhagic fever. J Res Med Sci 2016;21:84.

This is an open access article distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 3.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as the author is credited and the new creations are licensed under the identical terms.

For reprints contact: reprints@medknow.com
literature extensively to find out possible diagnostic and therapeutic lines in light of our understanding of Ebola virology, transmission, and pathogenesis. We also aim at developing solid prevention measures.

EBOLA VIROLOGY

Ebola virus belongs to filoviruses (family: Filoviridae) [Figure 1] that include a group of large viruses having a filamentary form (with characteristic filamentous particles) that may exceed 1000 nm (80–1400 nm) in diameter. Ebola virus is a nonsegmented negative-sense RNA virus. Ebola genome includes monocistronic genes that encode for a single protein, for example, nucleoprotein (NP), virion protein (VP) 35, VP40, VP30, VP24, and RNA-dependent RNA polymerase [Figure 2] in addition to the polycistronic glycoprotein gene. The genome of Ebola virus encodes for seven proteins and two nonstructural proteins.\[6-10\]

EPIDEMIOLOGY OF EBOLA HEMORRHAGIC FEVER

Filovirus hemorrhagic fever was first reported as Marburg virus in 1967 in Germany and former Yugoslavia. In\[11\] equatorial Africa and some African countries, for example, Gabon, Congo, Uganda, and Sudan, Ebola virus hemorrhagic fever still constitutes a big concern for the populations mostly due to increased numbers of outbreaks and cases that were mostly caused by Zaire Ebola virus. In the Philippines, in the far East, the emergence of Reston Ebola virus in pigs increases the concern of health authorities regarding public health, agriculture, and food safety, which may threaten the emergence of health problems in some parts of Asia.\[12\]

In Ebola-endemic areas, both apes and man and other mammals might be the end hosts rather than reservoir hosts. As Ebola represents a classic zoonosis, persistence of Ebola virus in reservoir animals is generally found. Bats are frequently encountered in equatorial Africa and hunted for food in many places. African fruit bats and insectivorous bats might be the reservoir hosts for Ebola [Figure 3] as documented by detection of viral RNA and antibodies in three tree-roosting species of fruit bats: hypsignathus monstrosus, epomops franqueti, and myonycteris torquata.\[13,14\] Further evidence was reported regarding filoviruses reservoir hosts where identification and isolation of Marburg virus from the cave-dwelling fruit bat Rousettus aegyptiacus was reported.\[15\] However, that may need further research confirmation.\[16\]

EBOLA TRANSMISSION

Ebola transmission occurs mainly through contact with infected subjects and blood products. Understanding the infectious routes and cycles through which Ebola virus can be transmitted seems critical to break the chain of transmission for future control and prevention against hemorrhagic fever viruses. Unlike respiratory viruses, both droplet and aerosol transmission regarding Ebola is thought to be rare. However, the blood-borne transmission may be critical as monocytes, macrophages, and dendritic
cells constitute the major replication sites for Ebola viruses. Therefore, blood and blood products transfusion may represent a big issue of concern. Ebola dissemination from the initial infection site may take place through monocytes, macrophages, and dendritic cells to regional lymph nodes reaching the lymphatic system, liver, and spleen through the blood.[19]

Contact with infected patients, infected human body fluids, dead human bodies, infected cadavers, and infected animal carcasses may be the most critical route for transmitting the Ebola virus infection. Lack of early diagnosis of Ebola in rural and forest areas in Africa in addition to the lack of patients’ isolation and application of quarantine measures may exaggerate the transmission of infection. Social habits regarding patient care, contact with patients’ belongings and personal clothes (e.g., fomites, towels, and sheets),[20] burial preparation (e.g., washing the dead body), and funeral ceremonies may increase the chance of Ebola transmission, particularly in epidemics.[21,22]

Extreme care should be given to patients’ body fluids even after recovery as Ebola virus was reported to be isolated from patients’ biological fluids, for example, sputum, saliva, vomitus, breast milk, tears, sweat, genital secretions, urine, and feces.[20] Moreover, Ebola virus was reported in breast milk and genital secretions for a long duration after recovery (13 weeks).[20,23]

Intact skin represents an immunological defense against infection with Ebola as the virus was reported to enter the body through skin abrasions, mucosal surface breaks, and through contaminated water for parenteral injection.[24,25] Laboratory-induced and blood-borne infections (via contaminated needle stick and blood) have been reported in the 1976 outbreaks of Ebola virus in some African countries, for example, Sudan and Zaire.[26,27]

As for the animal-borne transmission of infection, this was reported to occur through chimpanzees, bats, and nonhuman primates in equatorial Africa and may represent an important source of Ebola virus where reported organ infectivity titers reached $10^7$–$10^8$ pfu/g.[28] The bad nutritional habits of eating the flesh of chimpanzees and freshly killed bats (carriers of Ebola virus) or their slaughtering for food might be related to the emergence of outbreaks in Zaire, Congo, and Gabon. Even contact exposure with infected animals may cause Ebola virus transmission.[14,15,29] Moreover, undercooking infected animals and exposure to infected blood may enhance the possibility of infection and enhance virus transmission through the oral route as evidenced by the report that Zaire Ebola virus was highly lethal when given orally to rhesus macaques.[30]

**EBOLA PATHOGENESIS**

Pathogenesis of Ebola targets mainly the liver, adrenal cortex, lymphatic tissues, and some cells of the immune system causing many pathological effects [Figure 4]. Hemorrhagic fever is a descriptive pathological term for Ebola virus infection. The relatively large size of Ebola viruses may suggest a traumatic vascular injury to explain the origin of Ebola-induced hemorrhagic fever or the causes of inducing the hemorrhagic complications. However, this possibility was reported to be excluded upon vascular histological analysis of different tissues during autopsy.
Lack of evidence for the occurrence of substantial vascular lesions in nonhuman primates infected with Ebola virus was documented in many studies. Interestingly, infection of endothelial cells with Ebola virus in cynomolgus macaques occurred only in the terminal stages of the disease, which excludes vascular injury as a cause for hemorrhagic diathesis occurring during Ebola infection. Laboratory investigations of Ebola patients presenting with hemorrhagic fever confirmed the presence of coagulation abnormalities (consumption of clotting factors) that manifested clinically as petechiae, ecchymoses, mucosal hemorrhages, congestion, and uncontrolled bleeding at venipuncture sites during Ebola hemorrhagic fever that is clinically correlated with disseminated intravascular coagulation (DIC).

Importantly, Ebola-induced marked hepatic impairment and hepatocellular necrosis in both infected patients and nonhuman primates with the secondary disturbance in protein and coagulation factor synthesis might be the underlying factor for the hemorrhagic tendencies, fibrinolysis, consumptive coagulopathy, increased concentrations of fibrin degradation products, and thrombocytopenia causing infrequent blood loss that occurs mainly in the gastrointestinal tract. Ebola-induced coagulopathy may be due to the expression or release of tissue factors from infected monocytes and macrophages or rapid reductions in serum level of protein C (natural anticoagulant) that were recorded during the course of Zaire Ebola virus infection of cynomolgus monkeys. Moreover, coagulopathy-induced hemorrhages are not large enough to be the underlying cause of death.

Adrenal cortex may be the second affected tissue with Ebola virus where adrenocortical infection and necrosis were reported in patients and nonhuman primates during Ebola virus epidemics, which may explain the fluid and electrolyte disturbances ending in shock and fatal circulatory failure that are usually met with in end-stage infections with Ebola viruses.

Lymphatic tissues are also affected with Ebola virus infections where lymphoid depletion and necrosis were reported in the spleen, thymus, and lymph nodes of patients with fatal disease and in experimentally infected nonhuman primates causing impairment of both cell-mediated and humoral immunities. Lymphocyte apoptosis during Ebola virus pathogenesis may be the underlying cause for the progressive lymphopenia and lymphoid depletion and was reported to be due to activation of tumor necrosis factor (TNF)-related apoptosis-inducing ligand and factor of apoptotic signal death receptor pathways. That was evidenced by the premortem depletion of circulating T-lymphocytes and natural killer cell populations in the serum of patients who died of Ebola during fatal epidemics while in Ebola survivors, lymphocyte cell count did not decrease significantly. A similar hematological picture was noted in macaques infected with Zaire Ebola virus where the lymphocyte loss seemed to be greatest in T-lymphocytes and natural killer cells. Moreover, Ebola virus was reported to induce impairment in the dendritic cell function, which may be due to the immunosuppressive motif in the carboxyl-terminal region of the virus glycoproteins causing lymphocyte dysfunction or loss.

Ebola infection causes activation of antigen presenting cells and impairment of the coagulation systems causing multiorgan failure and septic shock. Blood and tissue chemistry may be severely affected by Ebola virus-induced inflammatory processes where released pro-inflammatory cytokines, chemokines, and other mediators (from antigen presenting cells), reactive oxygen, and nitrogen species help in the pathogenesis of Ebola hemorrhagic fever.

Moreover, there is reported increased levels of several inflammatory mediators and cytokines, for example, TNF-α, interleukin (IL)-2, IL-6, IL-8, IL-10, interferon (INF)-inducible protein-10, monocyte chemoattractant protein-1, and regulated upon activation normal T-cell expressed and secreted. Inhibition of the type-I INF response was also reported during Ebola virus infection. Interestingly, Ebola virus VP35 was reported to act as a type-I INF antagonist through blocking the activation of INF regulatory factor 3 and preventing the transcription of INF-β. Same INF inhibition was reported for VP24 of the Ebola virus that interferes with type-I INF signaling.

**BIOLOGY OF EBOLA HEMORRHAGIC FEVER**

The incubation period of Ebola hemorrhagic fever virus is not long (3–21 days, average 12.7 ± 4.3 days). Bats may be a natural reservoir, which still needs further research confirmation to isolate the virus from bats. Fruit bats (where viral RNA and antibodies were isolated) are resistant to Filoviridae. The newest member of filoviruses (Lloviucuevavirus) was discovered in 2010 in Spain and was retrieved from bats.

Seasonal variation in mortality among the African chimpanzees may suggest that climatic changes may affect the Ebola epidemics. A close relationship between the dry conditions at the end of the rainy season was found to correlate with the onset of epidemics, migration of bats, and human contamination that may induce a change in the behavior of fruit-eating mammals with enhancement in virus circulation.
CLINICAL PICTURE OF EBOLA VIRAL INFECTION

The main characteristic of Ebola symptomatology includes hematological, lymphatic, and immunological disturbances that should raise a high index of suspicion and should be differentiated from blood diseases. Patients usually present with a flu-like syndrome having fever, chills, abdominal pain, headache, myalgia, malaise, arthralgia, cough, and sore throat with dysphagia. However, hemorrhagic manifestations and complications characterize Ebola and differentiate it from other fevers. Infection with Ebola may present with gastrointestinal bleeding, uncontrolled oozing from venipuncture sites (denoting Ebola complicated with DIC), petechiae, ecchymoses, purpura, epistaxis, gingival bleeding, and mucosal hemorrhages. At autopsy, postmortem evidence of visceral hemorrhagic effusions confirms the cause of death to be Ebola hemorrhagic fever.

A maculopapular rash is a diagnostic sign that later desquamates (at days 5–7) in survivors. Nonspecific symptoms may make the diagnosis of Ebola hemorrhagic fever difficult, especially at primary health centers. However, nonspecific symptoms should be taken seriously in patients living in endemic areas, travelers to endemic areas, and at times of epidemics. Digestive disorders may be present, for example, nausea, vomiting, anorexia, and diarrhea. Respiratory symptoms may be presenting in the form of nasal discharge, conjunctival injection, postural hypotension, edema, chest pain, shortness of breath, and cough.

Neurological manifestations or complications may be dominating, for example, headache, confusion, and coma. Mortality rate was reported to decrease upon improving the prophylactic measures and decreasing the virus load. This is confirmed by data showing that viremia lower than $1 \times 10^{4}$–$10^{5}$ pfu/mL of blood was correlated with improved survival in patients and nonhuman primates infected experimentally.

LABORATORY DIAGNOSIS OF EBOLA VIRUS

Ebola hemorrhagic fever is often fatal, necessitating its early diagnosis to repress the progress of this vicious disease. However, the nonspecificity of early symptoms and limited laboratory facilities in endemic areas make the diagnosis challenging. A combination of some clinical manifestations in susceptible people should raise the possibility of Ebola infection and guide the laboratory investigations. Presumptive diagnosis includes nonspecific symptoms of fever, myalgia, malaise, headache, gastrointestinal complications, sore throat, and others. Conjunctivitis and maculopapular rashes may also appear. This is followed by bloody diarrhea, dyspnea due to pulmonary edema, and irritability that progresses to DIC, hepatic and renal dysfunctions, seizures, shock, coma, and eventually death.

Preliminary investigative markers include leucopenia, thrombocytopenia, asthenia, transaminitis (aspartate transaminase and alanine transaminase [ALT]), and elevated levels of blood urea nitrogen. However, these need to be corroborated with specific tests. Laboratory diagnosis is usually made through virus enumeration, serology, nucleic acid tests, or, rarely, viral culture. Virus or viral particles can be detected in the blood at the onset of infection.

ELISA was the mainstay for diagnosis that detects viral antigen, infectious virus proteins, and virus-specific IgM and IgG antibodies in the serum. Viral antigenemia could be detected in virtually all the patients using a polyvalent hyperimmune rabbit serum specific for the Ebola subtypes. However, its sensitivity ($\approx 93\%$) in the acute phase of illness wanes thereafter with the disappearance of the antigen. The serum levels of virus-specific IgM and IgG antibodies were detectable at approximately the same time after disease onset (8–10 days), but IgM persisted for a much shorter period than IgG among the surviving convalescent patients. While IgM was measured in a capture assay using modified capture and detection antigens with the polyvalent rabbit serum as the antigen detector. Antihuman IgG (gamma chain specific) was used to detect bound immunoglobulins in IgG ELISA.

Infectious virus isolation though attempted initially (using confluent layers of Vero 6 cells in biosafety level 4 containment facility) has now been discontinued due to the requirement of stringent conditions and slow growth of virus in culture. If the cytopathic effect was observed, the viral cultures were harvested, and antigen was tested using indirect fluorescent antibody test. Fluorescent focus assay (FFA) was also used to enumerate the virus by immunofluorescent staining of infected cells. Attempts to quantify virus by Plaque Assay using neutral red staining were less successful than FFA.

ELISA has been replaced by reverse transcription-polymerase chain reaction (RT-PCR) that is more sensitive to be deployed even in epidemic settings. RT-PCR surveillance of blood, sputum/saliva/throat swabs, conjunctival swabs, stool, urine, semen, and sweat (from axillary, forehead, and inguinal regions) has been reported. This is a rapid and sensitive technique targeting viral nucleic acid. Viral RNA remained undetectable in saliva, sputum, conjunctival swabs, and stools due to viral RNA shedding. However, urine and sweat samples have been reported to remain positive for viral RNA up to 30–40 days and semen for...
Moreover, NS was reported to inhibit the IL-1[28,89,90] and cyclooxygenase-2, which may suggest its use as a medicinal antisense oligonucleotides, and RNA synthesis in mammalian cells. Favipiravir is an antiviral agent that induces selective inhibition of viral polymerase in an animal model. TKM-Ebola is a combination of small interfering RNAs that target Ebola polymerase enzyme, membrane-associated protein (VP24), and the complex protein (VP35). It is designed to block the replication of the Ebola virus. An Ebola nucleoside analog was recently reported to protect against infection with Filoviridae through inhibiting the viral polymerase in an animal model.[90] Favipiravir is an antiviral agent that induces selective inhibition of viral RNA-dependent RNA polymerase without inhibiting RNA or DNA synthesis in mammalian cells. Favipiravir was approved in Japan in 2014 for treating influenza A virus disease. It was also reported to have activity against some RNA viruses, for example, influenza viruses.[92] Lamivudine may be helpful to patients having Ebola hemorrhagic fever. ZMapp is a potential therapeutic material[3] that is composed of three chimeric monoclonal antibodies.[93]

**SPECIFIC TREATMENT**

Ribavirin,[87,88] antisense oligonucleotides, and RNA interference may be promising based on their efficacy in animal studies.[52,99,100]

TKM-Ebola is a combination of small interfering RNAs that target Ebola polymerase enzyme, membrane-associated protein (VP24), and the complex protein (VP35). It is designed to block the replication of the Ebola virus. An Ebola nucleoside analog was recently reported to protect against infection with Filoviridae through inhibiting the viral polymerase in an animal model.[90] Favipiravir is an antiviral agent that induces selective inhibition of viral RNA-dependent RNA polymerase without inhibiting RNA or DNA synthesis in mammalian cells. Favipiravir was approved in Japan in 2014 for treating influenza pandemics. It was also reported to have activity against some RNA viruses, for example, influenza viruses.[92] Lamivudine may be helpful to patients having Ebola hemorrhagic fever. ZMapp is a potential therapeutic material[3] that is composed of three chimeric monoclonal antibodies.[93]

**TARGETING EBOLA BIOLOGY AS A FUTURE PERSPECTIVE**

Based on our understanding of the key points in Ebola transmission, pathogenesis, and complications, it may be strongly suggested that herbal and prophetic medicine remedies, for example, nigella sativa (NS) may play a role in both prophylaxis and treatment.

NS was reported to exert potent antiviral effects against many viruses, for example, viral hepatitis, coronavirus, and others,[94] which may suggest its use as a medicinal nutrition or nutritional supplement for treating Ebola virus disease.

NS was reported to possess potent anti-inflammatory effects where its active ingredient thymoquinone suppressed effectively the lipopolysaccharide-induced inflammatory reactions and reduced significantly the concentration of nitric oxide.[95] Moreover, NS was reported to inhibit the inflammatory processes through suppressing the activities of IL-1, IL-6, nuclear factor-xB,[96] IL-1β, cyclooxygenase-1, prostaglandin-E2, prostaglandin-D2,[97] cyclooxygenase-2, and TNF-α[98] that act as potent inflammatory mediators and were reported to play a major role in the pathogenesis of Ebola virus infection.

Immunostimulating effects of NS include increased natural killer cell activity, increased count, and activity of T helper-1 lymphocytes (immune stimulant effect) versus T helper-2 (immune suppressive effect). NS suppressed the production of IL-6 and TNF-α.[99]

All the above-mentioned effects may abolish and antagonize many steps in the pathogenesis of Ebola-induced hepatic impairment, hematological disturbances, and inflammatory reactions, which are responsible for the fatal outcomes in Ebola virus infection.

Moreover, many hepatoprotective effects of NS were recently reported. NS was reported to exert many hepatoprotective effects against ischemia-reperfusion injury where NS treatment restored the serum level of liver enzymes (ALT, aspartate aminotransferase, and lactate dehydrogenase), reduced oxidative stress index, and enhanced the total antioxidant capacity to near normal values.[100]

**Financial support and sponsorship**

This article is supported by the deanship of scientific research in Taibah University, Saudi Arabia.
CONFICTS OF INTEREST
The authors have no conflicts of interest.

AUTHORS’ CONTRIBUTION
SME: Drafted the article and figures, shared in the article preparation, and approved the submission. AAA: Prepared the biology part, shared in the article design, n and approved the submission. HAO: Wrote the diagnosis section, shared in the article preparation, and approved the submission. HAH: Prepared epidemiology and transmission sections, shared in the article preparation, and approved the submission. AMK: Wrote Ebola virology, shared in the article preparation, revised the article, and approved the submission. MBE: Shared in the article preparation, revised the article, wrote the abstract, and approved the submission. MMHN: Prepared the pathogenesis section, shared in the article preparation, and approved the submission. YFM: Critically reviewed the article, added important parts in the body of the article, and approved the submission. SME, AAA, HAO, HAH, AMK, MMHN, MBE, and YFM are the abbreviations for the names of the authors as enlisted in the author list section.

REFERENCES
1. Hartman AL, Bird BH, Towner JS, Antoniadou ZA, Zaki SR, Nichol ST. Inhibition of IRF-3 activation by VP35 is critical for the high level of virulence of Ebola virus. J Virol 2008;82:2699-704.
2. Wauquier N, Becquart P, Padilla C, Baize S, Leroy EM. Human fatal zaire Ebola virus infection is associated with an aberrant innate immunity and with massive lymphocyte apoptosis. PLoS Negl Trop Dis 2010;4: pii: e837.
3. Choi WY, Hong KJ, Hong JE, Lee WJ. Progress of vaccine and drug development for Ebola preparedness. Clin Exp Vaccine Res 2015;4:11-6.
4. WHO Ebola Response Team. Ebola virus disease in West Africa – The first 9 months of the epidemic and forward projections. N Engl J Med 2014;371:1481-95.
5. Chippaux JP. Outbreaks of Ebola virus disease in Africa: The beginnings of a tragic saga. J Venom Anim Toxins Incl Trop Dis 2014;20:44.
6. Ascenzi P, Boeddi A, Heptonstall J, Capobianchi MR, Di Caro A, Mastrangelo E, et al. Ebola virus and marburgvirus: Insight the filoviridae family. Mol Aspects Med 2008;29:151-85.
7. Feldmann H, Geisbert TW. Ebola haemorrhagic fever. Lancet 2011;377:849-62.
8. Groseth A, Marzi A, Hoenen T, Herwig A, Gardner D, Becker S, et al. The Ebola virus glycoprotein contributes to but is not sufficient for virulence in vivo. PLoS Pathog 2012;8:e1002847.
9. Feldmann H, Jones SM, Schnittler HJ, Geisbert T. Therapy and prophylaxis of Ebola virus infections. Curr Opin Investig Drugs 2005;6:823-30.
10. Sanchez A, Geisbert TW, Feldmann H. Filoviridae: Marburg and Ebola viruses. In: Knipe DM, Howley PM, editors. Fields Virology. Philadelphia: Lippincott Williams and Wilkins; 2006. p. 1409-48.
11. Siegert R, Shu HL, Slenczka W, Peters D, Müller G. On the etiology of an unknown human infection originating from monkeys. Dtsch Med Wochenschr 1967;92:2341-3.
12. Barrette RW, Metwally SA, Rowland JM, Xu L, Zaki SR, Nichol ST, et al. Discovery of swine as a host for the Reston ebolavirus. Science 2009;325:204-6.
13. Groseth A, Feldmann H, Strong JE. The ecology of Ebola virus. Trends Microbiol 2007;15:408-16.
14. Leroy EM, Epelboin A, Mondonge V, Pourrut X, Gonzalez JP, Muyembe-Tamfum JJ, et al. Human Ebola outbreak resulting from direct exposure to fruit bats in Luebo, Democratic Republic of Congo, 2007. Vector Borne Zoonotic Dis 2009;9:723-8.
15. Leroy EM, Kumulungui B, Pourrut X, Rouquet P, Hassanin A, Yaba P, et al. Fruit bats as reservoirs of Ebola virus. Nature 2005;438:575-6.
16. Pourrut X, Delicat C, Rollin PE, Ksiazeck TG, Gonzalez JP, Leroy EM. Spatial and temporal patterns of Zaire ebolavirus antibody prevalence in the possible reservoir bat species. J Infect Dis 2007;196 Suppl 2:S176-83.
17. Towner JS, Amman BR, Sealy TK, Carroll SA, Comer JA, Kemp A, et al. Isolation of genetically diverse Marburg viruses from Egyptian fruit bats. PLoS Pathog 2009;5:e1000536.
18. Swanepoel R, Leman BA, Burt FJ, Zachariaides NA, Braack LE, Ksiazeck TG, et al. Experimental inoculation of plants and animals with Ebola virus. Emerg Infect Dis 1996;2:321-5.
19. Geisbert TW, Hensley LE, Larsen T, Young HA, Reed DS, Geisbert JB, et al. Pathogenesis of Ebola hemorrhagic fever in cynomolagus macaques: Evidence that dendritic cells are early and sustained targets of infection. Am J Pathol 2003;163:2347-70.
20. Bausch DG, Towner JS, Dowell SF, Kaducu F, Lukwiya M, Sanchez A, et al. Assessment of the risk of Ebola virus transmission from bodily fluids and fomites. J Infect Dis 2007;196 Suppl 2:S142-7.
21. Hewlett BS, Amola RP. Cultural contexts of Ebola in Northern Uganda. Emerg Infect Dis 2005;9:1242-8.
22. Hewlett BS, Epelboin A, Hewlett BL, Formenty P. Medical anthropology and Ebola in Congo: Cultural models and humanistic care. Bull Soc Pathol Exot 2005;98:230-6.
23. Kibadi K, Mupapa K, Kuvula K, Massamba M, Ndaberey D, Ksiazeck TG, et al. Experimental inoculation of plants and animals with Ebola virus. Emerg Infect Dis 1996;2:321-5.
24. Bausch DG, Towner JS, Dowell SF, Kaducu F, Lukwiya M, Sanchez A, et al. Assessment of the risk of Ebola virus transmission from bodily fluids and fomites. J Infect Dis 2007;196 Suppl 2:S142-7.
25. Khan AS, Tshiokio FK, Heymann DL, Le Guenno B, Nabeth P, Kerstjens B, et al. The reemergence of Ebola hemorrhagic fever, Democratic Republic of the Congo, 1995. Commission de Lutte contre les Épidémies à Kikwit. J Infect Dis 1999;179 Suppl 1:S13-4.
26. Dowell SF, Mukuru R, Ksiazeck TG, Khan AS, Rollin PE, Peters CJ. Transmission of Ebola hemorrhagic fever: A study of risk factors in family members, Kikwit, Democratic Republic of the Congo, 1995. Commission de Lutte contre les Épidémies à Kikwit. J Infect Dis 1999;179 Suppl 1:S87-91.
27. Gallo JM, Desnoyer S, Roos JP, Roos JD, Le Guenno B, Heymann DL, et al. The reemergence of Ebola hemorrhagic fever, Democratic Republic of the Congo, 1995. Commission de Lutte contre les Épidémies à Kikwit. J Infect Dis 1999;179 Suppl 1:S87-91.
28. Ebola haemorrhagic fever in Sudan, 1976. Report of a WHO/International study team. Bull World Health Organ 1978;56:247-70.
29. Ebola haemorrhagic fever in Zaire, 1976. Bull World Health Organ 1978;56:271-93.
30. Geisbert TW, Lee AC, Robbins M, Geisbert JB, Honko AN, Sood V, et al. Postexposure protection of non-human primates against a lethal Ebola virus challenge with RNA interference: A proof-of-concept study. Lancet 2010;375:1896-905.
31. Georges-Courbot MC, Sanchez A, Lu CY, Baize S, Leroy E, Lansou-Soukate J, et al. Isolation and phylogenetic characterization of Ebola viruses causing different outbreaks in Gabon. Emerg Infect Dis 1997;3:59-62.
32. Jaax NK, Davis KJ, Geisbert TJ, Vogel P, Jaax GP, Topper M,
et al. Lethal experimental infection of rhesus monkeys with Ebola-Zaire (Mayinga) virus by the oral and conjunctival route of exposure. Arch Pathol Lab Med 1996;120:140-55.

31. Ryabchikova EI, Kolesnikova LV, Luchko SV. An analysis of features of pathogenesis in two animal models of Ebola virus infection. J Infect Dis 1999;179 Suppl 1:5199-202.

32. Baskerville A, Fisher-Hoch SP, Neild GH, Dowsett AB. Ultrastructural pathology of experimental Ebola haemorrhagic fever virus infection. J Pathol 1985;147:199-209.

33. Geisbert TW, Young HA, Jahrling PB, Davis KJ, Kagan E, Hensley LE. Mechanisms underlying coagulation abnormalities in Ebola hemorrhagic fever: Overexpression of tissue factor in primate monocytes/macrophages is a key event. J Infect Dis 2003;188:1618-29.

34. Isaacs M. Viral hemorrhagic fever hazards for travelers in Africa. Clin Infect Dis 2001;33:1707-12.

35. Levi M. Disseminated intravascular coagulation. Crit Care Med 2007;35:2191-5.

36. Murphy FA. Pathogenesis of Ebola virus infection. In: Pattyn SR, editor. Ebola virus Haemorrhagic Fever. Amsterdam: Elsevier/ North-Holland; 1978. p. 43-59.

37. Reed DS, Hensley LE, Geisbert JB, Jahrling PB, Geisbert TW. Depletion of peripheral blood T lymphocytes and NK cells during the course of Ebola hemorrhagic Fever in cynomolgus macaques. Viral Immunol 2004;17:390-400.

38. Geisbert TW, Young HA, Jahrling PB, Davis KJ, Larsen T, Kagan E, et al. Pathogenesis of Ebola hemorrhagic fever in primate models: Evidence that hemorrhage is not a direct effect of virus-induced cytolyis of endothelial cells. Am J Pathol 2003;163:2371-82.

39. Geisbert TW, Jaax NK. Marburg hemorrhagic fever: Report of a case studied by immunohistochemistry and electron microscopy. Ultrastruct Pathol 1998;22:3-17.

40. Zadek SR, Goldsmith CS. Pathologic features of filovirus infections in humans. Curr Top Microbiol Immunol 1999;235:97-116.

41. Baize S, Leroy EM, Mavoungou E, Fisher-Hoch SP. Apoptosis in fatal Ebola infection. Does the virus toll the bell for immune system? Apoptosis 2000;5:5-7.

42. Baize S, Leroy EM, Georges-Courbot MC, Capron M, Lansoud-Soukate J, Debri P, et al. Defective humoral responses and extensive intravascular apoptosis are associated with fatal outcome in Ebola virus-infected patients. Nat Med 1999;5:423-6.

43. Bosio CM, Aman MJ, Grogan C, Hogan R, Ruthel G, Negley D, et al. Ebola and Marburg viruses replicate in monocyte-derived dendritic cells without inducing the production of cytokines and full maturation. J Infect Dis 2003;188:1630-8.

44. Sanchez A, Lukwiya M, Bausch D, Mahanty S, Sanchez AJ, Wagener KD, Rollin PE. Analysis of human peripheral blood samples from fatal and nonfatal cases of Ebola (Sudan) hemorrhagic fever: Cellular responses, virus load, and nitric oxide levels. J Virol 2004;78:10370-7.

45. Yaddanapudi K, Palacios G, Towner JS, Chen L, Sariol CA, Nichol ST, et al. Implication of a retrovirus-like glycoprotein peptide in the immunopathogenesis of Ebola and Marburg viruses. FASEB J 2006;20:2519-30.

46. Volchkov VE, Blinov VM, Netesov SV. The envelope glycoprotein of Ebola virus contains an immunosuppressive-like domain similar to oncogenic retroviruses. FEBS Lett 1992;305:181-4.

47. Chepurunov AA, Tuzova MN, Ternovoy VA, Chernukhin IV. Suppressive effect of Ebola virus on T cell proliferation in vitro is provided by a 125-kDa GP viral protein. Immunol Lett 1999;68:257-61.

48. Villinger F, Rollin PE, Brar SS, Chikakala NF, Winter J, Sundstrom JB, et al. Markedly elevated levels of interferon (IFN)-gamma, IFN-alpha, interleukin (IL)-2, IL-10, and tumor necrosis factor-alpha associated with fatal Ebola virus infection. J Infect Dis 1999;179 Suppl 1:5188-91.

49. Harcourt BH, Sanchez A, Offermann MK. Ebola virus selectively inhibits responses to interferons, but not to interleukin-1beta, in endothelial cells. J Virol 1999;73:1349-6.

50. Basler CF, Wang X, Mühleberger E, Volchkov V, Paragas J, Klenk HD, et al. The Ebola virus VP35 protein functions as a type I IFN antagonist. Proc Natl Acad Sci U S A 2000;97:12289-94.

51. Basler CF, Mikulasova A, Martinez-Sobrindo L, Paragas J, Mühleberger E, Bray M, et al. The Ebola virus VP35 protein inhibits activation of interferon regulatory factor 3. J Virol 2007;77:7945-56.

52. Reid SP, Leung LW, Hartman AL, Martinez O, Shaw ML, Carboneille C, et al. Ebola virus VP24 binds karyopherin alpha and blocks STAT1 nuclear accumulation. J Virol 2006;80:5156-67.

53. Okware SI, Omaswa FG, Zaramba S, Opio A, Lutwama JJ, Kamugisha J, et al. An outbreak of Ebola in Uganda. Trop Med Int Health 2002;7:1068-75.

54. Eichner M, Dowell SF, Fierce N. Incubation period of ebola hemorrhagic virus subtype zaire. Osong Public Health Res Perspect 2011;2:3-7.

55. Pourrut X, Kumulungui B, Wittmann T, Moussaouv G, Delicat A, Yaba P, et al. The natural history of Ebola virus in Africa. Microbes Infect 2005;7:1005-14.

56. Olival KJ, Islam A, Yu M, Anthony SJ, Epstein JH, Khan SA, et al. Ebola virus antibodies in fruit bats, bangladesh. Emerg Infect Dis. 2013;19:270-3.

57. Pourrut X, Sours M, Towner JS, Rollin PE, Nichol ST, Gonzalez JP, et al. Large serological survey showing cocirculation of Ebola and Marburg viruses in Gabonese bat populations, and a high seroprevalence of both viruses in Rousettus aegyptiacus. BMC Infect Dis 2009;9:159.

58. Hayman DT, Emmerich P, Yu M, Wang LF, Suu Ire R, Fooks AR, et al. Long-term survival of an urban fruit bat seropositive for Ebola and Lagos bat viruses. PLoS One 2010;5:e107198.

59. Hayman DT, Yu M, Crameri G, Wang LF, Suu-Ire R, Wood JL, et al. Ebola virus antibodies in fruit bats, Ghana, West Africa. Emerg Infect Dis 2012;18:1207-9.

60. Smith CE, Simpson DJ, Bowen ET, Zlotnik I. Fatal human disease in vervet monkeys. Lancet 1967;2:1119-21.

61. Negroso A, Palacios G, Vázquez-Morón S, González F, Doppo H, Molero F, et al. Discovery of an ebolavirus-like filovirus in Europe. PLoS Pathog 2011;7:e1002304.

62. Kuhn JH, Bao Y, Bavar S, Becker S, Bradfute S, Brauburger K, et al. Virus nomenclature below the species level: A standardized nomenclature for filovirus strains and variants rescued from cDNA. Arch Virol 2014;169:1229-37.

63. Formenty P, Boesch C, Myers M, Steiner C, Donati F, Dind F, et al. Ebola virus outbreak among wild chimpanzees living in a rain forest of Côte d’Ivoire. J Infect Dis 1999;179 Suppl 1:5120-6.

64. Johnson BK, Wambui C, Ochicho D, Gichogo A, Oogo S, Libondo D, et al. Seasonal variation in antibodies against Ebola virus in Kenyan fever patients. Lancet 1986;1:1160.

65. Pinzon JE, Wilson JM, Tucker CJ, Arthur R, Jahrling PB, Formenty P. Trigger events: Enviroclimatic coupling of Ebola hemorrhagic fever outbreaks. Am J Trop Med Hyg 2004;71:664-7.

66. Gautier-Hion A, Michaloud G. Are figs always keystone resources for tropical frugivorous vertebrates? A test in Gabon. Ecology 1989;70:1826-33.

67. Pattyn SR. The epidemic of hemorrhagic fever in Zaire (August-November 1976) and its implications. Verh K Acad Geneeskd Belg 1983;45:201-25.

68. Pattyn, SR. Ebola virus haemorrhagic fever. Amsterdam, North-Holland: Elsevier; 1978.
69. Peters CJ, LeDuc JW. Ebola: The virus and the disease. J Infect Dis 1999;179 Suppl 1:S1-288.

70. Feldmann H, Geisbert T, Kawaoka Y. Filoviruses: Recent advances and future challenges. J Infect Dis 2007;196 Suppl 2:S129-30.

71. Baron RC, McCormick JB, Zubeir OA. Ebola virus disease in Southern Sudan: Hospital dissemination and intrafamilial spread. Bull World Health Organ 1983;61:997-1003.

72. Carod-Artal FJ. Illness due the Ebola virus: Epidemiology and clinical manifestations within the context of an international public health emergency. Rev Neurol 2015;60:267-77.

73. Report of an International Commission. Ebola haemorrhagic fever in Zaire, 1976. Bull World Health Organ 1978;56:271-93.

74. Bwaka MA, Bonnet MJ, Calain P, Colebunders R, De Roo A, Guimard Y, et al. Ebola hemorrhagic fever in Kikwit, Democratic Republic of the Congo: Clinical observations in 103 patients. J Infect Dis 1999;179 Suppl 1:S1-7.

75. MacNeil A, Farnon EC, Wamala J, Okware S, Cannon DL, Reed Z, et al. Proportion of deaths and clinical features in Bundibugyo Ebola virus infection, Uganda. Emerg Infect Dis 2010;16:1969-72.

76. Roddy P, Howard N, Van Kerkhove MD, Lutwama J, Wamala J, Yoti Z, et al. Clinical manifestations and case management of Ebola haemorrhagic fever caused by a newly identified virus strain, Bundibugyo, Uganda, 2007-2008. PLoS One 2012;7:e52986.

77. Hensley LE, Stevens EL, Yan SB, Geisbert JB, Macias WL, Larsen T, et al. Recombinant human activated protein C for the postexposure treatment of Ebola hemorrhagic fever. J Infect Dis 2007;196 Suppl 2:S390-9.

78. Fhogartaigh CN, Aarons E. Viral haemorrhagic fever. Clin Med (Lond) 2015;15:61-6.

79. Barry M, Traoré FA, Sako FB, Kpamy DO, Bah EI, Poncin M, et al. Ebola hemorrhagic fever in Kikwit, Democratic Republic of the Congo: Clinical observations in 103 patients. J Infect Dis 1999;179 Suppl 1:S177-87.

80. Ksiazek TG, Rollin PE, Williams AJ, Bressler DS, Martin ML, et al. Report of an International Commission. Ebola haemorrhagic fever caused by a newly identified virus strain, Bundibugyo, Uganda, 2007-2008. PLoS One 2012;7:e52986.

81. Kreuels B, Wichmann D, Emmerich P, Schmidt-Chanasit J, de Jong JWM, et al. Illness due to the Ebola virus: Epidemiology and clinical manifestations within the context of an international public health emergency. Rev Neurol 2015;60:267-77.

82. Baron RC, McCormick JB, Zubeir OA. Ebola virus. J Infect Dis 1999;179 Suppl 1:S1-7.

83. Liang H, Zhou Z, Zhang S, Zen K, Chen X, Zhang C. Identification of Ebola virus microRNAs and their putative pathological function. Sci China Life Sci 2014;57:973-81.

84. Clark DV, Jahrling PB, Lawler JV. Clinical management of filovirus-infected patients. Viruses 2012;4:1686-88.

85. Geisbert TW, Hensley LE, Jahrling PB, Larsen T, Geisbert JB, Paragas J, et al. Treatment of Ebola virus infection with a recombinant inhibitor of factor VIIa/tissue factor: A study in rhesus monkeys. Lancet 2003;362:1953-8.

86. Feldmann H, Jones SM, Daddario-Dicaprio KM, Geisbert JB, Striöher U, Grolla A, et al. Effective post-exposure treatment of Ebola infection. PLoS Pathog 2007;3:e2.

87. Huggins JW. Prospects for treatment of viral hemorrhagic fevers with ribavirin, a broad-spectrum antiviral drug. Rev Infect Dis 1989;11 Suppl 4:S750-61.

88. Ignatyev G, Steinkasserer A, Streltsova M, Atrasheuskaya A, Agafonov A, Lubitz W. Experimental study on the possibility of treatment of some hemorrhagic fevers. J Biotechnol 2000;83:67-76.

89. Geisbert TW, Hensley LE, Kagan E, Yu EZ, Geisbert JB, Daddario-Dicaprio K, et al. Postexposure protection of guinea pigs against a lethal Ebola virus challenge is conferred by RNA interference. J Infect Dis 2006;193:1650-7.

90. Warfield KL, Swenson DL, Olinger GG, Nichols DK, Pratt WD, Blrouch R, et al. Gene-specific countermeasures against Ebola virus based on antisense phosphorodiamidate morpholino oligomers. PLoS Pathog 2006;2:e1.

91. Warren TK, Wells J, Panchal RG, Stuthman KS, Garza NL, Van Tongeren SA, et al. Protection against filovirus diseases by a novel broad-spectrum nucleoside analogue BCX4430. Nature 2014;508:402-5.

92. Furuta Y, Gowen BB, Takahashi K, Shiraki K, Smee DF, Barnard DL. Favipiravir (T-705), a novel viral RNA polymerase inhibitor. Antiviral Res 2013;100:446-54.

93. Zhang Y, Li D, Jin X, Huang Z. Fighting Ebola with ZMapp: Spotlight on plant-made antibody. Sci China Life Sci 2014;57:987-8.

94. Ahmad A, Husain A, Mujeeb M, Khan NA, Najmi AK, Siddique NA, et al. A review on therapeutic potential of Nigella sativa: A miracle herb. Asian Pac J Trop Biomed 2013;3:337-52.

95. Alemi M, Sabouni F, Sanjarian F, Hagheen K, Ansari S. Anti-inflammatory effect of seeds and callus of Nigella sativa L. extracts on mix glial cells with regard to their thromboxone content. AAPS PharmSciTech 2013;14:160-7.

96. Shuid AN, Mohamed N, Mohamed IN, Othman F, Suhaimi F, Mohd Ramli ES, et al. Nigella sativa: A potential antiosteoporotic agent. Evid Based Complement Alternat Med 2012;2012:696230.

97. El Sayed, Mohsen A, Mohamed IN, Othman F, Suhaimi F, Mohd Ramli ES, et al. Nigella sativa: A potential antosteoporotic agent. Evid Based Complement Alternat Med 2012;2012:696230.

98. El Mezayen R, El Gazzar M, Nicolls MR, Marecki JC, Dreskin SC, et al. Anti-inflammatory effect of seeds and callus of Nigella sativa L. extracts on mix glial cells with regard to their thromboxone content. AAPS PharmSciTech 2013;14:160-7.

99. Majdalawieh AF, Hmaidan R, Carr RI. Nigella sativa modulates splenocyte proliferation, Th1/Th2 cytokine profile, macrophage function and NK anti-tumor activity. J Ethnopharmacol 2011;131:268-75.

100. Yildiz F, Coban S, Terzi A, Ates M, Aksoy N, Cakir H, et al. Nigella sativa relieves the deleterious effects of ischemia reperfusion injury on liver. World J Gastroenterol 2008;14:5204-9.