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Ebola virus disease: past, present and future

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ABSTRACT

Ebola virus disease is one of the most deadly ailments known to mankind due to its high mortality rate (up to 90%) accompanying with the disease. Ebola haemorrhagic fever (EHF) is an infectious disease of animal that can be transmitted to both human and non-human primates. The first epidemic of EHF occurred in 1976 in the Democratic Republic of the Congo. The incubation period of ebola is less than 21 days. Ebola virus infections are depicted by immune suppression and a systemic inflammatory response that leads to damage of the vascular, coagulation and immune systems, causing multi-organ failure and shock. Five genetically distinct members of the *Filoviridae* family responsible for EHF are as follows: *Zaire ebolavirus*, *Sudan ebolavirus*, *Côte d'Ivoire ebolavirus*, *Bundibugyo ebolavirus* and *Reston ebolavirus*. The ongoing 2014 West Africa ebola epidemic has been considered as the most serious panic in the medical field with respect to both the number of human cases and death toll. The natural host for ebola virus is unknown, thus it is not possible to carry out programs to regulate or abolish virus from transmission to people. The ebola virus infection provides little chance to develop acquired immunity causing rapid progression of the disease. It is pertinent to mention that at present, there is no antiviral therapy or vaccine that is helpful against ebola virus infection in humans. The impediment of EHF necessitates much better understanding of the epidemiology of the disease, particularly the role of wildlife, as well as bats, in the spread of ebola virus to humans.

1. Introduction

The new fatal diseases are being continuously reported in the past decade[1]. Ebola virus diseases (EVDs) have always been a challenge and a global menace since its discovery in 1976 by Dr. Peter Piotin in Zaire, Africa (now Democratic Republic of Congo) from the blood of a catholic nun who suspected of having yellow fever[2]. Ebola haemorrhagic fever (EHF) is a zoonotic disease transmitted accidentally by direct contact with infected live or dead animals. EHF is an acute viral syndrome with fever and subsequent bleeding diathesis marked by high mortality in human and nonhuman primates (monkeys, gorillas and chimpanzees). Ebola virus is a violent pathogen, a lipid-enveloped negatively stranded RNA virus that belongs to the viral family *Filoviridae*[3]. Exhaustive investigation on EHF in the equatorial region of the Democratic Republic of Congo between 1981 and 1985 pointed out that EHF episodically come out from nature to infected humans[4]. EHF is caused by any of five genetically different members of the *Filoviridae* family: *Zaire ebolavirus* (ZEBOV), *Sudan ebolavirus* (SEBOV), *Côte d'Ivoire ebolavirus*, *Bundibugyo ebolavirus* (BDBV) and *Reston ebolavirus* (REBOV). *Côte d'Ivoire ebolavirus* has been accompanied with only one human case[5]. REBOV has only caused disease in non-human primates (NHP) and was found in swine suffering from porcine reproductive and respiratory disease

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syndrome[6]. Zaire, Sudan and Bundibugyo Ebola viruses are accountable for most of the EHF epidemic but ZEBOV establish a particularly serious threat to both human and NHPs in sub-Saharan Africa. EHF has been associated with large human outbreaks, with case fatality rates for ZEBOV as high as 90%[7-9]. The 2014 West Africa ebola outbreak is an ongoing epidemic of the EVD in West Africa. The outbreak began in the republic of Guinea in February of 2014. Since its initial outbreaks, the virus has already spread to the republic of Liberia and the Sierra Leone. The 2014 West Africa Ebola outbreak is believed to be the most terrible in medical history with regards to both the number of human cases and fatalities[10-13]. Presently, there are no approved antiviral drugs or vaccines against filoviruses. The prevention of EHF requires more awareness of the pathology of the ailment, especially the role of wildlife, especially bats, in the spread of Ebola virus to humans. The present review is an attempt to summarize various essential aspects of EVD or EHF.

2. Virology

The EVD, previously known as EHF is a severe condition caused by a virus belonging to genus *Ebolavirus*, family *Filoviridae* and order Mononegavirales. The family *Filoviridae* comprises of one genus, Filovirus, which contains two species, morphologically identical but serologically distinct: Marburg virus and Ebola virus. There are five Ebola subtypes BDBV, ZEBOV, REBOV, SEBOV and *Taï Forest ebolavirus* (TAFV) which vary in pathogenicity, antigenicity and genomic constitution[16]. BDBV, ZEBOV and SEBOV have been accompanied with large EVD epidemic near the tropical rain forests of Central and West African distant villages; among these three ZEBOV are responsible for high mortality rates in humans. REBOV and TAFV were not accustomed for illness or mortality in human. The gene products of ebola and Marburg viruses exhibit a noteworthy degree of similarity and in some areas extensive identity, but are encoded in contradictory nucleotide sequences[17,18](Figure 1).

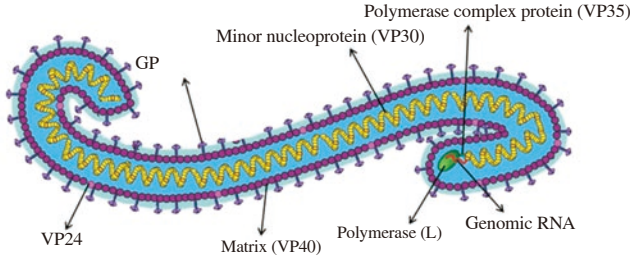

Figure 1. Schematic representation of the ebola virus[14,15,33].
VP: virion protein; GP: glycoprotein.

3. Ecology

Tropical rain forests in Africa provide a general ecosystem for ebola virus emergence, presenting a rich animal biodiversity and outbreak appears to be periodical. EVD is a conventional zoonotic disease. The evidences indicate that fruit bats are the reservoir for both ebola and Marburg virus. The first indication for the manifestation of Zaire ebola virus in naturally infected fruit bats was recorded by recognition of viral RNA and antibodies in three tree-roosting species: *Hypsignathus monstrosus, Epomops franqueti, Myonycteris torquata* and therefore have been associated as the potential fall over source for ebola virus[17]. Zaire ebola virus has not been well isolated from naturally infected animals. The identification and successful isolation of Marburg virus from the cave-dwelling fruit bat *Rousettus aegyptiacus* provide support to the idea that bats are a reservoir species for filoviruses[19]. Ebola virus might endure as an asymptomatic or subclinical infection in the reservoir species, with little or no transmission and might be intermittently provoked through a suitable stimulus. The stimulus might be stress, co-infection and change in food sources and pregnancy as displayed *in vivo* and *in vitro* investigations[20,21]. This hypothesis describes the infrequent nature and periodicity of EHF in Africa. Mammalian species including NHP vulnerable to infection are considered as the dead end hosts. The probabilities of seasonal outcome on introduction of ebola virus infection have also been proposed[22,23]. The future studies require consideration of the level of infections of ebola viruses in fruit or insectivorous bats in areas prevalent for these viruses. Issues such as virus pathology and perseverance in bats, conceivable activation process of insistent virus and possible transmission routes required to be monitored by field and experimental investigations. Other possible reservoir species and a role for potential augmenting hosts, particularly after the detection of Reston ebola virus in pigs in the Philippines should also be investigated[24].

4. Epidemiology

The first instance of filovirus haemorrhagic fever was reported in 1967 in Germany and the former Yugoslavia and the contributing agent was recognized as Marburg virus[25]. In 1976, epidemic of haemorrhagic fever was reported in two adjacent areas: first in Southern Sudan and consequently in Northern Zaire. The outbreak commenced with malaria like symptoms and transmitted due to application of unsterilized needles in the clinics. An uncertain causative agent was quarantined from patients in both epidemics and named ebola virus after a small ebola river in northwestern Democratic Republic of the Congo. These two outbreaks were caused by two distinct species of ebola virus, Sudan ebola virus (SEBOV) and Zaire ebola virus (ZEBOV)[26-28]. The third African ebola virus species, Tai forest ebola virus (earlier known as Ivory Coast or Cote d'Ivoire) was reported in 1994. The virus was isolated from a diseased ethnologist who had worked in the Tai forest reserve in Cote d'Ivoire and had performed a necropsy on a chimpanzee that had resided with a crowd where numerous members had expired due to EHF[29]. The recent breakthrough is the Bundibugyo ebola virus, existing in equatorial region of Africa[9]. Another ebola virus species, Reston ebola virus (discovered in Reston, Virginia, USA, in November 1989) has been recognized as non-pathogenic for humans[30]. Numerous outbreaks due to several species of ebola virus have been reported in diverse parts of Africa. Historically, EVD has occurred in areas around the rain forests of Central Africa.

Conversely, recent epidemics are now basically in distant villages of Central and Western Africa. The transmission of EVD to adjacent region is a crucial alarm. In July 2014, World Health Organization (WHO) delegates organized a crisis summit in Ghana to harmonize a more competent response to the ebola epidemic in West Africa. The Centers for Disease Control (CDC), WHO, the American Nurses Association, and many other national or international associations have supplied the most recent knowledge about EVD to clinicians, students, and travelers. EVD outbreaks have a fatality rate of 60% to 90%, depending upon time of diagnosis, accessibility of sympathetic medication, and EVD subtype[31].

5. Pathophysiology

A virus is an infectious, intracellular parasite. The genetic constitution of a virus is either DNA or RNA. A filovirus is a filamentous, enveloped particle with single stranded, non-segmented RNA molecule with diameter of 80 nm and varying length, which may be up to 1 400 nm, emerged as bacilliform particles that encode seven genes: nucleoprotein, VP35, VP40, GP, VP30, VP24, RNA-dependent RNA polymerase (L). The genomes of the five distinct ebolaviruses (BDBV, ZEBOV, REBOV, SEBOV and TAFV) vary in sequence and the number and location of gene overlaps. Amongst these, nucleoprotein, VP35, VP30 and RNA-dependent RNA polymerase are associated with viral replication and transactivation. VP40 is the matrix protein and involves in budding and delivery of viral particle, although VP24 is the minor matrix protein and accompanied with nucleocapsid formation. Both the matrix proteins, VP40 and VP24 are identified to obstruct interferon signaling. The only surface protein controlled by the filovirus is the GP, present as trimeric spikes comprising of GP1 and GP2. The non-structural soluble form of GP, sGP, a unique product of ZEBOV GP gene, gets secreted from infected cells[32]. EVD adhere to 6 phases of viral replication: attachment, penetration, uncoating, replication and expression, maturation and release/delivery of virus.

6. Pathogenesis and transmission

The wild animals like primates (chimpanzees, gorillas, baboons, duikers and African green monkeys) and fruit bats (*Hypsignathus monstrosus, Epomops franqueti, Myonycteris torquata* and *Pteropodidae*) are the natural hosts for the EVD and are responsible for transmission of ebola virus from animals to humans[34]. The animal-to-human spread happen when humans come into contact with tissues and bodily fluids of infected animals, particularly with infected nonhuman primates[18]. The natural reservoir for ebola has yet to be confirmed; however, bats are being recognized to be the most likely species and transmit the virus without getting ill. Plants, arthropods and birds have also been regarded as possible viral reservoirs[35]. Traces of ZEBOV were found in the carcasses of gorillas and chimpanzees during outbreaks in 2001 and 2003, which later became the source of human infections. Conversely, the high mortality rate in these species causing from ZEBOV infection accomplish unlikely; these species are a natural reservoir for the virus[36].

It is pertinent to mention that human-to-human spread of the ebola virus is basically accompanied with direct contact with the blood or numerous body fluids (saliva, mucus, vomit, feces, sweat, tears, breast milk, urine and semen) of a person who has developed warning sign of the ailment and indirect contact with environment contaminated with infected body fluids. The threat of ebola spread increases when there has been an interaction with a patient in the later stages of disease[37,38]. In the first ebola outbreak in the Democratic Republic of the Congo, in Yambuku, the reuse of unsterilized needles and syringes was an imperative factor in the transmission of the disease. In the Kikwit outbreak, several clinic employees got infected because of improper barrier measures[39,40]. The preliminary replication happens in monocytes, macrophage and dendritic cells and via these cells distribution of virus occurs to lymph nodes, spleen, liver and other organs. An evident increase of interleukin-2, interleukin-10, tumour necrosis factor, interferon-alpha and gamma were observed in fatal EHF cases. The virus is capable to persist on objects for a several hours in a dried state and can persist for a few days within body fluids[41,42]. More noteworthy effects are microvascular damage, changes in vascular permeability and activation of the clotting cascade. The impairment to platelets and endothelial cells cause disruption of fluid balance and homeostasis. In addition, the virus is considered to deal and hide immunological function[43]. The virus has been found in semen for up to 7 weeks after recovery from the illness, indicating the probability of sexual mode of transmission. Ebola virus infection may also spread through breast milk of women after recovery and it is unknown when it is prudent to breast feed again. Otherwise, person who recovered are not infectious[44]. Aerosol transmission has been put forwarded among monkeys infected with the Reston and Zaire subtypes of ebola virus. Ebola virus has also been recognized in alveoli of experimentally infected monkeys[45]. The spread array during epidemic in humans does not advocate respiratory route of transmission. No incidence proposes the role of insects in transmission of EVD[46,47]. Dead bodies remain infectious; thus, people handling human remains in procedure such as traditional burial rituals or more modern processes such as embalming are at threat[48](Figure 2).

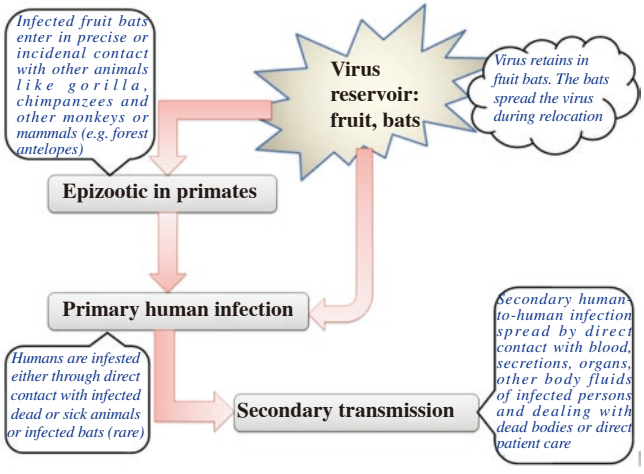

Figure 2. Modes of transmission of ebola virus infection[49].

7. Clinical manifestations

The harshness of different species has been found to be of variable grade. ZEBOV is the most severe with casualty rate approaching 90%, while Sudan ZEBOV (SEBOV) have 53%-66% mortality rate[50,51]. The disease has an incubation period of 2-21 days (average 4-10 days) followed by its symptoms like fever followed by headache, fatigue, dysphagia or odynophagia, abdominal pain, myalgia, sore throat, cough, anorexia, nausea, vomiting and diarrhea[44]. A conjunctival infection is often an early clinical sign and sometimes patients may also have hiccoughs, tachypnoea and bleeding and shock. Dermatological indications include a typical maculopapular rash on trunk, but this is more frequently observed on white people than darker skin people. Patients may also acquire neurological warning signs, namely, convulsions, delirium and coma. Patients normally die 6-9 weeks after the first indications but differences in death rates have been detected between epidemic and during an epidemic. In surviving patients, improvement may be gradual and is frequently illustrated by fatigue and arthralgia[45]. From these clinical indications, it is clear that EHF may mimic several other tropical ailments like malaria, typhoid fever or yellow fever at the start of the disease. In most outbreaks, identification of the disease is slow because physicians are not familiar to this new illness and the symptoms are generally non-specific (Figure 3).

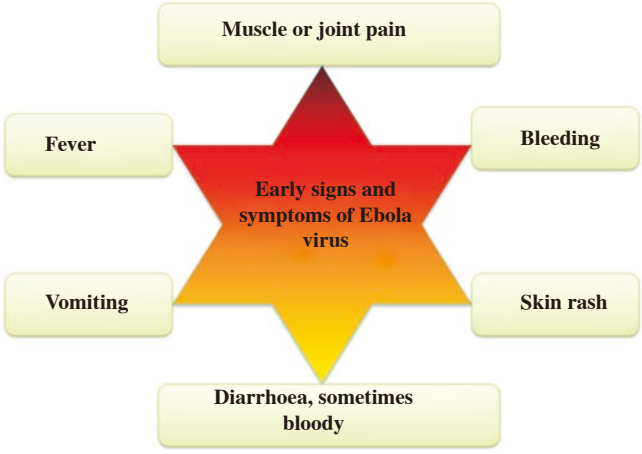

Figure 3. Early signs and symptoms of ebola virus[49].

8. Diagnosis

The diagnosis of EVD is very difficult as it needs a careful, complete history as well as a full examination. The acute febrile illness is the general appearance in the early part of the disease. In endemic countries, various parameters like travel to jungle, eating or hunting of bats or animals, contact to cave, close exposure with ill persons or dead bodies help in settling the apprehension for ebola virus infection. In non-endemic countries, patient with history of travel to disease prevalent countries or outbreak affected areas, if detecting acute febrile illness, should be assumed for ebola virus infection and diagnosed accordingly. The direct detection of the ebola virus can be performed using immunofluorescent methods, ELISA, immunohistochemistry and PCR. As enormous amount of ebola virus are found in dermal tissues, skin biopsies are considered for post-mortem confirmation of the ebola infection for investigations. Earlier to this serological testing of ebola virus infection was carried out using indirect fluorescent antibody method although this test has difficulty of unambiguity and sensitivity. An IgM capture ELISA assay is further helpful in the detection of acute infections and a direct IgG ELISA assay should substitute the indirect fluorescent antibody for seroprevalence evaluations[52-57]. In 1995, Dr. Sherif Zaki of the CDC established a colorimetric assay for identification of ebola virus in formalin-preserved skin biopsies from fatal cases of alleged EHF infection. Some supplementary tests, namely, complete blood count, metabolic panels, liver enzymes and coagulation studies are also helpful in the diagnosis[58-60]. Among the other serological tests, western blot and indirect immunofluorescence tests can also be employed for verification and examination, respectively. Sophisticated diagnostic tests like multiplex PCR and micro-array-based assay have also been developed on the basis of the common clinical disorder[46].

9. Treatment

There is no precise remedy for EHF. The management of patients with ebola virus infection was a main threat as there was no successful antiviral drug and no specific vaccine was available. The treatment is mainly based on supportive and symptomatic remedy focusing on supplying proper hydration and nutritional support with antibiotics, control of organ failure, and antimalarial drugs if required. Another essential measure includes avoidance of disease spread by severe quarantine of infected person and using impediment nursing operations. Personal protective instruments and surface cleaning, following the safety procedure suggested by CDC directions should be employed in the case where risk of infection is from dead bodies[61]. In a clinical investigation performed late in the 1995 ebola epidemic in Kikwit, blood transfusions of improving patients were administered to eight ebola patients for passive immunization and seven of them stayed alive[62]. Such type of investigations were not repeated in further outbreaks as *in vitro* assay indicated that antibodies against ebola had no neutralising action. Furthermore, monoclonal antibodies to the GP of ebola virus exhibited defensive and healing properties in mice but they were unable to protect NHP[63,64]. The animal studies suggest that ebola specific immunoglobulin of equine origin has little activity in hiding viraemia and slowing disease onset in NHP. Goat immunoglobulins were evaluated in pre-clinical test on laboratory animals and were administered to scientist assumed of gaining infection with EHF during their investigational work. It was suggested that these immunoglobulins might be beneficial for the emergency cure of persons inadvertently infected with EHF[65]. The ebola virus reproduction was shown to be hindered *in vitro* by a series of nine nucleoside analogue inhibitors of S-adenosylhomocysteine hydrolase

and carbocyclic 3-deazaadenosine was shown to avert death in mice infected with the ebola virus[66]. Various clinical aids like injections, catheters and parenteral interventions *etc* should be reduced to avert trauma and the increased challenge of disease spread. Several drugs specially aspirin, nonsteroidal anti-inflammatory drugs, anticoagulant therapies, and steroids should be contraindicated[67].

The recommendations of WHO for treatment at home have been comparatively effective if specialized care is not available[68]. The research work is going on to discover a drug for effective cure of patients infected with EVD. Currently, the main classes of drugs being evaluated for their potential against ebola infection include RNA inhibitor based (TKM-Ebola) agents, monoclonal antibodies (ZMapp), nucleoside analogs, positively charged phosphorodiamidate morpholino oligomers and antisense-based (AVI-7537) drugs. Favipiravir (T-705) a pyrazine carboxamide derivative, is another hopeful candidate, acting by prevention of viral replication. ZMapp is one of the most encouraging agents act by targeting the expression phase of viral replication. It comprises of 3 monoclonal antibodies created in tobacco plants. It binds to the protein of the ebola virus to inhibit replication of the virus once introduced into the host[69]. Another promising drug, BCX4430 possessing antiviral activity for marburg, ebola and yellow fever is also being tested for its capability to target an enzyme found in these specific viruses. BCX4430 has been found effective in small animals if treatment is initiated within 48 h after infection[70]. The off-label usages of angiotensin-converting enzyme inhibitors, angiotensin receptor blockers and statins have been advised for cure due to their ability to encourage immunity in the infected person. Ebola virus is normally considered as a conceivable biological weapon, thus there is a crucial need to develop effective antiviral drugs and vaccines.

10. Conclusion

Ebola virus has been a threat to human health due to dangerous, highly lethal and infectious behavior since its discovery in 1976. Ebola fever has come out as one of the most fatal identified forms of hemorrhagic fever, for which there is no specific remedy available. The spread among humans occurs mainly through the exchange of blood and body secretions. Other noticeable forms of transmission include hospital acquired infection and inadequate hygiene practices. There is an urgent requirement of dissemination of information to community and training programmes for doctors, nurses and other hospital staff.

The future endeavors require the emphasis on the understanding of the differences among species of ebola virus. There is an urgent demand for more field studies into the ecology of reservoir species and shedding procedures. The discovery of novel targets for intervention tactics requires more exhaustive research into the pathophysiology of ebola virus infections with laboratory animals. The best method to lower the cases and epidemic is to prevent the spread of the disease. The awareness programmers should be organized on large scale to develop the attentiveness about disease for its eradication. The research should also essentially be focused on establishment of rapid and simple diagnostic kits for ebola infection. It is anticipated that outcome of research investigations would result in development of easily available and affordable drug for the treatment of ebloa virus. A great effort with clear strategy is needed for transforming the potential drugs and vaccines from lab to clinical trials and ultimately for treatment of patients with ebola infection.

Conflict of interest statement

We declare that we have no conflict of interest.

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References

[1] Wiwanitkit V. Unprecedented scale ebola epidemic in Guinea: what we should know. *Asian Pac J Trop Biomed* 2014; **4**(2): 675.

[2] Centers for Disease Control and Prevention. Ebola in Liberia. Atlanta: Centers for Disease Control and Prevention; 2015. [Online] Available from: http://wwwnc.cdc.gov/travel/notices/warning/ebola-liberia [Accessed on 4th February, 2015]

[3] World Health Organization. WHO guidelines for epidemic preparedness and response to measles outbreaks. Geneva: World Health Organization; 1999. [Online] Available from: http://www.who.int/csr/resources/publications/measles/whocdscsrisr991.pdf [Accessed on 4th February, 2015]

[4] Jezek Z, Szczeniowski MY, Muyembe-Tamfum JJ, McCormick JB, Heymann DL. Ebola between outbreaks: intensified Ebola hemorrhagic fever surveillance in the Democratic Republic of the Congo, 1981-1985. *J Infect Dis* 1999; **179**: S60-4.

[5] Le Guenno B, Formenty P, Wyers M, Gounon P, Walker F, Boesch C. Isolation and partial characterisation of a new strain of Ebola virus. *Lancet* 1995; **345**(8960): 1271-4.

[6] 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**(5937): 204-6.

[7] Feldmann H, Geisbert TW, Jahrling PB, Klenk HD, Netesov SV, Peters CJ, et al. Filoviridae. In: Fauquet C, Mayo MA, Maniloff M, Desselberger U, Ball LA, editors. *Virus taxonomy: VIIIth report of the international committee on taxonomy of viruses*. London: Elsevier/

Academic Press; 2004, p. 645-53.

[8] Groseth A, Feldmann H, Strong JE. The ecology of Ebola virus. *Trends Microbiol* 2007; **15**(9): 408-16.

[9] Towner JS, Sealy TK, Khristova ML, Albariño CG, Conlan S, Reeder SA, et al. Newly discovered ebola virus associated with hemorrhagic fever outbreak in Uganda. *PLoS Pathog* 2008; **4**(11): e1000212.

[10] Bausch DG, Schwarz L. Outbreak of ebola virus disease in Guinea: where ecology meets economy. *PLoS Negl Trop Dis* 2014; **8**(7): e3056.

[11] Enserink M. Infectious diseases. ebola drugs still stuck in lab. *Science* 2014; **345**(6195): 364-5.

[12] Green A. Ebola emergency meeting establishes new control centre. *Lancet* 2014; **384**(9938): 118.

[13] Ansumana R, Bonwitt J, Stenger DA, Jacobsen KH. Ebola in Sierra Leone: a call for action. *Lancet* 2014; **384**(9940): 303.

[14] Centers for Disease Control and Prevention. Ebola (Ebola virus disease). Atlanta: Centers for Disease Control and Prevention; 2014. [Online] Available from: http://www.cdc.gov/vhf/ebola/ [Accessed on 4th February, 2015]

[15] HNGN news for a mobile generation. Ebola virus outbreak 2014: tobacco drug could be possible through genetic engineering-like process. US: HNGN news for a mobile generation; 2015. [Online] Available from: http://www.hngn.com/articles/38326/20140806/ebola-tobacco-drug-could-be-possible-through-genetic-engineering-like-process.htm. [Accessed on 4th February, 2015]

[16] Peters CJ, Sanchez A, Feldmann H, Rollin P, Nichol S, Kzaizek TG. Filoviruses as emerging pathogens. *Semin Virol* 1994; **5**: 147-54.

[17] Leroy EM, Kumulungui B, Pourrut X, Rouquet P, Hassanin A, Yaba P, et al. Fruit bats as reservoirs of Ebola virus. *Nature* 2005; **438**(7068): 575-6.

[18] Leroy EM, Rouquet P, Formenty P, Souquière S, Kilbourne A, Froment JM, et al. Multiple Ebola virus transmission events and rapid decline of central African wildlife. *Science* 2004; **303**(5656): 387-90.

[19] Towner JS, Amman BR, Sealy TK, Carroll SAR, Comer JA, Kemp A, et al. Isolation of genetically diverse marburg viruses from egyptian fruit bats. *PLoS Pathog* 2009; **5**(7): e1000536.

[20] Gupta M, Mahanty S, Greer P, Towner JS, Shieh WJ, Zaki SR, et al. Persistent infection with ebola virus under conditions of partial immunity. *J Virol* 2004; **78**(2): 958-67.

[21] Strong JE, Wong G, Jones SE, Grolla A, Theriault S, Kobinger GP, et al. Stimulation of ebola virus production from persistent infection through activation of the Ras/MAPK pathway. *Proc Natl Acad Sci U S A* 2008; **105**(46): 17982-7.

[22] Johnson BK, Wambui C, Ocheng D, Gichogo A, Oogo S, Libondo D, et al. Seasonal variation in antibodies against ebola virus in Kenyan fever patients. *Lancet* 1986; **1**(8490): 1160.

[23] Lahm SA, Kombila M, Swanepoel R, Barnes RF. Morbidity and mortality of wild animals in relation to outbreaks of ebola haemorrhagic fever in Gabon, 1994-2003. *Trans R Soc Trop Med Hyg* 2007; **101**: 64-78.

[24] 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**(5937): 204-6.

[25] Siegert R, Shu HL, Slenczka W, Peters D, Muller G. [On the etiology of an unknown human infection originating from monkeys]. *Dtsch Med Wochenschr* 1967; **92**(51): 2341-3. German.

[26] Report of a WHO/International Study Team. Ebola haemorrhagic fevers in Sudan, 1976. *Bull World Health Organ* 1978; **56**(2): 247-70.

[27] Report of an International Commission. Ebola haemorrhagic fever in Zaire, 1976. *Bull World Health Organ* 1978; **56**(2): 271-93.

[28] Cox NJ, McCormick JB, Johnson KM, Kiley MP. Evidence for two subtypes of Ebola virus based on oligonucleotide mapping of RNA. *J Infect Dis* 1983; **147**(2): 272-5.

[29] Le Guenno B, Formenty P, Wyers M, Gounon P, Walker F, Boesch C. Isolation and partial characterisation of a new strain of ebola virus. *Lancet* 1995; **345**(8960): 1271-4.

[30] Centers for Disease Control (CDC). Update: filovirus infections among persons with occupational exposure to nonhuman primates. *MMWR Morb Mortal Wkly Rep* 1990; **39**(16): 266-7; 273.

[31] World Health Organization. Ebola virus disease. Geneva: World Health Organization; 2014. [Online] Available from: http://www.who.int/mediacentre/factsheets/fs103/en/ [Accessed on 4th February, 2015]

[32] Sanchez A, Trappier SG, Mahy BW, Peters CJ, Nichol ST. The virion glycoproteins of ebola viruses are encoded in two reading frames and are expressed through transcriptional editing. *Proc Natl Acad Sci U S A* 1996; **93**(8): 3602-7.

[33] ASK Scientific. Ebola virus: life cycle and pathogenicity in humans. Cambridge: ASK Scientific; 2014. [Online] Available from: https://www.askscientific.com/ebola-virus-life-cycle-and-pathogenicity-in-humans/ [Accessed on 4th February, 2015]

[34] Groseth A, Feldmann H. Strong JE. The ecology of Ebola virus. *Trends Microbiol* 2007; **15**(9): 408-16.

[35] Centers for Disease Control and Prevention. Questions and answers about Ebola, pets and other animals. Atlanta: Centers for Disease Control and Prevention; 2015. [Online] Available from: http://www.cdc.gov/vhf/ebola/transmission/qas-pets.html [Accessed on 4th Feb, 2015]

[36] Pourrut X, Kumulungui B, Wittmann T, Moussavou G, Délicat A, Yaba P, et al. The natural history of ebola virus in Africa. *Microbes Infect* 2005; **7**: 1005-14.

[37] World Health Organization. West Africa - Ebola virus disease. Geneva: World Health Organization; 2014. [Online] Available from: http://www.who.int/ith/updates/20140421/en/ [Accessed on 4th February, 2015]

[38] Dowell SF, Mukunu R, Ksiazek 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. *J Infect Dis* 1999; **179**: S87-91.

[39] Khan AS, Tshioko FK, Heymann DL, Le Guenno B, Nabeth P, Kerstiëns B, et al. The reemergence of ebola hemorrhagic fever, Democratic Republic of the Congo, 1995. *J Infect Dis* 1999; **179**: S76-86.

[40] Muyembe-Tamfum JJ, Kipasa M, Kiyungu C, Colebunders R. Ebola outbreak in Kikwit, Democratic Republic of the Congo: discovery and control measures. *J Infect Dis* 1999; **179**: S259-62.

[41] Olejnik J, Ryabchikova E, Corley RB, Muhlberger E. Intracellular events

and cell fate in filovirus infection. *Viruses* 2011; **3**(8): 1501-31.

[42] 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 cytolysis of endothelial cells. *Am J Pathol* 2003; **163**(6): 2371-82.

[43] Casillas AM, Nyamathi AM, Sosa A, Wilder CL, Sands H. A current review of ebola virus: pathogenesis, clinical presentation, and diagnostic assessment. *Biol Res Nurs* 2003; **4**(4): 268-75.

[44] Feldmann H, Geisbert TW. Ebola haemorrhagic fever. *Lancet* 2011; **377**: 849-62.

[45] Colebunders R, Borchert M. Ebola haemorrhagic fever-a review. *J Infect* 2000; **40**(1): 16-20.

[46] Sanchez A, Giesbert TW, Feldmann H. Filoviridae: Marburg and Ebola viruses. In: Knipe DM, Howley PM, Griffin DE, Lamb RA, Martin MA, Roizman B, Straus SE, editors. *Fields virology*. 5th ed. Philadelphia: Lippincott Williams & Wilkins; 2007, p. 1409-48.

[47] Mahanty S, Bray M. Pathogenesis of filoviral haemorrhagic fevers. *Lancet Infect Dis* 2004; **4**(8): 487-98.

[48] Centers for Disease Control and Prevention. CDC Telebriefing on Ebola outbreak in West Africa. Atlanta: Centers for Disease Control and Prevention; 2014. [Online] Available from: http://www.cdc.gov/media/releases/2014/t0728- ebola.html [Accessed on 4th February, 2015]

[49] Caritas Internationalis. Response to ebola virus disease (EVD): guidelines for planning and provision of pastoral and social support services. Liberia: Caritas Internationalis; 2014. [Online] Available from: www.medbox.org/response-to-ebola-virus-disease-evd/download.pdf [Accessed on 4th February, 2015]

[50] Baron RC, McCormick JB, Zubeir OA. Ebola virus disease in southern Sudan: hospital dissemination and intrafamilial spread. *Bull World Health Organ* 1983; **61**(6): 997-1003.

[51] Towner JS, Rollin PE, Bausch DG, Sanchez A, Crary SM, Vincent M, et al. Rapid diagnosis of Ebola hemorrhagic fever by reverse transcription-PCR in an outbreak setting and assessment of patient viral load as a predictor of outcome. *J Virol* 2004; **78**(8): 4330-41.

[52] Guimard Y, Bwaka MA, Colebunders R, Calain P, Massamba M, De Roo A, et al. Organization of patient care during the ebola hemorrhagic fever epidemic in Kikwit, Democratic Republic of the Congo, 1995. *J Infect Dis* 1999; **179**: S268-73.

[53] Ksiazek TG, West CP, Rollin PE, Jahrling PB, Peters CJ. ELISA for the detection of antibodies to Ebola viruses. *J Infect Dis* 1999; **179**: S192-8.

[54] Zaki SR, Shieh W-J, Greer PW, Goldsmith CS, Ferebee T, Katshitshi J, et al. A novel immunohistochemical assay for the detection of ebola virus in skin: implications for diagnosis, spread, and surveillance of ebola hemorrhagic fever. *J Infect Dis* 1999; **179**: S36-47.

[55] van der Groen G, Johnson KM, Webb PA, Wulff H, Lange J. Results of ebola antibody surveys in various population groups. In: Pattyn SR, editor. *Ebola virus haemorrhagic fever*. Amsterdam: Elsevier/North-Holland Biomedical Press; 1978, p. 203-5.

[56] Tomori O, Bertolli J, Rollin PE, Fleerackers Y, Guimard Y, De Roo A, et al. Serologic survey among hospital and health center workers during the ebola hemorrhagic fever outbreak in Kikwit, Democratic Republic of the Congo, 1995. *J Infect Dis* 1999; **179**: S98-101.

[57] Busico KM, Marshall KL, Ksiazek TG, Roels TH, Fleerackers Y, Feldmann H, et al. Prevalence of IgG antibodies to Ebola virus in individuals during an ebola outbreak, Democratic Republic of the Congo, 1995. *J Infect Dis* 1999; **179**: S102-7.

[58] Centers for Disease Control and Prevention. Ebola virus disease information for clinicians in U.S. healthcare settings. Atlanta: Centers for Disease Control and Prevention; 2014. [Online] Available from: http://www.cdc.gov/vhf/ebola/hcp/clinician-information-us-healthcare-settings.html [Accessed on 4th February, 2015]

[59] Gatherer D. The 2014 Ebola virus disease outbreak in West Africa. *J Gen Virol* 2014; **95**(Pt 8): 1619-24.

[60] McElroy AK, Erickson BR, Flietstra TD, Rollin PE, Nichol ST, Towner JS, et al. Ebola hemorrhagic fever: novel biomarker correlates of clinical outcome. *J Infect Dis* 2014; **210**(4): 558-66.

[61] Centers for Disease Control and Prevention. Infection control for viral haemorrhagic fevers in the African health care setting. Atlanta: Centers for Disease Control and Prevention; 1998. [Online] Available from: http://www.cdc.gov/vhf/abroad/vhf-manual.html [Accessed on 4th February, 2015]

[62] Mupapa K, Massamba M, Kibadi K, Kuvula K, Bwaka A, Kipasa M, et al. Treatment of ebola hemorrhagic fever with blood transfusions from convalescent patients. *J Infect Dis* 1999; **179**: S18-23.

[63] Gupta M, Mahanty S, Bray M, Ahmed R, Rollin PE. Passive transfer of antibodies protects immunocompetent and imunodeficient mice against lethal ebola virus infection without complete inhibition of viral replication. *J Virol* 2001; **75**(10): 4649-54.

[64] Oswald WB, Geisbert TW, Davis KJ, Geisbert JB, Sullivan NJ, Jahrling PB, et al. Neutralizing antibody fails to impact the course of Ebola virus infection in monkeys. *PLoS Pathog* 2007; **3**(1): e9.

[65] Kudoyarova-Zubavichene NM, Sergeyev NN, Chepurnov AA, Netesov SV. Preparation and use of hyperimmune serum for prophylaxis and therapy of Ebola virus infections. *J Infect Dis* 1999; **179**: S218-23.

[66] Huggins J, Zhang ZX, Bray M. Antiviral drug therapy of filovirus infections: S-adenosylhomocysteine hydrolase inhibitors inhibit Ebola virus *in vitro* and in a lethal mouse model. *J Infect Dis* 1999; **179**: S240-7.

[67] Borio L, Inglesby T, Peters CJ, Schmaljohn AL, Hughes JM, Jahrling PB, et al. Hemorrhagic fever viruses as biological weapons: medical and public health management. *JAMA* 2002; **287**(18): 2391-405.

[68] World Health Organization. Annex 18. Transmission risk reduction of filoviruses in home-care settings. Geneva: World Health Organization; 2014. [Online] Available from: http://www.who.int/csr/disease/ebola/Annex_18_risk_reduction_home_care.pdf?ua=1 [Accessed on 4th February, 2015]

[69] World Health Organization. Ebola and Marburg virus disease epidemics: preparedness, alert, control and evaluation. Interim manual version 1.2. Geneva: World Health Organization; 2014. [Online] Available from: http://www.who.int/csr/disease/ebola/manual_EVD/en [Accessed on 4th February, 2015]

[70] Editorial. Ebola in West Africa. *Lancet Infect Dis* 2014; **14**(9): 779.