CURRENT STATUS OF THE BIOLOGY, PATHOGENESIS, AND IMPACTS OF EBOLA

VIRUS

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ABSTRACT

Ebola viruses (EV) are single-stranded negative-sense RNA viruses belonging to the Filoviridae family. There are 6 species of Ebola, and four of them can cause Ebola virus disease (EVD) in humans. Ebola viral hemorrhagic fever is one of the deadliest diseases known to infect humans and non-human primates.

The primary mode of transmission of Ebola has been identified as direct contact with infected animals, humans and body fluids. The early diagnosis of EVD is difficult because of similarities of the initial disease presentation to influenza-like symptoms such as high fever, myalgia, fatigue, headache, and chills. The most common symptoms that have been reported from previous outbreaks were fever, sore throat, abdominal pain, vomiting, bleeding, diarrhea, and chest pain. Several methods have been used to detect Ebola such as ELISA, conventional RT-PCR, and real-time RT-PCR. Scientists have been working on several therapeutics and vaccines to prevent and treat Ebola.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

BEBOV ....Bundibugyo EV

DRC ....Democratic Republic of Congo

EV ....Ebola virus

EVD ....Ebola virus disease

GP ....Glycoprotein

KPN-α1 ....Karyopherin α-1

NP ....Nucleoprotein

NC...Nucleocapsid

ORF...Open reading frame

PI3K ....Phosphoinositide-3 Kinase-Akt

PACT ....PKR-activating protein

REBOV ....Reston Ebolavirus

RLR...RIG-I like receptor

LP..RNA polymerase

SEBOV ....Sudan Ebola virus

ICEBOV..Tai Forest Ebola virus

VP ....Viral proteins

VSV...Vesicular stomatitis virus

YMH ....Yambuku Mission Hospital

BSL-4..Biosafety level 4 containment

APCs ....Antigen-presenting cells

EHF...Ebola hemorrhagic fever

NK...Natural killer

IgG ....Immunoglobulin G

1. INTRODUCTION

Filoviruses are negative sense RNA viruses belonging to the order mononegaviruses and contain two major members, Ebola viruses (EV) and Marburg viruses, which cause deadly hemorrhagic fevers. There are 6 species of Ebola: Zaire, Bundibugyo, Sudan, Taï Forest, Reston and Bombali. Four of them can cause Ebola virus disease (EVD) in humans, with Zaire and Sudan being the most important. Both species have been the cause of the majority of outbreaks and are the most pathogenic. The fatality rate is very high as 90%.

EVD first appeared in 1976 in 2 simultaneous outbreaks, one occurred in South Sudan and the other in Democratic Republic of Congo (DRC). Reston EV was discovered in the United States in 1989, among monkey species that were imported to Virginia. In 1994, EV re-emerged with a new species, Taï Forest ebolavirus, that was isolated from a person who developed the disease in the Parc National de Taï in Côte d'Ivoire (Murray, 2015). In 2007, the Bundibugyo ebolavirus, another subtype of EV that infects humans, was discovered in Uganda. The majority of outbreaks have been small, but EV captured the attention of the world because of high death rates (Centers for Disease Control and Prevention [CDC], 2021; World Health Organization [WHO], 2021).

When EV outbreaks occurred, it became a priority to contain the virus and prevent it from spreading across borders or between individuals. As a result of the epidemic, household incomes went down, and the poverty increased (Rohwerder, 2020). Ebola impacts the economy in several different ways. The outbreaks lead to the restriction on trade and transportation to prevent its transmission. Additionally, the outbreaks reduced tourism and travelers could not move to countries where an outbreak existed especially when countries rely on tourism economically. The epidemics had an adverse effect on agricultural market chains in the West African countries, particularly on populations that rely on agriculture as a main source of their income; the epidemics

impacted the transport of agricultural goods to consumption areas as workers were afraid of traveling to infected areas. With businesses closing their doors and households scared to go to work or sending their kids to school, it is easy to see how an Ebola outbreak puts a stop to productivity (Rohwerder, 2020).

EV spreads by human-to-human transmission, wildlife animal to human transmission, through direct contact with infected person's skin, secretions, blood and through burial and funeral ceremonies that does involve direct contact with the body of deceased the person (World Health Organization [WHO], 2016). Knowing how and from whom people acquired the disease can assist in providing information in responding to the disease by limiting the impact of outbreaks. Reducing the risk of wildlife to human transmission, human to human transmission, etc. has been the priority in order to contain the outbreaks of EVD. The symptoms of EV are varied and appear quickly, and this is one of the reasons why EV is very dangerous. The outbreaks provided an opportunity to evaluate the safety and efficacy of vaccines. currently, the EV vaccine is available and approved by the U.S. Food and Drug Administration (FDA). The numbers of Ebola cases have decreased significantly, although, in 2020 there have been small number of cases associated with the outbreak in DRC (Centers for Disease Control and Prevention, 2020). The question here is how we can prevent the Ebola and make Ebola history.

Researchers have only focused on the treatment of Ebola. Traditional qualitative methodologies for analyzing the effectiveness of interventions require the use of focus groups and interviews restricted to a particular community. These approaches restrict the applicability of results across populations and only help with getting a better understanding of treatment from the viewpoint of local residents, while pathogenesis is also an important aspect to consider.

EV and related filoviruses have been re-emerging across Africa for nearly 50 years, triggering epidemics of highly lethal hemorrhagic fever. The West African outbreak of 20142016, by far the most geographically widespread, fatal, and long-lasting in Ebola's history, posed a massive international public health challenge, but it also provided insights into Ebola's pathogenesis and natural history, clinical diagnosis, treatment, and prevention. Growing understanding of Ebola virus pathogenetic mechanisms and important new clinical observations of the disease course provide insights about prevention and treatment approaches. Although, EV disease often results in severe compromise of multiple organs, organ function recovery is expected when the patients receive supportive care. Major challenges for control EV in future include the establishment of early epidemics control and patient care. Furthermore, it will be critical to continue developing Ebola vaccines for all species and to establish policies for their use in epidemic and pre-epidemic situations.

This thesis will provide a broad understanding on the epidemiology of Ebola virus disease, the modes of transmission of the disease and the various measures available for preventing and controlling it. The various roles played by trained front line health care workers in preventing and controlling the disease are also analyzed, in addition to the modes by which the disease spreads and get people infected are through behavioral practices such as touching infected people, touching of items that are contaminated with bodily fluids of those with the Ebola Virus Disease, and eating and consuming infected animals.

In this thesis, I review information on the discovery and epidemiology, classification, molecular biology, transmission, pathogenicity. Additionally, I describe the different protocols for the diagnosis of virus in laboratory, and the immunity. Finally, I summarize the prevention and treatment of EV and its associated clinical disease.

2. VIRAL BIOLOGY

2.1. Classification of Ebola

Filoviruses are filamentous, enveloped viruses with a negative-sense RNA genome. They belong to family called Filoviridae. Three genera of Filoviridae have been identified which are *Marburgvirus, Cuevavirus, and Ebolavirus*. The Marburg virus and the Ebolavirus are related viruses, and both cause hemorrhagic fevers. They can infect human and nonhuman primates, but their pathogenicity is different from each other, and the Marburg is less deadly compared to Ebola.

Ebola belongs to order Mononegavirales (Jadav et al., 2015). Genus Ebolavirus has 6 different species (Fig1) which are Bundibugyo ebolavirus (BEBOV), Reston ebolavirus (REBOV), Sudan ebolavirus (SEBOV), Tai Forest ebolavirus (formerly Ivory Coast EV, ICEBOV), Bombali ebolavirus, and Zaire ebolavirus (ZEBOV) fig1 (Zawilińska & KoszVnenchak, 2014). Four of these species (Zaire, Sudan, Tai, and Bundibugyo) can cause disease in humans. Reston ebolavirus affects pigs and nonhuman primates such as monkey, and it does not cause disease in humans. It was discovered in 1989 in research monkeys imported from the Philippines into the U.S. (Zawilińska & Kosz-Vnenchak, 2014; Centers for Disease Control and Prevention, 2020). It is unknown yet if this species can cause disease in both human and animals (Kerlin, K, 2019; Forbes et al, 2019).

Figure 1

The Phylogenetic Tree of Ebola
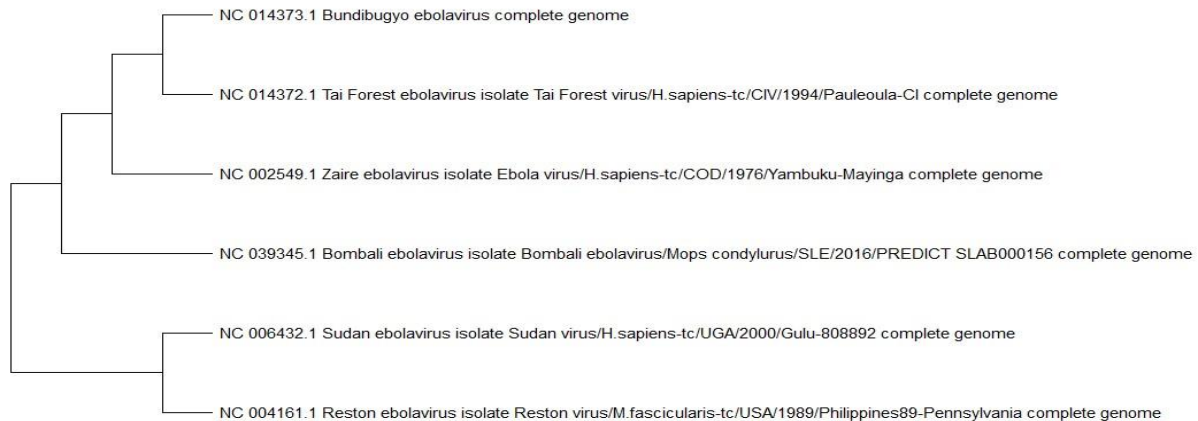
Note: Evolutionary relationships of Ebola complete genomes. The phylogenetic tree compared full length EV genomes from different species. The optimal tree is shown and represents the evolution of 6 species with time and the relation of different species of EV. The tree reflects how Ebola these 6 species evolve from common ancestor, and the lines are the branches that shows the difference. The numbers that shown in the tree that were download from NCBI website are the accession numbers of the genome of each EV.

2.2. Epidemiology and Outbreak

EV was discovered in 1976 near the Ebola River in Democratic Republic of Congo (DRC), West Africa, and associated with a widespread outbreak of the virus (Rivera & Messaoudi, 2016). The virus was named for the location where the virus appeared (Zawilińska & Kosz-Vnenchak, 2014). In 1976, outbreaks caused by Zaire and Sudan ebolaviruses occurred in those two countries. The Sudan outbreak started in two towns in Sudan, Nzara and Maridi, where 150 of 284 cases died, with a mortality of 53%. This outbreak was thought to have originated among cotton workers. It was reported that bats were present in the roof space of that factory. The second outbreak occurred in Zaire, and according to Bulletin of the World Health in September 1,1976, a patient was thought to have malaria due to his symptoms such as fever, sweating, and nausea being similar to malaria. He was injected with chloroquine for presumptive malaria at Yambuku Mission

Hospital (YMH) (Stein, 2014; Lever & Whitty, 2016). Within a week, other people who received the injection became infected with Ebola because the needle was not sanitized and was reused. The majority of confirmed cases occurred within the four weeks of epidemic, and the outbreak had 318 declared cases and 284 deaths (Stein, 2014; Lever & Whitty, 2016).

Since that time, outbreaks have occurred in isolated places across countries. Taï Forest ebolavirus was isolated from a person who developed the disease in the Parc National de Taï in Côte d'Ivoire in 1994 (Murray, 2015). Between 1994 and 1997, two outbreaks occurred in Gabon, with the virological confirmation of Ebola hemorrhagic fever. In this outbreak, the disease spread to countries such as Sierra Leone, Liberia, and Guinea, Nigeria, Mali, and other countries. During 1995 scientists recorded another outbreak spread through surrounding areas and families in the DRC, caused by Zaire ebolavirus. The Sudan ebolavirus outbreak started in the District of Uganda in 2000 and quickly spread to nearby districts. After this period, at least 12 outbreaks occurred between 2000 and 2013 caused by different subtypes. In 2007, the Bundibugyo ebolavirus, which is another subtype of EV that infects humans, was discovered in Uganda. The largest outbreak occurred in 2014-2016. It received international attention because news reports focused on the growing number of cases and its widespread effect. It involved the deadliest subtype, Zaire Ebolavirus, and it started in Guinea and spread to Sierra Leone, Liberia. Other minor outbreaks occurred between 2014 and 2020 and were caused mainly by the Zaire and Sudan Ebolaviruses (Fig 2).

Figure 2

The Outbreaks of Ebola
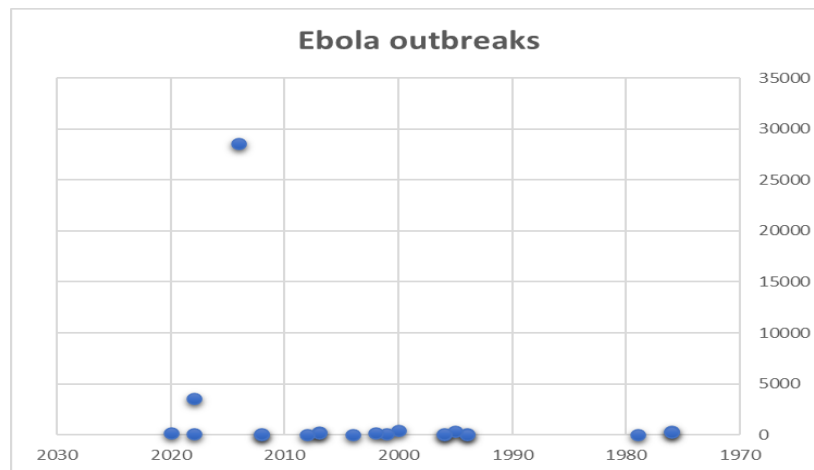
Note: The cumulative number of confirmed Ebola virus disease cases. There are the major and minor outbreaks since 1976. The outbreaks were caused by different subtypes of EV.

The outbreaks have occurred mainly in west Africa for various reasons. Scientists believe that people's interactions with infected bats might be behind the repeated outbreaks, and the transmission cycle begins when the people living in rural areas contact fruit bats (Centers for Disease Control and Prevention [CDC], 2021). in addition, the poverty is another factor for the presence of the disease because, people may directly contact with fruit bats and consume it as a source of foods. The lack health system and lack medical equipment are significantly factors for the occurrence of disease. The late diagnosis of disease leads to prevalence of cases. In addition, some families take their loves to traditional healers because they believe that their ways can cure them. The lack of education and lack of knowing the way to prevent spread of the disease are probably ways of its present in Africa.

EV, like any other virus undergoes mutations, which can be observed through its history of outbreaks. Initially, the virus infected animals however, mutations enabled it to become infectious for the human hosts. It is thought to originate from species of fruit bats and upon

transmitting to humans became a deadly outbreak. However, the mutation does not mean that the EV becomes more virulent. The EV mutation does not worsen the disease which means it without concern (Hayman et al., 2012).

EV affects both genders and people of different ages. Because the virus was unknown among people who lived in that area, the examination and identification of the virus were challenging, so previously there was no evidence about linkage between the epidemic of the hemorrhagic fever and the outbreak in northern Zaire. In 1989, Reston ebolavirus was discovered in the US among cynomolgus monkeys which were imported from Philippines to Reston, Virginia; the virus was named by the area where it emerged. The monkeys were intended for research. Humans were not infected with this subtype of Ebola. In 1994, Taï Forest ebolavirus was isolated from an ethnologist who probably developed the disease while performing an autopsy on a chimpanzee found dead in the Parc National de Taï in Côte d'Ivoire (Murray, 2015). Between 1994 and 1997, two outbreaks occurred in Gabon, and there was virological confirmation of Ebola hemorrhagic fever (Amblard et al, 1997). The three countries of Sierra Leone, Liberia, and Guinea had an acute transmission of Ebola. Additionally, countries across continents had isolated cases which were originally from West Africa. Since these periods, there have been minor outbreaks. In 2007, the Bundibugyo ebolavirus, a fifth subtype, was discovered in Uganda. The Bundibugyo ebolavirus caused an epidemic. 116 people were infected with the virus, and the deaths were 30. The fatality rate caused by this species has been estimated to be 25% (Stein, 2014; Murray, 2015). Since the discovery of EV disease in 1976, there have been >20 epidemics of almost all caused by Zaire ebolavirus and Sudan ebolavirus table 2 (Centers for Disease Control and Prevention [CDC], 2021).

Table 1

The Major Outbreaks of Ebola Species

Locations	Confirmed Cases	Death	Years	species
DRC	318	280	1976	*Zaire*
Sudan	284	151	1976	*Sudan*
Sudan	34	22	1979	*Sudan*
Gabon	52	31	1994	*Zaire*
Cote d'loire	1	0	1994	*Taï Forest*
DRC	315	254	1995	*Zaire*
Gabon	31	21	1996	*Zaire*
Gabon	60	45	1996	*Zaire*
Uganda	425	224	2000	*Sudan*
Gabon	65	53	2001-2002	*Zaire*
DRC	143	128	2002	*Zaire*
Sudan	17	7	2004	*Sudan*
DRC	264	181	2007	*Zaire*
Uganda	149	37	2007	*Bundibugyo*
DRC	32	14	2008	*Zaire*
Uganda	24	17	2012	*Sudan*
DRC	57	29	2012	*Bundibugyo*
West Africa	28,610	11,308	2014	*Zaire*
DRC,Uganda	3570	2287	2018	*Zaire*
DRC	54	33	2018	*Zaire*
DRC	130	55	2020	*Zaire*

Note: The major outbreaks of Ebola species since its discovery (CDC), caused by 4 species that infect humans. The majority of epidemics are caused by Zaire and Sudan species. The table represents the number of confirmed cases and deaths in each outbreak.

The ability of resource-limitations in these areas led to outbreaks. Additionally, the low quality, functioning of health systems in Africa is the one of main reasons outbreaks are not better controlled. The lack of healthcare system resources in west African countries increase the spread of EV in these countries. In the United States, availability of health-care workers, the facilities and equipment, disease surveillance mechanisms, and education are reasons to stop the spread of Ebola (Saeed, Hasan, Ahmad, & Masood, 2019). During the 2014-2016 epidemic, eleven cases exposed to the EV were detected in the United States, and they were medical workers who had

volunteered. According to CDC, Ebola transmission stopped in US because the of the steps to prevent the stops of the virus. The travelers and healthcare workers leaving West Africa were screened at the airports, and the screening helped identify who were at risk (Centers for Disease Control and Prevention [CDC], 2021).

2.3. Structure of Ebola Virus

The EV is an enveloped filamentous virus with a typical size of about 80 nm with 600 to1400 nm in length. Some particles tend to curve into an appearance looking like the number '6' under the electron microscope. The filamentous capsid contains seven genes encoding 7 proteins. The genes are nucleoprotein (NP), L (polymerase), glycoprotein (GP), and viral proteins VP24-VP30-VP35-VP40 (Falasca et al., 2015).

In 1980, the molecular weight of EV proteins was characterized and the virus was identified as a single-stranded RNA virus. Biochemical, cryoEM, and cryoET techniques have been used to identify the viral proteins in the Ebola nucleocapsid (Kiley et al, 1980). Moreover, after the structure of the nucleocapsid protein was determined, the function of the different proteins was studied. EV has7 genes encoding 7 proteins table 1. The viral structure is composed of 3 components, the nucleocapsid, the envelope, and the matrix. The structural proteins consist of glycoprotein (GP), polymerase cofactor VP35 (VP35), matrix protein 40 (VP40), membrane associated protein VP24 (VP24), RNA polymerase (L), nucleoprotein (NP), and hexametric zincfinger protein VP30 (VP30). The major viral surface protein is glycoprotein encoded by the GP gene, and it has two domains, GP1 and GP2. The GP functions in cell fusion and the binding of the receptor, and the GP1 and GP2 domains are important for regulating production and release of virus (Cantoni & Rossman, 2018; Rivera & Messaoudi, 2016; Falasca et al, 2015). NP is a key component of the viral ribonucleoprotein complex and it has a critical role in protecting RNA from

degradation. L is involved in transcription and regulation of genome and mRNA editing. L and VP30 form the RNA-dependent RNA polymerase (RdRp). They mediate transcription and replication of the viral genome. VP30 has a RNA-binding role in transcription and interferes with cellular RNA silencing. Additionally, it initiates ebolavirus transcription. VP40 is a structural protein that located under the membrane of virus, maintains the integrity of the viral particles. Additionally, it binds to the viral envelope and is required for the assembly of nucleocapsid (NC) (Cantoni & Rossman, 2018). The VP24 has a significant role in nucleocapsid formation, and it is known to inhibit interferon activation (IFN-α/β and IFN-γ). In addition, it blocks IFN signaling by binding to STAT1 thus preventing the phosphorylation, nuclear import, and transcription of IFN-stimulated genes. Also, VP24 has been known to function in capsid assembly and components of the viral nucleocapsid like VP, and it is necessary for incorporation of the viral genome into the nucleocapsid. VP35, a type-I IFN antagonist, inhibits the activation of interferon regulatory factor and blocks IFN production. It has more diverse functions during viral replication.

Table 2

The Functions of Ebola Viral Proteins

References	Proteins	Functions
Cantoni & Rossman., 2018	GP	Attachment and entry
	NP	Key component of ribonucleoprotein complex
		Encapsidation of viral genome
		Protection of viral mRNA from degradation
Rivera & Messaoudi., 2016	L	Involvement in transcription and regulation of viral genome Editing of mRNA
	VP30	Initiation of ebolavirus transcription
		Interference of cellular RNA silencing
	VP40	Viral assembly and budding
Falasca et al., 2015	VP24	Inhibition of interferons signaling
		Assembly and stability of Nucleocapsid
	VP35	Inhibition of type-I IFN signaling and production

Note: Each of 7 proteins has specific functions.

## 2.4. Entry and Viral Replication

2.4.1. Viral Entry

EV can enter the human body via direct contact with body fluids from infected individuals especially with injures to the skin or via direct contact. The virus can attack several human organs (Falasca et al, 2015). The virus enters the host cell using GP, which is the viral spike and achieves cellular attachment and fusion of the membranes (Beniac & Booth, 2017). After the binding of GP to C-type lectins receptor (CLECs) and phosphatidylserine receptors (PtdSer), the virion enters the host cell via endocytosis or lipid rafts. Viral fusion of envelope with the host cell membrane is mediated by GP2, which is produced as a cleavage product from GP1 by endosomal proteases cathepsin B and L. This leads to the release of the nucleocapsid to the cytoplasm (Pérez et al, 2014).

2.4.2. Genome, Transcription and Translation of Ebola

The negative sense RNA genome is approximately 19 kb in size, with 7 genes. The end of the genome contains trailer sequences, with promoters and encapsidation functions. The 3' end of the Ebola genome consists of short non-transcribed regions which contain cis-acting signals (fig 3). These are important for replication and transcription of the genomic RNA.

Figure 3

The Molecular Structure of Ebola
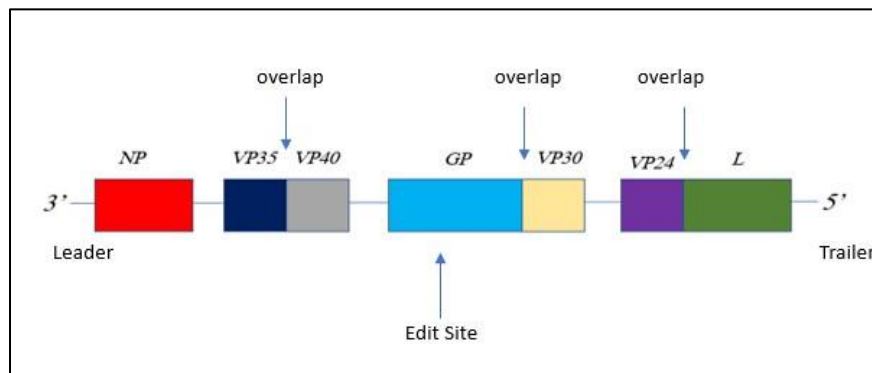
*Note*: Negative-stranded RNA linear genome of EV encodes seven proteins

2.4.3. Replication and Transcription

Viral replication and transcription occur in the cytoplasm of host cells. The transcription of the virus depends on the presence of VP30 and virally-encoded RNA-dependent RNA polymerase (RdRp) which can recognize the negative strand RNA as a template for replication and transcription. After transcription by RdRp, the viral proteins are translated. During transcription, seven mRNA molecules are transcribed from the negative-strand RNA genome in a 3′ to 5′ direction and are capped and polyadenylated. After the encapsidation of the genome, the ribonucleoprotein (RNP) serves as a template to produce the complement. Negative sense viral genomic RNA is generated and is packed into virions. The availability of the ribonucleoprotein (RNP) is critical for the transcription, replication and the assembly of EV. Following synthesis of

viral proteins, they are transported to the budding sites on the cell surface. The budding occurs at

the plasma membrane, releasing virions from surface of cells (fig 4) (Falasca et al, 2015; Rivera

& Messaoudi, 2016; Kruse et al, 2018).

Figure 4

The Replication of Ebola
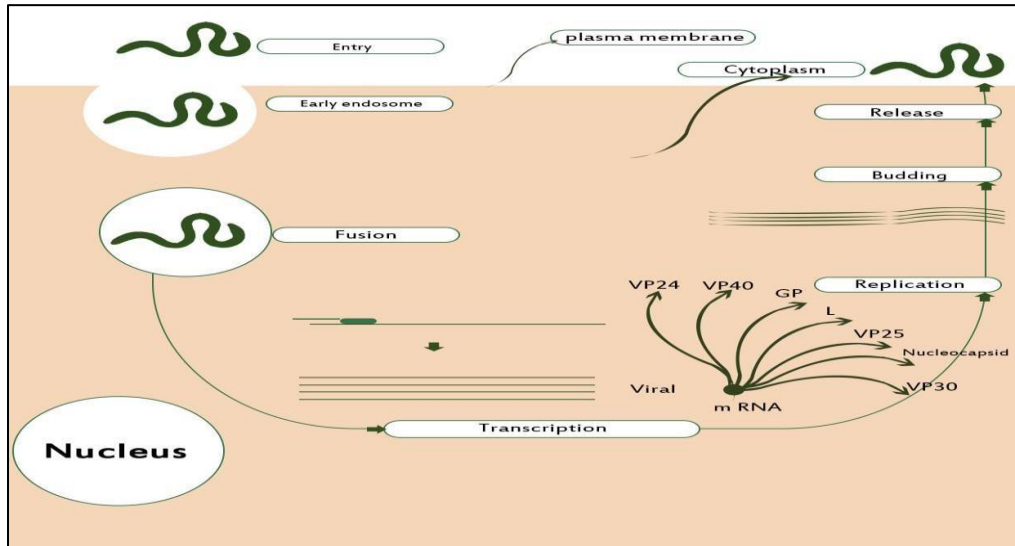
Note: Schematic diagram showing the replication cycle of EV. It enters host cell by receptor-mediated endocytosis, and acidification followed by fusion of virus and membrane. Then, nucleocapsid releases into cytoplasm. The RdRp transcribed mRNA which is then capped and polyadenylated in cytoplasm. During replication, the promoter drives the synthesis of positive sense RNA which then serves as a template to produce negative sense genomes. After the synthesis of viral proteins, the complete virions buds from surface of host cell.

3. PATHOGENESIS

3.1. Viral Ecology

The mode of transmission of Ebola is identified as direct contact with infected animals and humans (Saéz et al, 2014). According to scientists, EV is transmitted initially from infected zoonotic animals such as non-human primates or fruit and insectivorous bats. Active infection in the population has been detected in three species of fruit bats, which are *Epomops franqueti, Myonycteris torquata, and Hypsignathus monstrosus*. Based on PCR analysis Ebola RNA was found in the liver and spleen of fruit bats. In addition, immunoglobulin M (IgM) antibody was detected in the same species of bats (Calisher et al, 2006). In the Tai National Park an Ebola outbreak occurred in 1994 in wild chimpanzees. In addition, the outbreaks in Gabon and neighboring areas between 2001 and 2003 occurred due to infected non-human primates such as gorillas and chimpanzees (Osterholm et al, 2015). At that time, samples collected from the carcasses of gorillas and chimpanzees had Ebola specific antigen and were positive by immunohistochemical staining ($\geq$1 of the 3 laboratory tests). The laboratory researchers found that 14 of 21 total samples of animal carcasses (10 gorillas, 3 chimpanzees, and 1 duiker) were positive for EV (Rouquet et al, 2005). According to the CDC, an individual can develop Ebola disease after being exposed to an infected animal, with the current scientific opinion being that direct contact is the main mode of transmission (CDC, 2021).

3.1.1. Model of Human to Human Transmission

Examination of cases from outbreaks where Ebola spread between people in hospitals, factories and health care settings, transmission was determined to have occurred as a result of contact with primary cases (Osterholmet al, 2015). Transmission occurs through direct contact with Ebola contaminated fomites and bodily fluids (Judson et al, 2015). However, there is no

evidence that transmission of the virus occurs via mosquitoes or any other insects. According to CDC, EV cannot be transmitted to the individual via food; however, people might be infected by the hunting and consumption of infected animals (Centers for Disease Control and Prevention [CDC], 2021).

1. **Aerosol or Airborne Transmission.** Aerosol transmission of viruses occurs when the pathogen remains in the air in association with respiratory droplets. The nuclei can then easily travel through short or long distances from the infected person to the recipients. Two categories of droplets: small and nuclei droplets, with the diameter to be <20 µm and <5 µm respectively play a role in transmission. According to some experiments, EV remains in the air approximately 60100 minutes. After that time, it decays at relative humidity and at $22 \pm$ °C, under which conditions the virus degrades quickly. As a result of humidity increasing, aerosol recovery of EV might decrease. The virus can survive in dry air because it is hydrophobic, and its stability is higher in the absence of humidity (Mekibib & Ariën, 2016; Judson et al, 2015). However, people were infected through direct contact with infected people, wild animals, and contaminated subjects. The WHO states that there is no evidence of EV transmission via aerosol transmission and that Ebola is not an airborne virus (World Health Organization [WHO], 2021).

2. **Droplet Transmission.** This type of transmission refers to large droplets that do not evaporate and transfer across longer distances. However, these droplets can transfer within short distances, and transmission between the infected individual and other people can occur up to distances of 2 meters. The EV is stable inside large droplets, and when an individual is exposed to the droplets, they can become infected with the virus. Ebola droplets might be from human fluid such as blood, urine, and vomit. Based on

observations from previous Ebola outbreaks, 5 out of 19 cases were a result of droplet transmission in the absence of direct contact with infected patients (Judson et al, 2015).

3. **Fomites and Contact with Body Fluid**. EV persists on fomites or other surfaces. This mode of transmission occurs by touching infected objects or surfaces that are contaminated with infected patients' fluids (Centers for Disease Control and Prevention [CDC], 2021). Studies on viral stability on physical objects showed that viral loads reduce after 5 days. The virus can be recovered at 4°C for a maximum 50 days. The virus maintains viability for long periods, especially in controlled environments such as medical facilities and in water for almost 6 days (Fischer et al., 2015). In addition, EV remains viable in liquid blood for 14 days compared to dried blood where the virus can survive for 5 days. Virus isolation from blood, semen, sweat, and skin shows a wide transmission over a range of bodily fluids (Judson et al, 2015). Experiments with non-human primates have shown that the contact with pathogenic fluid through injection or infected saliva, sweat, and tears will transmit the virus. The first outbreak in 1976 in Yambuku Mission Hospital (YMH) occurred as a result of an unsterilized needle. Thus, contact with infected human fluids is the most likely mode of transmission of the virus within a population.

3.1.2. Factors Influencing the Spread

There are various factors that have influenced the Ebola epidemic, such as meteorological conditions, poverty, war, water supply, human mobility, and cultural features. For example, animals moving from one area to another increases the chance of contact within the animal reservoir, which is fruit bats. Moreover, one of the cultural behaviors that influences the transmission is burial practices, which caused the outbreak in Uganda. People taking part in burial

17

rituals were infected with the virus because the rituals involved washing and direct contact with the deceased (Alexander et al ,2015). Other factors like urbanization and deforestation played a major role in recent outbreaks. In Africa, especially in past years, when towns and villages were smaller than modern day the percentage of cases infected with virus was low. However, large cities and crowding provided a chance for the greater spread of Ebola. It is predicted that in 2050 the proportion of people who live in Africa will increase significantly from 36% to 60% (Wilson et al, 2014). Further, the rainforest has decreased to less than one-fifth of the original size (Saéz et al, 2014), which forced migration of non-primates and caused fruit bats to be concentrated in the remaining forest area (Saéz et al, 2014). As a consequence of increased urban area and decreased forests, humans come into increased contact with fruit bats. Fruit bats are also consumed as a source of food (Graham, 2014). Thus, cultural and economic practices have also had a significant influence on the spread of EV.

Direct contact with wild animals, human, and contaminated subjects is the main way to spread the EV, and the best way to avoid catching the disease is keeping away from areas where the virus presents. People in areas where Ebola is found should avoid contact with wild animals and humans. Additionally, health care workers may be exposed to EV by touching a patient's body fluids, contaminated supplies, or contaminated surfaces, and they can prevent infection and spread the virus to others by following control steps and wearing masks and gloves whenever they come into contact with infected people (Centers for Disease Control and Prevention [CDC], 2021).

There is a concern about the possible use of Ebola as a bioweapon. Some people have claimed that Ebola could be turned into bioterrorist weapon. Despite that, Ebola clearly is not an ideal bioweapon because it is highly debilitating and requires advanced handling equipment (Hummel, 2017). Additionally, Ebola is not an aerosolized virus, and its transmission is limited to

direct contact. This is why EV is not an ideal bioweapon; the ideal bioweapon would be aerosolized in order to infect massive numbers of people. Consequently, a terrorist would have to use direct delivery methods to infect others with EV, however, the victims would seek immediate medical attention after the exposure to EV. The claims that Ebola could easily be converted into a biological weapon are unfounded and sensationalized (Hummel, 2017)

3.2. Molecular Pathogenesis and Immune Subversion

The pathogenesis mechanism begins with the infection of the immune cells such as macrophages, dendritic cells, and monocytes. They are the first targets of EV. The monocyte and macrophage infections trigger expression of inflammatory mediators including interleukins (IL1β, IL-6, IL-8), macrophage inflammatory protein (MIP-1α, MIP-1β), monocyte chemoattractant protein (MCP-1), and tumor necrosis factor-α (TNF-α). EV initiates the infection of dendritic cells (DCs) which then impairs the maturation and suppression of Type I interferon (IFN) responses. This leads to the prevention of T cell activation. Following the exposure to EV, the inflammatory mediators are detected in the plasma (Rivera & Messaoudi, 2016). In addition, they can lead to impairment of the vascular system and intravascular coagulation. Secreted chemokines recruit monocytes that act as additional targets for viral infection. Inflammatory mediators and nitric oxide induce apoptosis which lead to death of lymphocytes. The lack of lymphocytes like CD4 T cells can inhibit the ability of the virus to induce antibody (Ab) responses. Eventually, inflammatory mediators and cytokines such as TNF-α increase endothelial permeability, leading to vascular leakage. EV can evade type I IFN responses and prevent the production of cellular responses to IFN. Some laboratory research suggested that VP35 blocks retinoic acid-inducible gene I (RIG-I)-like receptors (RLR) signaling by binding to dsRNA (double-stranded RNA). Moreover, VP35 prevents phosphorylation of interferon regulatory factor 3 (IRF-3). The virus disseminates to

19

organs such as liver, kidneys, adrenal glands, and endothelial cells, causing symptoms associated with hemorrhagic fever (Rivera & Messaoudi, 2016).

Ebola and Marburg viruses are distinct from each other but belong to the same family of filamentous filoviruses that cause clinically similar illnesses. Both viruses because diseases characterized by capillary leakage and hemorrhagic fevers. Their symptoms are very similar. Regarding the immunopathology, both diseases dysregulate the immune system. However, compared to the Marburg virus, EV is considered to be more virulent. The severity of the disease in EV infection is associated with Kupffer cell infection increase in the liver, along with skewing of macrophages and a high level of myeloid dysfunction as compared to Marburg virus infection (Martines et al., 2015).

3.3. Clinical Manifestations of Ebola

Clinical Manifestations of Ebola include fever, sore throat, abdominal pain, vomiting, bleeding, diarrhea, chest pain, and other symptoms. The percentage manifestation of symptoms include fever (87-90%), fatigue, (76%), vomiting and diarrhea (68% and 66% respectively), abdominal pain, headache, muscle pain, loss of appetite, rash, kidney and liver function failure, and sometimes bleeding (Beeching *et al*, 2016). According to clinical diagnosis, the incubation period of Ebola is 2 to 21 days: though the majority of cases manifest within 2 weeks of exposure (Kangbai, et al, 2019). There are three phases of disease. The first phase starts with a fever and headache which begin suddenly. After 2 days the previous symptoms are followed by diarrhea, vomiting, abdominal pain, and dehydration in the gastrointestinal phase. The final phase, which is fatal includes massive bleeding, with the majority of fatalities caused by loss of blood. In addition, some nervous system symptoms like stripping off clothes and climbing have been diagnosed in Nazara and Maridi. Physical examination of patients showed characteristic changes in a patient's

appearance such as sunken eyes, dry oral cavity, and stiff neck (Bulletin of the World Health Organization,1978; Centers for Disease Control and Prevention, 2019)

3.4. Detection and Diagnosis

Several techniques are used to detect EV infections and progression of Ebola. In addition, other diagnostic tests are designed to distinguish between the EV species. (Broadhurst et al, 2016). The detection of EV falls into 3 major categories; serologic tests which can detect host antibodies generated against the EV, molecular tests which be used to detect the RNA sequences, and antigen tests which can detect the viral proteins (Broadhurst et al, 2016). Antigen detection and molecular testing have been shown to be very effective for acute diagnosis because virus levels in the host blood are high within the first few days of symptoms (Broadhurst et al, 2016).

3.4.1. Diagnosis Methods

Detection of the EV is possible through blood test, however there is a limitation associated with it. Research suggests that EV reaches detectable levels three days after the onset of symptoms. Viral isolation in cell culture is the traditional gold standard approach for verifying the presence of EV. The virus can be visualized directly by electron microscope or indirectly by immunofluorescence microscopy. This method requires biosafety containment (BSL-4) and is restricted to studies in public health laboratories (Broadhurst et al, 2016).

ELISA has been used for both antibody and antigen detection. ELISA can be used to detect acute Ebola infection, by detecting Ebola virus-specific IgM and IgG antibodies. Furthermore, ELISAs detect both IgG and IgM antibodies; IgM antibodies develop earlier than IgG, so it can be used to detect the early stage of the disease while the IgG is commonly used to detect the virus in later stages (Broadhurst et al, 2016).

RT-PCR is used for detection of viral replication in infected tissues and body fluids. It is commonly used to diagnose acute Ebola infection. A significant advantage of using this test is that it can detect the virus with low levels in the blood. However, the ability of detecting the virus increases with the increase in infection, especially in the active stages. Additionally, this method can differentiate and quantify RNA of several viruses such as Ebola and Marburg Viruses, Rift Valley Fever Virus, Lassa Virus Dengue Virus, and others because it is difficult to clinically distinguish between these diseases (Broadhurst et al, 2016). PCR is used to amplify the GP, L, and nucleoproteins genes. Conventional RT-PCR has been found to be more sensitive than antibody or antigen detection ELISAs and was widely used during the outbreak in Gabon. It performed well for detection of virus in other body fluids like serum, saliva, and seminal fluid (Broadhurst et al, 2016). Real-time RT-PCR assays are capable of rapidly detecting viral RNA in blood specimens (Cherpillod et al, 2016). The assays are extremely sensitive and have a high throughput (Broadhurst et al, 2016) and are routinely used for laboratory diagnosis of EV (Ro et al, 2017).

4. PREVENTION AND TREATMENT

4.1. Immune Responses to Ebola

The innate immune system is the first line of defense against pathogens. The macrophages, monocytes, and dendritic cells play important roles in the activation and regulation of the innate immune response, in addition to the subsequent transition to adaptive immunity. The activation of the innate immune response limits the initial spread of the pathogen. Adaptive immunity is responsible for the prevention of viral replication, dissemination and eventual clearance of the virus from host. The primary goal of vaccines is to enhance adaptive immunity (Marcinkiewicz et al, 2015; Wong et al, 2014).

The outcome of each viral infection phase depends on the balancing of two variables, viral pathogenicity and host defenses. Innate, humoral, and cellular immunity are triggered at each step of EV replication throughout the body of the host. As stated above, macrophages, monocytes, and dendritic cells are the first targets of EV. (Marcinkiewicz et al, 2015; Wong et al, 2014), impairing the initial and subsequent adaptive immunes responses, promoting unregulated replication and spread of EV. The production of type 1 interferon is associated with protection against the viral infection and replication. However, Ebola viral proteins, VP24 and VP35, can block intracellular signaling by endogenous interferons (Marcinkiewicz et al, 2015; Wonget al, 2014).

The infection of macrophages and antigen-presenting cells (APCs) leads to inflammation and aberrant immune response in fatal Ebola hemorrhagic fever (EHF). Additionally, massive apoptosis of T lymphocytes and natural killer (NK) cells is also seen. Fatal Ebola hemorrhagic fever (EHF) is characterized by the lack of virus specific Immunoglobulin G (IgG) and by barely detectable Immunoglobulin M (IgM) (Marcinkiewicz et al, 2015; Wonget al, 2014). Studies indicate that impaired adaptive immunity with the associated cytokine storm, bleeding, organ

failure, and death is characteristic of severe infection with Ebola. The very high mortality rates in infected patients support the theory that vaccination is the best strategy for prevention of EV (Marcinkiewicz et al, 2015).

4.2. Antivirals

Viruses have a simple structure. To multiply, they invade cells and use host cellular biochemical pathways to make new viral proteins and genetic material. The virus and host cell are intimately linked, so antiviral drugs must be able to specifically target the virus and not the host cell. Antiviral treatment is effective in decreasing transmission, morbidity, and mortality (Wiltinket al,1991; Huggins et al,1999).

Strategies to manage EV include those that directly target the virus, approaches to modulate immune responses, and management of disease (Wiltink et al,1991). Targeting viral replication is the most popular strategy for therapeutics. It is done either by preventing the entry of EV into the host cells or by targeting viral replication and packaging.

Currently, FDA has approved two monoclonal antibody-based treatments, Inmazeb and Ebanga, against Zaire ebolavirus. However, they are only approved for this subtype. Both can be used by adults and children. In October 2020, Inmazeb was approved, and it is based on the combination of three monoclonal antibodies (mAbs). The Ab cocktail targets the glycoprotein on the surface of EV which mediates attachment to cell receptor, thus allowing the virus to enter the host cell. The three mAbs can bind the glycoprotein, and block attachment and entry of the virus to the cells. The second drug, Ebanga, which was approved in December 2020, is a single monoclonal antibody (mAb). The function of Ebanga is similar to Inmazeb, it blocks the binding of the virus to cell receptor, which then prevents viral entry into the host cell. Both antibody treatments are safe and effective. However, patients may experience symptoms include fever,

chills, tachycardia, tachypnea. and vomiting (Centers for Disease Control and Prevention [CDC], 2021; World Health Organization [WHO], 2021).

An experimental drug, ZMapp, was tested during the 2014-16 Ebola outbreak in West Africa. It is not approved by FDA. It is a combination of three monoclonal antibodies manufactured in tobacco plant. The results during the outbreak suggested that ZMapp was beneficial. However, not enough people were not treated to prove that ZMapp is effective (National Institutes of Health [NIH], 2016).

4.3. Passive Immunity with Antibodies

Passive immunotherapy or serum therapy has been used for more than 120 years for treatment of bacterial and viral infections. Passive immunization with neutralizing antibodies is done by transferring serum from an infected and recovered individual to the symptomatic patient. Serum / plasma from Ebola survivors are used to treat patients in several countries since the 1976 epidemics (Chippauxet al, 2015; Mire et al, 2016). The method does not provide l00% protection especially after 3 days post-exposure to Ebola. Passive immunotherapy has not been properly optimized in terms of use, limitation, and benefits. Considering the political and economic constraints of endemic countries, high end technology such as the production of monoclonal antibodies is expensive and often has low yields, which greatly decreases the number of possible beneficiaries (Chippauxet al, 2015Mire et al, 2016). However, passive immunotherapy is considered one is one of the more promising approaches to treat Ebola worldwide due to the limited immunization options which are available (Mire et al, 2016).

Alternately, the use of equine polyclonal IgG fragments may have significant potential benefits because they are less expensive to produce compared to monoclonal antibodies (Mire et al, 2016). The distribution of antibodies in the host is related to composition, whole or fragments

25

of IgG, and route of administration. Complete IgG and F(ab') tend to remain in the compartment of administration, while smaller fragments such as Fab or Fv have a high rate of systemic distribution. Generating immunotherapeutic agents from animals is considered to be a complex process because the success of the approach depends on many factors such as the quality of immunogens, species of host, and immune response. In addition, therapeutic dosage depends on the amount of antigen in the body and its tissue distribution (Mire et al, 2016).

4.4. Vaccines

Several EV vaccines have been developed and are undergoing evaluation in either 1st or 3rd phase clinical trials. Vectored vaccines where the EV GP is expressed in heterologous vectors include ChAd3.EBOZ based on an attenuated version of a chimpanzee adenovirus, Ad26.ZEBOV based on Ad26 which is a human adenovirus serotype 26, MVA BN Filo based on attenuated poxvirus and Ad5.EBOV which contains the human adenovirus serotype 5 vector.

Several other experimental vaccines are under development or clinical trials. Among the approved vaccine, rVSV-EBOZ provides protection against the Zaire ebolavirus (Centers for Disease Control and Prevention [CDC], 2020; World Health Organization [WHO], 2021). The rVSV-EBOZ consists of vesicular stomatitis virus (VSV), a rhabdovirus, expressing the Ebola Zaire glycoprotein, and is also a vectored recombinant vaccine (Centers for Disease Control and Prevention [CDC], 2020; World Health Organization [WHO], 2021). As a vector vaccine is designed in a way to increase the body's immunity against the infection by using a modified virus, and the vaccine contain a weaken virus which is advantageous. It triggers a strong immune response by infecting cells in a way that a real virus would and causes the immune system to produce large amount of antigen. Generally, vector vaccines may cause fetal infection, and it

possible to produce the disease. However, it is not possible for people to become infected with EV from the vaccines.

Based on clinical trials involving more than 11,000 persons in the Democratic Republic of Congo, Guinea, and Sierra Leone, rVSV-ZEBOV vaccine is safe, and it is well-tolerated and elicits a strong immune response (Mandal, 2019). Of the 11,000 trial volunteers, no cases of Ebola were identified among 5,837 people who received the vaccine (Belluz, 2018). People who are at risk of infection with Ebola are eligible to get vaccinated and the Advisory Committee on Immunization Practices (ACIP) recommends that adults responding to an outbreak, laboratorians, and healthcare employees are eligible for the pre-exposure vaccination against Ebola. The vaccine is not planned for commercial marketing, and only who in risk can get the vaccine.

Side effects of rVSV-EBOZ vaccination are minimal and may include fever, headache, nausea and others (Sridhar, 2015). As per the world health organizations (World Health Organization [WHO], 2021), Ebola outbreak response plan has been developed with respect to west Africa. Several strategic action plan for response to Ebola Outbreak is proposed in case of reoccurrences as well, some of which are specific to countries like Guinea, Liberia, and Sierra. The major aspect of Ebola pandemic plans is to stop the transmission of the virus and prevent new outbreaks in the country. Ebola can only be transmitted from symptomatic individuals. However, there are individuals that can test positive without having any symptoms of the disease. Contact tracing is done by identifying individuals that were in contact with Ebola infected individuals who are isolated for 21 days while under observation (World Health Organization [WHO], 2014).

It is not yet known how long the immunity lasts, but it may take several years. A research is being conducted in this regard by the Strategic Advisory Group of Experts (SAGE) at WHO.

The effectiveness rate of Ebola vaccine is estimated to be 97% (World Health Organization [WHO], 2020).

It is found that the patients infected with EV that do not survive the infection, stay for up to 7 days. However, more than half of the infected patients survive 3 weeks after infection. The mean survivor time in terms of hospital stay is 20.38 ± 7.58 days (Ji et al., 2016).

DRC (2019) data indicates that health care workers account for up to 5% of the victims in the Ebola outbreak. It shows that despite the precautions taken by the health care workers, they get affected by the outbreak of Ebola as well. Moreover, it is indicated that the survival rate is 64.7% for 8 days among the Ebola patients while it is 86.1% for 12 days or more in patients after the onset of symptoms (Qureshi et al., 2015). In a study, EV shedding was investigated, and findings suggest that EV sheds in different compartments in acute Ebola disease (Vetter et al., 2016). The EV was presented after days from recovery patients, and it can persist for months. The broad range includes, semen, tears, saliva, amniotic fluid, aqueous humor, breast milk, vaginal secretions, sweat, urine, blood, stool and cerebrospinal fluid (CSF). For example, the shedding may persist for 3 to 9 months in the semen.

Table 3

The Antivirals and Vaccines

References	Name	Vaccine/ Drugs	Status	Feature
(Centers for Disease Control and Prevention [CDC], 2021; World Health Organization [WHO], 2021)	Inmazeb	Drug	In use	3 monoclonal antibodies (mAbs)
(Centers for Disease Control and Prevention [CDC], 2021; World Health Organization [WHO], 2021)	Ebanga	Drug	In use	A single monoclonal antibody(mAb)
(National Institutes of Health [NIH], 2016)	Zmapp	Drug	Not approved	3 monoclonal antibodies (mAbs)
(Centers for Disease Control and Prevention [CDC], 2021; World Health Organization [WHO], 2021)	rVSV-EBOZ	Vaccine	In use	Attenuated VSV
(Sridhar, 2015)	ChAd3.EBOZ	Vaccine	Phase III	Attenuated chimpanzee adenovirus
(Sridhar, 2015)	Ad26.ZEBOV/ MVA BN	Vaccine	In use	Human adenovirus serotype 26 vector / Attenuated poxvirus
(Sridhar, 2015)	Ad5.EBOV	Vaccine	Phase I	Human adenovirus serotype 5 vector

4.5. Barriers to the Control and Prevention of Ebola

The Ebola virus disease outbreak in west Africa is still spreading at an alarming rate, with no end in sight, and rapid containment is hindered by various obstacles such as health system, fears, high population mobility, cultural beliefs (WHO, 2015). The weakness of local health systems made it difficult to maintain the Ebola and effectively respond to the outbreaks. A systemic weakness in training of healthcare professionals and lack of resources such as equipment, drugs, and medical supplies were reported.

Additionally, the standard containment steps like early diagnosis and isolation of cases, contact tracking, and management protocols, are difficult to enforce due to a lack of capacity. Since the early symptoms of EVD are similar to other diseases such as malaria and typhoid fever, the diagnostic capability is very important. Control of epidemics requires coordination of medical services which include early detection, contact tracing for people who have been exposed to the and treatment for people who have been contaminated to EV (WHO, 2015).

The infectious disease creates fear and psychological reactions. Fear played an indelible role in influencing behaviors among the public during the EVD outbreaks in West Africa. It is the most difficult obstacle to overcome. In addition, it leads to contacts of cases to escape from the surveillance system and families hiding symptomatic ones or taking them to traditional healers. It is not only accelerated the spread of Ebola, but it causes other serious problems as well and harming the mental health of people and communities (WHO, 2015).

West Africa is known for its high levels of human migration across porous borders. To an extent, poverty drives the mobility because people travel daily looking for food or work. additionally, West African families have relatives who living in different countries. Two major impediments to prevention and control, contact tracing and situation improvement in one country,

30

have arisen as a result of the mobility. First, the contact tracing is very difficult as a result of population movement across countries. Second, when one country experience intense spread of EV, other countries remain at risk of infection, no matter how their response are strong (WHO, 2015).

Cases were linked to cultural believes and funeral practices. People and healthcare workers are thought to have become infected when they contact with dead people. The EV spread through the networks that bind populations together in a culture that compassionate ritual care for dead bodies. Besides that, for many people, particularly the poor, traditional healers or self-medication are a preferred health care choice due to a lack of access to government-run health facilities or cultural believes. It can cause the spread of EV and impeded rapid containment (WHO, 2015).

5. CONCLUSION

The EV is a complex pathogen as well as a fatal virus. It is caused by several subtypes, and only 4 species (zaire, Sudan, Taï Forest, and Bundibugyo viruses) can infect both human and animals. It is unknown if the other 2 stains can infect humans. We do not know the origins of Ebola virus. However, we assume the virus is animal-borne, based on the existence of related viruses, with the most likely vector being bats or nonhuman primates like chimpanzees, monkeys, etc. EV can be transmitted to other animals and humans by direct contact with infected or dead animals carrying the virus. The virus spreads to other people through direct contact with the blood, body fluids, etc. of infected humans. Additionally, people can expose to the disease through direct contact with dead bodies through funeral practices.

The EV affects the innate and adaptive immune systems and then causes necrosis in various organs such as liver, kidney, spleen, and lung. The EV can be detected by cell culture, ELISA and PCR after the onset of symptoms. These tests can be used to detect the disease in early or late stages. The diagnostic tests can allow early triaging of infected people, which then reduces potential transmission and patients can get healthcare earlier.

Over the past decades, scientists have attempted to understand EV, and they understand the reasons why Ebola occurs repeatedly in west African countries. The understanding of Ebola and its interaction with the host immune system helped scientists to create a vaccine. The rVSVEBOZ vaccine and treatments are available and approved by FDA.

The majority of those infected had the disease in hospitals or funeral homes. Gowns, face masks, face shields, gloves, antiseptics, and boots are available. To stop the transmission of the disease, prohibitions and travel bans were enforced, as well as safe burials of those who died as a result of the outbreak by trained staff and medical services at Ebola Treatment Centers. The

education about the disease is very important so Nurses' importance as frontline health-care workers cannot be overstated in the fight against Ebola, and they should be involved in a number of activities related to the prevention and management of Ebola disease, including public education about the disease and how to keep a sanitary atmosphere to reduce transmission, treating the ill with evidence-based medicine in consultation with physicians, isolating the sick, and screening for people who have Ebola disease fever.

The EVD is lethal, and the sanitary atmosphere must be preserved to prevent an Ebola outbreak in west Africa and around the world. Any behavior that may result in the increase in outbreak must be avoided. The most effective ways to control the Ebola outbreaks is hand hygiene and avoiding contact with a potentially infected person. Additionally. we need to stay away from areas where EV is common, and travelers from that area need to be tested to diagnose whether they have been exposed to the virus. Following the precautions is significantly important to reduce the transmission and prevents more epidemics.

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