Review Article

Ebola Virus Disease- An Overview

Aadithya Tamilarasan, Meignanalakshmi Sundaram, Vijayarani Kumanan

Department of Animal Biotechnology, Faculty of Basic Sciences, Tamilnadu Veterinary and Animal Sciences University (TANUVAS) Madras Veterinary College, Chennai-7, India.

Abstract | Ebola virus disease (EVD), formerly known as Ebola haemorrhagic fever, is a severe, often fatal illness, with a case fatality rate of upto 90%. It is a severe contagious zoonotic disease affecting humans and non-human primates. It is caused by a virus belongs to the genus Ebola of Filoviridae family. This virus is a single stranded negative sense RNA consists of four glycoprotein which is required for replication and transcription. It can be transmitted to humans through direct contact with blood, tissue, body fluids and secretions from an infected animal or human. The Ebola virus (EBOV) is the cause of an emerging disease that may be harbored across a much larger geographic range then previously assumed. The present large outbreak of EBOV illustrates how an emerging disease may start and spread, the difficulty of containment and the socio-political factors that may appear during an emerging disease outbreak. There are no licensed specific treatments or vaccine available for use in people or animals.

Keywords | Ebola virus, Fruit bats, ELISA, RT-PCR, Reverse genetics.

Editor | Kuldeep Dhama, Indian Veterinary Research Institute, Uttar Pradesh, India.
Received | March 05, 2018; **Accepted** | April 27, 2018; **Published** | September 24, 2018
***Correspondence** | S Meignanalakshmi, Department of Animal Biotechnology, Faculty of Basic Sciences, Tamilnadu Veterinary and Animal Sciences University (TANUVAS) Madras Veterinary College, Chennai-7, India; **Email**: smeignanalakshmi@gmail.com
Citation | Aadithya T, Meignanalakshmi S, Vijayarani K (2018). Ebola virus disease- an overview. Adv. Anim. Vet. Sci. 6(11): 467-475.
DOI | http://dx.doi.org/10.17582/journal.aavs/2018/6.11.467.475
ISSN (Online) | 2307-8316; **ISSN (Print)** | 2309-3331

Copyright © 2018 Aadithya et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

INTRODUCTION

The epidemic of Ebola haemorrhagic fever (now called Ebola virus disease) observed in 2014 in West Africa is the largest outbreak which occurred since the first case of this disease in 1976. Compared with the previous epidemics the number of cases and death has been increased. According to the WHO (World Health Organisation) report of November 21, 2014, the total number of confirmed or suspected cases in the current outbreak is 15,351, with 5,459 reported deaths. Guinea, Liberia and Sierra Leone are the most affected countries (Barbara et al., 2014). EVD is transmitted to human through contact with blood, secretions, organs or other body fluids of infected animals including chimpanzees, gorillas, fruit bats, monkeys, forest antelopes and porcupines. Human-to-human transmission is possible through direct contact with blood, secretions, organs or other body fluids of infected people and indirect contact with environment contaminated with such fluids (Bausch et al., 2007). Healthcare workers are more prone for infection because the control measures are strictly practiced and they get infection frequently when they come in close contact with patients. The risk for person-to-person transmission of Ebola virus is highest during the latter stage of illness, when vomiting, diarrhoea and haemorrhage may lead to splash and droplet generation.

Historical Outbreaks and Studied Cases

First case of filovirus H.fever reported in 1967 in Germany and the former Yugoslavia and the causative agent was identified as Marburg virus. After that, 12 similar cases of H. fever were described in 1976 from outbreaks in two neighbouring locations first in Southern Sudan and Northern Zaire (now Democratic Republic of Congo (DRC).This strain cause three more epidemics in areas of Nezara, Sudan in 1979 (Borchet et al., 2011). An unknown causative agent was isolated from patients in both outbreaks and named Ebola virus after a small river in north western DRC. These two epidemics were caused by two distinct species of Ebola virus Sudan Ebola virus (SEBOV) and Zaire Ebola virus (ZEBOV) (Feldman et al., 2013). Outbreaks of ZEBOV, SEBOV and BEBOV (Bundibugyo Ebola Virus) hemorrhagic fever occur intermittently in Africa and are associated with more than 90% of Case fatality rates (Kuhl et al., 2011).

In Africa 1994, an ethnologist who used to work in tai forest has been reported with Tai forest virus (TAFV). Later it was identified that this newly discovered virus had the same aspect of Ebola haemorrhagic fever as the other two Ebola strains (Formenty et al., 1999). In 1994, an epidemic primarily reported as yellow fever outbreak in 44 cases with 28 deaths in Gabon. Later studies suggested that it was due to Ebola virus attack. Gabon was attacked twice by Ebola virus in 1996. First, outbreak began in February and out of 37 cases, 21 died. Second epidemic was from July to December. 40 deaths were reported among the 52 identified cases (Georges- Courbot et al.,1997). EHF (Ebola Haemorrhagic Fever) re-emerged in 1995 in Kikwit (DRC) Out of 300 affected, 231 people died. The second outbreak of Sudan Ebola Virus was observed in areas of Gulu, Mbarara, Masindi and Uganda in 2000 with total number of about 430 cases reported in Uganda in 2000-2001, this was considered as the largest epidemic described to date. The third Sudan Ebola Virus epidemic was observed in 2004 in Yambio and again in Sudan (Borchert et al., 2011).

Similar isolates were also observed during an outbreak of this fever in *Cynomolgus macaques* in Texas, Alice and Philippines in 1996. The second outbreak of Sudan Ebola virus observed in areas of Culu, Mubara, Masindi and Veganda in 2000. In 2000-2001, 430 cases reported in Uganda. Thirdly Sudan Ebola virus (SEBOV) observed in 2004 in Yambio and again in Sudan. Until, December 2013, a total of 23 outbreaks recorded 2388 human cases and 1590 deaths. (WHO, 2014) First appeared in 1976 in two simultaneous outbreaks in Nzara, Sudan and in Yambuku, Democratic Republic of Congo. It is the village situated near the Ebola river from which the disease takes its name. The first cases were reported from the forested region of South eastern Guinea. In, 1994-1996, the Gabon and small outbreaks in other parts of Africa. In 2007, a new strain was emerged in western Uganda un the township of Bundibugyo, which was considered as the fifth strain named as the Bundibugyo ebolavirus. This outbreak lasted 2 months with 149 suspected cases and 37 deaths. In March 21, 2014, large outbreak began in Guinea, Liberia, Nigeria and Sierra Leone. This is the first large outbreak in West Africa. In April 2014, in Guinea reported 151 clinically compatible cases of EVD of which 54 cases were confirmed by lab me PCR 195 of these patients were died. In Liberi, 21 cases reported of which 10 died. In Mali, six case was suspected as on April, 2014, of which two tested negative. The Ministry of Health and Social Welfare of Liberia has reported 27 clinical cases with associated deaths in 13 cases (48%) (WHO, 2014). However 6 and 12 suspected cases occurring in the neighbouring countries of Mali and Sierra Leone have been observed, but not been confirmed in laboratory (WHO, 2014) the largest outbreak is the ongoing epidemic in some specific areas of West Africa including main targets of Guinea and Sierrra Leone, WHO reported a total of 28,599 cases were observed of all 11,289 death have been observed from this outbreak by 10 November 2015. There were about 24 outbreaks of EVD , only 7 involved more than 100 cases. There were 15 generations of viral transmission in 1976 SUDV outbreak in Southern Sudan with 284 cases and 53% mortality while the outbreak of 4 generations of EBOV in Northern Zaire, and DRC had 315 cases and 86% mortality rate (Khan et al.,1999).

Ecology

The outbreak tend to occur when temperatures are lower and humidity is higher than usual for Africa. Even after a person recovers from the acute phase of the disease, Ebola virus survives for months in certain organs such as eyes and testes.

Table 1: Level of risk of transmission

Risk level	Type of contact
Very low or no recognised risk	Casual contact with a feverish, ambulant, self-caring patient. Examples: Sharing a sitting area or public transportation; reception tasks.
Low risk	Close face-to-face contact with a feverish and ambulant patient. Example: Physical examination, measuring temperature and blood pressures.
Moderate risk	Close face-to-face contact without appropriate personal protective equipment (including eye protection) with a patient who is coughing or vomiting, has nosebleeds or who has diarrhoea.
High risk	Percutaneous, needle stick or mucosal exposure to virus contaminated blood, bodily fuids, tissues or laboratory specimens in severely ill or known positive patients.

Genome and Structure

The Ebola virus (EBOV) particles contain a single negative strand ~19 kb, 18,959 to 18,961 nucleotides in length and helically wound RNA genome with seven linearly arranged genes encodes seven structural proteins. Four of these proteins nucleoprotein (NP), VP30, VP35 and L are required for the transcription and replication of the viral genomic RNA by constituting a helical nucleocapsid (Kevin and Valiquette, 2014). Other three proteins are glycoprotein (GP), VP(Viral Proteins) 24 and VP40 are associated with viral membrane to form the filamentous virions (Timmins et al., 2001). Expression of the virion associated proteins VP24 and VP35 led to assembly of nucleocapsid by transmission of electron microscopy and showed that it is involved in the assembly of EBOV nucleocapsid (Feldman and Geisbert, 2011). The matrix protein VP24 is also involved in the regulatory process of viral genome replica-

tion and transcription (Hoenen et al., 2010). The viral proteins VP24 and VP 35 are also important virulence factors, because they act as a type 1 Interferon9IFN) antagonists (Feldman et al., 2013). The 3'end is not Polyadenylated and the 5' ends are non-transcribed regions . The 5' ends carry important signals to control transcription, replication, and packaging of viral genome into new capsids. About 731 nucleotides from the 5' end and 472 nucleotides from the 3' end are enough for viral genome replication though not for infection. As Ebola virus is RNA coded, therefore it was found to mutate very rapid within the host and reservoir population. The observed mutational rate of Ebola virus is 2.0 x 10^{-3} substitutions per site per year that is as fast as seasonal influenza. That is why it is very difficult to develop vaccine against Ebola virus. The structure of Ebola is cylindrical/tubular that contains viral envelope, nucleocapsid and matrix components. The diameter of cylinders is approximately 80 nm and the length may be 14000 nm having a spike like virally encoded glycoprotein of 7-10 nm long projects from its surface of lipid bilayer (Ayithan et al., 2015). The overall shape f virions varies considerably ranging from simple cylinders to branches, loops and reverse direction. However, the characteristic threadlike structure is a more general shape of filoviruses.

Etiology

Ebola virus and its genus were both originally named for Zaire (now Democratic Republic of Congo), the country where it was first described and it was first suspected to new "strain" of closely related to Marburg virus. Renamed Ebola virus in 2010 to avoid confusion. 90% fatality rates and death rate 53%.Genus Ebola virus is 1 of 3 members of the filoviridae family (filovirus), along with genus Marburgvirus and genus Cuevavirus. Genus Ebolavirus comprises 5 distinct species:

Bundibugyo ebolavirus (BDBV)
Zaire ebolavirus (EBOV/ ZEBOV)
Reston ebolavirus (RESTV)
Sudan ebolavirus(SUDV)
Tai forest ebolavirus (TAFV)

BDBV, EBOV and SUDV associated with large EVD outbreaks in Africa, whereas RESTV and TAFV have not. The RESTV species, found in Philippines and the People's Republic of China, can infect humans, but no illness or death in humans from this species has been reported to date (Miranda et al., 2014). RESTV has been observed in animals in Asia but not as a cause of human disease (Choi and Croyle, 2013).

EV similar to Marburg virus strain isolated from South Africa in 1975 and Germany in 1967. BDBV, EBOV, SUDV (Sudan) are associated with large EVD outbreaks in Africa, whereas RESTV and TAFV are not. Outbreaks occur primarily in remote villages in Central and West Africa near tropical rain forests. RESTV strain is considered as not being pathogenic for humans but causes haemorrhagic fever in experimentally infected animals (Miranda et al., 1999, 2014).

Reservoir Host

Natural reservoir for Ebola virus is not yet confirmed. However, researchers suspect the fruit bats are thought to be natural reservoirs of Ebola virus (Leroy et al., 2014). Three different bat types are found to carry this virus without being affected that suggested bats as a primary natural reservoir host for Ebola virus (Beeching et al., 2014). Birds, plants and orthropods are also regarded as positive reservoir of this virus. Fruit bats belongs to Pteropodidae families such as *Hypsignathus monstrous, Epomops frangueti, Myonycteris torquate* considered as natural host. Due to higher sensitivity to the virus and high mortality rate in monkeys are not considered as an natural host. The role of pigs in EVD epidemiology is unclear. The domestic animals doesnot play any role in the transmission of disease to humans. A range of animal accidental hosts have been documented and Ebola virus has been implicated as one of the major causes of decline of African Chimpanzee and Gorilla population in recent decades (Walsh et al., 2003, Vogel et al., 2003). Human and other mammals serving as Accidental hosts (Leroy et al., 2014).

Transmission

In Ebola human to human transmission is by direct contact between mucous membranes eyes, nose or mouth or broken skin (cuts, wounds, abrasions), blood tissues and body fluids such as saliva, mucous, vomitus, urine, stool, semen, vaginal discharge, sweat, tears, breast milk, bile and phlegm of a symptomatic infected person (Leroy et al., 2014). By direct contact with the environment or objects contaminated with fluids room the infected person (clothes, bed linen and needles), direct contact with a person who has died from EVD (during funerals and burial rituals) (Legrand et al., 2006), through the semen of men who has recovered from the disease for upto 7 weeks after recovery through consumption of fruits contaminated with bat saliva and faeces (Murin et al., 2014).Through hunting, handling of dead and sick animals and consumption of uncooked bush meat, the disease is transmitted from wild animal to humans. The source of infection for non-human primates often remains unclear but most of the evident stated that the infection may be due to direct infection from one or more natural hosts. In rural areas fruit bats are popular source of forest meat for humans and are prepared by hand to be dried, smoked or cooked. Its is important to educate the people in affected countries to avoid contact with wild animals, including bats, rodents or monkeys because the communities having contact with these animals more prone for getting infection . It is thought that the

current epidemics throughout West Africa originated from a single animal-human transmission event that occurred in the forest at the border between Guinea, Sierra Leone and Liberia. There is no evidence that pet cat, dogs, mosquitoes and other insects can transmit ebola virus. It does not spread through air water or by food (Transmission Centers for Disease Control and Prevention, 2014). Latest studies shown that ebola viral transmission occurs when there is a high viral load of body fluids. Human-human transmission is considered as the principal mode of transmission for human. World Health Organization advises to obtain from sex or use condoms for a period of 3 months after the patient is cured (WHO, 2014). There is no evidence yet on when women recovering from the ebola virus can resume breast feeding (Centers for Disease Control and Prevention, 2014). Only few species of mammals (for eg. Humans, monkes and apes) have shown the ability to become infected and spread Ebola virus. The person remains infectious as long as the virus present in their blood and body fluids. Patients who have completely recovered from the Ebola virus cannot spread the virus.

Pathogenesis

Viral binding and entry: The primary target site is the mononuclear phagocytic system. The virus spreads through the organism and target the cells which includes endothelial cells, fibroplasts, hepatocytes and many other cells (Martinez et al., 2012). There is significant evidence that the Ebola outer glycoprotein (GP) plays an important role in this cell tropism and the spread and pathogenesis of infection (Feldman et al., 1999). Initially the Ebola viral spike glycoprotein (GP) mediates viral entry into both macrophages and dendritic antigen presenting cells (APC). Filovirus entry is by this spike glycoprotein binding to receptors on the target cell's surface (Feldman et al., 1999). Different strains of EBOV show variations in the processing of the cleavability of the glycoprotein and this may account for differences in pathogenicity, ass has been observed with influenza viruses and paramyxoviruses.

Receptors of viral glycoprotein: The Niemann-Pick C1 (NPC1) protein is a cholesterol transporter protein and appears to be the main receptor for Ebola GP binding and entry of Ebolavirions into the host cell for replication (Carollet al., 2013). The second candidate EBOV GP receptor is the TIM-1 (T-cell immunoglobin and mucin domain-1) protein. Silencing its effect with siRNA prevented infection of Vero cells. A monoclonal antibody against the lgV domain of TIM-1 blocked EBOV binding and infection (Kondratowicz et al., 2011).

The cells and tissues with high NPC1 and TIM-1 expression may be major sites of viral infection and sites of significant shedding of mature infectious viral progeny the virus affects the antigen-presenting cells and macrophages in the airway and skin. The tissues with high TIM-1 expression levels are seriously affected by the virus especially trachea, cornea and conjuctiva). This may be a factor in human-to-human virus transmission events and transmission from infected patients to medical workers (Marsh et al., 2011).

Initial stage of viral infection: Mucous membrane antigen-presenting cells (APCs) provide the initial targets and the Macrophages, monocytes, kupffer cells and dendritic cells (DCs) are all the targets of filovirus infection in vivo (Wauquier et al., 2010). The most specialised APCs in the body are the dendritic cells found in tissues that are in contact with the external environment, such as the skin (Langerhans cells) and the inner lining of the nose, lungs, stomach and intestines. They can also be found in an immature state in the blood.. This provides more targets for infection. Fatal EBOV infections are associated with the hypersecretion of numerous cytokines, chemokines and growth factors. This is not accompanied by an increase in interferon IFα2 secretion (Wauquier et al., 2010). Vascular permeability and expression of transmembrane glycoprotein which is called as Tissue factor (TF) promoted by the pro-inflammatory response. In non-survivors, the TF rises rapidly after the onset of symptoms and these mediators enters into the general circulation and reaches high levels just 2 days before the death by creating a " cytokine storm". The pro-inflammatory mediator levels range between 5 and 1,000 times higher than those observed in both healthy individuals and survivors shortly after death (Mc Elroy et al., 2014, Wong et al., 2014).

Systemic Inflammatory Response Syndrome (SIRS): Clinical and laboratory data suggest that EVD patients suffer from the systemic inflammatory response syndrome (SIRS), a generalised inflammatory state affecting the small blood vessels of the body, first described in 1983. SIRS is nothing but an immune response to a severe infection and is characterised as a subset of a "cytokine storm" which accompanied by the dysregulation and an abnormal production of lymphokines and cytokines that flood the systemic circulation and finally it precipitates the small blood vessels and vascular endothelium by the production of nitric oxide. Due to leakage of the capillary beds results in Hypotension and once it initiated may be progressively refractory to intravenous fluid therapy and vasopressors.

The abnormal systemic inflammation of the small blood vessels may progress to an active process of fibrin deposition, platelet aggregation, coagulopathies and liposomal release from stagnant leukocytes inside the vascular system. Due to inflammation of capillary beds which occluds the blood vessels by small fibrin microthrombi leading to organ microcirculatory damage and cellular hypoxemia. Finally it leads to the release of progressively increasing

amounts of TF into the circulation from dying and necrotic tissue areas (Enterleinet al., 2006) and the vascular inflammation of SIRS progressed into renal failure, the respiratory distress syndrome, gastrointestinal bleeding and central nervous system dysfunction as a part of multiple organ dysfunction syndrome or MODS.

Massive lymphocyte apoptosis: The viral replication occurs in the mononuclear phagocyte system and it will not replicate in human lymphocytes. The viral infection is associated with Lymphophenia and Lymphoid depletion which in turn resulted inlymphocyte apoptosis. The virus disseminated into other cell types throughout the body and cause severe illness which is resulted from a complex pathophysiology. These virus will supress innate and adaptive immune responses infect and kill a broad variety of cell types and elicit a strong harmful inflammatory response.

Multiple organ dysfunction syndrome: Multiple organ dysfunction syndrome (MODS) is the consequence of continuing severe systemic vasculo information with generalised increased capillary permeability, capillary leak and edema (Johnson and Mayers, 2001). In MODS, The organ dysfunction is precipitated by capillary changes in permeability, bood flow and the development of microvascular stasis and microthrombi. By definition, MODS is characterized by progressive dysfunction of six organ systems. Cytokine which induces damage to the capillaries thereby causing change in their permeability, resulting in water and serum proteins which leaks into the interstitial tissue spaces leads to the leakage of blood volume with increased blood pressure which is difficult to maintain with intravenous fluids and vasopressor drugs.

Hepatic dysfunction characterized by hyperbilirubinemia and depressed albumin production by the liver occurs early. As hepatocytes die, acute hepatic failure ensues,. Ammonia and amide levels in the body rise, and then end –stage encephalopathy may be induced as a result of elevated plasma NH_3, with reduction in the Glasgow Coma Score.

Renal risk increases from a combination of endothelial dysfunction, SIRS induced endothelial damage and progressive haemodynmic shock. This is characterized by oliguria as measured by hourly urine output less than 40-60 ml per hour and increased plasma BUN, Creatinine and Potassium with decreased urine urea, creatinine and potassium and multiple metabolic and endocrine abnormalities, including hyperglycema and increased insulin requirements. Eventually, lung interstitial spaces and alveoli may be involved. Some degree of myocardial depression may occur affecting the right side of the heart in particular. Blood supply to the bowel may become compromised with the resulting bloody diarrhoea and the patient may ischemic colitis. The resulting transudation of gram negative bacteria from the gut lumen into the general circulation may precipitate terminal septic shock and death. Because of the damage to the capillary micro circulation it is difficult to reverse the established organ failure. Therapy therefore is limited to maintaining adequate tissue perfusion and tissue oxygenation. The chance of survival diminishes as the number of different organs involved increases and the mortality rate of MODS has changed little since its recognition in the 1980s (Irwin et al., 2013).

Clinical Symptoms: The symptoms of the disease appear after 4-10 days of incubation period (range 2-21 days, depending on infective dose) (Goeijenbier et al., 2014). Severe acute viral illness is characterised with high temperature of about 39'C, haematemesis, diarrhoea with blood, retrosternal abdominal pain, prostration with heavy articulations and rapid evolution of heat after a mean of 3 days. Ebola virus was remain in the semen even after recovery for 3 months and also evident that a man who was infected in a laboratory, isolated the virus from semen 61 days after onset of illness. Haematoma at injection site, abdominal pain, cough, shortness of breath, postural hypotension, edema, headache, confusion and coma (Feldman et al 2013).

In some cases, a maculopapular rash develops after 5-7 days of symptoms (Hoenen et al., 2010). In several cases, the patient also develops haemorrhagic complications (such as mucosal haemorrhage, nose bleeding, vomiting/coughing up blood, bleed in stool, petechiae, ecchymoses, uncontrollable bleeding from vein puncture sites. And also severe metabolic disturbances, convulsion, shock, multiple organ failure, post partum vaginal bleeding, asthenia, myalgia, dysphagia and these complications are the most common cause of death in patients (Hoenen and Feldman, 2006). Gastro intestinal symptoms are the most common in the current outbreak (Goeijenbier et al., 2014). However, long term consequences of infection may persist as a recurrent hepatitis, spinal cord injury, uveitis, Psychosis or hair loss (Goeijenbier et al., 2014).

Diagnosis: Its very important to diagnose Ebola virus quickly to limit the spread of disease further. Diagnosis is difficult for few days because of early symptoms such as fever, are non-specific to Ebola and seen often in patients with more common disease such as malaria and typhoid fever. The presence of virus detected in blood only after the occurrence of the symptoms notably fever because during that time virus titre will be more in patients's body. It may take upto 3 days after symptoms start for the virus to reach thr detectable levels. ELISA (Enzyme Linked Immuno Sorbant Assay), antigen detection test, Serum Neutralisation test, RT-PCR (Reverse Transcriptase Polymerase Chain Reaction), Virus Isolation, done by cell culture (Geisber et al., 2006).

Fluorogenic 5' nuclease assay having one tube reverse transcription PCR was made and tested by the ABI PRISM 7700 sequence detection system that consisted of one common primer set and two differentially labelled fluorescent probes. This assay can detect and differentiate two subtypes of Ebola virus such as ZEBOV and SEBOV simultaneously (Wong et al., 2014). Ag captures ELISA. IgM and IgG Ab are used for diagnosis later in the disease course or after recovery . RT-PCR is sensitive, rapid, high specific testing result. Laboratory findings such as Leukopenia. Thrombocytopenia and elevated liver enzyme has been observed. Elevated thrombomodulin and ferritin levels have also been associated with death and haemorrhage in Ebola virus infected patients (Mc Elroy et al., 2014).

ELISA used to detect both antibodies as well as virus-specific antigens. But this assay is less useful because before the formation of specific antibodies the patient will die. Hence , this assay is carried out mainly for the epidemiological purpose. Positive results obtained by the ELISA can be confirmed by Western Blot. Sometimes IgM antibodies are detected only in sick person samples and these antibodies appear 2 days of symptoms onset and may last for 30-168 days. The IgG antibodies are detected between 6 and 18 th post onset of illness and it may persists for years. Usually , the antibody profile of the sera is significantly different for patients with lethal disease when compared with the survived person. Hence , this difference may play as an important prognostic marker for the management of patient. During outbreaks , ELISA methods used for the detection of specific viral antigens which is highly useful and frequently applied methods. The filovirus particles are usually present in the blood and tissue of patients in high titres at the early stage of illness. Several methods of ELISA have been developed to diagnose an acute viral infection (Barbara et al., 2014).

Virus isolation by cell cultures is one of the very sensitive methods. Acute phase patient sera or post-mortem samples considered to be a appropriate material for virus isolation. This virus is able te replicate in numerous cell lines and growth of virus can be detected by cytopathic effect. Vero or vero E6 cell lines are most commonly used for the isolation of virus. For confirmation of antigens in infected cells Fluroscently-labelled antibodies were used.

Treatment

Ebola filoviruses caused infectious haemorrhagic fevers and resulted in upto 90% mortality rates in humans while there is no useful vaccine and therapeutics available for clinical purposes. The repeated and continous outbreaks are the major concerns from public health and biodefense perspectives since no effective treatment is available (Kamata et al., 2014). Many drugs are being used as preventive medications for EVD, such as amiodarone, chlorquine and clomiphene. An effective vaccine is being devised such as recombinant vesicular stomatitis virus vaccine . It is the most promising but its efficacy has not been tested in humans (Maezi et al., 2011; Geisbert et al., 2010). A variety of DNA, protein subunit and Several viral vectors approaches both replicating and non-replication has been tested as potential vaccines and their efficacy has been tested in non-human primates.

Reverse Genetics: In the past several years, a number of laboratories and industry-based researchers have used reverse genetics to identify new targets within viral genomes for drug and vaccine development (Hoenon and Feldman, 2006). Reverse genetics allows development of recombinant filoviruses, such as EBOV, that contain key gene sequences but are nonreplicating and therefore noninfective and it has been applied in EBOV research to understand gene function as demonstrated in a study by Martinez and colleagues (Martinez et al., 2012). This team conducted studies of the EBOV VP30 and developed a model for VP30 phosphorylation that is dynamic and represents an important mechanism for regulation of the EBOV replication cycle. In another study by Borchetet al. (2011) reverse genetics was applied to express a Zaire EBOV (ZEBOV) glycoprotein. This team created a rabies virus (RABV) vaccine that efficiently expresses ZEBOV glycoprotein and induces humoral immunity against both RABV and ZEBOV and it confers protection against lethal RABV and EBOV challenge in mice.

Reverse genetics has also enabled creation of virus-like particles (VLPs) that have morphology identical to actual EBOV but are nonpathogenic (Watanabe et al., 2004). These VLPs, generated in a plasmid-based system, allow study of EBOV entry, replication, and assembly without the need for biosafety level 4 containment. Such a system has potential application in the development of EBOV vaccines. Finally, Reverse genetic techniques for EBOV have allowed high-throughput screening to identify potential drug targets on the virus without requiring biosafety level 4 containment facilities (Hoenen and Feldman, 2014b).

Nucleoside analog candidates: In 2014, antiviral research and development efforts have focused on identifying safe and efficacious agents that may be used as postexposure treatment . One such agent, T-705 (favipiravir), it has undergone animal trials to evaluate its efficacy against EBOV. Favipiravir is pyrazine carboxamide derivative and it was efficacious against ZEBOV both in vitro and in vivo (Oestereich et al., 2014). Animal studies have confirmed that favipiravir is effective in treating animals infected with the aerosolized E718 strain of EBOV (Smither et al., 2014). More recent studies suggest that favipiravir induces viral mutagenesis that leads to reduced viral infectivity and

replication (Arias et al., 2014).The agent has broad antiviral effects and was originally developed as an agent against influenza viruses.

Brincidofovir (BCV) whichis a lipid conjugate of the acyclic nucleotide phosphonate cidofovir and it was used to develope a antiviral against double stranded viruses (Florescu et al., 2014). Although clinical trials to study BCV's efficacy against human cytomegalovirus (CMV), smallpox, and adenovirus infections are ongoing, BCV has also been given Investigational New Drug status by the US FDA as a potential treatment for EBOV due to BCV's demonstrated in vitro activity against EBOV. An open-label, multicenter trial of BCV is now planned in humans to study safety and tolerability during treatment of EBOV infection.

In China, a novel antiviral compound, referred to as JK-05, has been approved for treatment of EBOV infections. This compound was been developed and tested by China's Institute of Microbiology Epidemiology of the Academy of Military Medical Sciences. This compound appears to act on RNA viral polymerase to inhibit viral replication and has undergone preclinical testing in animal models. JK-05 has been approved for emergency use only, and no human trial data are available at this time. Plans for clinical trials or distribution of this therapy in West Africa have not been announced at this time. Another viral nucleoside analog, BCX4430, has recently received substantial attention as a potential treatment for EBOV infections (Wong et al., 2014). BCX4430 is a novel adenosine analog that inhibits the viral RNA polymerase function by incorporating into new viral RNA chains and causing chain termination. When administered intramuscularly, BCX4430 has shown clinical protection in mouse and guinea pig models, even when administered after exposure. Administration is feasible at oral route , although pharmacokinetics data suggest that the intramuscular route provide more favorable therapeutic levels. Additional studies are needed to understand optimal dosing, routes of administration, and durations of therapy.

RNA Silencing Molecules: `RNA silencing (sometimes referred to as RNA interference [RNAi]) is a regulatory mechanism for gene expression and associated with control of cell differentiation and development. RNAi serves as an innate antiviral response in plants, nematodes, and insects. In mammals viruses code for RNA silencing suppressors (RSSs) that enable viral replication at higher titers. A number of human viruses have been shown to encode RSSs, including EBOV (Fabozzi et al., 2011). In fact, the EBOV VP35 protein is a suppressor of RNAi in mammals, and its RSS activity is functionally equivalent to that of the human immunodeficiency virus (HIV)-1 Tat Protein (Haasnoot et al., 2007; Zhu et al., 2012). Prior to the current EVD outbreak, scientists in one trial identified small RNAi molecules (sometimes referred to as small interfering RNAs [siRNAs]) that were effective in protecting guinea pigs against lethal challenge with EBOV (Geisbert et al., 2006). SiRNA molecules are fragments of double stranded RNA that inhibit viral messenger RNA (mRNA) and viral replication. In this trial, four siRNA molecules were created to target the polymerase (L) gene of ZEBOV. The siRNA molecules were complexed to polyethylenimine (PEI, a polymer composed of repeating amine units) or formulated in stable nucleic acid lipid particles. Administration of the RNAi molecules with PEI or the nucleic acid lipid particles provided greater protection against viremia and death in a guinea pig model when administered shortly after EBOV challenge. In a further study of siRNA molecules administered in nonhuman primates, molecules targeting EBOV L polymerase, VP 24, and VP35 were administered in a postexposure trial .Results suggest that the RNAi therapy provided complete protection and was well tolerated.In addition, studies are now initiated to evaluate lipid nanoparticle/siRNA referred to as TKMEbola TKMEbola (Choi et al., 2013). Phase I trials of TKM-Ebola were initiated . In March 2014, TKM-Ebola was given fast-track approval by the U.S. Food and Drug Administration. In addition, TKM-Ebola is now entering human clinical evaluation in Guinea for emergency use in treating patients with EBOV, in collaboration with a consortium led by the WHO. The TKM-Ebola molecule undergoing evaluation has been designed to target the Guinea variant of the ZEBOV species. An additional active area of research focused around development of small molecule therapeutics uses antisense phosphorodiamidate morpholino oligomers (PMOs) to target sequences of viral mRNAs corresponding to VP24, VP35, and the RNA polymerase L protected rodents in both preexposure and postexposure therapeutic regimens (Warfield et al., 2006: Enterlein et al., 2006).Two specific PMOs (AVI-6002 and AVI-6003]) have undergone phase I trials (AVI-6002 was evaluated for postexposure treatment of EBOV infection, and AVI-6003 was studied for Marburg virus infections. AVI-6002 is composed of two PMOs referred to as AVI- 7537 and AVI-7539, whereas AVI-6003 is composed of AVI-7287 and AVI-7288 .In two separate phase I safety and dose-escalation studies.

Immunotherapeutics: A novel immunotherapeutic, consisting of a combination of monoclonal antibodies (mAb) (Zhang et al., 2014) has undergone success in animal studies and is now undergoing further evaluation following its administration to approximately seven patients with EVD in the United States and other countries. Recently, the composition of the mAb combination (now referred to as ZMapp) was described (Arias et al., 2014).The results of this study suggest that individually, the mAbs bind the EBOV glycoprotein core. ZMapp is currently manufactured in a tobacco plant–based production facility where

plants are genetically modified to express monoclonal antibodies to EBOV glycoproteins. The present formulation of ZMapp is a combination of two mAb cocktails referred to as Mb-003 and ZMab. Currently, ZMapp is in short supply, but plans are under way to scale up production tomeet potential demand in the current West Africa EVD outbreak.

CONCLUSION

The public sector along with respective authorities, WHO, developing countries and Health care associations should endulge in devising the strategies for prevention and control. By keeping the available resources, researchers could involve in devising new vaccine strategies, drugs for treatment and also deals with preventive measures during outbreak before it occurs.

ACKNOWLEDGEMENTS

The authors are grateful to the Department of Animal Biotechnology, Madras Veterinary college, Vepery, Chennai-7, for providing financial support and technical assistance and facilities provided.

CONFLICT OF INTEREST

There is no conflict of interest.

AUTHORS CONTRIBUTION

All authors contributed in correcting the article by spending their valuable time and also supported by sharing valuable suggestions in writing an article.

REFERENCES

- Arias A, Thorne L, Goodfellow I (2014). Favipiravir elicits antiviralmutagenesis during virus replication in vivo. Elife,3:e03679. https://doi.org/10.7554/eLife.03679
- Ayithan N, Bradfute SB, Anthony SM, Stuthma KS, Bavari S, Bray M (2015).Virus-like particles activate type I interferon pathways to facilitatepost-exposure protection against Ebola virus infection. PLoS One. (2):e0118345. https://doi.org/10.1371/journal.pone.0118345
- Bausch DG, Towner IS, Dowell SF (2007). Assessment of the risk of Ebola virus transmission from bodily fluids and fomites. J. Infect. Dis. 196**:** 142-147. https://doi.org/10.1086/520545
- Barbara Z, MK Vnenchak (2014). General Introduction into the Ebola Virus Biology and Disease. Folia Medica Cracoviensia. 3**:** 57-65.
- Beeching NJ, Fenech M, Houlihan CF (2014). Ebola virus disease. BMJ. 349**.** https://doi.org/10.1136/bmj.g7348
- Borchert M, Mutyaba I, Van Kerkhov MD, Lutwama J, Luwaga H, Bisoborwa G (2011). Ebola haemorrhagic fever outbreak in Masindi District,Uganda: outbreak description and lessons learned. BMC Infect. Dis. 11:357. https://doi.org/10.1186/1471-2334-11-357
- Bray M, Geisbert TW (2005). Ebola virus: the role of macrophages and dendritic cells in the pathogenesis of Ebola hemorrhagic fever. Int. J. Biochem. Cell Biol. 37:1560-1566 https://doi.org/10.1016/j.biocel.2005.02.018.
- Choi JH, Croyle MA (2013). Emerging targets and novel approaches to Ebola virus prophylaxis and treatment. Bio. Drugs. 27:565–83. https://doi.org/10.1007/s40259-013-0046-1
- CDC, Ebola (Ebola Virus Disease) (2014).West Africa Outbreak.
- Caroll SA, Towner JS, Sealy TK (2013). Molecular evolution of viruses of the family Filoviridae based on 97 ehole genome sequences. J. Virol. 87**:** 2608-2616. https://doi.org/10.1128/JVI.03118-12
- Engelmann B, Luthe T, Müller I (2004). Intravascular tissue factor pathway—a model for rapid initiation of coagulation within the blood vessel. Thromb. Haemost.. 89:3-8.
- Enterlein S, Warfield KL, Swenson DL (2006). VP35 knockdown inhibits Ebola virus amplification and protects against lethal infection in mice. Antimicrob. Agents Chemother. 50: 984–93. https://doi.org/10.1128/AAC.50.3.984-993.2006
- Fabozzi G, Nabel CC, Dolan MA, Sullivan NJ (2011). Ebolavirus proteins suppress the effects of small interfering RNA by directinteraction with the mammalian RNA interference pathway. J. Virol. 85:2512–23. https://doi.org/10.1128/JVI.01160-10
- Feldmann H, Geisbert TW (2011). Ebola haemorrhagic fever. Lancet. 377:849–62. https://doi.org/10.1016/S0140-6736(10)60667-8
- Feldmann H, Volchkov VE, Volchkova VA, Klenk HD (1999). The glycoproteins of Marburg and Ebola virus and their potential roles in pathogenesis. Arch. Virol. 15(Suppl):159-169. https://doi.org/10.1007/978-3-7091-6425-9_11
- Feldman H, Volchko VE, Timons D (2013). The Ebola virus disease outbreak in West Africa. J. Gen. Virol. 95**:** 1619-1624.
- Florescu DF, Keck MA (2014). Development of CMX001 (Brincidofovir) for the treatment of serious diseases or conditions causedby dsDNA viruses. Expert Rev. Anti. Infect. Ther.12:1171–1178. https://doi.org/10.1586/14787210.2014.948847
- Formenty P, Leroy EM, Epelboin A (1999). Detection of Ebola virus in oral fluid specimens during outbreaks of Ebola Virus Haemorrhagic Fever in the Republic of Congo. Clin. Infect. Dis. 42**:** 1521-1526.
- Georges AJ, Baize S, Leroy EM, Goerges Courbot MC (1997). Ebola virus: What the Prectioner needs to know. Med. Trop. (mars). 58: 177-186. https://doi.org/10.1086/503836
- Geisbert TW, Lee AC, Robbins M (2010). Postexposure protectionof non-human primates against a lethal Ebola virus challengewith RNA interference: a proof-of-concept study. Lancet. 375:1896–905. https://doi.org/10.1016/S0140-6736(10)60357-1
- Geisbert TW, Lee AC, Smith MC, Robbins M (2006). Protection of non-human primates against Ebola. 430: 2501-2522.
- Goeijenbier M, Van Kampen JJ, Reusken CB (2014). Ebola virus disease a review on epidemiology, symptoms, treatment and pathogenesis. Neth. J. Med. 72(9):442-448.
- Haasnoot J, Geutjes EJ, Prins M, Haan P, Berkhout B (2007). The Ebola virus VP35 protein is a suppressor of RNA silencing. PLoS Pathog. 3:e86. https://doi.org/10.1371/

journal.ppat.0030086
- Hoenen T, Feldmann H (2014b). Ebolavirus in West Africa, and theuse of experimental therapies or vaccines. BMC Biol. 12:80. https://doi.org/10.1186/s12915-014-0080-6
- Hoenen T, Sunglien T, Feldmann H (2010). Ebolavirus and vaccine therapy. BMC Biol. 21: 122-135
- Hoenen T, Feldmann H (2006). Ebolavirus in West Africa, and theuse of experimental therapies or vaccines. BMC Biol.12:80. https://doi.org/10.1186/s12915-014-0080-6
- Irwin RS, Lilly CM, Rippe JM (2013). Manual of Intensive Care Medicine. Philadelphia, Pa.: Wolters Kluwer Health/ Lippincott Williams & Wilkins.
- Johnson D, Mayers I (2001). Multiple organ dysfunction syndrome; a narrative review. Can. J. Anaesth. 48:502-509. https://doi.org/10.1007/BF03028318
- Kamata T, Natesan M, Warfield M, Aman MJ, Ulrich RG (2014). Determination of specific antibody responses to the six species of ebola and Marburg viruses by multiplexed protein microarrays. Clin. Vacc. Immunol. 21: 1605-1612. https://doi.org/10.1128/CVI.00484-14
- Kevin BL, Valiquette L (2014). Adult Infectious Diseases Notes Ebola Virus Disease. Can. J. Infect. Dis. Med. Microbiol. 25:128-129. https://doi.org/10.1155/2014/527378
- Khan RC, McCormick JB, Zubeir OA (1999). Ebola virus disease in southern Sudan: hospital dissemination and intrafamilial spread. Bull. World Health Organ. 1:997-1003.
- Kondratowicz AS, Lennemann NJ, Sinn PL (2011). T-cell immunoglobulin and mucin domain 1 (TIM-1) is a receptor for Zaire Ebolavirus and Lake Victoria Marburgvirus. Proc. Nat. Acad. Sci. USA.108:8426-8431. https://doi.org/10.1073/pnas.1019030108
- Kuhl A, Hoffmann M, Muller MA, Munster VJ, Gnirss K, Kiene M (2011). Comparative analysis of Ebola virus glycoprotein interactionswith human and bat cells. J. Infect. Dis. 204 Suppl 3:S840–9.
- Legrand AU, Rouquet P, Formenty P (2006). Multiple Ebola virus transmission events and rapid decline of Central African wildlife .Science. 307:387-390.
- Leroy EM, Simmons AS, Renaut AA (2014). Ebola hemorrhagic fever outbreaks in Gabon,: epidemiologic andhealth control issues. J. Infect. Dis. 179(Suppl. 1):S65–75.
- Lopez AD, Mathers CD, Holmes EC, Gonzalez-Candelas F (2006). The population genetics and evolutionary epidemiology of RNA viruses. Natur. Rev. Microbiol. 2:279-288.
- Maezi TL, Triffith SM, Bunton UA (2011). Experimental aerosolized guinea pig –adapted Zaire Ebolaviru causes lethal pneumonia in guinea pigs. Vet. Pathol. 233: 1892-1910.
- Martinez O, Leung JW, Basler CF (2012). The role of antigen-presenting cells in filoviral hemorrhagic fever: gaps in current knowledge. Antiviral. Res. 93:416-428. https://doi.org/10.1016/j.antiviral.2012.01.011
- Marsh GA, Haining J, Robinson R (2011). Ebola Reston Virus infection of Pigs clinical significance and transmission potential. J. Infect. Dis. 204: 804-809. https://doi.org/10.1093/infdis/jir300
- McElroy AK, Erickson BR, Flietstra TD (2014). Ebola hemorrhagic fever: novel biomarker correlates of clinical outcome. J. Infect. Dis. 210:558-566. https://doi.org/10.1093/infdis/jiu088
- Miranda MEG, Miranda NLJ (2014). Reston ebolavirus in humans and animals in the Philippines: a review. J. Infect. Dis. 3:S757–60.
- Miranda ME, Ksiazek TG, Retuya TJ, Khan AS , Sanchezand CF, Fulhorst (1999). Epidemiology of Ebola (subtype Reston) virus in the Philippines. J. Infect. Dis. https://doi.org/10.1086/514314
- Murin CD, Fusco ML, Bornholdt ZA (2014). Structures of protectiveantibodies reveal sites of vulnerability on Ebola virus. Proc. Natl. Acad. Sci. USA. 111(48):17182-7. https://doi.org/10.1073/pnas.1414164111
- Oestereich L, Ludtke A, Wurr T, Rieger S, Munoz-Fontela C, Gunther S (2014). Successful treatment of advanced Ebola virus infection with T-705 (favipiravir) in a small animal model. Antiviral. Res. 105:17–21. https://doi.org/10.1016/j.antiviral.2014.02.014
- Qiu X, Wong G, Audet J (2014). Reversion of advanced Ebolavirus disease in nonhuman primates with ZMapp. Nature.514:47–53. https://doi.org/10.1038/nature13777
- Smither SJ, Eastaugh LS , Steward JA, Nelson M, Lenk RP, Lever M (2014). Post-exposure efficacy of oral T-705 (Favipiravir)against inhalational Ebola virus infection in a mouse model. Antiviral. Res. 104:153–5. https://doi.org/10.1016/j.antiviral.2014.01.012
- Timmins DJ, Leach RW, Bruenn J (2001). Filoviruses are ancient and integrated into mammalian genome. BMC Evol. Biol.10:193.
- Vogel YP, Zhang XE, Wei HP (2003). Laboratory detection and diagnosis of filoviruses. Vitol. Sin. 26: 73-80.
- Walsh PD, Abernethy KA, Bermejol M (2003). Catastrophic ape decline in western equatorial Africa. Nature. 422:611-4 https://doi.org/10.1038/nature01566.
- Watanabe S, Watanabe T, Noda T (2004). Production of novelebola virus-like particles from cDNAs: an alternative to ebolavirus generation by reverse genetics. J. Virol. 78:999–1005. https://doi.org/10.1128/JVI.78.2.999-1005.2004
- WHO. (2014). Ebola Virus Disease. Fact Sheet No. 103.
- Wong G, Kobinger GP, Qiu X (2014). Characterization of host immune responses in Ebola virus infections. Expert Rev. Clin. Immunol.10:781-790. https://doi.org/10.1586/1744666X.2014.908705
- Warfield KL, Swenson DL, Olinger GGI (2006). Gene-specific countermeasures against Ebola virus based on antisensephosphorodiamidate morpholino oligomers. PLoS Pathog. 2.
- Wauquier N, Becquart P, Padilla C, Baize S, Leroy EM (2010). Human fatal Zaire Ebola virus infection is associated with an aberrant innate immunity and with massive lymphocyte apoptosis. PLoS Negl. Trop. Dis. 4(10). https://doi.org/10.1371/journal.pntd.0000837
- Zhang Y, Li D, Jin X, Huang Z (2014). Fighting Ebola with ZMapp: spotlight on plant-made antibody. Sci. China Life Sci. 57:987–8. https://doi.org/10.1007/s11427-014-4746-7
- Zhu Y, Cherukuri NC, Jackel JN (2012). Characterization ofthe RNA silencing suppression activity of the Ebola virusVP35 protein in plants and mammalian cells. J. Virol. 86:3038–49 https://doi.org/10.1128/JVI.05741-11.