1. Introduction

Filoviruses, including Ebola and Marburg viruses, are recognized as a significant warning to public health, since they cause periodic human and non-human primate outbreaks with high mortality rates. Ebola virus disease is a severe disease that causes hemorrhagic fever in humans and animals. Ebola virus disease are often fatal as they affect the body’s vascular system. This can lead to significant internal bleeding and organ failure. Ebola virus was first discovered in 1976 near the Ebola River in Zaire what is now the Democratic Republic of the Congo (DRC). Since then, outbreaks of Ebola along with humans have appeared sporadically in Africa. Currently Ebola viruses are found in several African countries. It appears that Filoviruses are zoonotic, that is, transmitted to humans from ongoing life cycles in animals. Despite numerous attempts to locate the natural hosts, the reservoir of filoviruses was undetermined until recently when Marburg virus and Ebola virus were detected in bats in Africa. In 2005, the first direct evidence from different studies that bats were reservoir hosts for Ebola virus was reported[1], and since then many researches have focused on studying the role that bats play in the maintenance, transmission, and evolution of filoviruses. Until now, there are a number of interesting reviews on the virology of Ebola viruses, ecology and vaccine development [2-4]. This paper is an review which shows biological aspects of Ebola virus, transmission with focusing on reservoirs hosts, the most recents Ebola virus outbreaks, clinical features, symptoms of Ebola, diagnosis tests, prevention and treatment. Epidemics in cynomolgus monkeys (Macaca fascicularis) occurred in this facility and others through 1992 [5,6], and recurred in 1996, as reported by Rollin et al [7]. The control of two introduced virus outbreaks in 1989 and the 1990s stimulated laboratory studies to improve diagnosis of human transmission when barrier-nursing
measures of nonhuman primate infections[7-9]. However, the utility of these techniques for human’s diagnosis were deficient. During 1994-1996, no less than five independent active of Ebola virus transmission were identified: Ivory Coast in 1994[10], DRC in 1995[11], and Gabon in 1994, 1995, and 1996[12-14]. Ebola virus was also circulating in Gabon[15], and at least 3 separate outbreaks in humans and nonhuman primates occurred[12]. Until now, many outbreaks have been occurred. This study focuses on recent outbreaks between 2000 and 2014.

2. Virology

Filovirus is a filamentous enveloped particle with variable lengths. The genomes are composed of a non segmented, negative-strand RNA molecule with approximatively 19 Kb[16,17]. Following their discovery, filoviruses were initially grouped with Rhabdoviruses, based primarily on the appearance of virus particles. Furthermore, subsequent morphologic, genetic, physiochemical, and virologic studies of Marburg virus and Ebola virus isolates revealed unique properties and led to their placement into a separate family, the Filoviridae[18]. Further the revised filovirus taxonomy of the nineth report of the International Committee on Taxonomy of Viruses includes proposals by Kuhn et al. and Kuhn et al[19,20].

There are seven expressed proteins by filoviruses: nucleoprotein (NP), glycoprotein (GP), RNA-dependent RNA polymerase (RdRPs), and four structural proteins: VP24, VP30, VP35, and VP40[3].

Ebola virus and Marburg virus are two currently recognized genera of Filoviridae family. Ebola virus is able to express a truncated soluble glycoprotein (sGP) through RNA editing. The ribonucleoprotein is derived from the RNA genome, NP, VP30, VP35, and VP40[3].

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Historically, viruses in the genus Filoviridae are associated with hemorrhagic fever diseases that are often fatal[22,23]. The open reading frame region coding for this protein was recently discovered in the bat genomes suggesting that VP35 was a decisive element int he host-virus interactions and immunity[23].

However, there are five identified Ebola virus strains, four of which are known to cause disease in humans: Zaire ebolavirus (EBOV-Z), Sudan ebolavirus (EBOV-S), Tai Forest ebolavirus, formerly Ivory Cost ebolavirus, and Bundibugyo ebolavirus. The fifth, Reston ebolavirus (EBOV-R), has caused disease in nonhuman primates, but not in humans. However, it can still be fatal in monkeys and it has been recently recovered from infected swine in South-east Asia. Marburg virus is a member of the species Marburgivirus (formerly Lake Victoria marburgivirus)[24,25].

In contrast to the known variety of Ebola virus species, EBOV-Z have only 2.7% nucleotide difference between sequences, EBOV-S 5.2%, and EBOV-R 4.5%[26,27]. Even with increasing numbers of detected viruses, some species are represented by single viral lineage (e.g., Tai Forest ebolavirus by Tai Forest virus and Lloviu cuevavirus by Lloviu virus) reported by Olival and Hayman[21]. However, taxonomic classification will change with the monitoring of Ebola virus, which depends on the human host, wildlife and genome sequencing in different affected communities.

3. Reservoir hosts

Ebola can cause disease in humans and nonhuman primates (monkeys, gorillas, and chimpanzees). The natural reservoir host of Ebola virus remains unknown. However, on the basis of evidence and the nature of similar viruses, researchers believe that the virus is animal-borne and that bats are most likely reservoir. Four of the five virus strains occur in an animal host native to Africa. Bats are a major source of zoonotic viruses worldwide[28,29]. Molecular studies have demonstrated that bats are natural host reservoirs for several recently emerged high-profile zoonotic viruses, including Ebola and Marburg hemorrhagic fever filoviruses[1,25], sudden acute respiratory syndrome–like Coronaviruses[30], rabies and rabies-related Lyssaviruses; and many Paramyxoviruses, including Rubulavirus; Nipah and Hendra viruses[31,32].

Indeed, there are a few experimental studies sustaining the role of bats as reservoirs, but two key studies have investigated the ability of bats to be infected with filoviruses and to survive after infection. Swanepoel et al. showed that EBOV-Z could replicate and lead to seroconversion without disease in three species of infected bats (Tadarida condylura, Trichopsis pumila, and Epomophorus wahlbergi) and that virus could be isolated from feces[33].

Using captive bred Rousettus aegyptiacus (R. aegyptiacus) bats of known serological and infection status; viremia could be induced and Marburg virus was detected in multiple tissues from 2 to 9 d post infection[34]. Following viremia, immunoglobulin G (IgG) antibody was detected until 21 d post infection. Marburg virus could also be detected in different tissues, including salivary glands, female reproductive tract, intestines, kidney, bladder, lung. No clinical symptoms or gross pathology have been showed. Nevertheless, it is interesting to note that studies in R. aegyptiacus could not induce infection after oral or intra-nasal inoculation but only after intradermal or intra-peritoneal inoculation. No virus was isolated from secretions.

The bats in Tadarida spp. used by Swanepoel et al., was inoculated by sub-cutaneous injection[33]. Authors observed fecal shedding in one individual. However the results of Paweska et al. study were revealed that R. aegyptiacus was a reservoir host, and no potential mechanisms for bat-to-bat transmission had been demonstrated[34]. Additional experiments underway using a captive Rousettus colony housed at Centers for Disease Control and Prevention Atlanta will likely shed more light on some of these unresolved issues[35].

Recently, Albarino et al. point out that the virus used by Paweska et al., was passaged almost 40 times in primate Vero cells prior to infecting bats, and it is not known how this may affect the infectivity or virulence of this virus[35,36]. Reverse genetics can now be used to reconstruct “wild type” filoviruses strains from genome sequences obtained directly from bats, even in the absence of a viral isolate, and may be a useful tool more relevant than using human or vero-adapted viruses to understand viral dynamics in bats.

The new establishment of bat cell lines[37], including those of the most likely primary reservoir host for filoviruses, Rousettus aegyptiacus[38], has been invaluable to further unravel the
molecular mechanisms of filovirus cell entry and host range in bats. A recent study expressing filovirus envelope glycoproteins on the surface of vesicular stomatitis virus suggest that Llovii virus glycoprotein allows viral entry into bat cells more easily than other filoviruses, and thus may be an exceptionally bat-adapted virus[11]. This finding of evidence for adaptation suggests that the bat mortality that prompted the discovery of Llovii virus may be less likely due to this highly adapted virus, although lyssaviruses are a prime example of host-adapted viruses that remain highly virulent to bat hosts[39]. Additional investigations of host range in vitro also using vesicular stomatitis virus expressing glycoprotein surface protein, found that Marburgvirus was able to infect six different bat cell lines from four divergent bat genera (Eidolon, Rhinolophus, Carolia, Tadarida)[40].

4. Human transmission and incubation

The virulence of filoviruses in humans is quite variable, depending primarily on the species or strain of filovirus; a similar variability seems to recaptulate well in nonhuman primates. Among the Ebola virus species, EBOV-Z is the most virulent, and EBOV-R appears to be the least virulent. Infection of nonhuman primates with EBOV-Z usually progresses rapidly and is uniformly lethal with as little as one infectious unit being required to cause disease. The course of disease is influenced by the dose of filovirus. For example, cynomolgus macaques exposed to intramuscular injection of a low dose of EBOV-Z (10 PFU) and succumbed to infection 8 to 12 d post infection, whereas those exposed to a high dose (1 000 PFU) died within 5 to 8 d post infection[41,42]. In humans, the disease path and the outcome depend on the route of infection. The mean incubation period for EBOV-Z due to infection was 6.3 d versus 9.5 d for contact exposures[43]. Nonhuman primate models appear to be exquisitely sensitive to filoviruses compared to humans, particularly for EBOV-Z. Fewer studies have evaluated the pathogenesis of EBOV-S in nonhuman primates. The disease course in experimentally infected rhesus and cynomolgus macaques appears much slower than that seen in EBOV-Z infections, and the rates of survival appear consistent with human disease. Neither EBOV-S infection nor EBOV-R was not lethal in a small cohort of African green monkeys[44].

When an infection does occur in humans, the virus can spread in several ways to others. Ebola is spread through direct contact (through broken skin or mucous membranes) with blood or body fluids (including but not limited to urine, saliva, feces, vomit, and semen) of a person who is sick with Ebola virus, contact with objects (like needles and syringes) that have been contaminated with the virus, and contact with infected animals. Ebola is not spread through the air or by water or food. However, in Africa, Ebola may be spread through handling bushmeat (wild animals hunted for food) and contacting with infected bats[45].

However during outbreaks of Ebola, the disease can spread very quickly within healthcare settings (such as a clinic or hospital). Exposure to Ebola can occur in healthcare settings where hospital staff do not wear appropriate protective equipments, including masks, gowns, and gloves and eye protection.

Dedicated medical equipment (preferable disposable, when possible) should be used by healthcare personnel providing patient care. Proper cleaning and disposal of instruments, such as needles and syringes, are also important. If instruments are not disposable, they must be sterilized before being used again. Without adequate sterilization in instruments, virus transmission can continue and amplify an outbreak. Semen may be infectious in survivors for up to 3 months. It is not entirely clear how Ebola outbreak is initially started[18]. The initial infection is believed to occur by contact with an infected animal’s body fluids. Airborne transmission has not been documented during Ebola virus disease outbreaks[46]. They are, however, infectious as breathable 0.8- to 1.2-µm laboratory generated droplets[47]. The virus has been shown to travel, without contact, from pigs to primates, although the same study failed to demonstrate similar transmission between non-human primates[48].

5. Epidemiology

EBOV-Z, EBOV-S, and EBOV-IC are from the African tropical forest or nearby savanna and occasionally emerge during the rainy season, and EBOV-R has only been linked to a single export facility in the Philippines[49]. EBOV-R may be an Asian filovirus, possibly derived from infected macaques captured in the forests of Philippines, but alternatively it could represent a single introduction and subsequent circulate in the export facility from Philippines or foreign African source. The epidemiology of human infections in nature, besides internationally recognized outbreaks, is unknown. However, elapsed time between the occurrence of index cases and the recognition of subsequent large outbreaks suggested that sporadic cases of unrecognized filovirus infections could readily pass unnoticed[50]. Ebola virus infection, potentially with nonpathogenic strains/variants or strains/variants of low pathogenicity, may be quite frequent in rural African populations[15,51].

Whatever the source of the initial index case is, filovirus outbreaks have been propagated from person-to-person. This generally involves intimate contact; secondary attack rates have not exceeded 10% to 15%, indicating that transmission is not efficient. However, this risk increases by means of contact. For example, during 1976 Sudan outbreak, 23% of family members sleeping in the same room with the infected patients, compared to 81% of persons providing active nursing care to a patient[52]. The necessary intimacy is reflected in mildly infected children[53]. Nosocomial transmission is a special problem, and hospitals have often served as a source of disease amplification into the community and to health care workers.

There is unusual difference in the epidemics in Gabon/DRC compared to those caused by other filoviruses, particularly Ebola virus outbreaks. Most of the epidemics in this area are limited in case numbers and are related to contact with wildlife (chimpanzees, gorillas, and other species). Epidemiologic and genetic investigations showed that outbreaks resulted from the introduction of distinct strains, indicating that multiple EBOV-Z strains were co-circulating in this region. All index cases (mainly hunters) were infected by handling dead or wounded animals, and subsequently led to person-to-person transmission within their families. In many instances, human infections have been preceded by disease in wildlife, and these infected animals acted as either dead-end hosts or interim/ amplification hosts[54,55]. Aerosol transmission has
not been unequivocally implicated in human outbreaks to date. Interestingly, extremely efficient person-to-person transmission has been attributed to two individuals who may have been the source of infection for more than 50 cases in the 1995 EBOV-Z outbreak [56]. The mechanism of this heightened transmission was not identified, though contact with the patient and/or cadaver was strongly implicated. From 2000, kinds of outbreaks have been observed in different countries: Uganda, Russia, DRC, Philippines and others. Most of them have caused numerous deaths in population as reported in Table 1.

Ebola outbreak in 2014 is the biggest Ebola outbreak in West Africa in history, affecting multiple countries in West Africa. A small number of cases in Lagos and Port Harcourt, Nigeria, have been associated with a man from Liberia who traveled to Lagos and died in Ebola, but the virus does not appear to have been widely spread in Nigeria. The case in Senegal is related to a man who traveled there from Guinea, reported by CDC [57].

The risk of an Ebola outbreak in the United States was very low; however CDC confirmed on September 30, 2014, through laboratory tests, that the first case of Ebola to be diagnosed in the United States in a person who had traveled to Dallas, Texas from West Africa. The patient did not have symptoms when leaving West Africa, but developed symptoms approximately five days after arriving in the United States.

Currently, cases of Ebola virus disease have been reported from at least in four European countries. In Norway, a woman infected by Ebola virus in Sierra Leone is receiving treatment in Oslo according to the Norwegian Medicines Agency. The France nurse, who was diagnosed with Ebola virus disease while engaging in healthcare work in Monrovia, Liberia’s capital, and was repatriated to France for treatment, has been confirmed to have recovered from the disease by Doctors Without Borders with a confirmation from the French Health Ministry that the woman had survived the disease. She was evacuated from Liberia and cared for at a hospital near Paris. The University Medical Center Hamburg-Eppendorf in Germany also reported that a Senegalese scientist who was infected with Ebola in Sierra Leone has recovered and been discharged. On 6 October, Spanish authorities reported a confirmed case of Ebola virus disease of a healthcare worker who participated in the treatment of the second Spanish patient with Ebola infection repatriated to Spain. The patient arrived in Spain on 22 September and died on 25 September. The infected healthcare worker represented the first transmission of Ebola infection in the European Union. The healthcare worker was a woman working in La Paz-Carlos III hospital in Madrid. She reportedly developed fever on 30 September. According to the Spanish Ministry of Health, she participated in the medical care of the repatriated patient and was wearing the appropriate personal protection equipment. She was admitted to La Paz-Carlos III hospital (Madrid) on 6 October and was under strict isolation [58].

6. Clinical features and symptoms of Ebola

Filovirus infections are generally the most severe and have been learned from close observations of acute human cases to a great extent. Differences in the clinical syndromes caused by filoviruses may exist, but there have been few opportunities for close observation of the diseases under favorable conditions [9,59]. The abrupt onset follows an incubation period of 2 to 21 d, averaging 4 to 10 d, and is characterized by flu-like symptoms (fever, chills, malaise and myalgia). Recovery from Ebola depends on the patient’s immune response. People who recover from Ebola infection develop antibodies that last for at least 10 years.

The subsequent signs and symptoms include systemic (prostration), gastrointestinal (anorexia, nausea, vomiting, abdominal pain, diarrhea), respiratory (chest pain, shortness of breath, cough), vascular (conjunctival injection, postural hypotension, edema), and neurologic (headache, confusion, coma) manifestations. There is often a maculopapular rash associated with various degrees of erythema by Day 5 to 7 of the illness; this is a valuable differential diagnostic feature and is usually followed by desquamation in survivors. Abdominal pain is sometimes associated with hyperamylasemia and true pancreatitis. In later stages, shock, convulsions, severe metabolic disturbances will happen in patients, and more than half the cases will diffuse coagulopathy.
Nonfatal cases get fever for about 5 to 9 d, and improvement typically occurs around Day 7 to 11 with notable humoral antibody response[60]. Convalescence is prolonged and sometimes associated with myelitis, recurrent hepatitis, psychosis or uveitis[61]. There is an increased risk of abortion for pregnant women, and a high death rate for children whose mothers are infected. Fatal cases develop with clinical signs early during infection, and demise typically occurs between Day 6 and 16, due to hemorrhage and hypovolemic shock. The single observed Ivory Coast infection survived, as did a second serologically diagnosed case. The four EBOV-R infected individuals had no symptoms, but the virus was isolated from the serum of one patient.

Generally, the most common symptoms experienced by persons infected with the virus are the sudden onset of fever, intense weakness, muscle pain, headache and sore throat. This is followed by vomiting, diarrhea, rash, impaired kidney and liver function, and at advanced stage, both internal and external bleeding. Laboratory findings include low white blood cells and platelet counts and elevated liver enzymes. People are infectious as long as their blood and secretions contain the virus[46].

7. Diagnosis tests

Despite the capabilities of laboratory diagnostics, it should be kept in mind that initial diagnosis of filovirus infections will be based on clinical assessment.

Special care should be taken to avoid needle sticks and to immediately dispose of contaminated material in an appropriate manner. Collection of specimens should be done with a mind toward sterility and prevention of cross-contamination of specimens; this has become particularly important for ultrasensitive techniques such as reverse transcriptase-polymerase chain reaction (RT-PCR)[62,63]. Filoviruses are relatively stable, and infectious particles can survive less than favorable handling and shipping. Care should be taken to ensure the physical integrity for biosafety reasons and to maintain an adequate refrigerated or frozen state for biological integrity of the sample to maximize the reliability of diagnostic results.

Laboratory diagnosis of filovirus infections can basically be achieved in two ways: measurement of host-specific immune responses to infection, and detection of virus particles or particle components (RNA and protein) in infected individuals. RT-PCR[9,64], and antigen detection ELISA[65], are the primary test systems to diagnose acute infection. For antibody detection, the most commonly used assays are direct IgG and immunoglobulin M (IgM) ELISAs and IgM capture ELISA[66,67]. RT-PCR, antigen detection, and serology can be performed on materials that have been rendered sterile and prevention of cross-contamination of specimens; this has become particularly important for ultrasensitive techniques such as reverse transcriptase-polymerase chain reaction (RT-PCR)[62,63]. Filoviruses are relatively stable, and infectious particles can survive less than favorable handling and shipping. Care should be taken to ensure the physical integrity for biosafety reasons and to maintain an adequate refrigerated or frozen state for biological integrity of the sample to maximize the reliability of diagnostic results.

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In the literature, there are many RT-PCR protocol for the detection of filovirus which are practical and simple to implement in a laboratory of molecular biology[9,64,68,69]. However, the greatest problem in using these techniques is the lack of positive control to validate manipulations and diagnostics. Biosecurity regulatory restrictions make it almost impossible to obtain strains of Ebola or Marburg for laboratories especially for those in underdeveloped countries. Another alternative is then presented for such laboratories by employing a ready to use kit provided by international firms and including positive control. Thus in our lab for example, we have chosen to use the “quantification of Ebola virus (2014 outbreak)” kit developed by Primer Design Ltd company. The primers and probes of this kit target the nucleoprotein gene of the 2014 Ebola virus strain. We use this kit on the light cycler 2.0 from Roche® diagnostics or on the Applied biosystems® 7500. Several other molecular kits are available like “Ebola Virus (Zaire 2014) assay and control set” from applied biosystems® or “ZEOV Kit” from LIPSGENE®.

Serology can be useful for confirmation. However, a negative serology is inconclusive because filovirus-infected individuals often die without seroconversion. Based on past investigations, IgM antibodies can appear as early as 2 d post onset of symptoms and disappear between 30 and 168 d after infection. IgG-specific antibodies develop between Day 6 and 18 after onset and persist for many years[70]. A rising IgM or IgG titer constitutes a strong presumptive diagnosis. However, a single positive result should be confirmed on a follow-up sample, preferably a week later. Decreasing IgM and/or increasing IgG titers (fourfold) in successive paired sera are highly suggestive of a recent infection[70]. Standardization and evaluation of diagnostic procedures for filoviruses is difficult because of the restricted availability of virologic and clinical material. Recently, the European network for imported viral diseases provided an external quality assurance for filovirus PCR diagnostic procedures[71]. Although most laboratories in this study showed reasonable abilities to detect filoviruses by PCR technology, a small but significant fraction of laboratories demonstrated poor sensitivities.

According to CDC, diagnostic tests depend on timeline of infection. The diagnosis tests are different. Thus within a few days after symptoms begin, the recommended tests are different. The available tests are Antigen-capture ELISA testing, IgM ELISA, RT-PCR and Virus isolation. Later in disease course or after recovery, the diagnosis can be done by testing IgM and IgG antibodies. Retrospectively in deceased patients, available tests are immunohistochemistry testing, PCR, and virus isolation.

8. Prevention and treatment

Vaccination offers a promising intervention to prevent infection and limit spread. Vaccines have protected nonhuman primates.
Immunization takes six months, which impedes the counterepidemic use of the vaccines. The most promising candidates are DNA vaccines[72], or vaccines derived from adenviruses[42], vesicular stomatitis Indiana virus[73,74], or filovirus-like particles[75], because these candidates could protect nonhuman primates from Ebola virus-induced disease. DNA vaccines, adenovirus-based vaccines, and vesicular stomatitis virus-based vaccines have entered clinical trials[76,77]. However, a combination of DNA immunization and vesicular stomatitis virus-based vaccines have entered clinical trials[76,77]. However, a combination of DNA immunization and boosting with adenoviral vectors that encode viral proteins generated cellular and humoral immunity in cynomolgus macaques. Furthermore, some approaches have shown promising efficacy in macaque models of filoviruses, and some of them have completed or are at least nearing phase 1 clinical trials in humans[78].

Treatment approaches involving modulatory RNA (i.e. small interfering RNAs or phosphorodiamidate morpholino oligomers) are following close behind, along with a promising synthetic druglike small molecule, BCX4430[79]. The most promising vaccine approaches are based on recombinant technologies, such as viruslike particles produced through plasmid transfection and replication-incompetent and -competent viral vectors[78]. The current frontrunner for therapeutic intervention seems to be antibody treatment, which has been successful in macaques even when antibodies are administered more than 72 h after infection[50].

There is currently no specific licensed treatment for Ebola virus disease. Patients are treated for their symptoms. Treatment options include supportive care in an intensive care unit, maintenance of fluid levels and electrolytes, maintenance of oxygen status and blood pressure, replacement of lost blood and clotting factors and strict isolation to prevent the infection from spreading.

Researchers have investigated Ebola virus for many years. However, there is still not an effective vaccine that is able to be administered to those who are infected with the virus. Even if there are many candidate vaccines that have displayed very effective results on non-human primates, many of these potential cures may become a future vaccine to help save victims’ lives. Under the persistence and efforts of researchers in vaccine development, it is hopeful that related vaccine will be initially available in November or December 2014.

Conflict of interest statement

We declare that we have no conflict of interest.

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