GENERAL OVERVIEW OF EBOLA VIRUS

CHAPTER ONE

1.0 INTRODUCTION- A GENERAL OVERVIEW OF THE VIRUS

Ebola virus is a member of the family *Filoviridae,* which belongs to the order *Mononegavirales* (Ghanzanfar *et al*., 2015). It is also a member the genus *Ebolavirus*, and is known to cause fatal infection in humans (Hirokazu *et al*., 2015). The virus is a pleomorphic and filamentous virus that is enclosed in an envelope and consists of a lipid bilayer coat provides protection for the virus genome and facilitates its entry into host cells (Ansari, 2014). Its genome is a single negative-sensed RNA which is about 19Kb in length with a mean unit length of 1,200 nm (Sullivan *et al*., 2003; Hirokazu *et al*., 2015). Although the structure of the genome is similar among the species, it has been shown using phylogenetic analysis that the species have formed independent lineages with wide genetic divergence (Feldmann *et al*., 2011).

Presently, there are 5 species of the virus that have been identified as Zaire, Sudan, Bundibugyo, Reston, and Taï Forest while only three have been associated with massive outbreaks in Central Africa, with *Zaire ebolavirus* being the most virulent. The *Zaire ebolavirus* is known to have a mortality rate as high as 55%-88% and it is said to be the cause of the current outbreak in West Africa (WHO Ebola Response Team 2014; Ghanzanfar *et al*., 2015). Although the first outbreak of Ebola Virus Disease (EVD) was reported in 1976 in the Democratic Republic of the Congo (Burke *et al*., 1978), reports of small outbreaks in some countries in Central Africa, including Sudan and Uganda surfaced (Feldman *et al*., 2013), with cases estimated at 2350 between the 1970s and 2013 (Hirokazu *et al*., 2015).

In March 2014, the first case of the current EVD outbreak in West Africa was reported in Guinea (Baize *et al*., 2014) which then spread across land by land travel to Liberia, Sierra Leone, and Senegal while it spread to Nigeria by air travel. It was then declared a "Public Health Emergency of International Concern" on August 7, 2014 (Camacho *et al*., 2014; WHO Fact sheet, 2014). EVD is a very severe and often fatal illness in humans which is known to cause multiple, serial, and nonspecific-disease symptoms including high fever, headache, vomiting, anorexia, diarrhea,
And aching muscles (Sureau *et al*., 1989; Baize *et al*., 2014; Hirokazu *et al*., 2015). Upon initial human infection with Ebola virus through contact with an infected animal, such as a fruit bat or non-human primate (WebMD, 2018), it spreads from person to person via direct contact with the blood or body fluids of an infected symptomatic person or dead body. Entry of the body fluids such as urine, saliva, sweat, faeces, vomit, breast milk, vaginal fluid, and semen, through broken skin or mucous membranes in the eyes, nose, or mouth can lead to infection (Aceng *et al*., 2020).

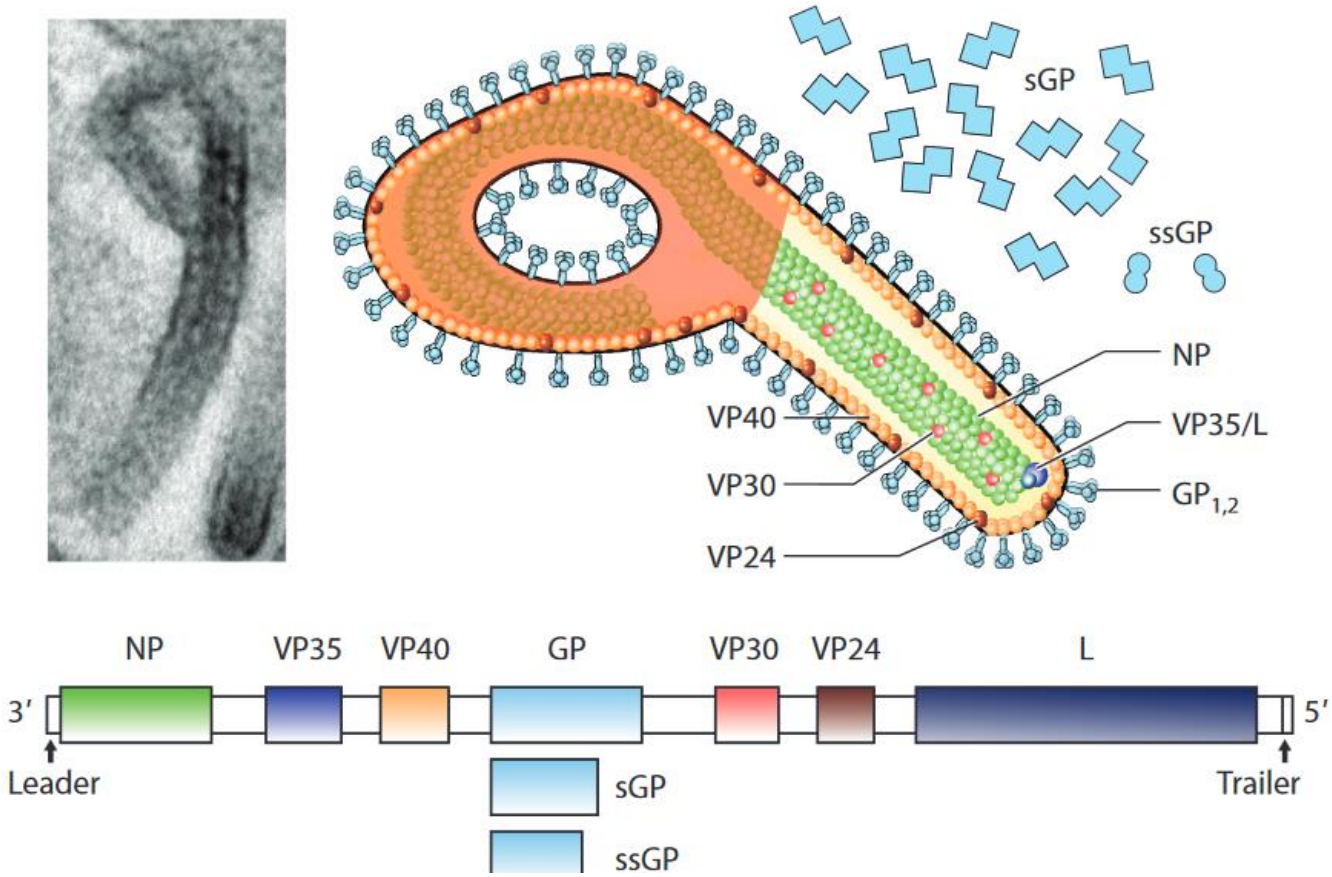

Figure 1. Ebolavirus structure indicating various proteins and the genes that code for them. The genome displays the following structure: 3′-leader→nucleoprotein (NP) gene → viral protein (VP) 35 gene→VP40 gene→ glycoprotein (GP) gene→VP30 gene→VP24 gene → polymerase (L) gene → 5′- trailer.
Baseler et al., 2017

1.1 EPIDEMIOLOGY

Since 1976, the outbreaks of Ebola virus have been occurring in forest ecotypes described as the 'Ebola Forest Belt' which provide habitats for diverse fauna (Nsio *et al*., 2019). Human influences and natural ecological processes, such as land use for agriculture and livestock grazing purposes or rural urbanization caused alterations of the natural ecosystem thereby increasing proximity between human population and wildlife, leading to enhanced contacts with the natural hosts of the virus (Rugarabamu *et al*., 2020). The first reported EVD outbreaks occurred in DRC (Johnson et al., 1977) and Sudan (Bres, 1978) in 1976 with Zaire ebolavirus (EBOV) and Sudan ebolavirus (SUDV) which were the first two human pathogenic Ebolavirus species to be isolated causing these two epidemics, respectively. However, the Zaire ebolavirus (EBOV) have been the leading cause of outbreaks in the past 4 decades, with 33,392 out of the total 34,356 cases in 34 EVD outbreaks which has caused 14 823 deaths in 11 countries in Sub-Saharan Africa including the DRC, South Sudan, Uganda, Mali, Nigeria, Sierra Leone, Guinea, Liberia, South Africa, Gabon and Côte d'Ivoire (MacNeil and Rollin, 2012; CDC, 2018; Nsio *et al*., 2019; Rugarabamu *et al*., 2020) (Table 1). Thus, Sub-Saharan Africa continues to face considerable challenges in EVD control

1.1.1 Ebola Virus Disease Occurrence and Epidemic from the 1970s Till Date

Prior to 2013, Ebola Virus Disease occurred mainly in the rainforest areas of Central Africa including DRC (1976, 1977, 1995, 2007, 2008, 2012), Sudan (2004), Gabon (1995–96, 2001), and Uganda (2000, 2007, 2011, 2012) which had its largest documented EVD outbreak in 2000–200, recording 425 cases and 224 deaths (CDC, 2018; Aceng *et al*., 2020). The disease is not known to be an immunogenic disease with specific natural seasonality as it can spread throughout the year

as suggested by historical data of Ebola epidemic outbreaks. So far, EVD is mainly endemic to the African continent, especially in West Africa whereas other countries, such as the United States, Thailand, United Kingdom, Canada, and Spain recorded sporadic and possibly imported cases (Feldmann *et al.*, 2011). The survival of the Ebola virus is favored by natural environment associated with the African continent because the natural and alternate hosts of Ebola virus such as fruit bats, apes, and monkeys are widely distributed in Africa. Also, according to the historical data (CDC, 2014), EVD mainly distributes between 10° North and South of the equator, with the temperature that benefits Ebola virus survival throughout the year (Liu *et al.*, 2015).

In Democratic Republic of Congo (DRC), the outbreak has been centered around the northeast of the country and with the number of cases having surpassed 3000, it is now by far the country's largest-ever Ebola outbreak. It is also the second biggest Ebola epidemic ever recorded, behind the West Africa outbreak of 2014-2016. However, in 2020, the number of cases recorded per week has declined drastically, with just a handful of cases recorded throughout January and February. As of 5 May 2020, a total of 3462 EVD cases, including 3317 confirmed and 145 probable cases have been reported, of which 2279 cases died (overall case fatality of 66%) (WHO, 2020).

Table 1 EVD outbreaks, virus type, morbidity, mortality and case fatality rate in affected countries

Year	Country	Virus	Cases	Deaths
1976	Democratic Republic of Congo	EBOV	318	280
1976	South Sudan	SUDV	284	151
1977	Democratic Republic of Congo	EBOV	1	1
1979	South Sudan	SUDV	34	22
1994	Gabon	EBOV	52	31
1994	Côte d'Ivoire	TAFV	1	0
1995	Democratic Republic of Congo	EBOV	315	250
1996	Gabon	EBOV	37	21
1996-1997	Gabon	EBOV	60	45
1996	South Africa	EBOV	2	1
2000-2001	Uganda	SUDV	425	224
2001-2002	Gabon	EBOV	65	53
2001-2002	Republic of Congo	EBOV	57	43
2002-2003	Republic of Congo	EBOV	143	128
2003	Republic of Congo	EBOV	35	29
2004	South Sudan	SUDV	17	7
2005	Republic of Congo	EBOV	12	10
2007	Democratic Republic of Congo	EBOV	264	187
2007-2008	Uganda	BDBV	149	37
2008-2009	Democratic Republic of Congo	EBOV	32	15
2011	Uganda	SUDV	1	1
2012	Uganda	SUDV	11	4
2012	Democratic Republic of Congo	Bundibugyo	36	13
2012	Uganda	SUDV	6	3
2014	Democratic Republic of Congo	EBOV	69	49
2014	Guinea	EBOV	3814	2544
2014	Liberia	EBOV	10678	4810
2014	Sierra Lone	EBOV	14124	3659
2014	Nigeria	EBOV	20	8
2014	Mali	EBOV	8	6
2017	Democratic Republic of Congo	EBOV	8	4
2018	Democratic Republic of Congo	EBOV	54	33
2018-2019	Democratic Republic of Congo	EBOV	3220	2150
2019	Uganda	EBOV	4	4

Adapted from Rugarabamu *et al.*

BDBV: Bundibugyo ebolavirus; EBOV: Ebola virus; EVD, Ebola virus disease; SUDV: Sudan Ebola virus; TAFV: Taï Forest ebolavirus.

1.1.2 Demographic distribution

Generally, people at any age group can be infected by this Ebola virus. Close to 80% of the EVD cases in some areas were in adults between ages 21 to 60 years old (Maganga *et al.,* 2014). This is probably because most people that fall within this age range are more likely to be physically active (particularly hunting for food) and therefore more liable to be exposed to the virus. The fatality rate of EVD for ages below 21 years is significantly lower than that in the age group of 45 years and older (57% vs. 94%, $P < 0.05$) as observed by Schieffelin *et al*., 2014. Due to the ease of transmission from human-to-human via close contact or via droplets by sneezing and coughing of patients, medical workers thus belong to a high-risk population. Some studies have found that the high-risk occupations for EVD are nurses, aid nurses, physicians, laboratory technicians, and physician assistants. Consequently, hospitals, clinics, and Ebola treatment units are considered and found to be fertile for Ebolavirus transmission (Matanock *et al*., 2014) Also, different genetic backgrounds of affected populations may play a major role in the different susceptibilities to EVD, but future mechanistic studies will have to be conducted to investigate the role of genetic factors on the development and progression of EVD (Rasmussen et al., 2014; Liu *et al*., 2015).

1.1.3 Outbreak in West Africa

Between December 30, 2013, and September 14, 2014— a 37-week period, a total of 4507 confirmed and probable EVD cases were reported to the WHO wile between September 8 and September 14, a total of 718 confirmed and probable cases and 289 deaths were reported (Nishiura and Chowell, 2014). The numbers of confirmed and probable cases reported by each country over time are shown in Figures 1 and 2. The median age of persons with EVD was 32 years (interquartile

range, 21 to 44), and there were no significant differences in the age distribution of persons with EVD among countries. Most persons with EVD (60.8%) were between 15 and 44 years of age (this age group makes up only 44% of the population) (Gomes *et al*., 2014). There were also no significant differences among countries in the total numbers of male and female persons with EVD reported (49.9% of the total were male patients). By September 14, a total of 318 cases, including 151 deaths, had been reported among health care workers, indicating how EVD took a heavy toll among health care workers in Guinea, Liberia, and Sierra Leone (WHO Ebola Response Team, 2014).

Since late 2013, the EVD outbreak that hit West Africa that was caused by the EBOV species of Ebola virus, represents an unprecedented event in terms of geographical spread and number of affected individuals. As of December 31, 2014, a total of 20,200 confirmed cases of EVD were reported. A total of 2707 confirmed cases were reported in Guinea, including 1708 deaths (case-fatality rate 63,1%); in Liberia a total of 8018 confirmed cases were reported, including 3423 deaths (case-fatality 42,7%); in Sierra Leone a total of 9446 confirmed cases were reported, including 2758 deaths (case-fatality rate 29,2%). In less affected countries including Nigeria, Mali, and Senegal, respectively, 20, 8, and 1 confirmed cases were reported, including, 8, 6, and 0 deaths respectively with their case-fatality rates being 40%, 75%, and 0%, respectively (WHO, 2014).

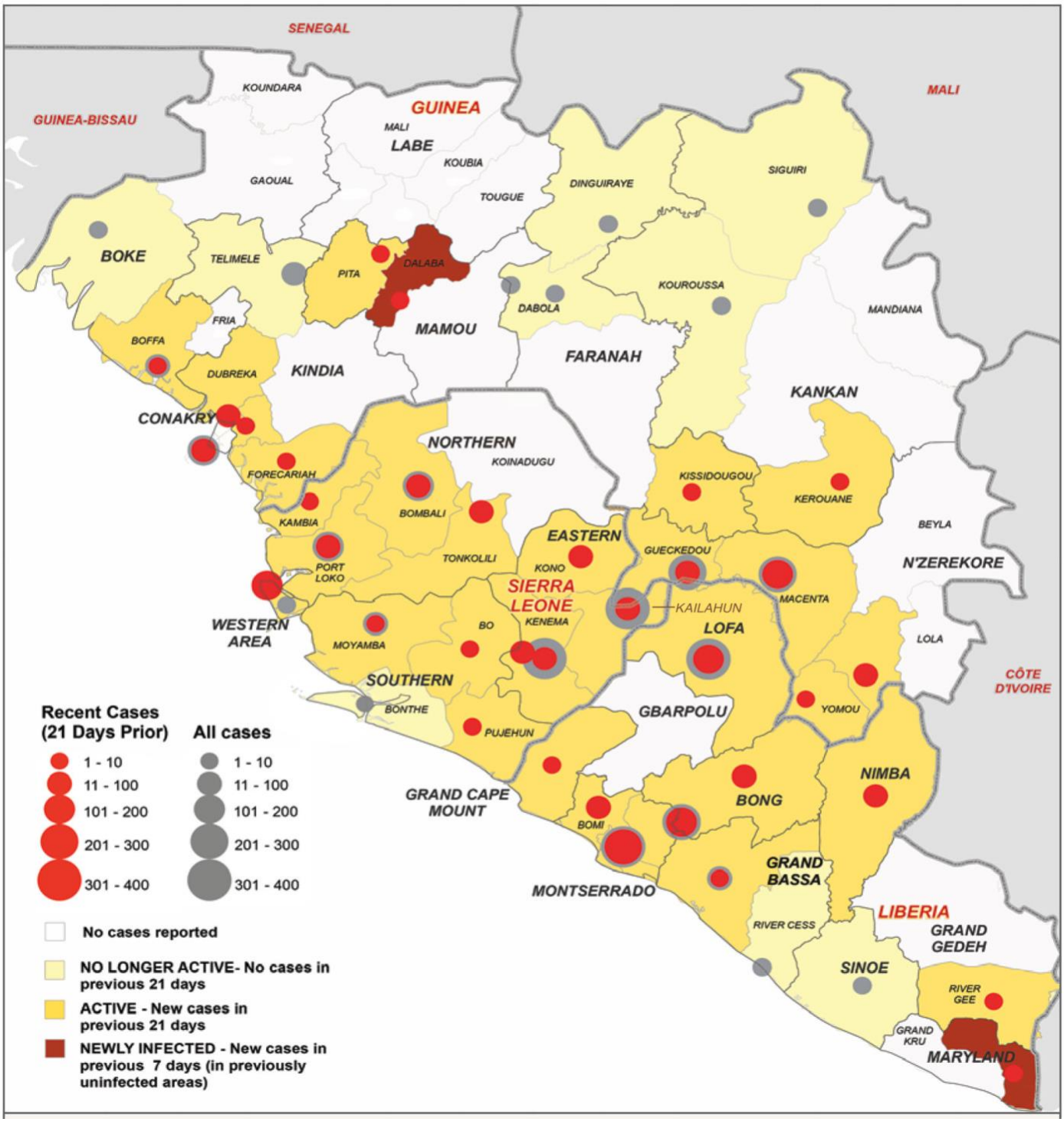

Figure 2. Districts Affected by Ebola Virus Disease in Three Countries in Africa

The map shows the districts that have been affected by Ebola virus disease in Guinea, Liberia, and Sierra Leone. Gray circles indicate the total numbers of confirmed and probable Ebola cases reported in each affected district, and red circles the number reported during the 21 days leading up to September 14, 2014.

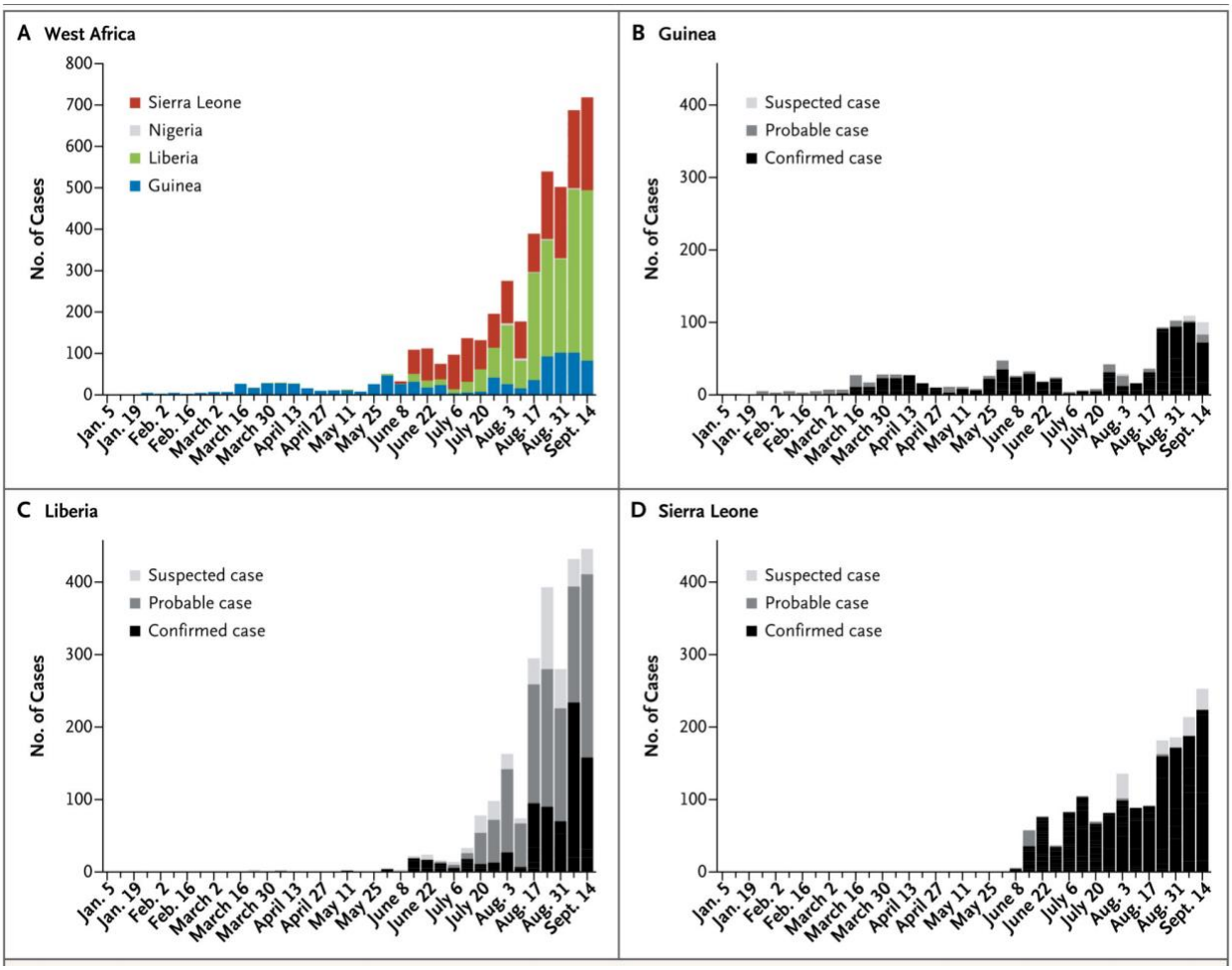

Figure 3. Weekly Incidence of Confirmed, Probable and Suspected of Ebola Virus Disease Cases.

- **Geographic Origin and the Spread of Infection in West Africa**

In December 2013, the first cases occurred in Guéckédou and Macenta districts (Briand *et al*., 2014), which happened to be the focus of the epidemic in Guinea. In March 2014, an increase in the numbers of cases in these two districts, in addition to the first reports from Lofa and other districts in Liberia, was followed by the discovery of cases in Conakry, the capital. Then, in May and June of 2014, another rise in case incidence in Guinea — first in Guéckédou and Macenta and then in the capital — occurred. During this period, particularly in May, the focus of the epidemic in Guinea expanded to the neighboring districts of Kenema and Kailahun in Sierra Leone, and in June further cases were reported in Lofa district in Liberia. These five districts remained the focus of transmission in the border areas of the three countries and from July 2014 onward, there were sharp increases in case numbers at the epidemic foci in all three countries, at other sites away from the epicenter, and in the capital cities of Conakry, Freetown, and Monrovia (WHO Ebola Response Team, 2014). However, although EVD has spread to many parts of Guinea, Liberia, and Sierra Leone, it was not reported in all districts in the countries: among the total of 67 districts in the three countries, only 43 reported one or more confirmed, probable, or suspected cases, and more than 90% of cases were reported from just 14 districts at that time (WHO, 2014).

CHAPTER TWO

2.0 PATHOGENESIS

EVD is a typical zoonotic disease, but the wild reservoir of EBOV is still unclear. Non-human primates, like apes or monkeys, have long been considered as important sources of infection to humans; however, these primates might not be original reservoir species because they could be killed by this infection (Groseth *et al*., 2007). The viruses can exist in body fluids such as blood, semen, and genital secretions as well as the skin of contagious patients (Rodriguez *et al*., 1999; Liu *et al*., 2015) where they can survive for hours at room temperature (20°C−25°C), and for weeks at low temperature (Okware *et al*., 2002). Infection in human can occur by direct contact with blood and body fluids of infected animals such as apes, gorillas, fruit bats, and monkeys (Leroy *et al*., 2005; CDC, 2014). While there is no evidence that pet cat and dogs, mosquitoes, or other insects can transmit Ebola virus (CDC, 2014), human-to-human transmission occurs via direct contact with the blood, organs, secretion, and other bodily fluids (such as urine, feces, semen, breast milk, mucus, vomit) of an infected person, and via surface and materials contaminated with these fluids (Bausch *et al*., 2007; Ghazanfar *et al*., 2015). This virus is known to enter the human body through mucosal surfaces, abrasions and injuries in the skin or even by direct parental transmission. Although infection through intact skin is considered unlikely, it is still not excluded (Hofmann-Winkler *et al*., 2012; Goeijenbier *et al*., 2014).

In both humans and in nonhuman primates, high-level EBOV replication that is associated with systemic dissemination to multiple cell types results in a complex pathogenesis that includes both damaging immune suppression and immune overactivation in different aspects of the immune response, disordered coagulation, and tissue damage due to direct viral and indirect host-mediated effectors. Once the virus gets inoculation into mucous membranes or broken skin, it targets the

dendritic cells and other cells of the macrophage lineage (Geisbert *et al*., 2003). These infected cells then travel via the lymphatic vessels to lymph nodes and nodal chains, where virus replication and dissemination occur prior to the start of symptoms. After symptom onset, estimation of viral load by quantitative reverse transcriptase polymerase chain reaction (q-RT PCR) assay increases exponentially in blood, from undetectable levels to often >105 viral particles/ml (Edwards *et al*., 2015; Lanini *et al*., 2015). This increase in viral load is consistent with the virus overcoming and evading the host's innate immune defenses and becoming widely disseminated in the blood. Some of the major mechanisms by which the virus evades the immune defenses are listed out in table 2 (Baseler *et al*., 2017).

Table 2. Mediators of Ebolavirus Immune Evasion

Viral Mediator	Mechanism of Immune Evasion
sGP	Binds anti-GP neutralizing antibodies
VP24	Inhibits type I IFN signaling
VP35	Inhibits type I IFN production
	Inhibits dendritic cell maturation

GP, glycoprotein; IFN, interferon; sGP, soluble glycoprotein; VP, viral protein.

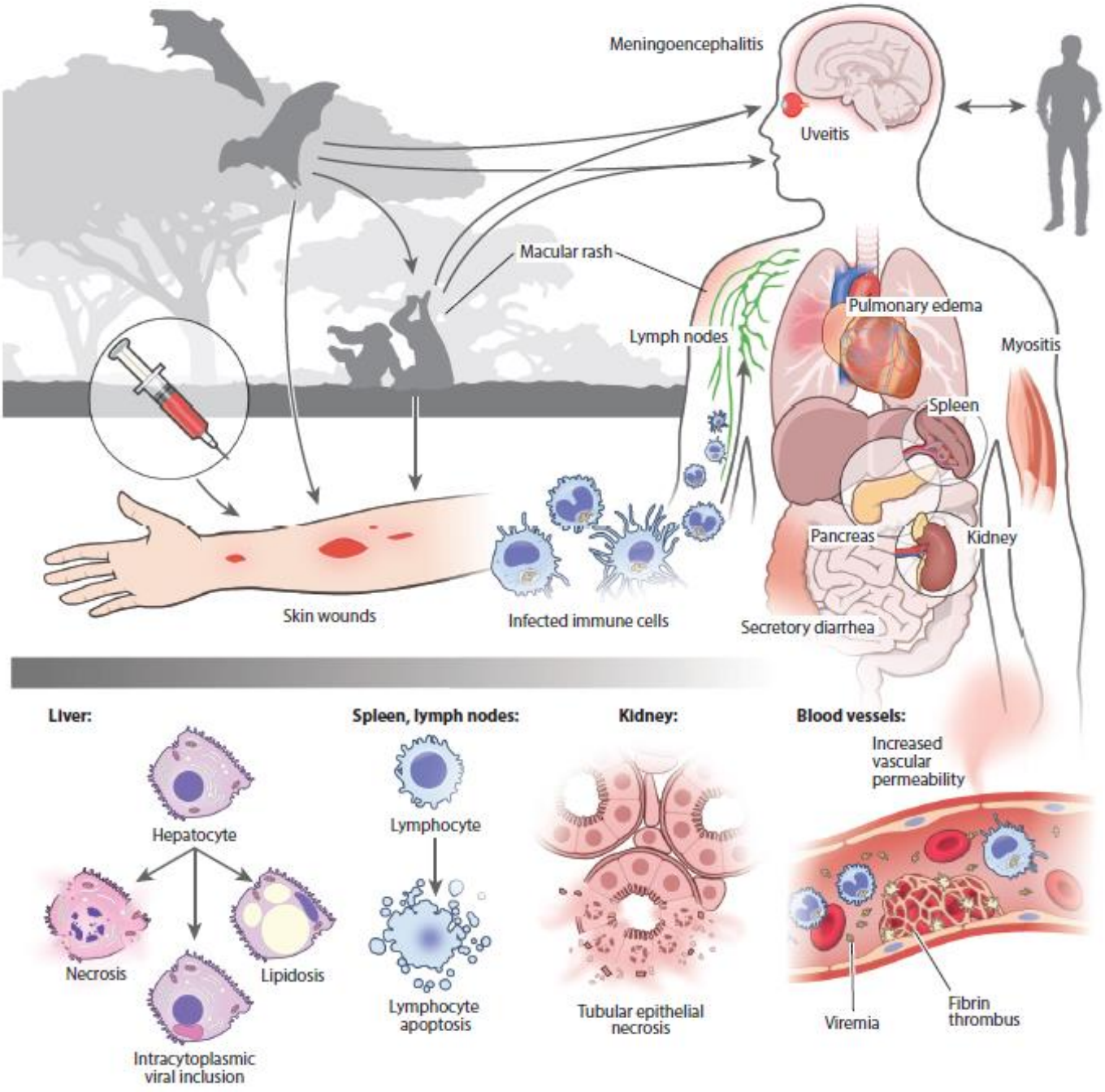

Figure 4. The transmission and pathogenesis of ebolavirus infection. Zoonotic, nosocomial, or person-to-person transmission of ebolavirus leads to viral infection of mononuclear phagocytes, which transport the virus to regional lymph nodes. Virus replication is followed by viremia with widespread viral dissemination, leading to tissue and vascular damage.
Baseler et al., 2017

2.1 Pathophysiologic Mechanisms

EBOV employs multiple mechanisms to achieve its early extensive replication and dissemination which is predicated upon an effective evasion of the host immune responses. Example of this is the antagonism of the type I interferon (IFN) response, mediated by the EBOV structural proteins VP24 and VP35 (Basler *et al*., 2000; Leung *et al*., 2010; Yen *et al*., 2014; Xu *et al*., 2014). The EBOV VP24 prevents the dimerization of tyrosine kinases and nuclear translocation of signal transducer and activator of transcription 1 (STAT1), this blocking IFN signaling (Reid *et al*., 2006) while The IFN inhibitory domain (IID) of VP35 binds dsRNA in host cells and prevents it from binding the cytoplasmic receptors *retinoic acid-inducible gene-I* (*RIG-I*) and *melanoma differentiation-associated gene 5* (*MDA-5*), causing an impairment of the phosphorylation of IFN regulatory factor (IRF)-3 and IRF-7, thereby inhibiting type I IFN expression (Basler *et al*., 2000; Leung *et al*., 2011). Additionally, VP35 inhibits maturation of dendritic cells by interfering with the RIG-I signaling pathway to prevent upregulation of MHC I and MHC II and the costimulatory molecules CD40, CD80, and CD86, thus impairing antigen presentation to CD8+ and CD4+ T cells and T-cell activation (Yen *et al*., 2014; Jin et al., 2010) and thereby impeding linkage of the innate and adaptive immune responses. Data from the study by Yen et al. (2014) showed that by expressing VP35-WT, the virus has sufficient ability to impair several aspects of human monocyte-derived dendritic cells (MDDCs) maturation beyond IFN-α/β and proinflammatory cytokine production. In the absence of other viral factors, VP35 also hindered the expression of chemokine genes after infection with Sendai virus (SeV). Additionally, the upregulation of CD40 and CD80 which are cell surface markers of DC maturation, as was T cell activation was found to be

impaired. The findings from the study are like that of Bosio et al. (2003) in which the expression of VP35 was shown to suppress IFN-α production in human MDDCs. As also reported by Jin et al. (2010), delivery of VP35 had pleiotropic suppressive effects on mouse DCs. Yen et at. (2014) clearly revealed that, even when VP35 is expressed in the absence of other viral proteins, it can greatly suppress the MDDCs, however, its suppressive activities do not extend to TLR signaling pathways. Therefore, in the context of DCs, VP35 suppresses multiple aspects of DC maturation when maturation is induced through RLRs. However, the effects of VP35 upon TLR-induced maturation are more limited

On the other hand, sGP, a viral protein which is released in abundance during acute illness and thus circulating at high levels in the serum, acts as a decoy by binding EBOV-neutralizing antibodies to impair a protective humoral immune response (Ito *et al*., 2001). It is hypothesized that glycosylation of transmembrane GP may sterically impede the binding of neutralizing antibodies to it (Francica *et al*., 2010), as is the case with several unrelated viruses. Although lymphocytes are not infected by the EBOV, lymphocyte apoptosis results in early lymphopenia and lymphoid depletion in the spleen and lymph nodes, further affecting adaptive immune responses (Baseler *et al*., 2017). The mechanism underlying lymphocyte apoptosis is not well understood, although intrinsic and extrinsic apoptotic pathways are both thought to be involved (Wauquier *et al*., 2010; Baseler *et al*., 2017).

Ebola virus invades host tissues through infected fluids that come in contact with the mucosal or skin breaks (Ansari, 2014), and preferably replicate in the monocytes, macrophages, and dendritic cells (Beeching *et al*., 2014). Tissue damage in EBOV-infected individuals is usually caused by

multiple interrelated mechanisms, including but not limited to direct viral-induced cytopathic effects and indirect organ injury mediated by host inflammatory responses, endothelial dysfunction, and disordered coagulation (Baseler *et al*., 2017). It has been shown by In vitro studies that cytopathic effects, including cell rounding and detachment, are directly mediated by viral GP (Yang *et al*., 2000). Proinflammatory cytokines and chemokines such as tumor necrosis factor (TNF)-α, interleukin (IL)-1, IL-6, monocyte chemoattractant protein (MCP)- 1, macrophage inflammatory protein (MIP)-1α, andMIP-1β, as well as increased TNF-related apoptosis-inducing ligand (TRAIL) expression and production of nitric oxide (NO), are among the features that have been observed in fatal EVD and are likely contribute to lymphoid cell apoptosis (Cheepsattayakorn and Cheepsattayakorn, 2015) and organ injury that is associated with the disease (Hensley *et al*., 2002; Wauquier *et al*., 2010; Baseler *et al*., 2017). These proinflammatory cytokines and chemokines recruit leukocytes to infected tissues in order to restrict the virus dissemination and kill the already virus-infected host cells, thus contributing to tissue damage (Topham *et al*., 2009; Baseler *et al*., 2017). Additionally, Nitric oxide released by activated macrophages may trigger parenchymal cell apoptosis or necrosis (Wei *et al*., 2000). In vitro studies suggest that increased TRAIL expression and increased Fas–Fas ligand interactions can trigger lymphocyte apoptosis, leading to severe lymphoid depletion in the spleen and lymph nodes (Gupta *et al*., 2007; Baseler *et al*., 2017).

When EBOV infects mononuclear phagocytes and immune system activation occurs, increased tissue factor expression on monocytes/macrophages is observed, which then triggers the extrinsic coagulation pathway leading to fibrin thrombus formation (Geisbert *et al.*, 2003). TNF-α may decrease thrombomodulin levels, and liver injury may impair production of proteins C and S; these effects jointly contribute to decreased anticoagulant effects (Baseler *et al*., 2017). Coagulopathy,

a bleeding disorder, in EVD is usually characterized by thrombocytopenia, formation and deposition of fibrin thrombi, and increased fibrin degradation products (Geisbert *et al*., 2003; Rollin *et al*., 2007). Microvascular fibrin thrombi contribute to tissue ischemia and necrosis; clotting factor consumption predisposes the infected individual to bleeding within tissues and from mucosal surfaces (Taylor *et al*., 2001). Classical signs of EVD, including conjunctival and gingival hemorrhages and hemorrhages from body orifices and venipuncture sites, are thought to represent disseminated intravascular coagulation (DIC) (Baseler *et al*., 2017).

Endothelial cells are also infected by EBOV, causing swelling, necrosis, and loss of the endothelial cells which have been reported in a few fatal cases and likely contribute to vascular permeability and intravascular coagulation (Martines *et al*., 2015). Also, endothelial cell rounding, detachment, and loss are caused by EBOV GP in vitro. This potentially compromises intercellular tight junctions, eventually leading to increased vascular permeability (Takada *et al*., 2000; Baseler *et al*., 2017). Release of Nitric Oxide (NO) by endothelial cells and monocytes/macrophages as well as the release of TNF-α by monocytes/macrophages, induces vasodilation and increased vascular permeability, thus contributing to intravascular volume depletion (Jerca *et al*., 2002).

2.2 Clinical Manifestation

The initial symptoms of EVD include fever, headache, fatigue, sore throat, and muscle pain, which are followed by anorexia, nausea, diarrhea, vomiting, rash, abdominal pain, cough, shortness of breath, postural hypotension, edema, headache, confusion, and coma while in some cases, a maculopapular rash develops after 5–7 days of symptoms (Ghazanfar *et al*., 2015). In the 2014

outbreak, the most common symptoms reported between symptom onset and case detection were fever (87.1%), fatigue (76.4%), vomiting (67.6%), diarrhea (65.6%), loss of appetite (64.5%), headache (53.4%) (Cheepsattayakorn and Cheepsattayakorn, 2014). This disease can rapidly evolve into a severe state with a rapid clinical decline. This disease phase is characterised by potential haemorrhagic complications and multiple organ failure (Goeijenbier *et al.*, 2014).

The second week of EVD, characterized by stable or declining viral RNA in the blood and presumably decreasing viremia, is paradoxically associated with maximal organ injury leading to death in a subset of cases; those who survive this stage rapidly develop life-saving adaptive immune responses. Sequential organ failure may occur despite meticulous fluid and electrolyte replacement and absent hypotension, apparently as a result of direct viral and indirect host-mediated organ injury. Hypoglycemia during EVD likely reflects depleted liver and muscle glycogen stores following high metabolic demand associated with severe illness (Baseler *et al.*, 2017).

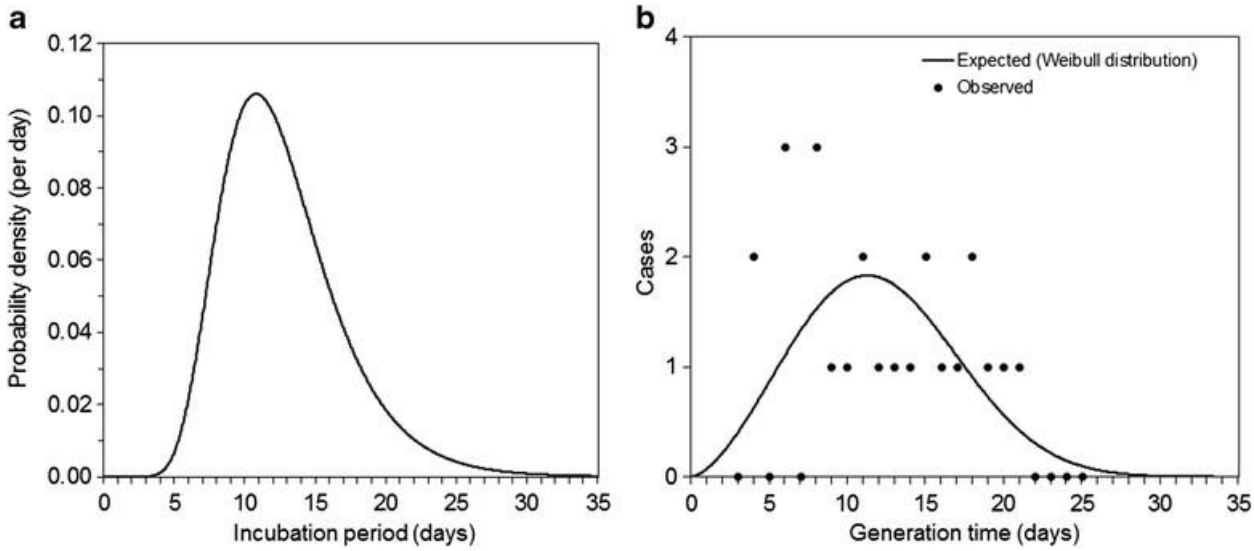

Figure 5. Incubation period and generation time of Ebola virus disease (EVD).
Gerardo and Hiroshi, 2014

The incubation period for Ebola infection is 2 to 21 days, although most cases manifest within 2 weeks after exposure (WHO, 2014). Symptoms, signs, and laboratory test abnormalities commonly observed in patients with Ebola virus disease are summarized in Table 3. Approximately 95% of the case patients had symptom onset within 21 days after exposure, which is the recommended period for follow-up of contacts. The estimated mean (±SD) serial interval was 15.3±9.3 days, which is the same as the estimated mean generation time. The mean time from the onset of symptoms to hospitalization, a measure of the period of infectiousness in the community, was 5.0±4.7 days, and was no shorter for health care workers than for other case patients. The mean time to death after admission to the hospital was 4.2±6.4 days, and the mean time to discharge was 11.8±6.1 days. The mean length of stay in hospital was 6.4 days in Guinea, Liberia, and Sierra Leone (WHO Ebola response team, 2014). Due to the atypical manifestations in the initial phase, EVD mimicks other diseases including dengue fever, typhoid fever, malaria, meningococcemia, and other bacterial infections, and as such pose diagnostic dilemmas (Beeching et al., 2014). A variety of hemorrhagic manifestations forms an integral component of the late disease phase of the disease (WHO Ebola Response Team, 2014). The Gastrointestinal tract bleeding associated with EVD usually manifests as petechiae, hematuria, melena, conjunctival bleeding, contusion, or intraperitoneal bleeding. Additionally, mucous membrane and venipuncture site bleeding, along with excess clot formation may occur. Over time, the infected individual begins to experience dehydration, confusion, stupor, hypotension, and multiorgan dysfunction which results in fulminant shock and ultimately death (Wiwanitkit, 2014). Although EVD has several similar features with other viral hemorrhagic fevers (e.g., dengue), there are differences that set them apart which are highlighted in table 4.

Maculopapular exanthema constitutes a characteristic manifestation of all Filovirus infection, including EVD. The rash usually appears during the 5th to 7th day of disease and occur in 25–52% of patients in the past EVD outbreaks (Kortepeter et al., 2011).

Table 3. Common symptoms, signs, and laboratory test abnormalities in Ebola

Symptoms	Signs	Laboratory Test Abnormalities
Fever (87%)	Elevated temperature	Leukopenia→leukocytosis, atypical lymphocytosis
Fatique (76%)	Pulse temperature dissociation	Thrombocytopenia
Vomiting (68%)		Transaminitis (AST.ALT)
Diarrhea (66%)		Hyponatremia
Loss of Appetite (65%)		Hypokalemia
Headache (53%)		Hypocalcemia
Abdominal Pain (44%)		Elevated BUN and creatinine
Arthralgias (39%)		Lactic acidosis
Myalgias (39%)		Prolonged INR and PTT
		Hypoalbuminemia

Definition of abbreviations: ALT = alanine transaminase; AST = aspartate transaminase; BUN = blood urea nitrogen; INR = international normalized ratio; PTT = partial thromboplastin time.
Data adopted from WHO Ebola Response Team.
Ref: Fowler *et al*., 2014; Sonneville *et al*., 2013.

Table 4 Differentiating features of Ebola and dengue virus infection

Differentiating features	**Dengue**	**Ebola**
Incubation period	3-14 days	2-21 days
Etiology	RNA virus belongs to the genus Flavivirus of family Flaviviridae	RNA virus belongs to the genus Ebola virus of family Filoviridae
Mode of transmission	Arthropod borne	Direct contact with infected blood/body fluids and environment contaminated with these secretions
Human-human transmission	No	Yes
Mortality	0.04%-0.05%	50%-90%
Typical signs and symptoms		
Fever	Common severely high fever (≥40°) lasts for 4-7 days	Common High fever (≥38°)
Headache	Common and high intensity (usually retrobulbar)	Common and high intensity
Muscle ache and pain	Common and severely intense (known as break bone fever)	Common
Nausea and vomiting	Common	Common
Ocular involvement	Nonpurulent conjunctivitis	Conjunctival injection; subconjunctival hemorrhage
Diarrhea	Uncommon	Common estimated 5 L or more of watery diarrhea per day, lasting for up to 7 days and sometimes longer
Bleeding	Unusual	Usual Bleeding from body orifices is a prominent feature
Rash (maculopapular exanthema)	Moderately elevated; initial rash occurs before or during 1-2 days of fever; 2nd rash is seen 3-5 days later	Elevated; occurs during the 5^{th}-7^{th} day
Course of disease	Dengue can be divided into undifferentiated fever, dengue fever, and dengue hemorrhagic fever	Features can be divided into 4 main phases: Early febrile phase, gastrointestinal phase, shock or recovery phase and late complications

CHAPTER THREE

3.0 DIAGNOSIS

Identifying patients who are at risk is very crucial to the early diagnosis of EVD. Thus, screening and active case finding are essential and as developed by WHO and the CDC, case definitions rely on a history of exposure and clinical evidence of illness. (Beeching et al., 2014**).** Early diagnosis of EVD is very important to initiate therapy before the development of shock and multiorgan failure in order to alert public health authorities, and to institute infection control procedures. The virus is generally detectable after 48 hours of infection, meaning that a negative test result within the first 48 hours after exposure does not rule out EBOV infection (Goeijenbier *et al*., 2014; West and Amelie, 2014). Reverse transcriptase polymerase chain reaction (RT-PCR) is the main confirmatory test. Ebola viral RNA can be detected in the blood by the RT-PCR from day 3 to day 6-17 of the symptoms (Beeching *et al*., 2014; Cheepsattayakorn and Cheepsattayakorn, 2015). If the RT-PCR test reveals negative, the test should be repeated within 48 hours (Beeching *et al*., 2014). IgM enzyme-linked immunosorbent assay (ELISA), antigen-capture ELISA, polymerase chain reaction (PCR), and virus isolation are the diagnostic tests available to diagnose a patient who presents within a few days of showing symptoms. IgG response is generally considered to start between day 6 and 18 post onset of illness and remains detectable for years (Goeijenbier *et al*., 2014; Ghazanfar *et al*., 2015). Serum amylase, coagulation studies, renal function tests, liver function tests, blood cultures, chest radiography, arterial blood gases, and complete blood count are other uselful investigations that can be used to diagnose EVD (Cheepsattayakorn and Cheepsattayakorn, 2015).

The antibody profile of the sera from patients with lethal disease as compared with those that survive is markedly distinct. This difference can serve as a prognostic marker for the management of the patient since antibody responses strongly differ between lethal and survivor cases and it has been shown that deceased patients show a much lower or even absent antibody response compared with survivors (Baize *et al*., 2002; McElroy *et al*., 2014; Goeijenbier *et al*., 2014).

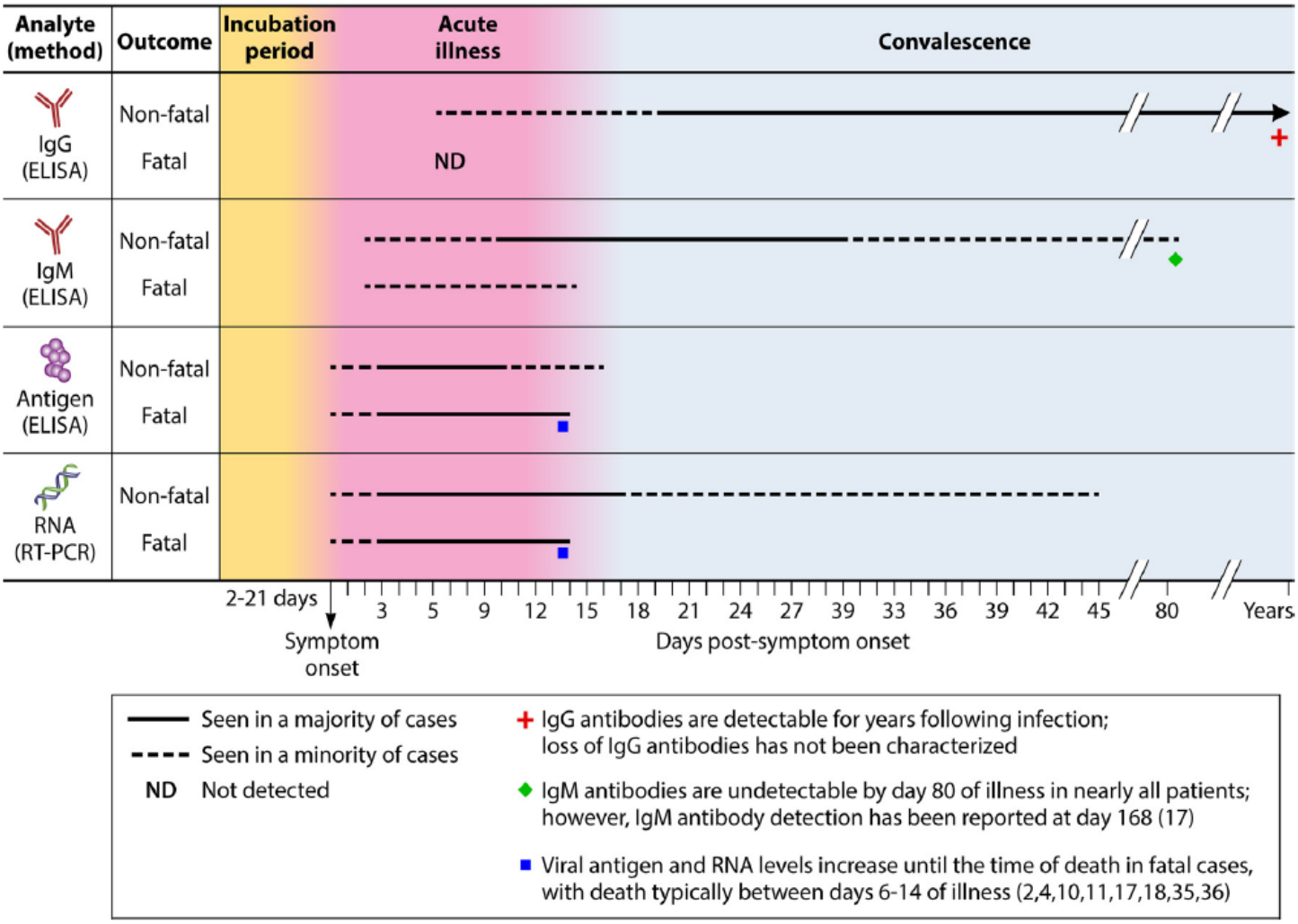

Figure 6. Detection of Ebola virus infection in nonfatal versus fatal cases. Solid lines indicate that the analyte of interest is detected in most cases at the corresponding time point (days post-symptom onset); dashed lines indicate that the analyte of interest is detected in the minority of cases at that time point. Data from Ksiazek *et al.*, 1999; Towner *et al.*, 2004; Wauquier *et al.*, 2009; de La *et al.*, 2015; Uyeki *et al.*, 2016.

RT-PCR: reverse transcription-PCR; ELISA: enzyme-linked immunosorbent assay.

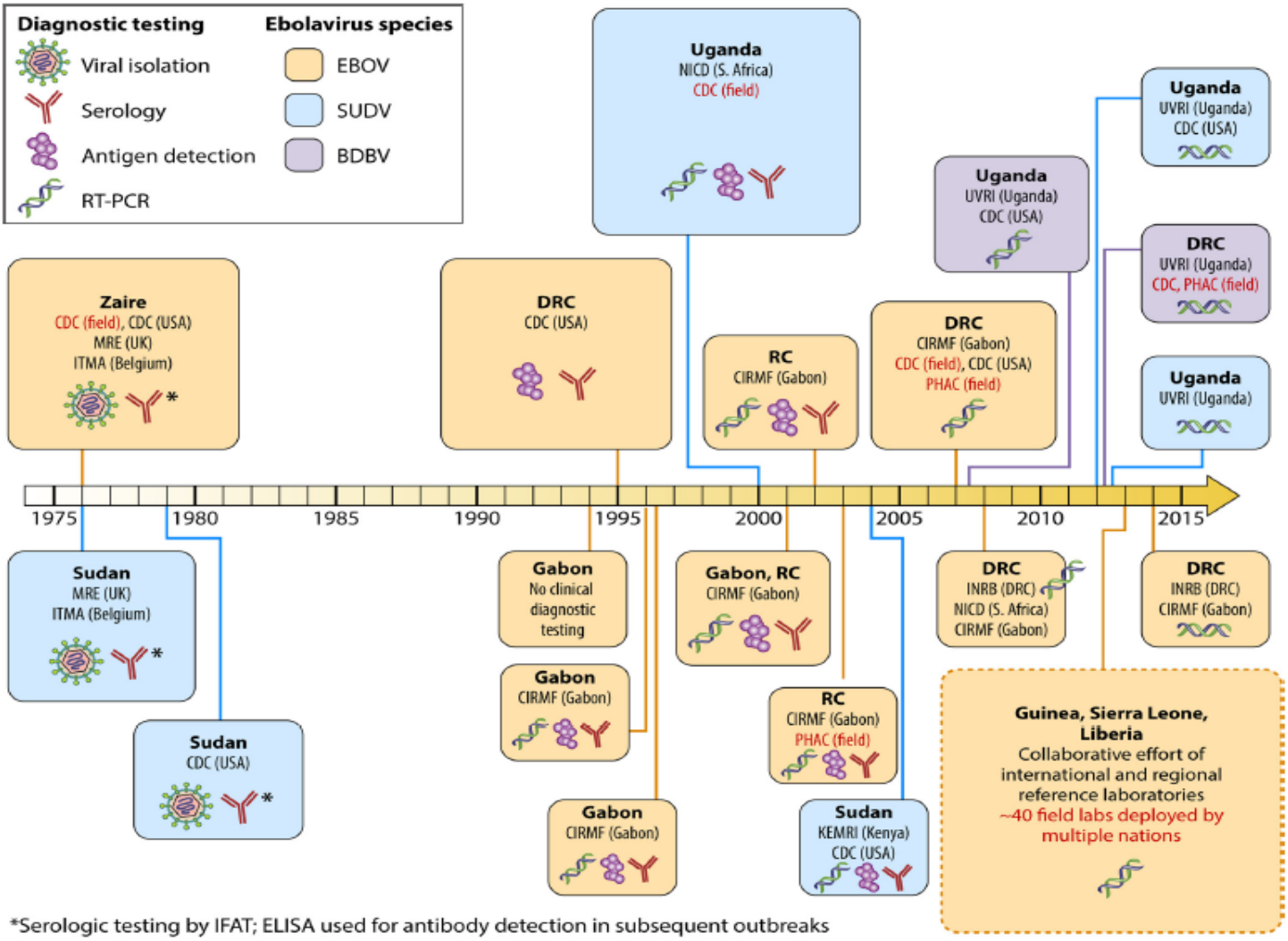

Figure 7. Diagnostic testing in Ebola virus outbreaks.

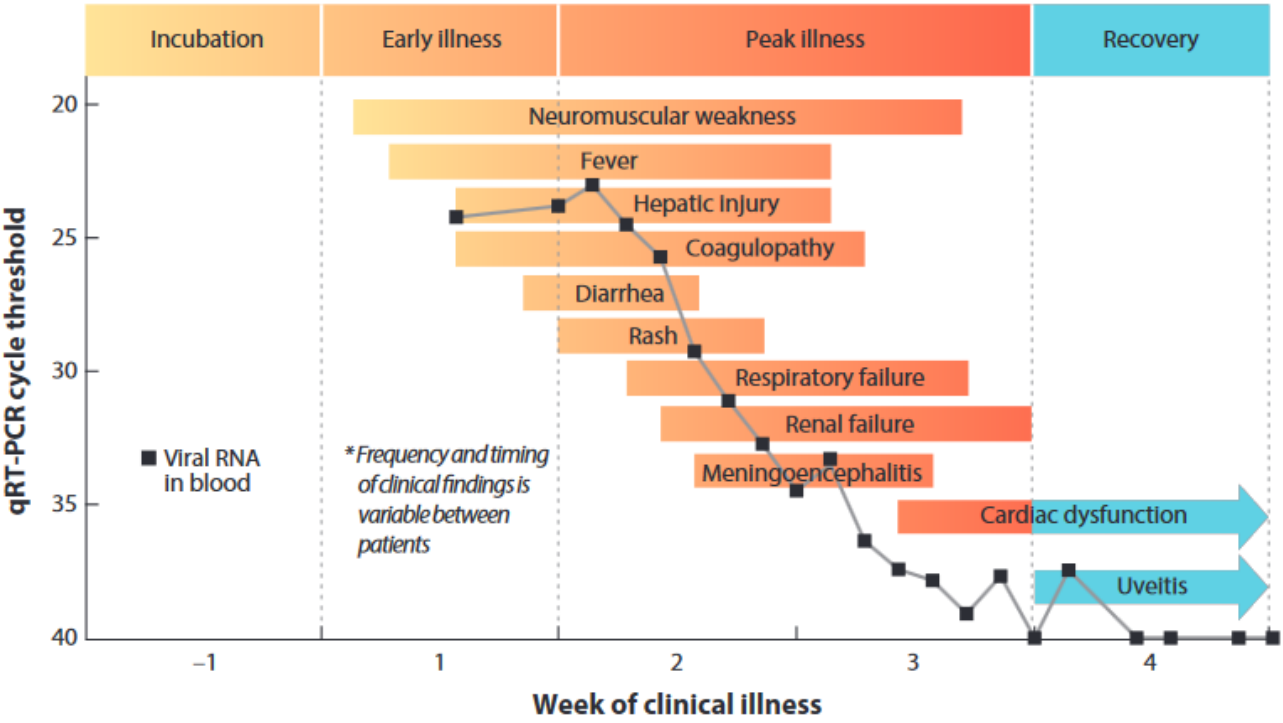

Figure 8. Clinical course of a typical case of severe Ebola virus disease.
Baseler et al., 2017

CHAPTER FOUR

4.0 PREVENTION

Currently there are no specific medications that are broadly available to cure EVD. (Liu et al., 2015) Thus, preventing susceptible people from the infection and restricting the spread of it stands as the most important thing. Doing this will require the concerted efforts of governments, public health facilities, medical units, and individuals. Additionally, in the case of a more serious outbreak in which local public health systems are overwhelmed, the use of military forces comes in as another possibility. In the case of spreading of the infection to other regions and countries, screening at all the exit portals of afflicted countries would be a very effective way of containing the outbreak. However, implementing such screening protocols is beyond the abilities of individual countries without international aide. (Ghazanfar *et al*., 2015) Nonetheless, one thing that can be implemented is the banning of all forms of transportation from affected countries.

In preventing the spread of the virus, different reasons have been named to be the cause for the proportional increase in the spread of the disease. These reasons include lack of preparedness, delayed initial response, weak public health care delivery system, disruption in the ecology of forests, poverty, migration and international travel of infected persons, human resource constraints, poor awareness among people, minimal trust on public authorities, development of a sense of fear among people, non-utilization of the public health sector, funeral rituals involving intense body contact with the deceased and absence of specific treatment or vaccine. However, absence or insufficient implementation of adequate infection prevention and control measures in hospital and community settings are other factor that have contributed to the continued spread of the disease beyond epidemic proportions. (Brian et al., 2014; WHO, 2014; Baize et al., 2014). From the case

in countries like Nigeria and Senegal, strengthening of infection prevention and control, along with other measures was a major factor in achieving their Ebola-free statuses. (Saurabh *et al*., 2015).

To prevent the large scale spread of the disease, preventing individual infections is a fundamental cause that must be ensured. Doing this will require that individuals avoid contact with blood and body fluids (such as urine, feces, saliva, sweat, vomit, breast milk, semen, and vaginal fluids) of persons who are ill; avoid contact with semen from a man who has recovered from EVD, until testing verifies the virus is gone from the semen; avoid contact with items that may have come in contact with an infected person's blood or body fluids (such as clothes, bedding, needles, and medical equipment); avoid partaking in funeral or burial rituals that require handling the body of someone who died from EVD; avoid contact with bats and nonhuman primates' blood, fluids, or raw meat prepared from these animals (bushmeat) and avoid contact with the raw meat of an unknown source. Also, since Ebola virus spreads through direct contact with the blood or body fluids of an infected person and the virus from blood and body fluids can enter the body through broken skin or mucous membranes in the eyes, nose, or mouth by touching one's face with contaminated hands, Hand hygiene such as the use of alcohol-based hand rubs, washing of hands with soap and water, and correct use of hand gloves becomes a basic component of personal and community hygiene and is an important way to prevent the spread of infections while providing healthcare. This lowers the number of germs on the hands and limits the opportunity for spread of the virus. (CDC, 2019)

4.1 **Present Status of Therapeutic Drug Development**

The first case of drug development for the treatment of Ebola virus started in 2002 and was supported by the National Institute of Health (NIH), Biomedical Advanced Research and Development Authority and Defense Threat Reduction agency of United States, as well as the Public Health Agency in Canada. (Woo-Young Choi *et al.*, 2015) ZMapp, an antibody cocktail mixing the humanized monoclonal antibodies (mAbs) with the selected composition of c13C6 from MB-003 (human-mouse chimeric mAbs developed by Mapp Biopharmaceutical Inc. located in San Diego, CA, USA) and c2G4 and c4G7 from ZMAb (mouse mAbs developed by DeFyrus located in Toronto, Canada) was the first antibody therapeutics under development far back in 2004. (Olinger *et al*., 2012; Qui *et al*., 2014). However, apart from this, other EVD therapeutic candidates including TKM-Ebola (drug using RNAi form designed to block the replication of the Ebola virus) and favipiravir (an RNA polymerase inhibitor) were also developed by Tekmira, a Canadian company and Toyama Chemicals, Japanese company respectively. In addition to these candidates, there are reports that other antiviral drugs such lamivudine (an anti-HIV) drug) may show efficacy to help EVD patients. (Woo-Young Choi et al., 2015) Table 4 shows experimental therapies for treatment of Ebola virus diseases from different reports. However, while there is currently no antiviral drug licensed by the U.S. Food and Drug Administration (FDA) to treat EVD in people, two treatments, called regeneron (REGN-EB3) and mAb114 which were among four investigational treatments initially available to treat patients with confirmed Ebola during the 2018 eastern Democratic Republic of the Congo outbreak remain in use for patients with confirmed Ebola as a result of their high survival rates. (CDC, 2019)

Table 3 Experimental treatments for Ebola viral disease

Drug	Drug Type	Mode of Action	Drug tested in humans	Drug tested in Ebola infected humans	Approval status
Favipiravir (T-705) (Fujifilm Holdings Corp)	Nucleoside analogue – broad spectrum activity against RNA viruses	RNA chain termination and/or lethal mutagenesis	Phase-2 completed (influenza) and phase-3 ongoing (influenza)	No	Not approved
TKM-Ebola (Tekmira Pharma-ceuticals Corp)	Lipid nanoparticle with siRNA – Ebolavirus specific compound	Gene silencing	Phase-1 study partially on hold	No	Not approved
BCX-4430 (BioCryst Pharma-ceuticals)	Nucleoside analogue – broad spectrum activity against RNA viruses	RNA chain termination	No	No	Not approved
ZMapp (Mapp Biopharma-ceuticals)	Cocktail of 3 monoclonal antibodies – Ebolavirus specific compound	Most likely virus neu-tralisation	Currently being used to treat small number of victims of the current EBOV outbreak	Yes	Not yet approved

Ref: Furuta *et al*., 2013; Goeijenbier *et al*., 2013; Goeijenbier *et al*., 2014

4.2 Emerging Treatments: Vaccines

While there is no EBOV-specific therapy with proven efficacy yet, multiple antiviral compounds, many of which were administered to EVD patients or to persons undergoing prospective clinical trial evaluation during the West African epidemic have shown therapeutic potentials in in-vitro and animal studies. (Baseler *et al*., 2017) Additionally, more compounds including AVI-7537, which consists of antisense phosphorodiamidate morpholinooligomers (PMOs) that target the Ebola virus VP24 gene; AVI-602 which consists of two PMOs (AV-7537 and AV-7539), targeting the VP35 gene (Beeching *et al*., 2014); BCX-4430, an adenosine analogue that is active against Ebola virus in rodents by inhibition of viral RNA dependent RNA polymerase of paramyxoviruses, arenaviruses, bunyaviruses, and flaviviruses (Cheepsattayakorn and Cheepsattayakorn, 2015); FAvipiravir or T-705 selectively viral RNA dependent RNA polymerase of the foot and mouth disease virus, alphaviruses, bunyaviruses, arenaviruses, flaviviruses, yellow fever virus, West Nile virus, and influenza viruses (Beeching *et al*., 2014); TKM-Ebola which is made of a combination of small interfering RNAs that target Ebola virus RNA polymerase L, formulated with lipid nanoparticle technolog; and Brincidofovir or CMX-001 that have demonstrated activity against Ebola virus in vitro. (Beeching *et al*., 2014; Cheepsattayakorn and Cheepsattayakorn, 2015) have all been highlighted for clinical trials for Ebola virus treatment.

The ideal candidate vaccine for Ebola virus disease will be one that is able to confer interspecies cross-protection against Zaire ebolavirus, Bundibugyo ebolavirus, Sudan ebolavirus, and even unknown species of the virus. The possibility of developing this cross-protective vaccines for the Ebola viruses has also been demonstrated earlier in 2015 by Sobarzo et al. (Muyembe-Tamfum et al., 2012**;** Sobarzo *et al*., 2015) Some important preventive vaccines include the human parainfluenza virus 3 that revealed 100% protection after a single vaccination in guinea pigs.

However, this vaccine required 2 vaccinations in order to induce protective immunity in non-human primates. The other preventive vaccine- rabies virus-recombinant Ebola virus vaccine, demonstrated 100% of protection in mice model after infection with Zaire Ebola virus. (Cheepsattayakorn and Cheepsattayakorn, 2015) In 2015, two experimental vaccines were tried. The first was cAd3-ZEBOV, a chimpanzee derived adenovirus vector with an Ebola virus gene inserted while the second was rVSV-ZEBOV, an attenuated vesicular stomatitis virus with one of its genes replaced by an Ebola virus gene. These pre-clinical trials were performed in the United States, United Kingdom, Switzerland, and some African countries, whereas the clinical trials were launched in the United States. (Cheepsattayakorn and Cheepsattayakorn, 2015). Recently, in 2019, the U.S. Food and Drug Administration (FDA) approved the second vaccine that underwent trial- the Ebola vaccine rVSV-ZEBOV (tradename "Ervebo"). This rVSV-ZEBOV vaccine is a single dose vaccine regimen, and it has been found to be safe and protective against only the *Zaire ebolavirus* species of ebolavirus. This approval became the first FDA approval of a vaccine for Ebola. However, another investigational vaccine was developed and introduced under a research protocol to battle an Ebola outbreak in the Democratic Republic of the Congo in 2019. This vaccine leverages two different vaccine components (Ad26.ZEBOV and MVA-BN-Filo) and it requires two doses with an initial dose followed by a second "booster" dose after 56 days. This second vaccine is also designed to protect against only the *Zaire ebolavirus* species of Ebola. (CDC, 2019)

4.2.1 Immunomonitoring of human responses to the rVSV-ZEBOV Ebola vaccine

The rVSV-ZEBOV vaccine remains the only Ebola vaccine that has shown clinical efficacy in a ring-vaccination clinical trial, with more than 236,000 people who have been vaccinated (WHO, 2019). Its immunogenicity, though reactogenic, has been confirmed in several Phase I clinical studies, and it has been confirmed to be safe. While conventional serology and cellular immunology could give vital insights into vaccine induced protective immunity, the mechanisms of action for protection by the rVSV-ZEBOV vaccine largely remain unknown (Medaglini and Siegrist, 2019). Integrating clinical and immunological read outs with distinct 'Omics' analyses, including transcriptomics and metabolomics, may provide better insight into the mechanisms of vaccine-induced protective reactogenicity and immunity (as illustrated in Figure 9). Ultimately, this may help in identifying early signatures/biomarkers that can predict the magnitude, quality and duration of vaccine-induced adaptive immune responses as well as the markers of adverse effects. Systems biology approaches have recently been used to determine signatures of immunogenicity for some human vaccines (Hagan et al., 2015). Using these techniques and models, it is possible to assess the intricate interactions between the different components of the human immune system in response to vaccination. Ultimately, this is of great significance to adequately uncovering the immunological signature of rVSV-ZEBOV (Li S et al., 2014; Li W et al., 2015).

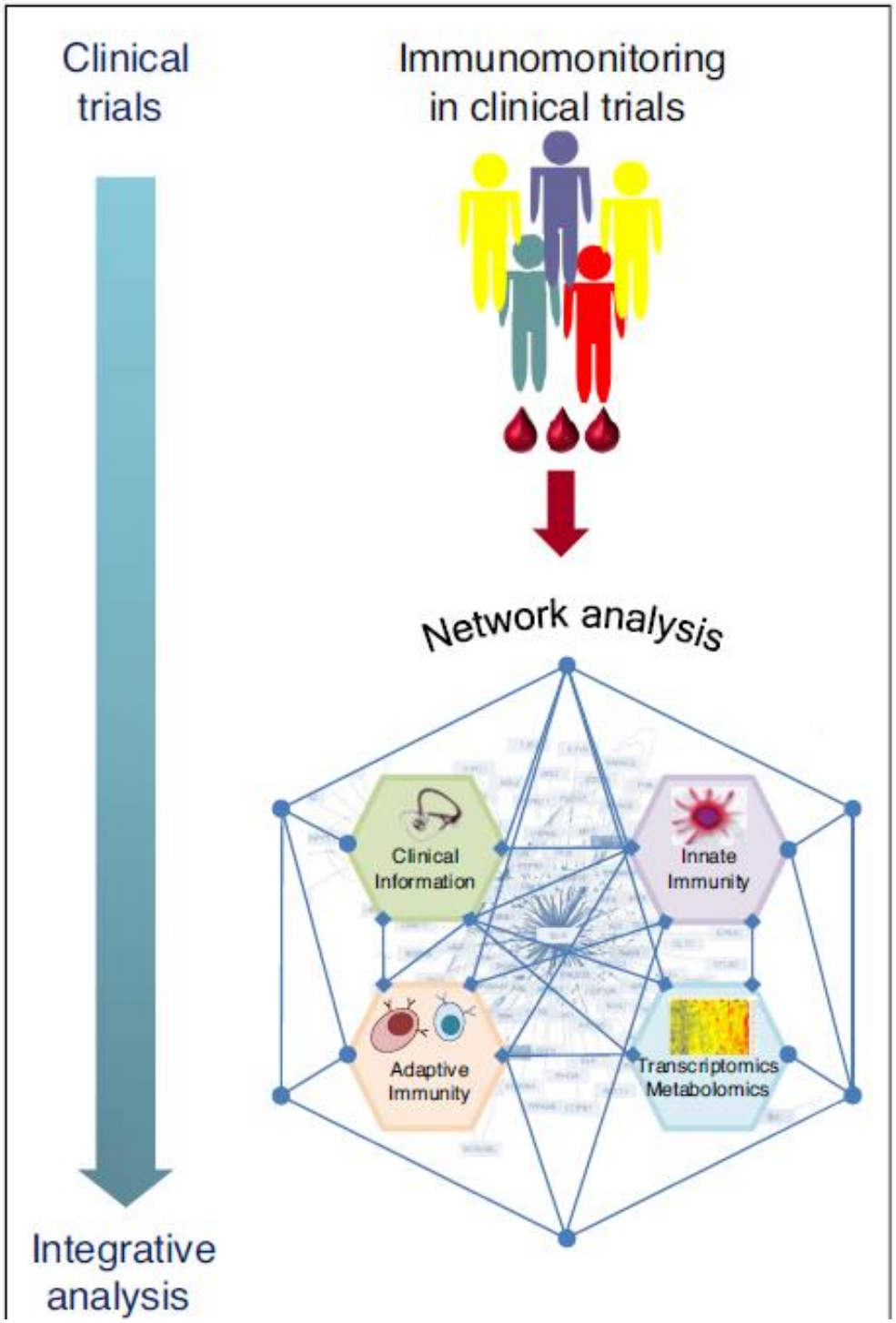

Fig 9. Integrative immunological analysis of rVSV-ZEBOV.

In a phase I clinical trial, it has been shown through serological analyses that EBOV– glycoprotein (GP)–specific IgG antibody responses were detected in nearly all participants. These responses were with increased titers of EBOV neutralizing antibodies when higher vaccine doses were administered (Huttner et al., 2015). The importance of a predominantly anti-GP IgM response for EBOV neutralization was shown as well as an independent evolution of antibody immune responses – in terms of antibody epitope repertoire diversity, affinity maturation, durability and isotype switching – after vaccination with rVSV-ZEBOV in three dose groups (Khurana et al., 2016). Also, a strong association between in vitro neutralization of the virus and serum GP-binding antibody titers was noted. However, after a second dose of the vaccine, there was no boost in antibody or virus neutralization titers while there was antibody affinity maturation (Li S et al., 2014).

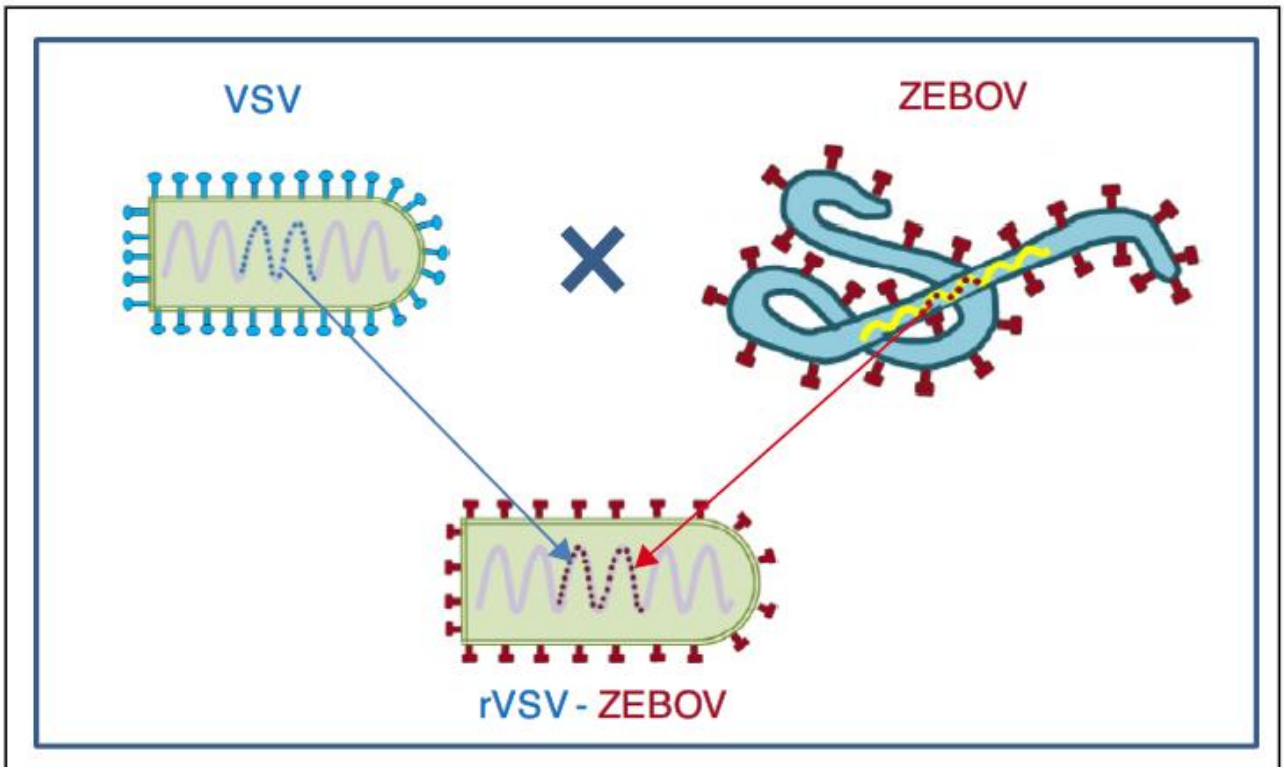

Figure 10. Schematic representation of rVSV-ZEBOV. The rVSV-ZEBOV vaccine is a recombinant virus in which the VSV envelope glycoprotein (in blue) is replaced with the Zaire strain Ebola virus (ZEBOV) glycoprotein (in red) giving rise to the chimeric virus rVSVDG-ZEBOV-GP (rVSV-ZEBOV).

In 2017, a validation cohort study conducted within the phase I randomized, placebo controlled, and dose-escalation trials noted an early plasma signature of the safety and immunogenicity of the rVSV-Ebola vaccine that depended on the vaccine dose (Huttner et al., 2017). Thus far, results obtained from the rVSV-ZEBOV clinical trials have revealed differences among volunteers receiving different doses of the vaccine with regards to the degree of antibody response as well as the extent and time period of adverse reactions. In Geneva, the rVSV-ZEBOV phase I trials generated an exclusive set of immunological and vaccine safety observations, and the biological bases of these observations are now being studied using biological samples (Donata and Claire-Anne, 2017). It is now important to take a step past the state-of-the-art, and this can be achieved harmonizing and involving an in-depth integrated analysis of the data generated by all different clinical and immunological/molecular read outs including safety, immunogenicity, innate and adaptive immunity, immunological memory, transcriptomics and metabolomics. Doing this will facilitate a maximum leverage of the Phase I Ebola vaccine trials- characterizing the immune responses induced by rVSV-ZEBOV and ensuring that all information from clinical studies is fully exploited and shared (Henao-Restrepo et al., 2016).

Table 4 Clinical trials with the rVSV-ZEBOV vaccine

Phase	**Dose**	**Subjects (n)**	**Status**	**Country**	**References**
1 – Safety, and immunogenicity	2.5×10^4 2.5×10^5 2.5×10^6	38	Completed	USA	Regules et al., 2017
1- Safety, tolerability and immunogenicity	3.0×10^5 3.0×10^6 2.0×10^7	30	Completed	Germany	Agnandji et al., 2015
Safety, tolerability and immunogenicity	1.0×10^5 5.0×10^5 3.0×10^6	40	Completed	Canada	Donata and Claire-Anne, 2017
Safety, tolerability and immunogenicity	3.0×10^6 1.0×10^7	40	Ongoing	Kenya	Agnandji et al., 2015
Safety, immunogenicity and efficacy	3 consistency lots and a high-dose lot	1198	Ongoing	USA	Donata and Claire-Anne, 2017

4.3 Other Control Measures

Incident management systems (IMSs), such as the one adopted by the CDC for the control of the current epidemic, have proven to be efficient in preventing the spread of the virus and adequately controlling the disease. (Pillai *et al*., 2014) This Incident management system has been employment in Nigeria, and it successfully limited the outbreak and no further cases have been reported since August 31, 2014. (Nyenswah *et al*., 2014) The employment of such a system in Liberia also resulted in a decrease of EVD patients. (Ghazanfar *et al*., 2015) Thus, an IMS may be adopted and modified, keeping in view the available resources and infrastructure of the country. This IMS system involves authorizing representative healthcare personnel to fulfill specific tasks such as international correspondence, setting forth important measures to respond to the event, supporting affected families, devising plans to curtail chaos among the public, and monitoring healthcare providers. (Ghazanfar *et al*., 2015)

Table 5 Infection control measures to prevent Ebola virus spread

Personal protective equipment (PPE)	Ebola virus infection may be transmitted through broken skin and mucosa	Gown, gloves (possibly double gloves), surgical mask, eye visor/goggles, or face shield to protect conjunctival, nasal, and oral mucosae at the same time. use additional personal protective equipment (such as double gloving, leg covers and disposable shoe covers, when there is contact with blood and bodily fluids. Choose PPE of exact size. Gloves or other PPE that becomes contaminated by blood or bodily fluids must be cleaned or changed before touching other instruments or surfaces.

		Gloved/ungloved hand hygiene. Use alcohol-based hand rub or soap and running water. undertake scrupulous hand cleaning before and after glove use
Sharp instruments	Sharp instruments are extremely dangerous because they become contaminated by blood or bodily fluids and may break skin/mucosae even if protected by PPE.	Use of needles and other sharp instruments must be limited. These instruments must be handled with extreme care and disposed after use in dedicated seal containers
Nonsharp instruments	Indirect transmission through nonsharp contaminated instruments is not demonstrated Preventive measures are recommended under the Precautionary Principle	Use of disposable medical equipment is recommended or, alternatively, nondisposable medical equipment must be cleaned and disinfected after use according to manufacturer's instructions
Droplets	Airborne transmission is not demonstrated preventive measures are recommended	If aerosol generating procedures or events, such as coughing or sputum induction,

	under the precautionary principle	occur, the use of powered air-purifying respirator or respirator (FFP2 or EN certified equivalent or US NIOSH-certified N95) is recommended
Environmental Surfaces	Environmental surfaces do not pose a risk of infection. However, Ebola virus is nonenveloped and can survive in the environment for long time. Preventive measures regarding surfaces visibly contaminated with blood and bodily fluids are recommended under the precautionary principle.	Use of standard hospital detergents and disinfectants (e.g., 0.5% chlorine solution or a solution containing 5000 ppm available free chlorine), preceded by cleaning to prevent inactivation of disinfectants by organic matter, is recommended

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