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REVIEW ARTICLE

Resurgence of Ebola Virus: Transmission, Pathogenesis, Prevention and Cure

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ABSTRACT

Ebola virus (EBOV) disease is a zoonotic disease that is caused by four species of genus Ebolavirus. The EBOV disease has outbreaks (1976-2022) in West and Central African countries, high mortality rate has made it a public health concern. EBOV is a filamentous virus having negative stranded and non-segmented RNA. The EBOV genome has the unique capability of forming soluble glycoprotein. Since its first emergence in human history, several effective therapeutics and vaccines are developed. These vaccines include replicative or non-replicative vectored vaccines, DNA vaccines and monovalent or polyepitopic vaccines. This article reviewed the structure of EBOV and pathophysiology as well as the immune response of EBOV infection. In addition to diagnosis and vaccinations available to cure EBOV at acute and chronic stages.

Keywords: Zoonotic disease, Filovirus, Soluble glycoprotein, DNA vaccines, Polyepitopic, Vectored vaccines, Pathophysiology, Immune response

INTRODUCTION

Ebola virus disease (EVD), also conferred as Ebola hemorrhagic fever and Ebola, is caused by Ebola virus. The first Ebola virus disease outbreak was documented in Zaire in 1976, which renamed to Democratic Republic of Congo (DRC), near the river Ebola. A 53% mortality rate was recorded in DRC[1]. The second outbreak recognized occurred in South Sudan at the same period. From first case reported from Yambuku town on August 22, 1976 till today, Ebola virus outbreak has affected African countries having variable arial magnitude. The 2013 and 2016 outbreak caused 11,000 expiries with 28,000 reported cases[2]. Since 1994, a virus wave has been observed in Africa every 1.5 years. Some researchers believe that the rapid spread of the Ebola virus in 1976 occurred partly due to the vaccination program, virus contaminated sanitary items and contact in different ways, such as sharing meals, conversation, touch or while sharing bed[3]. The Ebola virus has affected Sudan, DRC, the USA, UK, Philippines, Italy etc., with a high magnitude of fatality rate in West and Equatorial African countries[4].

Classification and Structure: The researchers have described 12 filoviruses based on molecular peculiarity. Two genera, genus Ebolavirus; Bundibugyo virus (BDBV), Ebola virus (EBOV), Reston virus (RESTV), Sudan virus (SUDV) and Tai Forest virus (TAFV) and genus Marburgvirus; Ravn virus (RAVV) and Marburg virus (MARV), have been detected in humans. Two subgroups of filoviruses categorized by WHO's International Classification of Disease under Revision 11 (ICD 11) include; BPBU, EBOV, SUDV and TAFU which cause Ebola virus disease and RAVV and MARV which cause Marburg disease[5] (Figure 1).

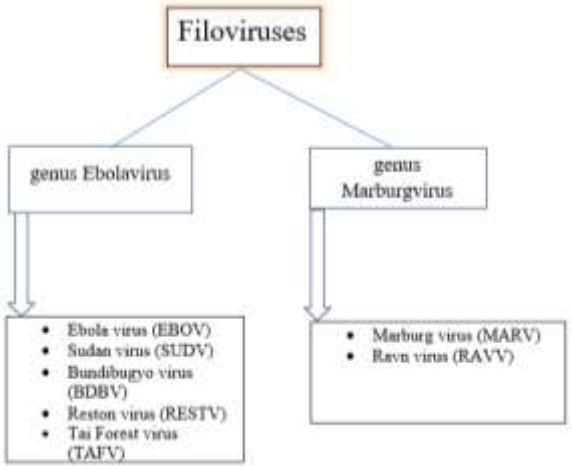

Figure 1: Classification of Ebola virus

Ebola virus disease (EVD) is hemorrhagic fever, only caused by EBOV. Ebola virus disease is a zoonotic disease ascribed by genetic, anthropogenic, ecological and socio-economic parameters. EBOV is recognized as a filamentous enveloped particle containing a negative single stranded RNA that is not segmented. The 18.9kb genome of Ebola virus encompasses eight mRNAs for encoding seven structural proteins. The genome of Ebola virus encapsidated with a nucleoprotein (NP) , that is associated with different viral proteins to form ribonucleoprotein complex. These viral proteins include Vp30, RNA polymerase protein (L) and polymerase matrix protein acting as interferon antagonists (Vp35). The Vp24 minor matrix protein (membrane allied protein interfering in interferon signalling) and Vp40 major inner matrix protein, originate particle formation and control virus morphogenesis. The glycoprotein GP; containing GP1 and GP2, two disulfides bonded furin cleavage fragments; is glycosylated and assists in particle-cellular attachment and entry, Ebola virus has encoding for the production of soluble glycoprotein. The spikes on the outer surface are composed of trimetric transmembrane protein[6,7].

The Ebola virus disease affects the coagulation process, it causes tissue damage and inflammation due to internal bleeding and clotting issues. At the initial stage, it causes headache, high fever, joint, muscular and abdominal pain, malaise, gastrointestinal problems (i.e., vomiting and diarrhoea), respiratory issues (sore throat, cough), weakness and fatigue[8].

At a later stage, it causes red eyes, hiccups and skin rashes etc. the hemorrhagic symptoms i.e., melena, hematochezia, and hematemesis cause gastrointestinal problems after 5-7 days of disease onset[9].

Ebola Virus Disease Outbreaks: The 1st Ebola virus outbreak in human population emerged in 1976, two simultaneous outbreaks in Zaire and Sudan. The 1st outbreak in Zaire (now DRC) had 88% mortality rate. Since 1976, according to WHO, EBOV has 34,710 cases globally[10].

The EBOV variant isolated in Kikwit in 1995 is closely related to EBOV strain, responsible for current Ebola virus disease in DRC. Total of 319 confirmed cases of EBOV infection were reported in Kikwit. On the basis of geographical location and genetic characterization, the strain causing outbreak in DRC has no epidemiological association with strain prevailing in South Africa. In 2007, Uganda and DRC reported 395 cases, the 1st occurrence of Bundibugyo virus. The largest outbreak occurred in 2014 and WHO referred to it as Public Health Emergency. The 7th outbreak of Ebola virus was observed in 2014 in Congo as an epidemic disease continued to spread to equatorial Africa[11].

The 8th Ebola virus outbreak in DRC was observed in 2017. The novel variant Ebola Muyembe was identified, with a 50% fatality rate; the 2017 outbreak was 2nd well-managed outbreak, followed by 2014 Ebola virus disease outbreak with the controlled

transmission of viral species. The bush meal was the main source of 2017 outbreak[12,13].

Table 1: According to WHO, till today the Ebola virus has affected following countries.

Period	Geographical Location	Cases Reported
1976-79	Sudan, UK, DRC	638
1989-90	USA, Philippines, Italy	7
2000-2007	Sudan, DRC, Russia, Gabon, Uganda	1194
2011-19	Guinea, Uganda	609

The 10th Ebola virus outbreak in DRC was the deadliest, affecting 3,481 people with 1,162 survivors. The 12th outbreak was declared over in May 2021 in Congo; however, Guinea observed an outbreak of Ebola virus in Feb 2021[14].

On June 1st 2020, six cases of Ebola virus disease were reported in Wangata health zone, Mbandaka. On 2nd June, 3,317 confirmed cases were reported. On 9th August, the outbreak reached to highest rate of infected/ suspected ratio. The outbreak was declared over on 18th November by recommendation of WHO[15]. On April 22, the Ministry of Health of Congo discovered two confirmed cases of EBOV and announced 13th outbreak[10].

Replication Cycle of EBOV: The exact source of Ebola virus outbreak in 1976 is unknown, but it is considered an animal source. The transmission pathways of Ebola virus include infected blood, body fluid, mucous membrane, tissues, tears, and secretion of infected people such as stool, saliva, urine, swabs of vagina, breast milk, rectum and seminal fluid. Direct human-bat contact is also a source of Ebola virus disease. The infection mainly spread in/via burial ceremonies, medical care and neighbour contact. The natural host i.e., infected animals and contaminated food, cut skin, and injection, are modes of rapid viral transmission in humans[9].

The structure of EBOV play role in characteristic epidemiology. Several surface proteins facilitate entry and attachment of virion into the host cell. The asialoglycoprotein receptor on hepatocytes; C-type lectin, human macrophages (i.e., lectin for galactose); folate receptor α on epithelial tissues are main host receptors that are used by virus to gain entry. The receptor (C-type lectin) interacts with N and O-linked glycan present on GP. Similarly, phosphatidylserine (PtdSer) of host cell binds PtdSer of viral envelope. The proteolytic processing of endosomal compartment is way making filovirus/host interaction possible and GP RBD remain covered while viral entry in host[16].

As GP binds receptors, virus enters the host cell, micropinocytosis is the main pathway, and internalization also occurs via caveolin-clathrin endocytosis. The uptake mechanism fluctuates with receptor and different cell type i.e., in vero cell entry is mediated by T-cell immune globulin and mucin domain 1 (TIM 1) and Phosphoinositide 3-kinase signalling pathway. In contrast, TAM tyrosine kinase phospholipase signalling is needed for SNB-19 cells. The viral moves from cell surface to lysosomal and endosomal compartments, followed by viral/host membrane fusion[17].

To expose its RBD and form fusion, virus needs GP proteolytic cleavage, occurs in endosomal/lysosomal pathway. The acidification and endosomal cysteine proteases activation, low pH protease, remove glycan cap of GP1. The cathepsins L and B are not required for this cleavage and formation of 17-19 kDa protein. Now GP can interact with an intracellular receptor (NPC1). The process of EBOV entry through NPC1 is independent of its role in cholesterol trafficking. The luminal C domain of NPC1 interacts with GP cleavage EBOV. Some researches reveal that NPC1 colocalizes TIM-1, linking two receptors. the TCP/NCP1 compartments provide EBOV particle accumulation. The low pH triggers hydrophobic conformational changes in loop of GP2, allowing its fusion in host membrane[18]. In this way, the EBOV is released in cytoplasm as a result of fusion of host and viral membrane. Endosomal protein NPC1 receptor that bind EBOV GP, forming changes in GP, and triggering membrane fusion[19,20].

The conformational change in GP2 causes the complete fusing of the host & viral endosomal membranes, the viral RNA & its associated proteins are liberated in the host cell cytoplasm. This EBOV inhibits the innate immune response via the VP24 and VP35 proteins. It also takes over the control of transcription and translation for genome replication and the new virion formation after entering into the host cell's cytoplasm. The L protein, NP, VP30, and VP35 make up the ribonucleoprotein complex, which facilitates viral genome transcription and replication. Dynamic phosphorylation of VP30 during the EBOV replication cycle is crucial for replicating and preserving the equilibrium between transcription and replication[21].

After the EBOV RNA polymerase binds negative-sense genome, it transcribes individual genes in 3` to 5` direction. Each gene is defined by a transcriptional start and polyadenylation site marking stop. EBOV mRNA is capped at 5`. Initially, VPs are backlogged by transcription and translation, triggering viral replication. During this, 3` synthesizes antigenomic RNA, that act as a template for production of a negative-sense genome. As they replicate to a sufficient level, these particles assemble in a plasma membrane[22].

However, VP30 is not necessary for viral replication but is crucial for the start of transcription. The matrix proteins VP40 and VP24 help to control viral genome replication, transcription, and the vital stage of virus budding before viral emersion. These matrix proteins are important for the structure and stability of the viral lipid coat. A desirable target for the development of EBOV therapies is this distinct EBOV replication cycle[23].

Immune Response Towards the EBOV: The struggle between the virus's ability to evade host immune responses and the host's ability to limit the virus reproduction determines the viral infection in any host. In order to replication in the kidney, liver and spleen, the EBOV manipulates both adaptive and innate immunity as well as develops methods for dodging the host's immunological defenses. The host's antiviral response is hypothesized to be suppressed by a number of VPs, including VP and GP[24].

After gaining entry, EBOV has the ability to infect different types of cells; antigen-presenting cells are the first target. The EBOV replicates rapidly, causing necrosis, and releasing virus in the extracellular matrix. The virus suppresses type-1 interferon response, making a pathway to lymph nodes. The infected hepatocytes and necrosis of lymphocytes leads to loss of immunity. If the virus enters in the gastrointestinal tract, it results in gastrointestinal dysfunction[25].

Thus, EBOV causes damage to adrenal cortical and endothelial cells, fibroblast, hepatocytes, epithelial cells etc. Macrophage tissue factor expression trigger overactivation of coagulation process resulting in coagulopathy[26].

Monocytes, myeloid dendrite cells, and macrophages are the EBOV's primary targets. EBOV alters the adaptive immune response by decreasing antigen presentation and destroying antigen-presenting cells. The EBOV's mononuclear phagocyte differentiation and inhibition of dendrite cells (DC) maturation affect adaptive as well as innate immune response. EBOV can proliferate inside macrophages; due to the release of reactive oxygen species and cytokines; which may facilitate an inflammatory response and, in certain instances, result in hemorrhagic syndrome. Due to T-cell depletion or death, the production of chemokines and cytokines would not excite T-cells in extreme situations. Similar to how the 1918 influenza virus altered the immune system, this suppression of interferons and stimulation of inflammation cause viremia[27].

The infected cells' migration from the lymph and spleen eventually cause the spread of the virus in large quantities. Following infection, macrophages become activated, which causes a large release of inflammatory cytokines. The antiapoptotic genes are activated in phagocytes and DCs, in order to survive and spread the virus after infection. Although maturation is inhibited by blocking co-stimulatory surface markers (such as CD80 and CD86), as well as by decreased production of IFN-α/β and other inflammatory cytokines, DCs still replicate the EBOV. After a

cytokine and chemokine release-induced altered immune response, intravascular thrombosis or multi-organ dysfunction may occur. The EBOV can successfully spread by binding to the Siglec-1 receptor on DCs. In Guinea the immune profiles of 2013–16 EVD survivors a revealed elevated level of proinflammatory cytokines and signs of chronic immunological activation. In nonhuman primates, protection from the EBOV is closely associated with the expression of type I interferon. Additionally, the expression of type I and type III IFN signalling is downregulated by VP24 and VP35. According to a recent study using single-cell transcriptomics in monkey models, pro-viral genes are increased while interferon signalling and antiviral genes are inhibited in infected cells. Whereas tetherin, an IFN-stimulated gene for virus budding, is inhibited by GP, STAT1, a transcription factor required for the expression of IFN-stimulated genes, is inhibited by VP24. Natural killer (NK) cell death is produced by the EBOV, which also prevents NK cell activity and NK cell-driven differentiation of DCs[28].

Response of Host: Many host factors have a substantial impact on the EOBV infection's virulence and infectious process. It has been shown that a number of calcium-dependent (C-type) lectins, including folate receptor-alpha, Tyro3 receptor tyrosine kinase family membersdAxl, Dtk, and Mer, serve as cofactors for EBOV cellular entry. Host protein interactions are necessary for the viral replication cycle's latter stages to take place. Additionally, host proteins are needed for VP alteration, which is necessary for their functionality. Dephosphorylation of the VP30 protein is one such observed alteration, and protein phosphatase 1 directly controls it. Several host proteins that interact directly with VPs have been shown to play a role in transcription and genome replication. When the VP35 interferon inhibitory domain and double-stranded RNA-binding protein 76 come together, the transcription/replication complex is harmed. At a later stage of the infection cycle, the budding domain of VP40 attracts the endosomal sorting complex required for transport-related proteins to initiate membrane fission. The pathogenesis of the EBOV is still poorly understood despite the identification of various host variables. Targeting macrophages and DCs during primary Ebola infections prevents the adaptive immune response from being triggered. All antigen-specific immune responses are triggered by DCs, which also transmit foreign antigens to T cells and create cytokines that stimulate T lymphocytes. The DC must mature through numerous complex maturation signals in order to carry out all of these specific roles. T lymphocyte dysfunction is brought on by the VPs' suppression of DC development. Although the precise processes for the host immune system to be manipulated are yet unknown, infection is typically linked to the antagonistic nature of interferon-based responses. The VP24 and VP35 mediate this reduction in defensive responses. The interferon regulatory factors IRF-3 and IRF-7 are the only two mechanisms by which the VP35 limits the type I interferon expression. As Major Histocompatibility Complex II is mostly in charge of antigen presentation to CD4-positive T cells and T cell activation, it must be up-regulated as part of the host's initial immunological response. The VP35 protein, an antagonist of retinoic acid-inducible gene 1 (RIG-1) signalling that influences T cell activation, prevents the elevation of co-stimulatory markers in DCs[29].

Diagnosis of EBOV: Several techniques for identifying Ebola virus infection and/or sickness have been developed in recent years that can be applied in clinical laboratory settings. Reverse transcriptase-polymerase chain reaction (RT-PCR) assay, loop-mediated isothermal amplification (LAMP), Rapid Diagnostic Test (RDT), viral RNA detection, virus isolation by cell culture, and antibody (Ab) - capture ELISA are all employed. EBOV can be found in a biopsy or blood sample by looking for viral nucleic acid. In this RT-PCR, cDNA synthesis and PCR amplification take place in the same tube. The single-tube RT-PCR stated above was made to detect the Reston Ebola virus and the Zaire Ebola virus in bodily tissues and fluids from individuals who had been diagnosed with EBOV. RT-PCR is crucial because it enables early diagnosis and has a variety of advantages and special qualities. The viral genome sequence can be determined by RT-PCR during the early stages of infection[30].

Furthermore, because it can detect the patient's viral load, RT-PCR is precise, sensitive, and calculable. The RT-PCR technology, however, consistently produces false-positive and false-negative results. When transferring materials, caution must be taken to prevent denaturation of viral RNA, and RT-PCR requires a high level of proficiency. In comparison to RT-PCR, a very precise and delicate RT-qPCR is more dependable. The viral target RNA is identified using the Q3-Plus device, which is an integrated, miniature thermal cycler with specialised software, in a one-step reaction. Due to the possibility of false-positive results from rapid-antigen (Ag) testing, the WHO states that RT-qPCR is the preferred method. The multistep criteria, experienced laboratory employees, skilled laboratory facilities, proper training, and cutting-edge technology are the RT-qPCR tests' key drawbacks[31].

Coris EBOLA Ag K-SeT rapid test, QuickNavi-Ebola and OraQuick Ebola Rapid Antigen Test are three RDTs were compared to the industry-recognized Cepheid GeneXpert Ebola assay. The findings indicated that the three RDTs evaluated did not meet the WHO target product profile's requirements for sensitivity and specificity. In spite of the fact that they are unable to triage and exclude Ebola virus infection among clinical suspicions, RDTs can nevertheless help in categorizing persons with suspected Ebola virus disease into low-risk and high-risk groups while waiting for GeneXpert Ebola assay reference testing[32].

WHO advises using RT-qPCR tests for confirmatory testing since they are typically thought to be more precise than RDTs. The WHO advises repeat testing after 72 hours since patients may test negative during the first three days following the onset of symptoms[33].

As real-time RT-PCR testing is a precise and high-throughput procedure, it has become the gold standard for EVD diagnosis. Four of these real-time RT-PCR tests are sold as kits, and the WHO and FDA have approved the use of many of these tests in emergency conditions. Standard real-time RT-PCR diagnosis in an outbreak environment necessitates the use of field laboratories with extensive infrastructure, the ability to operate and maintain sophisticated equipment, and knowledge of molecular techniques[34].

Prevention and CURE: The world's longest Ebola virus disease pandemic was successfully stopped in 2016 by efforts from Guinea, Liberia, and Sierra Leone. Case isolation, tracking of contact, fast identification, and contact isolation were crucial elements of the effective and coordinated public health measures utilised to contain the pandemic[35].

Early diagnosis of infected cases, tracing of contact and management, safe and respectable funerals, and the avoidance of new infections are all part of the control tactics used during an Ebola outbreak. With more information on the attitudes, practices and knowledge of populations afflicted by the EVD, control plan attempts may be improved. Although social resistance, violent conflict, and burial customs can obstruct efforts to contain the Ebola virus in eastern DRC, the new vaccine is widely accepted. The recurrent intra-communal strife in eastern DRC was likened to the Ebola virus outbreak, and it was decided that it would be beneficial to mobilize teams to eliminate the insecurity with the same amount of effort[36].

The 2014–2016 Ebola epidemic in Guinea was successfully contained in part through health communication and social mobilisation activities to increase the public's knowledge, attitudes, and practices regarding the disease caused by the Ebola virus[37].

Vaccination: The first licensed vaccine for Ebola virus illness prevention is called ERVEBO®. Over 200,000 people have received the vaccine, which was initially created by the Public Health Agency of Canada, as part of a disease outbreak that is still ongoing as of 2018–2020.

A live, attenuated vaccine created from the backbone of a recombinant vesicular stomatitis virus (rVSV), in which the VSV

envelope glycoprotein has been switched out for the glycoprotein found on the envelope of the Ebola virus. The immunization promotes the production of antibodies against the glycoprotein found on the envelope of the Ebola virus. Antibodies attach to a variety of known target sites on the Ebola virus's glycoprotein surface. By tagging virions and infected cells for destruction and removal by the immune system, antibodies can mechanically destroy or inactivate virions. Additionally, the Ebola virus glycoprotein elicits the production of T lymphocytes, in particular CD8+ T cells[38].

A safe and effective vaccination for human usage is VSV-EBOV. Currently, the vaccine for EVD that has received FDA approval is VSV-EBOV (rVSV-ZEBOV, Ervebo by Merck)[39]. Human clinical trials conducted between the outbreaks in the DRC in 2018–2020 and West Africa between 2013–2016 showed strong safety and efficacy. The VSV-EBOV has a distinct advantage over other EBOV vaccine platforms due to its quick protection following vaccination and possible post-exposure efficacy, despite the fact that multiple other EBOV vaccine platforms have been developed and advanced to clinical trials. The VSV-EBOV vaccine's post-exposure therapy capabilities have been applied multiple times in laboratory and by healthcare personnel exposed to and possibly infected with EBOV. It's significant because the VSV vaccine platform is adaptable to additional infections that are developing or re-emerging as threats, such as other paramyxoviruses, filoviruses, and arenaviruses[40].

A severely attenuated variety of rVSV that expresses EBOV-GP is being evaluated in phase 1. In order to reduce rVSV, the N gene was initially positioned at genome position 4 (N4) as part of an HIV vaccine project. Moreover, only one amino acid, rather than the wild-type 29 amino acids, made up the G-cytoplasmic protein's tail (CT1). Together, these changes had the impact of decreasing the virus's ability to replicate in culture and considerably reducing pathogenesis in mouse and non-human primate neurovirulence models. The resulting vectors, which expressed HIV-1 gag from genome position 1 and HIV-1 env from genome position 5, showed immunogenicity and safety in several phase 1 investigations. These results led to the creation of rVSVN4CT1-EBOVGP1, an Ebola virus vaccine, which replaced the gag gene with the GP gene from the Mayinga strain of the virus. This vaccine demonstrated single-dose protection against lethal challenges with low passage Ebola virus in non-human primates, either as a monovalent vaccine or as a component of a trivalent, pan-filovirus vaccine formulation containing analogous rVSVs expressing Sudan virus and Marburg virus GP proteins. Various studies have described the immunogenicity of different viral vaccines[41-44]. Our goal was to assess the rVSVN4CT1-EBOVGP1 vaccine's immunogenicity and safety in healthy individuals[45].

Recommendation:

- It is necessary to provide healthcare workers with information on the risks connected with this sickness as well as training on how to prevent this illness during outbreaks and how to follow preventive measures.
- Since the Ebola virus disease symptoms appear suddenly, it is urgent to centralize care for people who are suspected of having the disease in order to stop the outbreak. Healthcare workers must protect themselves by wearing the proper gloves, medical robes, goggles and masks when performing procedures on patients who have EVD and when handling Ebola virus samples.
- The government is required to create surveillance posts for the response, equip medical staff with protective gear, and establish specialized facilities for the treatment of Ebola patients. There must be a green phone number accessible for correspondence or alerts.
- Visitors entering a location where EVD is on the rise must be immunized against it and follow hygienic precautions to prevent exposure to the virus.
- The general public must abide by hygienic regulations and refrain from approaching animals during Ebola virus outbreak.

CONCLUSION

Although the first EBOV cases were first discovered in Sudan and Zaire in 1976, scientists only started paying serious attention to the virus in 1995. The need for quick, easy, accurate, low-cost, and effective diagnostic techniques is expanding as a result of the rising frequency of EBOV infection, the treatment gap, and the high fatality rate. In order to stop the spread of the disease, it must be followed and monitored closely. Numerous commercially accessible detection tests exist, such as RT-PCR, antigen-based test kits, and enzyme-linked immunosorbent assay kits. However, none of them fully satisfy all requirements for a perfect Point-of-care testing (POCT)study.

Due to the 2014 Zaire ebolavirus outbreak in African nations, a vaccine is now required to stop the virus's spread and protect the population from its lethal effects. The access to the remote area, complexity of the disease, The access to the remote area, complexity of the disease, and lack of interest on the part of firms and all hindered the development of the vaccine and its successful clinical testing. Further challenges included a lack of experience and knowledge in the pathophysiology of EBOV. The FDA, government agencies of African nations, the European Medicines Agency, and the World Health Organization have just approved the ERVEBO® vaccine because it is highly effective at eliciting an immune response. However, due to the fast rate of virus mutation, constant genetic makeup monitoring is necessary to preserve the efficacy of these created vaccinations. Several social, geopolitical, and financial obstacles still need to be removed to speed up the response to an outbreak. These obstacles include the lack of human resources and specialized infrastructures for treatment and diagnosis, the delay in referring infected people for care because of cultural or physical restrictions, and the delay in involving and empowering affected communities. The management of future epidemics in underdeveloped and rural places, where fatal infectious illnesses like Ebola could frequently reemerge, would depend on the awareness of these challenges and providing them with proper attention.

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