Ebola Virus Disease: The Global Worry

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Abstract: *Ebola virus disease caused by the Ebola virus was identified in 1976 and is an emerging and re-emerging zoonosis. Outbreaks have occurred in Africa in the ensuing years, with mortality rates ranging from 50 to 90%. The 2014 outbreak of Ebola virus disease in West Africa is the largest outbreak of Ebola virus disease in history. It has claimed more lives than all the previous epidemics combined. This single stranded Ribonucleic acid virus has five known species: Zaire Ebola virus, Sudan Ebola virus, Tai Forest Ebola virus, Reston Ebola virus, and Bundibugyo Ebola virus. Ebola virus is characterized by high lethality, high infectivity, and lack of effective treatment or prophylaxis. The causes of death are multifactorial and include massive haemorrhage, hypovolemia and electrolyte imbalance, severe sepsis and multi-organ failure. The diagnosis of Ebola virus disease is done on the basis of clinical symptoms, detection of antigen/virus and the antibodies. Present treatment strategies are mainly symptomatic and supportive. No licensed human or animal vaccine is currently available for the prevention and control of the lethal Ebola virus disease. Several experimental trials are underway in various parts of the world to find an efficient vaccine to prevent Ebola virus infection and spread. This review describes the morphology, classification, pathogenesis, clinical features, epidemiology, diagnosis, prevention and vaccines.*

Keywords: *Ebola virus*, Ebola virus disease, Ebola haemorrhagic fever, Filovirus, outbreak, vaccine, Epidemiology, prevention, diagnosis.

1. Introduction

Ebola virus disease (EVD) caused by the *Ebola virus* (EBOV), was identified four decades ago in 1976 and is an emerging and re-emerging zoonosis.[1]*Ebola virus* disease , formerly known as Ebola haemorrhagic fever (EHF), is an acute, severe and fatal disease in humans.[2] The World Health Organization (WHO) describes Ebola as one of the world's most virulent diseases.[3]The 2014 outbreak of EVD in West Africa, caused by *Ebola virus*, is the largest outbreak of EVD in history.[4] EBOV is characterized by a high degree of lethality, high infectivity, as well as a lack of effective treatment or prophylaxis.[2] Considering the possibility of imported infection and the use for biological terrorism, it becomes not only a public health problem to Africa, but also a worldwide bio-threat.[2] This pathogen has been listed by the U.S. Centre for Disease Control and Prevention (CDC) as a Category A Bioterrorism Agent .[2] Outbreaks have occurred in Africa in the ensuing years, with mortality rates ranging from 25 to 90% .[1] This high fatality, combined with the absence of treatment and vaccination options, makes *EBOV* an important public health pathogen.[5]

The extraordinarily high fatality, multisite haemorrhaging, and the specificity to African ecosystems enhanced the fascination of Ebola for the entire global community. [6] This partly explains why research on these viruses has markedly advanced in the last 15 years, leading to the development of potential antiviral agents and probable vaccines. [6]

2. Biology of Virus

Ebola virus belongs to the family Filoviridae, which comprises three genera: Marburg virus, Cuevavirus andEbolavirus.[7] This family belongs to the order Mononegavirales.[7] *Ebola virus* is the type species of the genus. [8] The name Ebola was derived from the name of a river in Zaire valley from where this deadly virus was reported for the first time.[1]The virus was initially named Ebola-like virus, which was changed to *Ebola virus* in 2002.[1]

Filoviruses are non-segmented, negative-stranded RNA enveloped viruses of varying morphology.[5] These viruses have characteristic filamentous particles that give the virus family its name (Filo- derived from the Latin *filum* i.e. thread).[5] Morphologically, when studied under an electron microscope, the viral particles look like long stretched filaments with some particles tending to curve into an appearance looking like the number 6.[9] The unstable RNA genome of EBOV leads to a high mutation rate.[2]

The virus genome of EBOV is almost 19 kb long and encodes seven viral proteins, namely, nucleoprotein (NP), polymerase cofactor (VP35), matrix protein (VP40), glycoprotein (GP), replication-transcription protein (VP30), matrix protein (VP24), and RNA-dependent RNA polymerase (L), with an additional soluble glycoprotein (sGP) produced from an edited GP mRNA. [10]

The taxonomy has been modified several times since their discovery in 1976. [8] The five known species of the genus *Ebola virus* have nucleotide sequences that differ by 35 to 40% and are named for the region where each was originally identified.[8]

The genus *Ebola virus* contains five species: Zaire *Ebola virus* (ZEBOV), Sudan *Ebola virus* (SEBOV), Tai Forest *Ebola virus* (TEBOV), Reston *Ebola virus* (REBOV), and Bundibugyo *Ebola virus* (BEBOV) .[1]

Ebola viruses are susceptible to alcohol-based products and dilutions (dilutions of 1:10 to 1:100 for at least 10

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min) of 5-25% household bleach (sodium hypochlorite) and calcium hypochlorite (bleach powder) as well as 3% acetic acid and 1% glutaraldehyde.[8] In blood and other body fluids, or even on contaminated surfaces, EBOV can survive for hours at room temperature (20°C−25°C), and for weeks at low temperature (4°C) .[2] EBOV is only moderately heat resistant and can be inactivated by heat treatment (>60°C) for at least 1 hour.[2] EBOV is also sensitive to ultraviolet light, gamma rays.[2]

3. Pathogenesis

The host immune system and vascular bed is directly affected by EBOV entering macrophages and dendritic cells.[1] *EBOV* replicates at an unusually high rate which overwhelms the protein synthesis apparatus of infected cells and host immune defences. Both the adaptive immune and inflammatory systems respond to infection at the same time.[11] EBOV-primed host macrophages and monocytes release the inflammatory cytokines in very high levels in blood stream, which destroy the normal tissues and microcirculation as well as cause extensive damage to the endothelial vessels, thereby leading to massive blood loss, which is the major feature of EBOV infection. The coagulation mechanism inside the blood vessels is also activated to cause intravascular coagulation. Profound capillary leakage, disseminated intravascular coagulation and renal failure are observed in people suffering with severe EVD. Death occurs either due to the intravascular coagulation or due to the severe blood loss. Unlike normal circumstances wherein antibodies developed against any infectious agent help to get rid of that particular pathogen from body, EBOV uses these antibodies along with the complement factor C1 to cause severe cell damage, thereby exaggerating its pathogenesis.[1] The antibodies attach to EBOV and subsequently their Fc region gets attached to the C1q complement region, which in turn aids in their attachment to the target receptors on macrophages and dendritic cells, and thus the adherence of EBOV to the host cells occurs. Antibody-enhanced infection pathways are utilized by the EBOV to reach various organs where it causes extensive damage.[1] Pathological lesions seen include necrosis of various organs, including liver, ovaries, testes, and kidneys; haemorrhages in mucosa.[1]

4. Clinical Features

The onset of the disease is abrupt after an incubation period of 2 to 21 days.[4] The incubation period is up to 21 days, which is the epidemiological basis for quarantine. Fever and other EVD symptoms such as headache, fatigue, and diarrhoea often appear at the earlier contagious stages and before there is any significant alteration in the laboratory indexes, allowing for identification of infected patients in time. However, the initial clinical signs of EVD lack specificity. Therefore, laboratory test are indispensible for a confirmative diagnosis.[2]

The clinical features can be segregated into four main phases which are as follows:

Phase 1 – where the patient show a Flu-like syndrome. The onset is abrupt with nonspecific symptoms or signs such as high pyrexia, headache, nausea, myalgia, arthralgia, and sore throat.[4]

Phase 2- Acute (days 1-6) Headache and intense fatigue followed by diarrhoea and abdominal pain and vomiting, accompanied by a persistent fever which does not respond to antimalarials or antibiotics.[4]

Phase 3- Pseudo-remission (days 7-8): In this phase the patient feels better and seeks food. The health situation appears somewhat improved. Some patients may recover during this phase and survive from the disease.[4]

Phase 4- Aggravation (day 9): In many if not most cases, the health status becomes worse. The following symptoms may be observed: Skin manifestations like petechiae (not so obvious on black skin), purpura, and morbiliform skin rash, Respiratory symptoms like dyspnoea, cough, hiccups, throat and chest pain, Cardiovascular distress and hypovolemic shock.[4]

Usually, the symptoms of EBOV infection begin suddenly, similar to influenza in the initial stages.[1] Based on these clinical manifestations, it is obvious that in the initial stage of EHF, the disease can also mimic many other tropical diseases such as malaria or typhoid fever. In most outbreaks, recognition of EHF is delayed because physicians are not accustomed to seeing this illness and its symptoms are generally nonspecific.[4]

EVD is a quickly progressive disease with multisystem involvement, causing bleeding and coagulopathy. The causes of death are multifactorial and include massive haemorrhage, hypovolemia and electrolyte imbalance, severe sepsis and multi-organ failure. Survivors often show improvement from the 6th day to the 11th day, which coincides with the development of the neutralizing antibodies.[12]

5. Epidemiology

EVD is a typical zoonotic disease, but the wild reservoir of EBOV is not clear. Non-human primates, like apes and monkeys, have been considered as important sources of infection to the humans. The natural environment of the African continent provides a favourable condition for the survival of *EBOV*. First, the natural and alternate hosts of EBOV such as apes, fruit bats, and monkeys are widely distributed in Africa. Second, according to the historical data, EVD mainly distributes between 10° north and south of the equator, with the temperature that benefits *EBOV* survival throughout the year.[2]

EBOV was first discovered in 1976 when two outbreaks of hemorrhagic fever occurred in northern Zaire (now the Democratic Republic of Congo [DRC]) and southern Sudan. Since then *EBOV* has appeared sporadically in Africa, with small to large EVD outbreaks. The five Ebola viruses which are known: Zaire *Ebola virus* (ZEBOV) and Sudan virus (SEBOV), discovered in 1976; Reston virus (REBOV), in 1989; Tai Forest virus (TEBOV), in 1994; and Bundibugyo virus (BEBOV), in 2007. Only four *Ebola virus* species, ZEBOV, SEBOV, TEBOV, and

BEBOV, are pathogenic in humans.[13] The first 3 subtypes have been responsible for large outbreaks in Africa, with the Zaire strain causing the most fatalities.[14] Other than the RESTV, which is only found in the Philippines, all the other four members are the causative agents of EVD and are endemic to West Africa. ZEBOV is the most virulent species, followed by SEBOV. The REBOV is the mildest among these 5 species, and only causes disease in non-human primates.[2]

Apes, man, and perhaps other mammalian species that are susceptible to *Ebola virus* infection are regarded as end hosts and not as reservoir species. Rodents and bats have long been thought to be potential reservoir species.[5] In 2005 Leroy *et al.* managed to detect anti-*Ebola virus* antibodies and *Ebola virus* RNA in three fruit bat species: *Epomops franqueti* , *Hypsignathus monstrosus* and *Myonycteris torquata* and research has since grown to understand the role played by bats in the maintenance, transmission, and evolution of filoviruses.[15]

The major mode of transmission of EBOV infection in human beings is by direct contact with body secretions/fluids (saliva, urine, faeces, blood, semen) or tissue/organ of infected or sick persons, as well as organ transplantation from such cases.[1] The main routes of EBOV infection are through mucous membrane, conjunctiva, small skin breaks, infected needles or syringes, unhygienic practices such as unsterile burial customs, including cleansing of infected corpse.[1] The risk is high because these rituals, which bring several hundreds of persons together and also in contact with highly infectious dead bodies, most often require people to follow long-standing rituals, including keeping the corpses for up to 3 days and communal hand washing in water that was used to bathe corpses.[14] Aerosol dissemination of EBOV has not been established as a mode of transmission in humans.[4] According to CDC, surface transmission may be possible, but is not considered a high risk.[1] EBOV has also been transmitted following accidental infection of workers in Biosafety-Level-4 (BSL-4) facilities during investigational studies.[16]

6. Recent Outbreak

The WHO declared the present 2014 *Ebola virus* disease (EVD) outbreak as a "Public Health Emergency of International Concern" on 7th August , 2014 .[17] WHO classified as confirmed any suspected or probable case with a positive laboratory result; as probable any suspected case evaluated by a clinician, or any deceased suspected case having an epidemiological link with a confirmed case where it has not been possible to collect specimens for laboratory confirmation; and as suspected any individuals suffering or having suffered from sudden onset of high fever and having had contact with a suspected, probable, or confirmed case, or a dead or sick animal, or any person with sudden onset of high fever and at least three of the EVD symptoms .[13]

The current West African outbreak was caused by ZEBOV, which is also the most virulent among the five species.[7] Evidence from the limited-sequence studies that

have been published suggests that the outbreak resulted from a single reservoir-to-human transmission event and subsequent human-to-human spread.[18]

It was the 25th known outbreak and occurred in areas that were not previously the prime centres of occurrence of the disease.[19] The current EBOV outbreak in West Africa is the largest ever recorded epidemic considering the number of affected people, countries involved, and the longest persistent transmission. [14] It has claimed greater number of lives than all the previous epidemics combined.[19]

The introduction of an EVD case into unaffected countries remains a risk for as long as cases are reported in any country.[20] With sufficient levels of preparedness, however, such introductions of the infection can be contained with a rapid and adequate response.[20] WHO's preparedness activities aim to ensure all countries are ready to effectively and safely detect, investigate and also report potential EVD cases, and to mount an effective response.[20] WHO provides this support through country visits by preparedness-strengthening teams (PSTs), direct technical assistance to countries, and also provides technical guidance and tools.[20]

7. Diagnosis

The diagnosis of EVD is mainly done on the basis of clinical symptoms, detection of antigen or virus and the antibodies .[1] Antigen/virus detection can be utilised to detect the infection at the initial stages while antibody detection can be done at the later stage of infection.[1] Blood parameters like low white blood cell count and platelet count along with an elevated level of hepatic enzymes are indicative of EVD.[1]

Laboratory diagnosis of *Ebola virus* is achieved by two ways: measurement of immune response to the infection and detection of the viral particles, or particle components in infected patients.[5] Laboratory tests done on the active virus – experimental animal inoculation , cell cultures or sample – present an extreme biohazard risk and WHO recommends that all such tests are to be conducted in BSL-4 laboratories only.[21] Viral antigen and nucleic acid is detectable in blood from day 3 up to 7–16 days after the onset of symptoms.[5] A very efficient way to inactivate the virus for antigen and antibody detection tests is by the use of gamma irradiation from a cobalt-60 source, heat inactivation or formaldehyde.[5] Similarly, the nucleic acid can be amplified by purification of the virus RNA from materials which have been treated with guanidinium isothiocyanate—a chemical which denatures the virus proteins and renders the sample non-infectious.[5]

Antigen/virus detection

Virus isolation is the essential key for confirmatory diagnosis, which can be done in Vero or Vero E6 cell lines.[1] Plaque assay is the method of choice to detect the viruses in the cell culture system.[1] The main disadvantage of the isolation of virus in cell culture is the requirement of BSL-4 facility to handle the sample and organism as EBOV is highly lethal.[1] Specimens like blood/serum must

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be sent only to BSL-4 laboratories, which are situated in the developed countries most of the time , usually very far from the outbreak area.[10] The shipment of the requisite samples for virus culture under required conditions (cold chain right from the period from shipment till arrival) is often tedious.[10] Hence, diagnostic criteria based on virus culture alone may not yield an etiologic diagnosis.[10] Antigen detection methods are mainly useful in the early phase of the infection, when the virus load is high.[1] The antigen-capture ELISA has been found to give satisfactory results for detecting the *Ebola virus* in patients' serum, plasma, and whole blood.[1] Polymerase-chain-reaction (PCR) test for EBOV nucleic acid and the detection of viral antigen in the blood can become reliably positive from day 2 to day 16 after the onset of symptoms.[2] The sensitivity of the assay can be further improved using SYBR Green or TaqMan-based real-time quantitative RT-PCR assays.[1] A multiplex real-time fluorescence quantitative PCR is also available to detect the Ebola viruses.[1] Though RT-PCR is more effective in detecting EBOV, the disadvantage of this technique is the requirement of sophisticated instruments.[1] The developed RT-LAMP assay has been reported to be rapid enough to detect the virus within 26 minutes.[1] Due to the various advantages of the test being rapid, simple, highly sensitivity, specificity and efficiency, this test can have high diagnostic use in the field as well as laboratories for testing EBOV in EVD outbreak-affected areas.[1]

Antibody detection-

Detection of antibodies can be done in the late symptomatic phase, when the patients are in recovery .[1] Antibody detection tests usually involve detection of IgG or IgM antibodies by an ELISA test.[1] Positive results in antibody detection tests reveal EVD, but a negative result will not always indicate the patient to be free of EBOV as antibodies are developed only in the later stage of the disease; due to high fatality of the disease, the chances of a person escaping the early phase of infection are low.[1] Immunoglobulin M (IgM) can be detected as early as 2 days after the onset and immunoglobulin G (IgG) is usually seen between 6 and 18 days after the appearance of the clinical signs.[2] In successive paired serum samples, either a decrease of IgM titre or an increase (more than four-fold) of IgG titre, or both can indicate a recent infection .[2] IgM is seen to disappear within 30 to 168 days after the infection, while the IgG can persist for several years .[2]

An indirect Immunofluorescence assay developed with original virus antigens obtained from virus-infected cells has been used as a diagnostic test for the detection of antibodies to the EBOV.[10] During early convalescence period the test has shown a high sensitivity for antibodies and has even been used extensively in epidemiological surveys of EBOV antibody prevalence.[10]

Another test is the phage-antibody spot test, which is capable of detecting antibodies against the EBOV Nucleoprotein antigen in the tested sera by inhibiting the reaction of the Fab antibody with the EBOV antigens, has also been developed.[10] These assays are considered apt for

the diagnosis of *Ebola* infections and for epidemiological studies.[10] Cross-reactivity with other organisms is one disadvantage in most of the serological assays.[1]

To establish a post-mortem diagnosis, autopsy tissues can be tested for the presence of *Ebola virus* antigen using *in situ* hybridization techniques or immunohistochemical staining. Virus culture or PCR-based methods can be used when fresh tissue is available.[8]

Laboratory-confirmed cases are those which test positive for the presence of the *Ebola virus*, by detection of *Ebola* antigen by a specific Antigen detection test or by the detection of virus RNA by RT-PCR, and/or by detection of Immunoglobulin M (IgM) antibodies directed against *Ebola*.[22] Two negative RT PCR test results, at least 48 hours apart, is a requirement for a clinically asymptomatic patient to be discharged from the hospital.[22] The search is on for an ideal diagnostic assay which can immediately identify the patients who need quarantine and can discriminate them from the cases that do not need to be placed in quarantine.[1]

8. Treatment

Present treatment strategies are mainly symptomatic and supportive.[5] There is no proven *Ebola virus* specific treatment presently; however, there are certain measures which can be taken to improve a patient's chances of survival.[4]

In developing countries which have minimum health-care provision, the strategies include isolation, antipyretics, malaria treatment and broad spectrum antibiotics before diagnosis.[5] Fluid substitution, given by intravenous administration, and analgesics should be given as needed.[5] In developed health-care systems with appropriate isolation units, proper intensive care treatment can be advised and is to be mainly directed towards the maintenance of effective blood volume and electrolyte balance.[5] At present, no single strategy has proved successful in specific pre-exposure and post exposure treatment of *Ebola virus* infections in man .[5]

Case management is based on the isolation of patients and use of strict barrier nursing procedures, such as protective clothing and respirators.[5] Such procedures have been seen to be sufficient to rapidly interrupt the transmission in hospital settings even in rural Africa.[5]

The WHO declared that, taking into fact the magnitude and the severity of the present outbreak, it is considered ethical to use experimental drugs for treatment as well as prevention of EVD.[9] Due to the life-threatening situation during EBOV outbreaks and the lack of known treatments, experimental therapies are being deployed for compassionate use. [14]

9. Prevention

The United Nations (UN) made a rapid response to the current Ebola epidemic by creating a mission named as UN Mission for Ebola Emergency Response (UNMEER)

for effectively tackling this public health emergency.[1] The mission pooled and oriented vast resources of the UN agencies for reinforcing the WHO's technical expertise and experience in managing Ebola disease outbreaks.[1]

However, in the absence of an effective vaccine in hand, the only way left with global health agencies is to keep the spread of EBOV under check by strengthening and strictly implementing appropriate prevention and control measures, including regular monitoring, tracking, and surveillance of the circulating viruses, infected persons, potential suspects, and people inhabiting or visiting epidemic affected areas, especially African countries.[1]

The local and global strategies framed to combat the Ebola epidemic had involved state-of-the art early warning systems for tracking global movement of people travelling through outbreak-affected countries, rapid screening of the patients and suspects, rapid medical care to patients, safe disposal of dead persons and their discharges through dedicated health personnel, and disaster management planning and implementing agencies operating at national and international levels.[1]

Without an approved vaccine, prevention of EVD predominantly involves behaviour modification, proper personal protective equipment and sterilization.[3] This is further complicated by the fact that the natural reservoir of the virus remains unknown, thereby negatively impacting primary prevention.[16] In absence of primary prevention, epidemic management focuses mainly on educating the masses and instituting secondary strategies during outbreaks and in the aftermath.[16] The success of secondary prevention strategies requires good understanding of the public's views about Ebola as a disease.[16] The experience in developed countries has shown that with appropriate resources, case mortality of EV infection can be decreased.[14]

10. Vaccines

No licensed human or animal vaccine is currently available for the prevention and control of the fatal EVD.[1] Several experimental trials are ongoing in several parts of the world to find an effective vaccine to prevent EBOV infection and spread.[1] A protective vaccine will be very valuable not only for at-risk medical personnel, first responders, military personnel, and researchers, but even for targeted vaccination in the affected populations, especially during epidemics, for use in the ring vaccination strategy.[5]

A bivalent vaccine (adenovirus-based vaccine, cAdVax) has been reported to be developed against the Sudan and Zaire Ebola viruses (ZEBOV and SEBOV), using the GP genes of SEBOV and ZEBOV, and its trial in mice has shown 100% protection with effectively inducted virus-specific antibodies and cell-mediated immunity.[1] In non-human primates, a single intramuscular injection of the vaccine has induced both humoral and cellular immune responses.[1]

A vaccine based on the recombinant vesicular stomatitis virus (r-VSV) which expresses a single filovirus GP is found to be promising to protect nonhuman primates from the three species of *Ebola virus* infection.[1] VSV-based vaccines have also shown protection in macaques against the BEBOV, with results to indicate that complete protection requires an incorporation of BEBOV GP or a prime-boost vaccine regime.[1] The blended heterologous (SEBOV/ZEBOV) rVSV-based filovirus vaccine vectors, if used in the prime-boost approach, can provide protection.[1]

Vaccination strategy is also being tried with EBOV-like particles (eVLPs) like EBOV nucleoprotein (NP), glycoprotein (GP), and also the VP40 matrix protein.[1] In non-human primates, it has provided complete protection against challenge with lethal EBOV.[1] Based on safety and efficacy, the eVLPs have shown a way towards an effective EBOV vaccine for human use.[1]

The WHO has announced that the two promising candidate vaccines (chimpanzee adenovirus vaccine and VSV-based vaccine) which are currently undergoing phase III trials, are expected to be released by the end of 2015.[1]

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