Global Outbreaks of Ebola Virus Disease and Its Preventive Strategies

Ameer Khusro and Chirom Aarti

Abstract Ebola virus disease (EVD) has always been a challenge for humankind since its discovery. Ebola virus is transmitted from human-to-human via direct contact with the body fluids of infected people, and with surfaces and materials contaminated with these fluids. Fever, profound weakness, diarrhoea, cramping, abdominal pain, nausea, and vomiting are typical symptoms of EVD. The African fruit bat (*Rousettus aegyptiacus)* is considered the natural reservoir for EVD. Ebola virus epidemic emphasized the necessity for developing auspicious antiviral drugs and vaccines to combat the disease. Over the past two decades, few effective vaccines have been developed using DNA, virus-like particles, recombinant viral vectors, and recombinant proteins. These vaccines were tested in rodents and non-human primates which showed high efficacy towards the prevention of EVD. Currently, rVSV-ZEBOV vaccine is the only approved vaccine for EVD based on its high efficiency rate. In addition, few vaccine candidates are in preclinical stage. Considering the dearth of promising antiviral drugs and approval of limited vaccines for humans, prevention is a leading strategy to eradicate EVD at present. This chapter highlights not only the biology and pathogenesis of EVD but also discusses the epidemiology, disparate vaccines development, and other common preventive strategies to combat EVD outbreak in future.

Keywords Ebola virus · Ebola virus biology · Ebola virus disease · Epidemic · Non-human primates · Outbreak · Pathogenesis · Prevention · Transmission · Vaccination

A. Khusro (✉) · C. Aarti
Research Department of Plant Biology and Biotechnology, Loyola College, Chennai 600034, TN, India

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1 Introduction

Ebola virus disease (EVD) is considered as one of the deadliest diseases to humankind because of its high mortality (Wiwanitkit 2014). This disease was first discovered in 1976 by Dr. Peter Piotin in Zaire, Africa (now Democratic Republic of Congo) from the blood of a catholic nun who was a suspected case of yellow fever (Centres for Disease Control and Prevention 2015). It was formerly called as Ebola haemorrhagic fever and has been a colossal challenge since then for communities worldwide. It is a zoonotic disease which is transmitted accidentally due to direct contact with infected live or dead animals. Ebola virus contains a lipid-enveloped negatively stranded ribonucleic acid (RNA) virus that belongs to the family Filoviridae (World Health Organization [WHO] 2015). Ebola haemorrhagic fever is caused by any of five genetically different members of the Filoviridae family: *Zaire ebolavirus* (ZEBOV), *Sudan ebolavirus* (SEBOV), *Côte d'Ivoire ebolavirus*, *Bundibugyo ebolavirus* (BDBV), and *Reston ebolavirus* (REBOV). Le Guenno et al. 1995 in the Côte-d'Ivoire had isolated a new strain of Ebola *Côte d'Ivoire ebolavirus* from a non-fatal human case infected during the autopsy of a wild chimpanzee. It was suggested that this animal has been responsible for the outbreaks of haemorrhagic syndromes. This is the first time that a human infection has been connected to naturally-infected monkeys in Africa. REBOV infects non-human primates (NHPs) only (Barrette et al. 2009). ZEBOV, SEBOV, and BDBV account for most of the Ebola haemorrhagic fever epidemic but ZEBOV is threat to both human and NHPs in sub-Saharan Africa (Groseth et al. 2007). Recent EVD outbreak began in the republic of Guinea in February 2014 and spread to the republic of Liberia and the Sierra Leone (Enserink 2014).

Now we understand that the virus is transmitted to people from wild animals including fruit bats, porcupines, and NHPs, and then spreads in the human population through direct contact with the secretions such as, saliva, mucus, vomit, faeces, sweat, tears, blood, breast milk, urine, and semen or other bodily fluids of infected people, and with surfaces and materials (e.g. bedding, clothing) contaminated with these fluids (Moghadam et al. 2015).

The African fruit bat (*Rousettus aegyptiacus)* is considered the natural reservoir for Ebola virus. These bats transmit the virus to apes, monkeys, and other animals' species of forest. Humans living in these areas come in contact with such infected animals and transmit the infection (Rajak et al. 2015) (Fig. 1).

The incubation period of the virus is 2 to 21 days. Symptoms of EVD include fever, fatigue, muscle pain, headache, and sore throat followed by vomiting, diarrhoea, rash, impaired kidney and liver function, low white blood cell and platelet counts, and elevated liver enzymes (Fig. 2).

Unfortunately, the symptoms of EVD are similar to malaria, typhoid fever, and meningitis which make it difficult to diagnose in the primary stage of infection. The present chapter summarizes not only various essential aspects of EVD such as virus biology, pathogenesis, and epidemiology but also role of vaccination towards its prime preventive strategies in future outbreak.

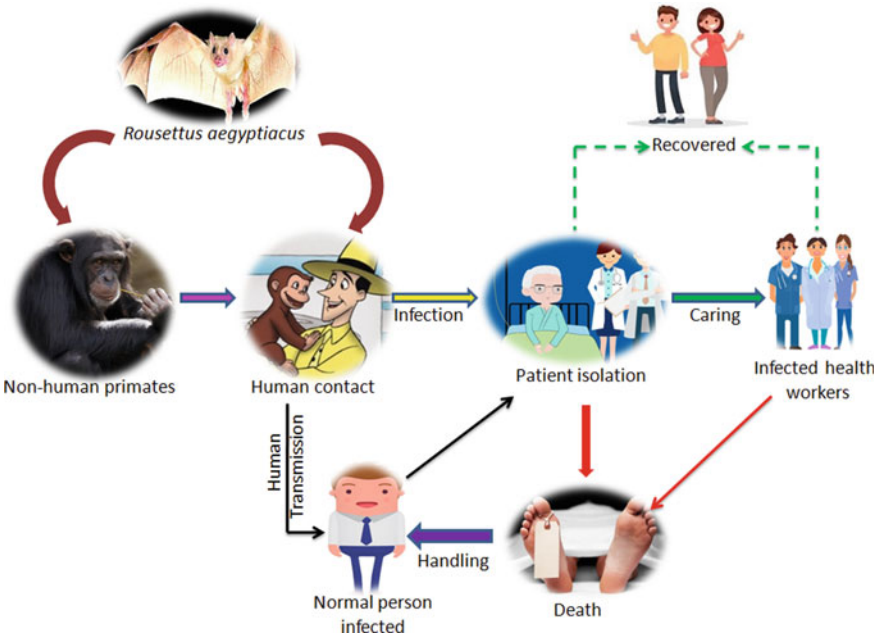

Fig. 1 Transmission cycle of EVD

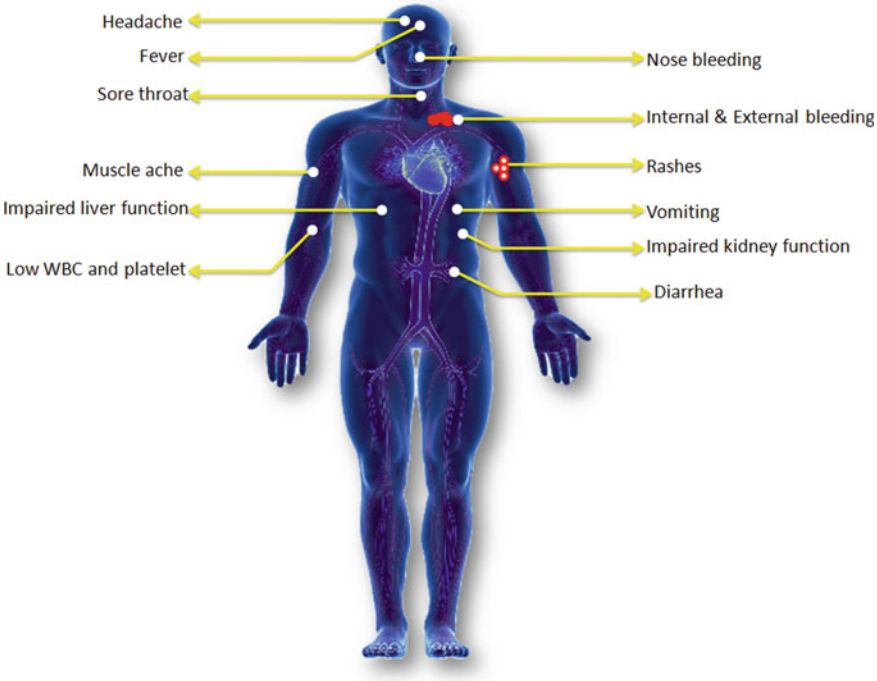

Fig. 2 Symptoms of EVD

2 Ebola Virus Biology

Ebola virus is an enveloped virus containing non-segmented negative-strand ribonucleic acid (RNA) genome of approximately 19 kb.

The viral genome encodes seven different genes which are known to encode nine proteins: nucleoprotein (NP), polymerase cofactor (VP35), matrix protein (VP40), glycoprotein (GP), soluble GP (sGP), small soluble GP (ssGP), transcription activator (VP30), minor matrix protein (VP24), and RNA-dependent RNA polymerase (L) (Fig. 3).

The genome shows following structure: 3'-leader→*nucleoprotein (NP)* gene→*viral protein (VP) 35* gene→*VP40* gene→*glycoprotein (GP)* gene→*VP30* gene→*VP24* gene→*polymerase (L)* gene→5'-trailer. Genome replication promoters and packaging signals are present in conserved leader and trailer regions. Each gene is flanked by 3' and 5' untranslated regions (UTRs) including conserved transcriptional start and stop signals. In spite of overlapping of few genes in UTRs, most genes are separated by intergenic regions of varied lengths. All genes are monocistronic except *GP*, which encodes three GP. The sGP is the preliminary product of *GP*. The virus down-regulates transmembrane GP expression produces a second small ssGP through RNA editing by polymerase complex (Furuyama and Marzi 2019) (Fig. 4).

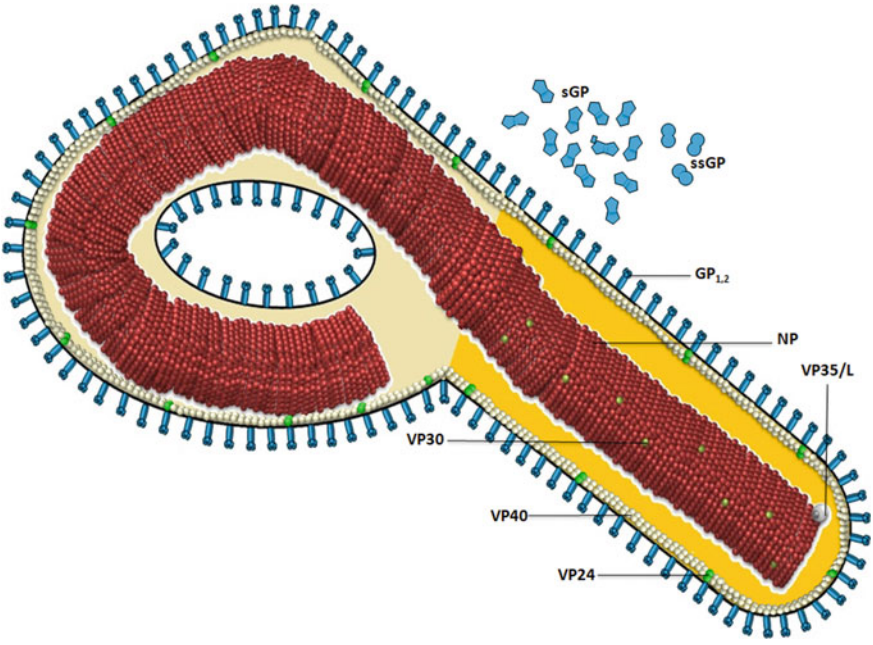

Fig. 3 Structure of Ebola virus showing various proteins

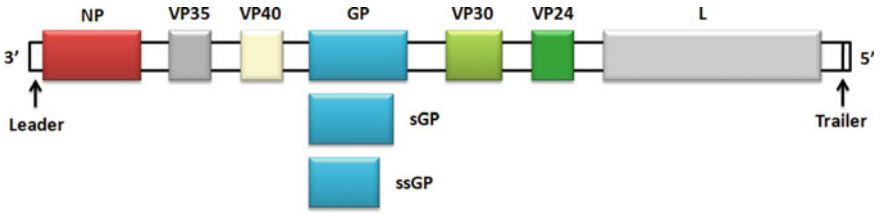

Fig. 4 Ebola virus genome displaying 3'-leader→*nucleoprotein (NP)* gene→*viral protein (VP) 35* gene→*VP40* gene→*glycoprotein (GP)* gene→*VP30* gene→*VP24* gene→*polymerase (L)* gene→5'trailer

The size of Ebola virus particle i.e. virion varies from 50 to 80 nm. The length of virus ranges from 10000 to 14000 nm. The most common shape of the virus is filamentous. Both the sGP are non-structural proteins and are secreted from infected cells. The membrane-associated matrix protein (VP40) forms the filamentous structure of viral particles and connects the nucleocapsid with the host-derived lipid membrane (Hoenen and Feldmann 2014). The virus proteins perform distinct function in the replication cycle (Banadyga et al. 2016).

Nucleoprotein is a prime constituent of ribonucleoprotein complex (RNP) which is known to encapsidate the genome and protects RNA degradation. It is composed of three major domains: N-terminal, core (located in the centre), and C-terminal domains. N and C-terminal as well as lobes of the core domain recognize and clamp to RNA binding groove regions. NP oligomerization and NP tube structure formation are mainly carried out by N-terminal portion (Rojas et al. 2020).

VP35 (a polymerase cofactor) is a phosphoprotein which interferes with the early innate response of host. VP35 interacts with NP and forms VP35-NP complex. This complex is known to prevent premature NP oligomerization and causes variations in NP in viral RNA synthesis machinery. VP30 is a transcriptional activator which supports primary transcription and RNA editing. It plays pivotal role in the stimulation of RNA synthesis. Leucine-zipper motif is present in N-terminal region which helps in homo-oligomerization (Hartlieb et al. 2007). The basic amino acid cluster of C-terminal region interacts with NP and control VP30 activity (Kirchdoerfer et al. 2016). This protein is not essential for the replication of virus.

The RNA-dependent RNA polymerase L carries the enzymatic functional domains for genome transcription and replication. It also causes activation of RNA synthesis and edits messenger RNA (mRNA). It binds to genomic RNA at 3′ leader promoter and alters virus negative-sense RNA into positive-sense messenger RNA for producing new virions. The L protein is responsible for mRNA polyadenylation and capping too because of its catalytic nature (Martin et al. 2017). The editing trait of L protein leads to generate GP transcript and regulates proteins expression (Volchkova et al. 2011).

VP24 plays an important role in RNP assembly. The protein-protein interaction is carried out by N-terminal region of this protein and leads to the formation of

capsid. Protein aggregation occurs due to the mutation in this particular region (Han et al. 2003). C-terminal deletion inhibits the formation of nucleocapsid-like structure formation, thereby indicating the importance of both domains in the assembly of nucleocapsid (Han et al. 2003). VP24 interacts with NP via conserved loop by depending on VP24 amino acids (V170 and N171) that plays important role in nucleocapsid condensation, thereby producing new virions (Banadyga et al. 2017). VP24 determines the virulence property of virus and inhibits IFN-α/β/γ trigger by sequestering proteins belonging to the karyopherin α (KPNA) family viz. KPNA α1, α5, and α6 proteins, thus, inhibiting activated signal transducer and activator of transcription 1 (STAT1) (Reid et al. 2006). VP24 also binds to STAT1 and inhibits its phosphorylation, nuclear transport, and interferon (IFN)-associated gene activation (Zhang et al. 2012). VP24 is also known to inhibit the production of IFN via both Interferon regulatory factor 3 (IRF3) and early tumour necrosis factor (TNF)-induced nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) signalling (Guito et al. 2017).

The coordination of virion assembly, maintenance of structural integrity, and budding of viral particles from infected cell is performed by VP40 (Pleet et al. 2017). The N-terminal domain of VP40 helps in dimerization and oligomerization in the cytoplasm and translocates to the plasma membrane. The C-terminal domain causes the interaction with the plasma membrane. Additionally, this protein induces exosomes formation (Madara et al. 2015).

Glycoprotein is essential in viral pathogenesis. It is the only observable protein on the surface of virus. It initiates the entry of virus into the host by fusion with receptor (Lee and Saphire 2009). The GP gene encodes three different proteins: GP with two subunits—GP1 (for receptor binding) and GP2 (for viral fusion); sGP lacks transmembrane domain and is formed from the unedited transcript. The third protein is called as ssGP and its activity is undetermined (Mehedi et al. 2011). The fusion between virus and target cell is generally mediated by GP1. GP1 contains three domains: the receptor binding domain (RBD) (interacts with one or more receptors), the glycan cap (protects the RBD from antibody recognition and interacts with GP2 to prevent premature fusion), and the O-linked glycosylated mucin-like domain (protects RBD from immune recognition and has a cytotoxic influence through extracellular signal-regulated kinase and mitogen-activated protein kinase pathways) (Zampieri et al. 2007). GP2 causes the fusion of membrane. It contains five domains: the N-terminal heptad repeat region (HRR), C-terminal HRR, fusion loop, the transmembrane region, and the cytoplasmic tail (Mohan et al. 2012). The membrane fusion is carried out by fusion loop due to the presence of a hydrophobic sequence. Subsequently, GP2 HRR forms a transmembrane six-helix bundle that serves as an opening in the membrane (Malashkevich et al. 1999). sGP is known for evading the immune system (Mohan et al. 2012).

3 Ebola Virus Pathogenesis

Ebola virus enters into the host cells by interacting with fluids of infected body through mucous membranes or skin lesions (Rojas et al. 2020). In vivo studies in NHPs models revealed that endothelial cells, adrenal cells, immature dendritic cells (DCs), monocytes, macrophages, and Kupffer cells are mainly infected by virus in the liver because of the potentiality of virus GP1 to interact with varied host-cell proteins (Hensley et al. 2011). Several attachment factors have been reported and may cause binding of the virus.

Lectins viz. C-type lectins dendritic cell-Specific intercellular adhesion molecule-3-grabbing non-integrin (DC-SIGN), and L-SIGN are prime receptors for virion attachment (Matsuno et al. 2010). The increased infectivity of Ebola virus may be because of an increase in the glycan extension on the viral GP from the virus, which shows high affinity for the "macrophage galactose-type calcium-type lectin" (Fujihira et al. 2018). Other co-receptors such as β1-integrin (Schornberg et al. 2009), folate receptor-α (Chan et al. 2001), glycosaminoglycans (O'Hearn et al. 2015), Tyro3, Axl, and Mer (TAM) receptor tyrosine kinases (Dahlmann et al. 2015), and T-cell immunoglobulin and mucin 1 (TIM-1) (Dahlmann et al. 2015) are also responsible for virus entry. After binding, the virion is taken up into the target cells by macro-pinocytosis (a non-specific endocytosis mechanism) (Aleksandrowicz et al. 2011). The host cell membrane interacts with the phosphatidylserine present on the virion and stimulates the reorganization of cytoskeleton, triggers the ruffling of the plasma membrane, and invagination of the virion with further internalization into the early endosome (Moller-Tank and Maury 2015). After host-cell membrane fusion process, the nucleocapsid is released into the cytoplasm. At this stage, mRNA is formed from virus RNA via translation mechanism. Virus proteins viz. GP, NP, VP24, VP35, VP40, and L are then translated from viral mRNA by the host machinery. After that, NP, VP35, and L proteins act as a viral polymerase complex and allow the replication of viral genome (Muhlberger 2007). The new virion is released due to the interaction of VP40 with the host machinery (Jasenosky and Kawaoka 2004) (Fig. 5).

The secretion of pro-inflammatory cytokines such as interleukin 6 (IL-6), TNF, and IFN-β as well as the release of tissue factor and vasoactive peptides are enhanced due to the infection of Ebola virus (Rogers and Maury 2018). Additionally, the virus infection shows impairment in DC activation by the action of VP35 and VP24. Ebola virus-infected cells lack type-I IFNs production and does not show maturation process (Bosio et al. 2003). Ebola virus hijacks IFN-I response by inhibiting its production and by blocking its signalling mechanism, and thus, causes impairment in the maturation of DCs, thereby inactivation of adaptive immune response. Ebola virus primarily targets lymphoid organs. As a matter of fact, the virus initially infects DCs and macrophages, and these immune cells guide the virus towards the proximal lymph nodes and infect those (Steele et al. 2009). As per in vivo NHPs models study, the virus destructs lymphocytes, follicular

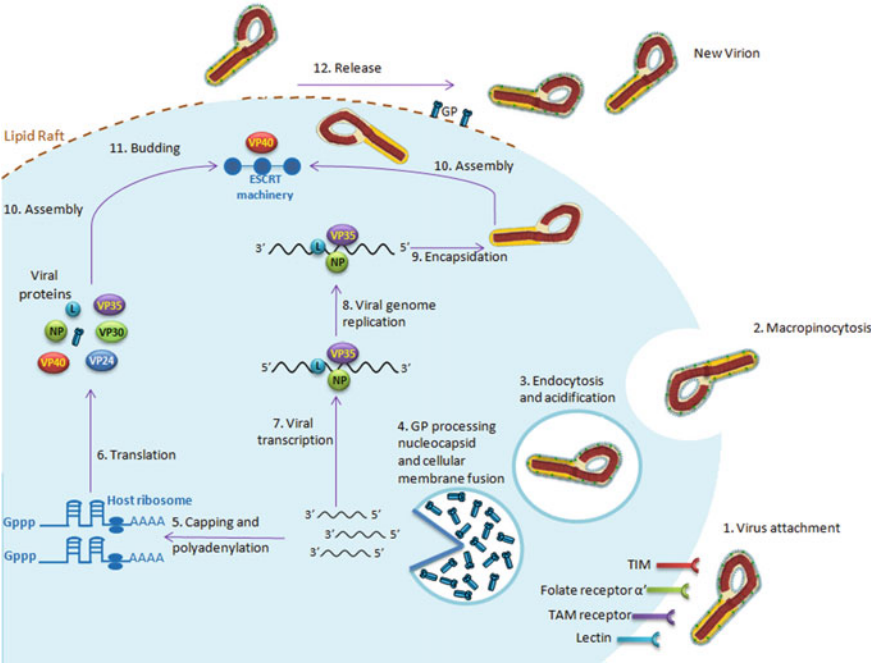

Fig. 5 Ebola virus replication cycle showing the binding of the host cell through different co-receptors, followed by its invasion into the host cytoplasm and transcription of viral RNA into protein. Further, the viral genome is replicated, followed by encapsidation, assembly, and release of a new virion. [*Note* ESCRT: Endosomal sorting complexes required for transport; GP: Glycoprotein; TIM-1: T-cell immunoglobulin and mucin domain 1; TAM receptors: Tyro3, Axl, and Mer receptors; VP: Virion protein; and NP: Nucleoprotein]

structure, and parenchyma (Twenhafel et al. 2013). The virus is known to disrupt vascular endothelium too, causing bleeding, partial blood circulation, and intravascular coagulation blood-tissue barrier permeability (Rasmussen 2017).

4 Epidemiology of Ebola Virus Disease

From 1976 to 2013, the WHO reported 2387 confirmed cases with 1590 deaths. The Ebola virus epidemic in West Africa is considered to be the largest outbreak, which caused thousands of mortalities in Guinea, Sierra Leone, and Liberia. In March 2014, the WHO reported a major EVD outbreak in Guinea, a West African nation. The disease rapidly spread to the neighbouring countries of Liberia and Sierra Leone. It was the largest EVD outbreak ever documented, and the first recorded in the region. On 8 August 2014, the WHO declared this epidemic an international public health emergency (Moghadam et al. 2015). On 29 December

2015, Guinea was declared free of Ebola virus transmission. On 14 January 2016, Ebola virus was detected again in Sierra Leone but emergency was not declared. On 11 May 2017, the DRC Ministry of Public Health notified the WHO about an outbreak of Ebola which killed 4 people. On 2 July 2017, the WHO again declared the end of the outbreak. On 1 August 2018, the world's 10th EVD outbreak was declared in North Kivu province of the Democratic Republic of the Congo. By November 2018, nearly 200 Congolese died of Ebola. By March 2019, this became the second largest EVD outbreak ever recorded, with more than 1000 cases. As of 4 June 2019, the WHO reported 2025 confirmed and probable cases with 1357 deaths. In June 2019, 2 people died of EVD in neighbouring Uganda. On 17 July 2019, the WHO declared the EVD outbreak a global health emergency (World Health Organization 2019).

From 13 to 19 January 2020, 15 new confirmed cases of EVD were reported from North Kivu Province in the Democratic Republic of the Congo. From 30 December 2019 to 19 January 2020, 37 confirmed cases were reported from 12 of the 87 health areas in 6 active health zones in North Kivu and Ituri Provinces. As of 19 January 2020, a total of 3414 EVD cases, including 3295 confirmed and 119 probable cases have been reported, of which 2237 patients died. Of the total confirmed and probable cases, 56% were female, 28% were children aged less than 18 years, and 5% were health workers. As of 10 March 2020, a total of 3444 EVD cases were reported, including 3310 confirmed and 134 probable cases, of which 2264 patients died. Of the total confirmed and probable cases, 56% were female, 28% were children aged less than 18 years, and 5% were health care workers. As of 30 March, 2020, a total of 3453 cases (3310 confirmed and 143 suspected) were reported which includes 2264 deaths, 1169 survivors, and rest of the patients are still under care (World Health Organization 2020).

5 Ebola Virus Vaccination

Over the past few centuries, vaccination has been considered as a prime strategy to prevent and control outbreaks of certain deadly human diseases. The development of efficacious vaccine for EVD was started in the late 1970s. The inactivated virus based vaccine was the first vaccine developed against EVD, which prevented guinea pigs from this deadly infection (Lupton et al. 1980). Since then, a variety of vaccines has been developed against EVD including deoxyribonucleic acid (DNA), virus-like particles (VLPs), recombinant viral vectors, and recombinant proteins (Reynolds and Marzi 2017). The effectiveness of those vaccines was assessed in rodents or NHPs (Reynolds and Marzi 2017). At present, 8 vaccine candidates are in clinical trials stages which target viral GP (Table 1).

Plasmid encoding sGP and GP were used for the development of first DNA vaccine for EVD. The vaccine was known to elicit humoral and T cell responses (Xu et al. 1998). This vaccine is safer to use, produce, and shows advantages over live attenuated vaccines. Previous study revealed that the administration of DNA

Table 1 Vaccine candidates in clinical trial phases

Vaccines	Class	Immunogen	Trial phases
DNA	DNA	GP and NP	Phase 1
Protein	Subunit	GP	Phase 1
Ad26	Replication-deficient virus expressing EBOV protein	GP	Ad26/cAd3—phase I Ad26/MVA—phases I, II, III
Ad5	Replication-deficient virus expressing EBOV protein	GP	Ad5—phases I, II Ad5/VSV—phases I, II, IV
chAd3	Replication-deficient virus expressing EBOV protein	GP	chAd3—phases I, II chAd3/MVA—phase I
MVA	Replication-deficient virus expressing filovirus protein	GP and NP	Ad26/MVA—phases I, II, III chAd3/MVA—phase I
VSV	Replication-competent virus expressing EBOV protein	GP	Phases I, II, III
HPIV3	Replication-competent virus expressing EBOV protein	GP	Phase I

Abbreviation: Ad—adenovirus; chAd—chimpanzee adenovirus; GP—glycoprotein; HPIV3—Human parainfluenza virus 3; MVA—modified vaccinia Ankara; NP—nucleoprotein; VSV—vesicular stomatitis virus

vaccine encoding viral GP and NP protected all the mice from EVD (Vanderzanden et al. 1998). In another study, the vaccination of DNA vaccine with optimized antigen expression showed 83% efficiency in NHPs (Grant-Klein et al. 2015). A combination of DNA prime together with an adenovirus boost (both encoding viral GP) exhibited 100% protection in NHPs (Sullivan et al. 2000). The Ebola virus GP-based DNA vaccination depicted protection to NHPs (Patel et al. 2018).

Ebola VLPs have also been used for the development of vaccine by co-expressing Ebola virus GP and VP40 in transfected cells. The VLP vaccination stimulated NK cells for protecting against the virus (Warfield et al. 2004). Additionally, VLP vaccination induces host responses through Toll-like receptor and type I IFN signalling, thereby leading to the initiation of early innate protective immune responses (Ayithan et al. 2014). VLPs containing VP40 and GP showed 100% protection against viral infection in rodents (Swenson et al. 2005). The vaccination of VLPs along with RIBI adjuvant stimulated viral GP-specific antibodies and strong T cell responses (Warfield et al. 2007).

The replication-deficient Ebola virus lacking the VP30 gene (EBOV_VP30) is a subunit vaccine. Study showed that the administration of mice with EBOV_VP30 showed robust Ebola virus GP-specific antibody and Ebola virus NP-specific T cell responses with high survival rate of animals. Guinea pigs immunized twice with EBOV_VP30 showed protection from Ebola virus (Halfmann et al. 2009). In

another report, the immunization of NHPs with EBOV_VP30 showed protection from the infection (Marzi et al. 2015b).

The viral vectors encoding Ebola virus GP is another EVD vaccine in preclinical stage. A replicon system based on Venezuelan equine encephalitis virus (VEEV) is effective against Ebola virus. VEEV replicons expressing Ebola virus GP protected guinea pigs and mice from Ebola virus infection (Pushko et al. 2000).

Human adenovirus serotype 1, serotype 2, and serotype 5 (Ad5) cause mild upper respiratory tract infections. Replication-deficient human Ad5 has been used as a vaccine candidate for EVD. Ad5 stimulates cellular and humoral immunity against the encoded antigen. Replication-deficient recombinant Ad5 revealed protection in NHPs against viral infection (Sullivan et al. 2003).

The vaccination of Ad26-Ebolavirus showed effectiveness against Ebola virus in NHPs. Further, its effectiveness was enhanced when used with an Ad35-Ebola virus boost (Geisbert et al. 2011). In another study, a single dose of recombinant chAd3 expressing Ebola virus GP depicted 100% protection in NHPs against EVD. Unfortunately, the protection reduced to 50% when animals were challenged 10 months post vaccination. Hence, a chAd3- Ebola virus prime was boosted with a modified vaccinia Ankara (MVA) vector encoding the Ebola virus GP (MVA-BN Filo) for improving the protection (Stanley et al. 2014).

Vesicular stomatitis virus (VSV) belongs to *Rhabdoviridae* family and infects livestock as well as other animals. It causes mild infection in humans (Letchworth et al. 1999). Thus, a VSV-based vaccine is considered crucial for protection against EVD. The VSV-G coding sequence was replaced in the wild-type VSV genome with a sequence encoding Ebola virus GP for engineering recombinant VSV-Ebola virus vaccine (also called as rVSV-ZEBOV) (Mire et al. 2012). rVSV-ZEBOV showed high protection in NHPs (Marzi et al. 2015a). Headache, fatigue, and muscle pain were commonly reported as adverse events in this period across all age groups. Overall, rVSV-ZEBOV offered substantial protection against EVD.

Recombinant rabies virus (RABV) has also been used for developing EVD vaccine. The vaccine showed complete prevention against Ebola virus in NHPs (Blaney et al. 2013). However, a replication-deficient or an inactivated version of this vaccine candidate showed only 50% protection (Blaney et al. 2013). The inactivated RABV vaccine vector was improved using a codon-optimized antigen which showed complete prevention against Ebola virus (Willet et al. 2015).

The EVD vaccine was also developed using recombinant paramyxovirus-based vectors. Multiple GPs were inserted into the genome of Human parainfluenza virus 3 (HPIV-3) (Skiadopoulos et al. 2002). Recombinant HPIV-3 expressing viral GP alone or along with NP prevented guinea pigs against EVD (Bukreyev et al. 2006). Two-dose immunization process using this very vaccine showed protection in NHPs (Bukreyev et al. 2007). Mucosal (IN/intratracheal) administration of the HPIV-3-Ebolavirus GP vaccine prevented NHPs from EVD (Meyer et al. 2015).

Prime-boost regimen with a combination of the Ad26-Ebolavirus and MVA-BN Filo vaccines are in clinical trial phases. In one phase I trial, immunization with Ad26-Ebolavirus or MVA-BN Filo did not show any side effect (Milligan et al. 2016). GamEvac-Combi, the combination vaccine of VSV-Ebola virus and

Ad5-Ebola virus was developed in Russia. The vaccine induced viral GP-specific antibodies in 95% of the volunteers, and neutralizing antibodies were detected in 93.1%. Furthermore, CD4+ and CD8+ T cells were detected in 82.8% and 58.6% of participants, respectively (Dolzhikova et al. 2017). This vaccine is being tested in an ongoing phase IV clinical trial in Guinea and Russia.

After EVD epidemic, several human clinical trials with VSV-Ebola virus are ongoing or have been completed in North America, Europe, and Africa (Feldmann et al. 2018), and still others are planned. Ten phase I clinical trials of VSV-Ebola virus were evaluated to determine the safety and immunogenicity of the vaccine as well as to identify doses and regimes for further clinical trial phases (Agnandji et al. 2017). Five phase II trials using the VSV-Ebola virus vaccine in healthy adults, HIV-positive adults, and adults at risk for Ebola virus infection were completed in the United States, Canada, Liberia, Sierra Leone, and Guinea (Kennedy et al. 2017). The efficacy of the VSV-Ebola virus vaccine was tested to prevent EVD which showed serious side effects (Henao-Restrepo et al. 2017). Further trials suggested the administration of VSV-Ebola virus as an emergency vaccine for the prevention of EVD (Halperin et al. 2017; Carter et al. 2018). During the recent outbreak in North Kivu of the Democratic Republic of the Congo (DRC), VSV-Ebola virus was administered to over 93000 individuals for preventing EVD (World Health Organization 2019).

In December 2019, the U.S. Food and Drug Administration (FDA) approved 'Ervebo' or rVSV-ZEBOV, the first FDA-approved vaccine for the prevention of EVD, caused by Zaire Ebola virus in individuals 18 years of age and older (Fig. 6). As discussed earlier, it is a live attenuated vaccine that has been genetically engineered to contain a protein from the Zaire Ebola virus. The approval of this vaccine was granted to Merck & Co., Inc. (Centers for Disease Control and Prevention 2019).

According to Anna Abram, FDA Deputy Commissioner for Policy, Legislation, and International Affairs, "While the risk of EVD in the U.S. remains low, the U.S. government remains deeply committed to fight devastating Ebola outbreaks in Africa, including its current outbreak in the Democratic Republic of the Congo. Today's approval is an important step in our continuing efforts to fight EVD in close coordination with our partners across the U.S. Department of Health and Human Services, as well as our international partners, such as the WHO. These efforts, including today's landmark approval, reflect the FDA's unwavering dedication to leveraging our expertise to facilitate the development and availability of safe and effective medical products to address urgent public health needs and fight infectious diseases, as part of our vital public health mission" (Centers for Disease Control and Prevention 2019).

The approval of 'Ervebo' is a colossal step towards the prevention of EVD outbreaks. As a matter of fact, the vaccine is an outcome of the study conducted in Guinea during the 2014–2016 outbreak in individuals, 18 years of age and older. The safety of 'Ervebo' was assessed in approximately 15,000 individuals in Africa, Europe, and North America. This vaccine is administered as a single-dose injection. However, side effects such as pain, swelling and redness at the injection site,

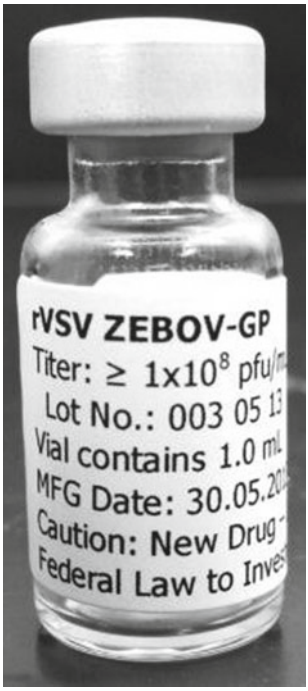

Fig. 6 FDA approved only vaccine for prevention of Ebola virus infection

headache, fever, joint and muscle aches, and fatigue were reported (Centers for Disease Control and Prevention 2019).

6 rVSV Technology for EVD Vaccination

VSV is an attenuated vector backbone which is used to develop vaccines. It causes asymptomatic infections in humans. VSV as a vector has several advantages such as its reduced prevalence of immunity to the vector in most populations targeted for immunization, lack of viral RNA integration into the host, expression of large foreign transgenes, and pseudo-typing of virus with heterologous viral glycoproteins (Monath et al. 2019). VSV-Indiana and VSV New Jersey are two serotypes of VSV. VSV-Indiana (VSV-I) is the basis for current vaccine candidates. The VSV genome contains 11000 nucleotides which encode five major proteins. The VSV glycoprotein (G) is present in the envelope of virus which causes cellular attachment, fusion with endosomal membranes, and release of viral RNA into the cytoplasm (Monath et al. 2019). This protein provides protective immunity against VSV. VSV vectors are constructed expressing genes from diversified species such as Ebola virus, Marburg virus, Lassa fever virus, and influenza virus, bacteria (Zhang et al. 2017), and tumour antigens (Blanchard et al. 2015) using reverse

genetic process. Sometimes, a portion of the VSV G protein is retained for expression as well as enabling fusion and internalization of the recombinant virus (Clarke et al. 2016). VSV vectors lacking the VSV G gene (VSVDG) must reconstitute the attachment, fusion, and budding functions with one or more proteins encoded by the heterologous envelope gene. The rVSVDG-ZEBOV-GP produced by reverse genetic system constitutes plasmids containing the VSV genome with G deleted and replaced with ZEBOV GP, together with helper plasmids expressing the VSV N, P, and L genes (Huttner et al. 2015). The rVSVDG-ZEBOV-GP is constructed with full-length GP. On the other hand, native ZEBOV expresses sGP (de La Vega et al. 2015). rVSVDG-ZEBOV-GP is more efficiently neutralized by antibody than wild-type ZEBOV due to lack of sGP (Ilinykh et al. 2016). The full length heterologous GP is incorporated into the rVSV particle, showing bullet shaped morphology. ZEBOV and rVSV pseudo-typed with ZEBOV GP enter the cell by macropinocytosis in a GP protein-dependent manner (Nanbo et al. 2010). The deletion of VSV G protein and replacement by the heterologous transgene in rVSVDG-ZEBOV-GP shows highly attenuated phenotype (Monath et al. 2019).

7 Preventive Approaches Other Than Vaccination

Plethora of strategies such as case management, surveillance, and laboratory testing at wide scale are essential to prevent any outbreak. Raising awareness of risk factors for EVD can certainly be an effective approach to reduce human-to-human transmission. Reducing transmission of EVD relies on diversified factors as mentioned below:

- Contact with infected fruit bats, monkeys, apes, forest antelope or porcupines should be avoided maximally in order to reduce wildlife-to-human transmission. Animals should be handled with gloves and other appropriate protective clothing. Animal's raw products (blood and meat) should not be consumed, and if unavoidable, cooked thoroughly before consumption.
- **C**lose contact with EVD infected people or people with EVD symptoms should be avoided in order to reduce human-to-human transmission. Contact with the body fluids of infected people should be avoided. Gloves and appropriate personal protective equipment should be worn when taking care of ill patients. Regular hand washing is required after visiting patients in hospital, as well as after taking care of patients at home.
- Safe and dignified burial of the dead person should be carried out.
- People who may have been in contact with someone infected with EVD should be identified and monitored for 21 days during quarantine.
- Risk of possible sexual transmission should be reduced. Male survivors of EVD should practice safer sex and hygiene for at least a year from onset of symptoms or until their semen tests negative twice for Ebola virus.

- Pregnant women who have survived EVD need to be monitored.
- Health-care workers should always take standard precautions while caring the patients. The health-care workers should wear face protective mask, goggles, and gloves.
- Samples received for Ebola virus confirmation should be handled by trained staff and processed in equipped laboratories.
- After sex, used condoms should be handled safely, and safely disposed of in order to prevent contact with seminal fluids.

8 Conclusion

Since the discovery of Ebola virus, the EVD has been a serious threat to humankind due to its human-to-human transmission feature. The epidemics, unavailability of effective antiviral drug, and reduced research activities makes this disease fatal and colossal health concern of recent times. Few vaccine candidates have shown tremendous outcomes in rodents and NHPs at experimental stages. The use of those vaccines in human based on NHPs results will not only be a time consuming approach but also expensive process. Currently, rVSV-vector based vaccine is the only FDA approved vaccine for EVD based on its high efficacy rate. Hence, at present, the best approach to reduce the epidemic of EVD is the prevention only. The quarantine and care of the infected patients, avoiding close contact with NHPs and infected persons, early diagnosis based on sign and symptoms, and safe burial of the dead can be prime preventive options to stop the human-to-human transmission. The disease can be eradicated by initiating awareness programmes at large scale. The rapid, cheap, and simple diagnostic kits should be made available for health care workers in nearby medical centres and hospitals. A significant effort should be undertaken by the government to test the suspected cases free of cost in order to examine maximum number of people. The research funding should be provided to prestigious institutes or research centres for the development of ideal vaccine and antiviral drugs against Ebola virus. Most importantly, the government and community should clarify any misconception among people regarding role of any religious factor or groups towards the outbreak of EVD.

Conflict of Interest None declared.

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