

SJMLS-3(1)-009

Ebola Virus-A ReviewRogo, L.D.¹, Auwal, I.K.^{2,3*}

Department of Medical Laboratory Sciences, Faculty of Allied Sciences, College of Health Sciences, Bayero University, PMB 3011, Kano Nigeria¹, Department of Virology, School of Public Health, Tehran University of Medical Sciences-*International Campus*, P.O. Box 14155-6446, Tehran², Department of Medical Microbiology and Parasitology, Faculty of Clinical Sciences, College of Health Sciences, Bayero University, PMB 3011, Kano Nigeria³.

Author for Correspondence*: auwalkabuga76@gmail.com; +98-930-690-2127; +234-806-816-1470.

Summary

Ebola virus (EBOV) is a single stranded zoonotic RNA virus of positive sense polarity under Filoviridae family, with no geographic, socio-economic, ethnic, gender or age boundaries, introduced to humans via contact with body fluids and products of wild animals. Direct human-to human transmission is equally important as it results to high transmission and mortality rates. EBOV primarily attack macrophages and dendritic cells with suppression of type-I interferon response with early systemic spread following release of large progeny particles into the extracellular fluid. Due to the complex genetic evolution, strange properties and functional profile, researchers are still struggling to comprehend the pathogenic capacity of this virus and its interaction with the host cellular machineries. This comprehensive review is an overview of what is known about Ebola virus and encompasses biology of the virus including its interaction with the host, epidemiology, disease presentation, diagnosis, therapeutics and progress in vaccine development.

Keywords: Ebola; Haemorrhagic fever; Ebola virus disease; Pathogenesis; Therapeutics.

Introduction

Ebola virus (EBOV) is a linear, single stranded, negative-sense RNA virus (ssRNA) of the order *Mononegavirale* and *Filoviridae* family, with five (5) species under the genus Ebola virus. These includes, *Sudan ebolavirus* (SUDV), *Zaire ebolavirus* (EBOV), *Tai Forest (Ivory Coast) ebolavirus* (TAFV), *Reston ebolavirus*

(RESTV) and *Bundibugyo ebolavirus* (BDBV) (Feldmann *et al.*, 2005; Mayo, 2002). These viruses together cause the severe and often fatal haemorrhagic fever in both humans and non-human primates known as Ebola Virus Disease (EVD). *Zaire ebolavirus* is the most ancient and was first discovered in Ebola River - Zaire (currently Democratic Republic of Congo) following the 1976 outbreak, hence the name "*Ebola virus*" (Kuhn *et al.*, 2010).

Zaire ebolavirus is associated with high transmission and very high case fatality rate. This was further testified by the fatal outbreak between 2013 and 2015 that reported 28,603 cases and 40% mortality (Liu *et al.*, 2015). Mucosal surfaces or skin abrasions are the main porta of entry; however, most of human infection occurs after direct contact with infected patients or cadavers. In addition, eating contaminated meat of wild animal sources have been found to be associated with the disease (Chowell and Nishiura, 2015).

The symptoms of high fever, chills and myalgia occur following an incubation period of 2-21 days, followed by the multisystem presentation involving gastrointestinal tract, respiratory, central nervous system, clotting and tegumentary systems, resulting in the classical disease with bleeding diathesis (Baize *et al.*, 2014). Infected individuals develop strong immune response during the first stage of the infection as evidenced by the high level of specific immunoglobulin G (IgG). Following virus clearance from the circulation, there is consistent immune activation with persistently

activated CD8 T cells (McElroy *et al.*, 2015). Management is mainly supportive and aimed at maintaining adequate circulation; however, specific symptoms management is associated with increased chance of survival. There is no available chemotherapeutic agent or vaccine against EBOV yet, hence other public health measures like contact tracing, good surveillance system, state of the art laboratory services, careful burials of the deceased and good social

mobilization programs, remained the main stay of prevention and control.

Virion structure

A particle of EBOV is made up of seven structural proteins locked together, giving a strand of cooked spaghetti-like appearance. It measures 80nm by 1000nm with approximate nucleotide size of 19 kb (Fig. 1) (Nanbo *et al.*, 2013).

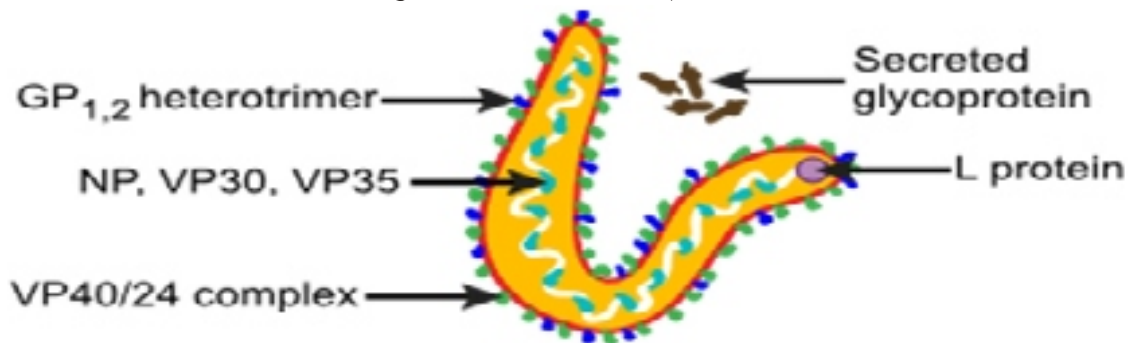


Figure 1. Cylindrical/tubular structure of Ebola virus. Nucleoprotein (NP), polymerase co-factor (VP35), (VP40), GP, transcription activator (VP30), VP24, and RNA-dependent RNA polymerase (L).

The virion is cylindrical or somehow tubular with envelope, matrix, and nucleocapsid components. A complete cylinder is roughly 80 nm in diameter with viral encoded glycoprotein (GP) protruding as 7-10 nm long spikes from its lipid bilayer surface (Tran *et al.*, 2014). The cylinders are of variable length, typically 800 nm, but sometimes up to 1000 nm long. The outer most viral envelope is obtained through budding from domains of host cell membrane into which the GP spikes have been fitted at the time of their biogenesis (Nanbo *et al.*, 2013). Individual GP molecule appear with spacing of about 10 nm (Erin *et al.*, 2014). Viral proteins VP40 and VP24 are stationed in the *matrix space*, in between the

envelope and the nucleocapsid. The nucleocapsid is made up of series of viral proteins joined to an 18–19 kb linear, negative-sense RNA without 3'-polyadenylation or 5'-capping (Fig. 2) (Fieldmann, 1993). The viral RNA is helically wound in complex with NP, VP35, VP30, and L proteins; this helix has a diameter of 80 nm and contains a central channel of 20–30 nm in diameter (Nanbo *et al.*, 2013). It is important to note that NP, VP35 and L gene sections from filoviruses have been realized as endogenous in the genomes of many groups of small mammals as seen in the figure 2 above (Taylor *et al.*, 2014; Hulo *et al.*, 2011).

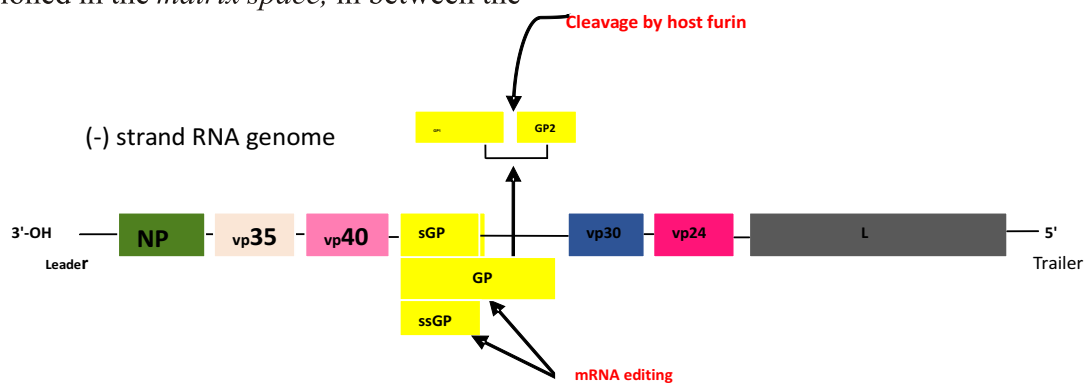


Figure 2: Genome structure of Ebola virus. The gene order is 3'-leader-NP-VP35-VP40-GP/sGP-VP30-VP24-L-trailer-5'; with the leader and trailer being non-transcribed regions, which contains vital signals for transcription, replication, and packaging of the viral genomes into progeny virions.

Functional genomics of EBOV

The seven EBOV genes are split by variable gene borders, including short (4- or 5-nucleotide) intergenic regions (IRs), a single long (144-nucleotide) IR, and gene overlaps, where the adjacent gene end and start signals share five conserved nucleotides (Castilletti *et al.*, 2015). Each gene encodes for one protein, except for the GP gene that encodes three glycoproteins.

The nucleoprotein (NP) is connected to the viral genome and assembles into a helical nucleocapsid (NC) together with the polymerase co-factor (VP35), the transcription activator (VP30), and RNA-dependent RNA polymerase (L) (Brauburger *et al.*, 2014). Together encapsidate and protect the genome from the endonucleases. The encapsidated genomic RNA produce the nucleocapsid and serve as a template for both transcription and replication.

Vp35 of EBOV is a multifunctional dsRNA binding protein (d-RBP) that play significant role in viral replication, innate immune evasion and pathogenesis. It is also cofactor of viral RNA polymerase complex and together with NP and VP24 take part in nucleocapsid biosynthesis. VP35 communicate with helical NP-RNA complexes via C-terminus of NP and their co-expression decrease production of NP-RNA complexes. Consequently, represses the emergence of NP-induced inclusion bodies that usually form in EBOV-infected cells. Muhlberger *et al.* (1999) have also established significant correlation between the NP: VP35 ratio and viral replication.

Vp40 is situated straight under the viral lipid bilayer and thus provide structural support. It is the most prolific protein in the virus particle and is considered inevitable for assembly and budding of new virus particles. VP40 is equally important in the movement of NCs to the cell surface and its incorporation into virions. The interaction between nucleoprotein and the matrix protein VP40 is probably vital for these processes (Noda *et al.*, 2011). EBOV replicate in the cytoplasm, while assembly of new virus particles and budding occur at the plasma membrane. At this stage, VP40 connect with cellular membranes and are brought together via

interactions with the C-terminal ends of viral glycoproteins (Noda *et al.*, 2011). It has been shown that VP40 in solution forms an octameric ring with a central pore. The two farthest N and C end tail regions have been shown to stabilize the monomeric state via a direct connection. The tails seem to be functioning as a latch, released upon a particular molecular stimulation such as RNA ligation. The stimulated release of the tails allows coordination of late-stage activities in the viral life cycle at the inner membrane of the host cell. The N-tail release in particular, exposes the L-domain motifs PTAP/PPEY to the transport and budding complexes while stimulated C-tail release enhance connection with the site of budding (Dessen *et al.*, 2000).

Glycoprotein (GP) of EBOV, the only viral protein expressed on the virion surface play an important role for binding and enhances membrane fusion. The GP gene encodes seven consecutive adenosine nucleotides within a predicted hairpin loop (Silva *et al.*, 2012). Initial pre-GP is cleaved by furin at a multi-basic motif into two subunits, GP1 and GP2, which remain connected via a disulfide linkage between Cys53 of GP1 and Cys609 of GP2 (Sanchez *et al.*, 1996). The virion-bonded GP is important in the EBOV life cycle as it is exclusively important for binding, fusion and entry into the target cells. GP is also important for main in pathogenicity and specie differentiation. GP1 subunit is important for cellular binding and contains supposed receptor-binding regions as well as an excessively glycosylated region, named the mucin-like domain (Jeffers *et al.*, 2002). GP2 subunit play a key role in viral-host membrane fusion. It bears the hydrophobic internal fusion loop, two heptad repeats (HR1 and HR2), a CX6CC disulfide bond motif, a membrane proximal external region and a transmembrane anchor (Lee and Saphire, 2009). In addition to its surface glycoprotein (GP 1 and 2), EBOV manipulate the production of large amount of a truncated glycoprotein isoform (sGP) which is released into the extracellular space. Generation and secretion of antigens was studied in many viruses and was found to be a means of host immune evasion by removing antibodies and meddling with antibody-mediated clearance. It has been found that sGP easily subvert a

previously immunized host's anti-GP 1 and 2 responses resulting in strong cross-reactivity with sGP (Gallaher, 1996).

VP30 is known to be a transcription activation factor necessary for the viral life cycle. Although the mechanism is not completely understood, it is proposed that this protein is connected with initiation of transcription because VP30-dependent transcription is controlled by RNA of the first transcription start signal. The starting 23 nucleotides of this NP mRNA are concerned in stem-loop structure formation that might meddle with the progression of transcription by physically interfering with polymerase movement (Mohan *et al.*, 2012). In addition, the N-terminal of VP30 contains a Zn⁺² binding Cys-His motif (Weik *et al.*, 2002) and high amount of basic amino acids, allowing it to straight ahead interact with RNA (Modrof *et al.*, 2003). Consequently, VP30 could either resolve or cover this secondary structure and permit transcription to continue. Many works also reported that VP30 is vital in transcription re-initiation and may attach to stem-loops generated by the promoter of each Ebola virus gene (John *et al.*, 2008). It has been shown that phosphorylation of VP30 prevents viral transcription but viral replication is enhanced at the same time. Phosphorylation of VP30 in return controls a recently explained interaction of VP30 with VP35 and fortifies the interaction with NP. VP30 phosphorylation was reported to be critical for viral transcription and replication, implying a means by which VP30 phosphorylation regulate the composition of the viral polymerase complex, thereby probably forming a transcriptase in the existence of non-phosphorylated VP30 or a replicase with phosphorylated VP30 (Martinez *et al.*, 2008).

Another important protein is VP24, this works to prevent transcription and replication of the EBOV genome. Though the exact means is still not yet clarified, it may be that VP24 attaches to NP and interfere with the function of VP35, VP30 or L. This interference may be vital in converting the viral genome from a transcription or replication competent form to one that is ready for virion assembly and exit (Biedenkopf *et al.*, 2013). It has been shown that VP24 is essential for the accurate assembly of a functional nucleocapsid, as lack of VP24 leads to decreased transcription/translation of VP30 (Watanabe *et al.*, 2007).

It was found that 472 nucleotides from the 3' end and 731 nucleotides from the 5' end are enough for a viral "mini-genome" replication, although not enough for infection (Hoenen *et al.*, 2006). In accordance with the stop-start model describing NNS RNA virus transcription, the viral polymerase complex attaches to a single transcription promoter at the 3' end of the genome and moves toward the 5' end, sequentially synthesizing mostly mono-cistronic mRNAs (Klenk *et al.*, 2004).

An L gene and 5' trailer region nucleotide sequence of Ebola virus has been resolved, as such finalized the sequence of the Ebola virus genome. The L gene carries a single ORF encoding a polypeptide of 2212 amino acid. Messenger RNAs are capped and polyadenylated by the L protein at the time of biogenesis. The 5'-trailer sequence of the genomic RNA is made up of 676 nucleotides and discloses self-complementary sequence at the farthest end, and may play a vital part in virus replication (Whelan *et al.*, 2004). The functions of these genes are summarized in table I.

Table I: Ebola virus proteins and their functions

Gene	Function/role
Nuclear protein (NP)	Connect with the viral genome and assemble into a helical nucleocapsid (NC), also protect the genome from nuclease activities.
Viral protein-35 (VP35)	Serve as a polymerase co-factor. As a multifunctional dsRNA binding protein, it plays important role in viral replication, innate immune evasion and pathogenesis.
Viral protein-40 (VP40)	A membrane connected protein considered inevitable for assembly and budding of new virus particles.
Glycoprotein GP/sGP	Plays important role for binding to host cells and for fusion. Is responsible for pathogenic differences among the viral species.
Viral protein-30 (VP30)	A known transcription activation factor necessary for viral life cycle.
Viral protein-24 (VP24)	Prevent transcription and replication of the Ebola virus genome. Essential for accurate assembly of functional nucleocapsid.
L gene	Carries a single ORF encoding polypeptide of 2212 aa. Responsible for capping and polyadenylation of mRNAs at the time of biogenesis.

(Castelletti *et al.*, 2015; Nanbo *et al.*, 2013; Noda *et al.*, 2011; Jeffers *et al.*, 2002; Fieldman *et al.*, 1993)

Ebola virus receptor and internalization

Two proteins have been reported as candidates for host cell entry. First, is a cholesterol transporter protein, the host-encoded Niemann-Pick C1 (NPC1), that appears to be vital for entry of Ebola virions into the host cell and for its final replication (Carette *et al.*, 2011; Volchkov *et al.*, 1999) and the second candidate is TIM-1 (Côté *et al.*, 2011). Report shows that NPC1 play vital role in entry of Ebola virus due to the fact that it interfere in the infection by attaching directly to viral GP and TIM-1 was shown to attach to the

receptor binding domain of the EBOV glycoprotein, to enhance the receptivity of vero cells (Hofmann-Winkler *et al.*, 2015; Kondratowicz *et al.*, 2011; Saeed *et al.*, 2010). Mechanism of cell entry between the Ebola Virus from Zaire, 1976 and Sierra Leone, 2014, where reported to be the same (Noda *et al.*, 2006). The virus attach to the host cell surface via glycoprotein (GP) surface peplomer and enter by endocytosis in macropinosomes. To penetrate the cell, the viral membrane fuses with vesicle membrane to release the nucleocapsid into the

cytoplasm. Encapsidated, negative-sense genomic ssRNA is used as a template for the biogenesis (3'-5') of polyadenylated, monocistronic mRNAs, and using the host cell's ribosomes, tRNA molecules, the mRNA is translated into respective viral proteins. These proteins are dealt with: a glycoprotein precursor (GP0) is split to GP1 and GP2, which are then greatly glycosylated by cellular enzymes and substrates. These two molecules join, first into heterodimers, and then into trimers to produce the surface peplomers. Released glycoprotein (sGP) precursor split to sGP and delta peptide, both of which are released from the cell (Cox *et al.*, 1983). More so, as viral protein levels increase, a switch occurs from translation to replication. Using the negative-sense genomic RNA as a template, a complementary (+) ssRNA is produced; which is then used as a template for biogenesis of new genomic (-) ssRNA, which is immediately encapsidated. Examination of virus-infected cells revealed virions carrying NCs to appear horizontally from the cell surface, whereas empty virions usually bud vertically, indicating that horizontal budding is the main form of Ebola virus budding (Muyembe-Tamfumet *et al.*, 2012).

Epidemiology

The first outbreak of EVD occurred in small villages close to Ebola River - Zaire (currently Democratic Republic of Congo) in 1976 located in tropical rainforests (Feldmann and Geisbert, 2011). This partly explained why first outbreak of EVD was due to EBOV and SUDV and remained restricted to a limited area (Goeijenbier *et al.*, 2014). Since then, there was no another outbreak until in 1994, but after 1994 the number increases, leading to the discovery of two new Ebola virus species (BDBV and TAFV) (Kreuels *et al.*, 2014). This was attributed to increased bush meat consumption and movements to previously inaccessible areas (Baize *et al.*, 2014). There is no certainty on how the index case became infected in the 2013 outbreak that took place in Guinea (Park *et al.*, 2015). Animals implicated in introduction of EBOV into the human population are chimpanzee, gorillas, duikers and specific species of fruit bats, all found to be infected with EBOV during the targeted studies (Folarin *et al.*, 2016). Recent outbreak was the largest ever-documented

epidemic of EVD in terms of numbers and geographical spread throughout EVD history with case fatality rate of 90%. EBOV is capable of actively circulating worldwide without ethnic or geographical boundaries, albeit with some regional differences (Folarin *et al.*, 2016).

A seven-month epidemiological study of EBOV Makona genome showed both host-to-host transmission and recurrent emergence of intra-host genetic variants and increasing impact of purifying selection in suppressing accumulation of nonsynonymous mutations over time (Varkey *et al.*, 2015). A report by Christie *et al.* (2015) confirmed a single source for the Nigerian cases indicating that the Nigerian EBOV lineage nests within the previously but genetically distinct form seen in Liberia and supports the notion that transmission from Nigeria to other places did not occur. By May 2016, 28,657 clinically suspected or confirmed cases were reported including 11,325 mortalities. Over 870 of infected individuals were healthcare workers and a significant number have lost their lives in three most prevalent (Guinea, Liberia and Sierra Leone) and other affected countries (Mali, Nigeria, Senegal, Spain and USA) (Christie *et al.*, 2015).

Pathogenesis and immune response

Ebola virus enters the host primarily through mucosal surfaces or skin abrasions, with most human infections occurring after direct contact with infected humans. The virus is shed in body fluids such as urine, semen, saliva, aqueous humor, breast milk, tears and sweat (Osterholm *et al.*, 2015; Bray and Geisbert, 2005). Several cases were attributed to ingestion of meats from infected wild animals with hunters being the most affected. Airborne transmission between monkeys was demonstrated during the outbreak in Virginia, but there is limited evidence to support this in humans (Baize *et al.*, 2014). Ebola virus can fit in as an opportunistic pathogen transmitted via aerosols under certain conditions, since infectious aerosols (either large or small droplet), could be generated and emitted during the course of disease (Basler, 2005).

Due to the problem of conducting clinical studies at the time of outbreak situations, virtually all information concerning the pathogenesis of Ebola virus disease was derived from laboratory

experiments using non-human primates, guinea pigs and mice. However, observational studies/case reports involving large-scale patients in recent outbreaks shed more lights regarding the pathogenesis in humans (Basler, 2005).

Following viral entry, many different cell types including Macrophages and dendritic cells are mostly affected. Filoviruses replicate easily within these omnipresent "sentinel" cells, resulting in their necrosis and freeing large numbers of progeny particles into extracellular fluid (Hensley *et al.*, 2002). Type I interferon response suppression result in an early systemic spread (Hutchinson and Rollin, 2007). Spread to regional lymph nodes result in another round of replication, with secondary haematogenous extension to dendritic cells and mobile/fixed macrophages located in some reticuloendothelial organs such as liver, spleen and thymus gland. Further studies have also established the involvement of other cell types, including endothelial cells, fibroblasts, adrenal cortical and epithelial cells. In fatal infection, there is multifocal necrosis involving hepatic and splenic tissues (Geisbert *et al.*, 2003). Gastrointestinal symptoms such as diarrhoea and vomiting may result in severe dehydration and consequently hypovolaemic shock (Geisbert *et al.*, 2000). However, there is dilemma as to whether this is because of direct involvement of the gastrointestinal tract, induced by cytokines or both (Geisbert *et al.*, 2003). The virus also induces systemic inflammatory response with release of inflammatory cells as well as pro-inflammatory mediators from macrophages and other cells (Baize *et al.*, 2000). Tumor necrosis factor (TNF- α), interleukin (IL)-1 β , IL-6, macrophage chemotactic protein (MCP)-1, and nitric oxide (NO) among others were recovered in blood samples of Ebola-infected macaques and severely infected humans in Africa (Bradfute *et al.*, 2007). Breakdown products of necrotic cells also trigger the release of the same mediators (Parrino *et al.*, 2007).

Ebola virus appears to be involved indirectly in inducing coagulation defect because of host inflammatory response. Infected macrophages synthesize cell-surface tissue factor (TF), stimulating the extrinsic coagulation pathway; pro-inflammatory cytokines also trigger macrophages to release TF (Bradfute *et al.*, 2008). These simultaneous phenomena explain the rapid development and severity of platelet dysfunction in Ebola virus infection. Furthermore, there are other supporting evidences that explained coagulopathy in Ebola virus disease. For examples, D-dimers were recovered in blood samples of infected monkeys within 24 hours of virus challenge. This was also demonstrated in plasma of infected humans. Activated protein C was found to decrease on the 2nd day of infection in infected macaque, but with a sustainable level of platelets count until after the 3rd or 4th day of virus challenge, implying that activated platelets remained attached to the endothelial cells. With disease progression, hepatic injury has also been associated with decrease plasma levels of certain coagulation factors (Geisbert *et al.*, 2003). Flaviviruses were frequently associated with severe fatal disease due to failure of host adaptive immunity because of incapacitated dendritic cells and lymphatic apoptosis. Furthermore, with decrease levels of lymphocytes, adaptive immunity tends to be impaired (Fowler *et al.*, 2014; Crary *et al.*, 2003). Although these cells appear to remain uninfected, they undergo "bystander" apoptosis, presumably induced by inflammatory mediators and/or loss of support signals from dendritic cells. This was similarly observed in septic shock (Kibadi *et al.*, 1999). Report shows that virus-specific lymphocyte proliferation occurs despite the surrounding massive apoptosis in infected mice, but arrives too late to stop fatal outcome (Kibadi *et al.*, 1999). The pathogenesis of EBOV has been summarized in figure 3 and 4 below.

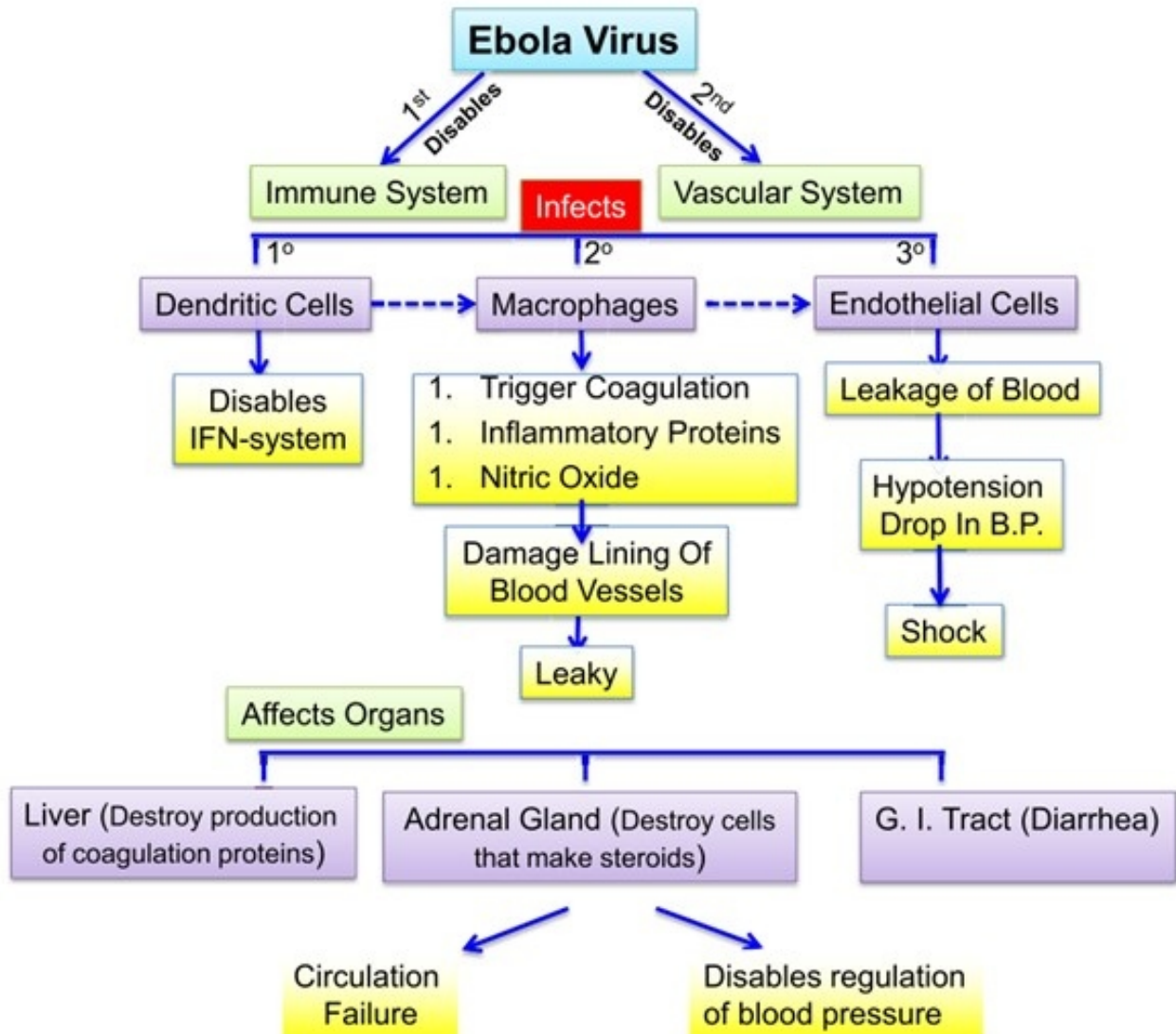


Figure 3: Schematic representation of pathogenesis of Ebola virus (Vingolo *et al.*, 2015).

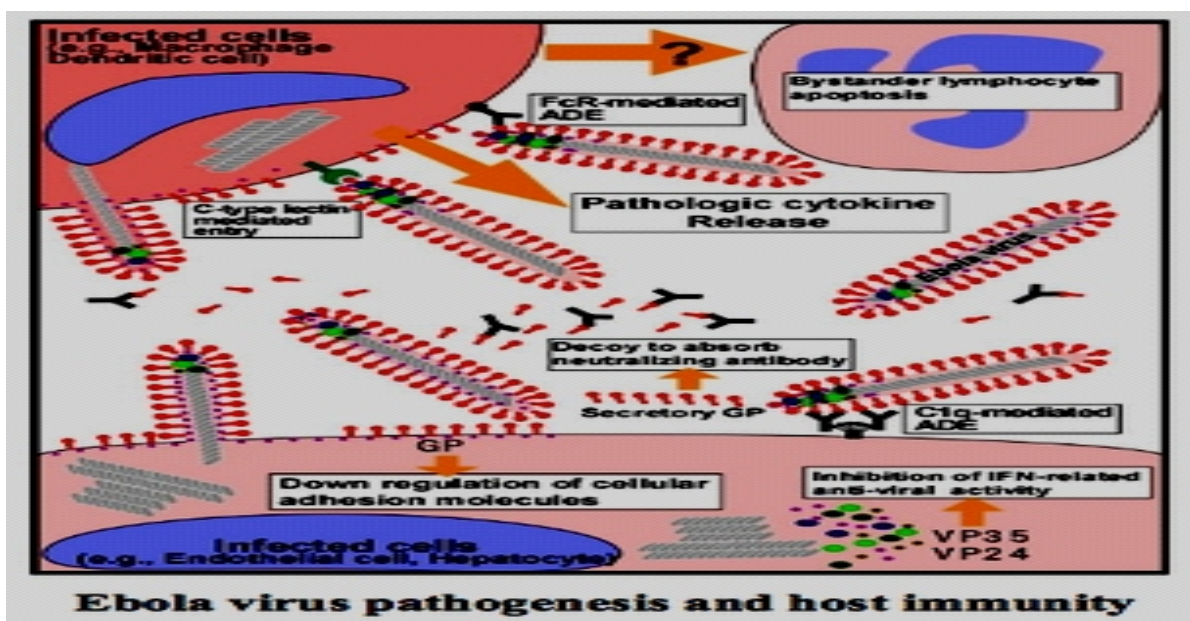


Figure 4: Immune response and inhibition pathways in Ebola virus infection (Vingolo *et al.*, 2015).

Clinical manifestation and complications

The spectrum of clinical disorders that are connected with EBV infection ranges from mild to life threatening. Initial symptoms of fever, chills, malaise and myalgia occur 8-12 days after exposure (Vingolo et al., 2015). These non-specific symptoms often may be confused with other more common infectious diseases such as malaria, typhoid fever, meningococemia among others most especially in the early course of the disease. Individuals can advance (in five days) to more specific presentations like terrible diarrhoea, nausea, vomiting and abdominal pain. Other systemic symptoms such as angina, dyspnea, cephalalgia or perplexity can also begin. Conjunctival injection, hiccups, seizures and cerebral edema have been reported too (Vingolo et al., 2015). Bleeding is not universally present but can manifest late in due course as purpura, ecchymosis/bruises, mucosal haemorrhages or oozing from venipuncture sites and. Frank haemorrhage is less common, but unexplained bleeding has been reported in 18% and bloody stool in 6% of affected individuals in the 2015 outbreak in Sierra Leon (Park et al., 2015). Patients may develop diffuse erythematous maculopapular rash by day 5 to 7 (usually around the neck, trunk, and arms) which may desquamate.

Analysis of symptoms in the 2014 outbreak from onset to full-blown disease revealed the following; fever (87%), fatigue (76%), vomiting (68%), diarrhoea (66%), and loss of appetite (65%). Fatal disease cases usually develop more severe clinical signs immediately and die typically of multiple organ failure and shock within 6 to 16 days (average 7.5 days) (CDC, 2015; Baize et al., 2014; WHO, 2014). A constellation of symptoms including unexplained bleeding; and many more other signs and symptoms example diarrhoea, chest pain, cough, dyspnoea, dysphasia, conjunctivitis, sore throat, confusion, hiccups and coma were associated with fatal outcomes, especially in those above the age of 45 years in West Africa (Chua et al., 2015; Martin et al., 2015). Ocular complications were reported in three (15%) of 20 survivors of the 1995 outbreak in the DRC. Ocular pain, photophobia, increased lacrimation, decreased visual acuity and uveitis

were among the ocular presentations (Martins et al., 2015). Survivors have also reported long-term sequelae including myalgia, asymmetric and migratory arthralgia, headache, fatigue, bulimia, amenorrhoea, hearing loss, tinnitus, unilateral orchitis and suppurative parotitis (Basler, 2005).

Diagnosis

In early stage of the disease, diagnosis is often strenuous due to non-specific features that mimic common illnesses in the endemic zones (Towner et al., 2004). Suspected individual, especially with strong history of contact should be quarantine and reported to the appropriate public health authorities for immediate prompt action. Emphasis should be giving to people who come in contact with: blood or body fluids from a suspected individual or died from Ebola, fomites that have been contaminated with blood or body fluids of suspects or someone who died of Ebola, infected fruit bats and primates (apes and monkeys), or semen from recovered patients (Beeching et al., 2014; Rollin et al., 2007). Presumptive diagnosis can be made in the appropriate clinical context based on history of exposure, symptomatology, and/or laboratory findings in keeping with the disease (Kortepeter et al., 2011). Laboratory parameters that are paramount in the diagnosis of EVD are summarized in table II and III. Low platelet count with significant lymphopenia, even though is not diagnostic can be observed in the early phase. Often followed by neutrophil leukocytosis in patients who eventually recovered along with normalization of thrombocytopenia. However, leukocytosis may persist and show immature cells. Progressive decrease in platelet count in patients with severe disease could suggest disseminated intravascular coagulation (DIC). Low haemoglobin concentration indicates significant blood loss (Bishop, 2014; McElroy et al., 2014; Kortepeter et al., 2011). Prolonged prothrombin time or activated partial thromboplastin time is associated with more severe infection and bleeding manifestation such as DIC. The value of D-dimers may rise four times higher on day 6-8 of infection as evidenced by post-mortem results in comparison with that of the survivors (Oswald et al., 2007). A raise in serum creatinine, urea or

derange electrolytes may indicate acute kidney injury and may be seen at the end of first week of infection (King et al., 2015). Hypokalaemia, Haematuria and proteinuria indicate fatal disease. Elevated serum lactate is in keeping with tissue hypoperfusion and shock. Derange liver enzymes such as AST and ALT with marked AST: ALT ratio up to 15:1 was observed on day 6-8 in fatal cases as compared with 5:1 in non-fatal (King et al., 2015). Bilirubin, γ -glutamyl transferase, and alkaline phosphatase are often slightly raised. Higher concentrations of serum amylase may indicate pancreatitis, also suggestive of fatal infection (King et al., 2015;

Bray et al., 2000). Negative blood culture is helpful and rule out other non-viral infectious causes such as sepsis or enteric fever (Kortepeter et al., 2011).

Detection of viral genome in peripheral blood or other tissue fluid is considered more direct and appropriate diagnostic approach (King et al., 2015). As a progress towards rapid and accurate molecular diagnosis, a wide array of molecular hybridization and nucleic acid amplification techniques are continuously being developed (table II and III) (Goodman, 2014; Bray et al., 2000).

Table II: Specific diagnostic tests used at various stages of the disease

Time of infection	Laboratory Diagnostic Tests
Few days after symptoms develop	Antigen-capture ELISA, IgM, Nucleic acid detection (PCR), virus isolation
Later in disease course or after recovery	IgM and IgG antibodies
Retrospectively in deceased patients	Immunohistochemistry testing, PCR, virus isolation

(Beeching *et al.*, 2014; McElroy *et al.*, 2014; Kortepeter *et al.*, 2011; Rolling *et al.*, 2007)

Key: PCR- polymerase chain reaction, IgM- immunoglobulin M, IgG-immunoglobulin G.

Table III: Other supportive diagnostic approaches

Investigation	Outcome/Remark
Full Blood Count	<p>Decreased platelet count with marked lymphopenia in initial stage, often followed by neutrophil leukocytosis in later stage in patients who eventually recovered along with normalization of thrombocytopenia. Leukocytosis may persist and show immature form.</p> <p>Progressive decrease in platelet count in patients with severe disease may indicate DIC. □</p> <p>Low haemoglobin in patients with bleeding manifestation.</p>

Coagulation Studies	<p>*Prolonged prothrombin or activated partial thromboplastin time is associated with more severe infection and bleeding manifestation such as DIC.</p> <p>D-dimers value may rise four times higher on day 6 -8 of infection in patients who die compared with the survivors.</p>
Renal functions and Serum electrolytes values	<p>Raised serum creatinine or urea and deranged electrolytes may indicate acute kidney injury which may be seen at the end of first week of infection.</p> <p>Hypokalaemia (associated with vomiting and diarrhoea) in about half of all cases were found in some studies.</p> <p>Hypocalcaemia has been associated with fatal infection.</p> <p>Haematuria and proteinuria may also be observe in fatal cases.</p> <p>Oliguria that do not respond to fluid resuscitation is a poor prognostic sign.</p>
Blood Gases	<p>Venous or arterial blood lactate, pH, and bicarbonate can give clue to the level of systemic hypoperfusion and guide fluid resuscitation in extremely sick patients with signs of sepsis.</p> <p>Raised lactate is an indicator of tissue hypoperfusion and shock.</p>
Liver functions	<p>ALT and AST are usually raised with more AST than ALT. This is more suggestive of tissue damage rather than hepatocellular injury.</p> <p>The AST: ALT ratio peaked at 15:1 on days 6 -8 of infection in fatal cases compared with 5:1 in non-fatal cases.</p> <p>Bilirubin, γ-glutamyl transferase, and alkaline phosphatase are often slightly raised. Greatly raised ALT and serious jaundice indicate alternative diagnosis (e.g. viral hepatitis).</p>
Serum amylase	<p>Higher concentration have been reported in several studies and indicate pancreatitis.</p>
Blood culture	<p>Negative blood culture rule out other non-viral infections.</p>
Chest Radiography	<p>Important in patients with respiratory symptoms.</p> <p>Pulmonary infiltrates are not typical EVD and indicate alternative diagnosis.</p>

(King *et al.*, 2015; Oswald *et al.*, 2007; Bray *et al.*, 2000)

Therapeutics

Specific antiviral therapy is not available to treat EBV infection. The treatment approach depends on clinical presentation, host immune status and other underlying conditions (Zhang *et al.*, 2014).

Previous study shows that EBOV has a mechanism of infection propagation in vivo in macaques that is distinctively insensitive even to increased concentration of neutralizing antibody (McCarthy, 2014; Qiu *et al.*, 2014). Post-exposure protection with rNAPc2 in primates provides a new foundation for therapeutic regimens that target the disease process rather than viral replication (Qiu *et al.*, 2014). Evidence from past outbreaks shows that transfusion of blood from recovering patients might be beneficial in the acute phase of infection and was associated with decreased mortality; now at the stage of clinical trials (Choi and Croyle, 2013). Adenosine analogues were found to inhibit in vitro, probably by blocking the cellular enzyme, S-adenosyl-L-homocysteine hydrolase, thereby indirectly limiting methylation of 5' cap of the viral messenger RNA (Thi *et al.*, 2014; Choi and Croyle, 2013). ZMapp, a mixture of three humanized monoclonal antibodies targeted against three of the virus glycoprotein epitopes was engineered for expression in tobacco plant, currently may be the best option for treatment (WHO, 2014). Before the 2014 outbreak, ZMapp had established its protective role when given to non-human primates 24-48 hours after infection. Another study reported that the drug could save non-human primates if treatment is extended up to five days post infection (Furuta *et al.*, 2009). Even though its safety or efficacy is not yet proven in humans, very small quantity (seven doses) was made available to infected individuals and only one of them died. Regardless of its potential, numbers are very little to make any inferences on the drug's safety and efficacy. However, additional doses are being developed with support from the US government (Warren *et al.*, 2014).

TKM-Ebola is another drug, consisting of a mixture of small interfering RNAs targeted against EBOV RNA polymerase L and is produced using lipid nanoparticle technology. This is also shown to be promising in non-human primates and is equally effective against

Marburg virus in guinea pigs and monkeys (Turone, 2014; Iversen *et al.*, 2012). Brincidofovir, previously known as CMX-001, presently at phase III trial for the treatment of cytomegalovirus and adenovirus, is reported to be active against Ebola virus in vitro. Has been used in individuals with EVD in US under Emergency Investigational New Drug applications approved by the FDA, trials are underway in West Africa (Towner *et al.*, 2004). Favipiravir (T-705), a potent antiviral, selectively inhibits viral RNA dependent RNA polymerase of many viruses including influenza viruses, West Nile virus, yellow fever virus, foot and mouth disease virus, other flaviviruses, arenaviruses, bunya viruses, and alphaviruses. The drug was approved in Japan for influenza pandemics and show potency against EBOV in mouse models (Fletcher *et al.*, 2014). Another adenosine analogue (BCX-4430), shown to be active against Ebola virus in rodents, and thought to work by inhibiting viral RNA-dependent RNA polymerase. Is also active against flaviviruses, bunya viruses, arenaviruses, and paramyxoviruses. The drug has been shown to be protective in non-human primates and rodents, even when given 48 hours after infection with filoviruses; however, no study was carried out on humans (Xu *et al.*, 1998). AVI-7537 is another antiviral agent consisting of antisense phosphorodiamidate morpholino oligomers (PMOs) that target Ebola virus VP24 gene and provide survival advantage to Ebola virus infected non-human primates (Ye and Yang, 2015). AVI-6002 consists of two PMOs (AV-7537 and AV-7539) and targets both VP24 and VP35 genes. AV-6002 have also undergone phase I clinical studies (Ledgerwood *et al.*, 2017). Therapeutic agents used for other diseases, such as amiodarone, clomiphene, and chloroquine inhibit Ebola virus interactions with human cells in models and amiodarone will shortly be trialed in West Africa (Ledgerwood *et al.*, 2017).

Prevention and vaccine development

Currently, no any vaccine is licensed against EBOV in humans; however, two experimental vaccines are at various stages of clinical trial. cAd3-ZEBOV, a chimpanzee derived adenovirus vector with an Ebola virus gene

inserted (Ledgerwood *et al.*, 2017) is undergoing further evaluation in the United Kingdom, United States, Switzerland, and some African countries. rVSV-ZEBOV is attenuated form of vesicular stomatitis virus and has one of its genes replaced by an Ebola virus gene, is being evaluated on humans in the US. Another vaccine candidate, which uses a prime-boost protocol to increase immunogenicity with two distinct viral vectors administered at different doses (Ad26-EBOV and MVA-EBOV) is also available (Marzi *et al.*, 2011). Report showed addition of Matrix-M adjuvant to the EBOV/Mak GP nanoparticles enhanced both B and T-cell immune stimulation, which may be critical for an Ebola subunit vaccine with broad and long lasting protective immunity (Bengtsson *et al.*, 2017).

Considering high pathogenicity and rapid spread of EBOV and lack of appropriate vaccine, quarantine measures should be the first line of action and continued until an individual is tested negative (Marzi *et al.*, 2011). Careful hygiene practices should be employed, hand washing with soap and water or an alcohol-based hand sanitizer is necessary. Always avoid contact with blood and body fluids (urine, feces, saliva, sweat, urine, vomit, breast milk, semen or vaginal secretions from suspected or established case (Ledgerwood *et al.*, 2014). Handling of items that may have encountered infected person's blood or body fluids (clothes, bedding, needles, and medical equipment) should be avoided. Avoid contact with bats and nonhuman primates or blood, fluids, and raw meat prepared from these animals. Health care workers should wear appropriate personal protective equipment (Marzi *et al.*, 2011). Report show good correlation between protection with antibody titer and antigen-specific T-cell responses to sGP or GP.

Conclusions

Infection with EBOV is connected with broad spectrum of clinical diseases of which majority lack pathogenetic explanations. The form of its genetic evolution, strange properties and functional profile coupled with poorly understood complicated connection with the host are all subjects far from absolute

comprehension. Extended awareness and definition of the actual pathogenetic role of EBOV in human diseases, development of better diagnostic methods and algorithms including prophylaxis and drug development are crucial issues worth of efforts by the scientific community.

References

- Baize, S., Leroy, E.M., Mavoungou, E. and Fisher-Hoch, S.P. (2000). Apoptosis in fatal Ebola infection. Does the virus toll the bell for immune system? *Apoptosis*; 5(1): 5-7.
- Baize, S., Pannetier, D., Oestereich, L., Rieger, T., Koivogui, L., Magassouba, N.F., Soropogui, M, Sow, M.S., Keita, S., De Clerck, H., Tiffany, A., Dominguez, G., Loua, M., Traoré, A., Kolié, M., Malano, E.R., Heleze, E., Bocquin, A., Mély, S., Raoul, H., Caro, V., Cadar, D., Gabriel, M., Pahlmann, M., Tappe, D., Schmidt-Chanasit, J., Impouma, B., Diallo, A.K., Formenty, P., Herp, M.V. and Günther, S. (2014). Emergence of Zaire Ebola virus disease in Guinea. *New England Journal of Medicine*; 371: 1418–1425.
- Basler, C.F. (2005). Interferon antagonists encoded by emerging RNA viruses. In: *Modulation of Host Gene Expression and Innate Immunity by Viruses*, Palese P (Ed), Springer, Dordrecht, Netherlands:197.
- Beeching, N.J., Manuel, F.M., Houlihan, C.F. (2014). Ebola virus disease. *British Medical Journal*; 349: 7348.
- Bengtsson, K.L., Song, H., Stertman, L., Liu, Y., Flyer, D.C., Massare, M.J., Xu, R.H., Zhou, B., Lu, H., Kwilas, S.A., Hahn, T.J., Kpamegan, E., Hooper, J., Carrion, R. Jr., Glenn, G. and Smith, G. (2016). Matrix-M adjuvant enhances antibody, cellular and protective immune responses of a Zaire Ebola/Makona virus glycoprotein (GP) nanoparticle vaccine in mice. *Vaccine*; 34(16): 1927-1935.
- Biedenkopf, N., Hartlieb, B., Hoenen, T. and Becker, S. (2013). Phosphorylation of Ebola Virus VP30 Influences the Composition of the Viral Nucleocapsid Complex: Impact on Viral Transcription

- and Replication. *The Journal of Biology and Chemistry*; 288(16): 11165–11174.
- Bishop, B.M. (2014). Potential and emerging treatment options for Ebola virus disease. *Annals of Pharmacotherapy*; 49(2): 196–206.
- Bradfute, S.B., Braun, D.R., Shamblin, J.D., Geisbert, J.B., Paragas, J., Garrison, A., Hensley, L.E. and Geisbert, T.W. (2007). Lymphocyte Death in a Mouse Model of Ebola Virus Infection. *Journal of Infectious Disease*; 196(2): S296–S304.
- Bradfute, S.B., Warfield, K.L. and Bavari, S. (2008). Functional CD8+ T cell responses in lethal Ebola virus infection. *Journal of Immunology*; 180(6): 4058–4066.
- Brauburger, K., Boehmann, Y., Tsuda, Y., Hoenen, T., Olejnik, J., Schümann, M., Ebihara, H. and Mühlberger, E. (2014). Analysis of the highly diverse gene borders in Ebola virus reveals a distinct mechanism of transcriptional regulation. *Journal of Virology*; 88(21): 12558–12571.
- Bray, M. and Geisbert, T.W. (2005). Ebola virus: the role of macrophages and dendritic cells in the pathogenesis of Ebola hemorrhagic fever. *International Journal of Biochemistry and Cell Biology*; 37(8): 1560–1566.
- Bray, M., Driscoll, J. and Huggins, J.W. (2000). Treatment of lethal Ebola virus infection in mice with a single dose of an S-adenosyl-L-homocysteine hydrolase inhibitor. *Antiviral Research*; 45(2): 135–147.
- Carette, J.E., Raaben M., Wong, A.C., Herbert, A.S., Obernosterer, G., Mulherkar, N., Kuehne, A.I., Kranzusch, P.J., Griffin, A.M., Ruthel, G., Dal, C.P., Dye, J.M., Whelan, S.P., Chandran, K. and Brummelkamp, T.R. (2011). Ebola virus entry requires the cholesterol transporter Niemann-Pick C1. *Nature*; 477(7364): 340–343.
- Castilletti, C., Carletti, F., Gruber, C.E.M., Bordi, L., Lalle, E., Quartu, S., Meschi, S., Lapa, D., Colavita, F., Chiappini, R., Mazzarelli, A., Marsella, P., Petrosillo, N., Nicastrì, E., Chillemi, G., Valentini, A., Desideri, A., Di Caro, A., Ippolito, G. and Capobianchi, M.R. (2015). Molecular Characterization of the First Ebola Virus Isolated in Italy, from a Health Care Worker Repatriated from Sierra Leone. *Genome Announcements*; 3(3): e00639–15.
- Centers for Disease Control and Prevention (2015). Ebola Virus Disease (EVD) Diagnosis. <http://www.cdc.gov/vhf/ebola/diagnosis> [Accessed 19 August 2015].
- Choi, J.H. and Croyle, M.A. (2013). Emerging targets and novel approaches to Ebola virus prophylaxis and treatment. *Biology and Drugs*; 27(6): 565–583.
- Chowell, G. and Nishiura, H. (2015). Characterizing the Transmission dynamics and control of Ebola virus disease. *PLoS Biology*; 13(1): e1002057.
- Christie, A., Davies-Wayne, G.J., Cordier-Lasalle, T., Blackley, D.J., Scott Laney, A., Williams, D.E., Shinde, S.A., Badio, M., Lo, T., Mate, S.E., Ladner, J.T., Wiley, M.R., Kugelman, J.R., Palacios, G., Holbrook, M.R., Janosko, K.B., de Wit, E., van Doremalen, N., Munster, V.J., Pettitt, J., Schoepp, R.J., Verhenne, L., Evlampidou, I., Kollie, K.K., Sieh, S.B., Gasasira, A., Bolay, F., Kateh, F.N., Nyenswah, T.G. and De Cock, K.M. (2015). Possible sexual transmission of Ebola virus-Liberia. *Mortality Weekly Report*; 64(17): 479–481.
- Chua, A.C., Cunningham, J., Moussy, F., Perkins, M.D. and Formenty, P. (2015). The Case for Improved Diagnostic Tools to Control Ebola Virus Disease in West Africa and How to Get There. *PLoS Neglected Tropical Disease*; 9(6): e0003734.
- Côté, M., Misasi, J., Ren, T., Bruchez, A., Lee, K., Filone, C.M., Hensley, L., Li, Q., Ory, D., Chandran, K. and Cunningham, J. (2011). Small molecule inhibitors reveal Niemann-Pick C1 is essential for Ebola virus infection. *Nature*; 477(7364): 344–348.
- Cox, N.J., McCormick, J.B., Johnson, K.M. and Kiley, M.P. (1983): Evidence for two subtypes of Ebola virus based on oligonucleotide mapping of RNA. *Journal of Infectious Diseases*; 147(2): 272–275.
- Crary, S.M., Towner, J.S., Honig, J.E., Shoemaker, T.R. and Nichol, S.T. (2003).

- Analysis of the role of predicted RNA secondary structures in Ebola virus replication. *Virology*; 306(2): 210-218.
- Dessen, A., Dolnik, O., Klenk, H. and Weissenhorn, W. (2000). Crystal structure of the matrix protein VP40 from Ebola virus. *The European Molecular Biology Organization Journal*; 19(16): 4228-4236.
- Feldmann, H. and Geisbert, T.W. (2011). Ebola haemorrhagic fever. *Lancet*; 377(9768): 849-862.
- Feldmann, H., Geisbert, T.W., Jahrling, P.B., Lenk H.D., Netesov, S.V., Peters, C.J., Sanchez, A., Swanepoel, R. and Volchkov, V.E., FAMILY FILOVIRIDAE. (2005). In: Fauquet, C.M., Mayo, M.A., Maniloff, J., Desselberger, U. and Ball, L.A. (eds): Virus Taxonomy. Eighth Report of the International Committee on Taxonomy of Viruses. Elsevier/Academic Press, San Diego, USA: 645–653.
- Feldmann, H., Klenk, H.D. and Sanchez, A. (1993). Molecular biology and evolution of filoviruses. *Achieves of Virology*; 7: 81-100.
- Fletcher, T.E., Brooks, T.J. and Beeching, N.J. (2014). Ebola and other viral haemorrhagic fevers. *British Medical Journal*; 349: 5079.
- Folarin, O.A., Ehichioya, D., Schaffner, S.F., Winnicki, S.M., Wohl, S., Eromon, P., West, K.L., Gladden-Young, A., Oyejide, N.E., Matranga, C.B., Deme, A.B., James, A., Tomkins-Tinch, C., Onyewurunwa, K., Ladner, J.T., Palacios, G., Nosamiefan, I., Andersen, K.G., Omilabu, S., Park, D.J., Yozwiak, N.L., Nasidi, A., Garry, R.F., Tomori, O., Sabeti, P.C. and Happi, C.T. (2016). Ebola Virus Epidemiology and Evolution in Nigeria. *Journal of Infectious Disease*; 214(3): S102-S109.
- Fowler, R.A., Fletcher, T., Fischer, W.A., et al. (2014). Caring for critically ill patients with Ebola virus disease. Perspectives from West Africa. *American Journal of Respiratory and Critical Care Medicine*; 190(7): 733-737.
- Furuta, Y., Takahashi, K., Shiraki, K., Lamontagne, F., Jacob, S., Brett-Major, D., Lawler, J.V., Jacquerioz, F.A., Houlihan, C., O'Dempsey, T., Ferri, M., Adachi, T., Lamah, M.C., Bah, E.I., Mayet, T., Schieffelin, J., McLellan, S.L., Senga, M., Kato, Y., Clement, C., Mardel, S., Vallenias Bejar De Villar, R.C., Shindo, N. and Bausch, D. (2009). T-705 (favipiravir) and related compounds: novel broad-spectrum inhibitors of RNA viral infections. *Antiviral Research*; 82(3): 95-102.
- Gallagher, W.R. (1996). Similar structural models of the transmembrane proteins of Ebola and avian sarcoma viruses. *Cell*. 85(4): 477–478.
- Geisbert, T.W., Young, H.A., Jahrling, P.B., Davis, K.J., Larsen, T., Kagan, E. and Hensley, L.E. (2003). Pathogenesis of Ebola hemorrhagic fever in primate models: evidence that hemorrhage is not a direct effect of virus-induced cytolysis of endothelial cells. *American Journal of Pathology*; 163(6): 2371-2382.
- Goeijenbier, M., van Kampen, J.J.A., Reusken, C.B.E.M., Koopmans, M.P., van Gorp, E.C. (2014). Ebola virus disease: a review on epidemiology, symptoms, treatment and pathogenesis. *Netherlands Journal of Medicine*; 72(9): 442-448.
- Goodman, J.L. (2014). Studying secret serums: toward safe, effective Ebola treatments. *New England Journal of Medicine*; 371(12): 1086-1089.
- Hensley, L.E., Young, H.A., Jahrling, P.B. and . (2002). Pro-inflammatory response during Ebola virus infection of primate models: possible involvement of the tumor necrosis factor receptor superfamily. *Immunology Letter*; 80(3): 169-179.
- Hoenen, T., Groseth, A., Kolesnikova, L., Theriault, S., Ebihara, H., Hartlieb, B., Bamberg, S., Feldmann, H., Ströher, U. and Becker, S. (2006). Infection of naïve target cells with virus-like particles: Implications for the Function of Ebola Virus VP24. *Journal of Virology*; 80(14): 7260-7264.
- Hofmann-Winkler, H., Gnirß, K., Wrensch, F. and Pöhlmann, S. (2015). Comparative Analysis of Host Cell Entry of Ebola Virus from Sierra Leone, 2014 and Zaire, 1976. *Journal of Infectious Disease*; 212(2): S172-180.
- Hulo, C., de Castro, E., Masson, P., Bougueleret,

- L., Bairoch, A., Xenarios, I. and Le Mercier, P. (2011). Viral Zone: a knowledge resource to understand virus diversity. *Nucleic Acids Research*; 39: D576-582.
- Hutchinson, K.L. and Rollin, P.E. (2007). Cytokine and chemokine expression in humans infected with Sudan Ebola virus. *Journal Infectious Disease*; 196(2): S357-363.
- Iversen, P.L., Warren, T.K., Wells, J.B., Garza, N.L., Mourich, D.V., Welch, L.S., Panchal, R.G. and Bavari, S. (2012). Discovery and early development of AVI-7537 and AVI-7288 for the treatment of Ebola virus and Marburg virus infections. *Viruses*; 4(11): 2806-2830.
- Jeffers, S.A., Sanders, D.A. and Sanchez, A. (2002). Covalent modifications of the Ebola virus glycoprotein. *Journal of Virology*; 76(24): 12463–12472.
- John, S.P., Wang, T., Steffen, S., Longhi, S., Schmaljohn, C.S., Jonsson, C.B. (2007). Ebola Virus VP30 Is an RNA Binding Protein. *Journal of Virology*; 81(17): 8967-8976.
- Kibadi, K., Mupapa, K., Kuvula, K., Massamba, M., Ndaberey, D., Muyembe-Tamfum, J.J., Bwaka, M.A., De Roo, A. and Colebunders, R. (1999). Late Ophthalmologic Manifestations in Survivors of the 1995 Ebola Virus Epidemic in Kikwit, Democratic Republic of the Congo. *Journal of Infectious Disease*; 179(1): S13-S14.
- King, J. and Rafeek, H. (2015). Ebola Virus Infection, Treatment & Management. *M e d s c a p e* ; <http://emedicine.medscape.com/article/216288-treatment> [Accessed 19 August 2015].
- Klenk, H.D. and Feldmann, H. (2004). *Ebola and Marburg Viruses: Molecular and Cellular Biology*. Horizon Bioscience, Canada, ISBN: 978-1-904933 (hardback). ISBN: 13:978-1-904933-49-6 (ebook).
- Kondratowicz, A.S., Lennemann, N.J., Sinn, P.L., Davey, R.A., Hunt, C.L., Moller-Tank, S., Meyerholz, D.K., Rennert, P., Mullins, R.F., Brindley, M., Sandersfeld, L.M., Quinn, K., Weller, M., McCray, P.B. Jr., Chiorini, J. and Maury, W. (2011). T-cell immunoglobulin and mucin domain 1 (TIM-1) is a receptor for Zaire Ebolavirus and Lake Victoria Marburgvirus. *Proceedings of the National Academy of Science USA*; 108(20): 8426-8431.
- Kortepeter, M.G., Bausch, D.G. and Bray, M. (2011). Basic clinical and laboratory features of filoviral hemorrhagic fever. *Journal of Infectious Disease*; 204(Suppl 3): S810-816.
- Kreuels, B., Wichmann, D., Emmerich, P., Schmidt-Chanasit, J., de Heer, G., Kluge, S., Sow, A., Renné, T., Günther, S., Lohse, A.W., Addo, M.M. and Schmiedel, S. (2014). A case of severe Ebola virus infection complicated by gram-negative septicemia. *New England Journal of Medicine*; 371(25): 2394-401.
- Kuhn, J.H., Becker, S., Ebihara, H., et al. (2010). Proposal for a revised taxonomy of the family Filoviridae: Classification, names of taxa, viruses, and virus abbreviations. *Archives of Virology*; 155(12): 2083-2103.
- Ledgerwood, J.E., DeZure, A.D., Stanley, M.S., Coates, E.E., Novik, L., Enama, M.E., Berkowitz, N.M., Hu, Z., Joshi, G., Ploquin, A., Sitar, S., Gordon, I.J., Plummer, S.S., Holman, L.A., Hendel, C.S., Yamshchikov, G., Roman, F., Nicosia, A., Colloca, S., Cortese, R., Bailer, R.T., Schwartz, R.M., Roederer, M., Mascola, J.R., Koup, R.A., Sullivan, N.J., Graham, B.S. and VRC 207 Study Team (2017). Chimpanzee Adenovirus Vector Ebola Vaccine. *New England Journal of Medicine*; 376(10): 928-938.
- Lee, J.E. and Saphire, E.O. (2009). Ebolavirus glycoprotein structure and mechanism of entry. *Future Virology*; 4(6): 621–635.
- Liu, W.B., Li, Z.X., Du, Y. and Cao, G.W. (2015). Ebola virus disease: from epidemiology to prophylaxis. *Military Medical Research*; 2(7): DOI: 10.1186/s40779-015-0035-4.
- Martin, P., Laupland, K.B., Frost, E.H. and Valiquette L. (2015). Laboratory diagnosis of Ebola virus disease. *Intensive Care Medicine*; 41(5): 895-898.
- Martínez, M.J., Biedenkopf, N., Volchkova, V.,

- Hartlieb, B., Alazard-Dany, N., Reynard, O., Becker, S. and Volchkov, V. (2008). Role of Ebola Virus VP30 in Transcription Re-initiation. *Journal of Virology*; 82(24): 12569-12573.
- Marzi, A., Ebihara, H., Callison, J., Groseth, A., Williams, K.J., Geisbert, T.W. and Feldmann, H. (2011). Vesicular Stomatitis Virus-Based Ebola Vaccines with Improved Cross-Protective Efficacy. *Journal of Infectious Disease*; 204(3): S1066–S1074.
- Mayo, M.A. (2002). ICTV at the Paris ICV: Results of the plenary session and the binomial ballot. *Archives of Virology*; 147: 2254–2260.
- McCarthy, M. (2014). US signs contract with ZMapp maker to accelerate development of the Ebola drug. *British Medical Journal*; 349: 5488.
- McElroy, A.K., Akondy, R.S., Davis, C.W., Ellebedy, A.H., Mehta, A.K., Kraft, C.S., Lyon, G.M., Ribner, B.S., Varkey, J., Sidney, J., Sette, A., Campbell, S., Ströher, U., Damon, I., Nichol, S.T., Spiropoulou, C.F. and Ahmed, R. (2015). Human Ebola virus infection results in substantial immune activation. *Proceedings of the National Academy of Science*; 112(15): 4719-4724.
- McElroy, A.K., Erickson, B.R., Flietstra, T.D., Rollin, P.E., Nichol, S.T., Towner, J.S. and Spiropoulou, C.F. (2014). Biomarker correlates of survival in pediatric patients with Ebola virus disease. *Emerging Infectious Disease*; 20(10): 1683-1690.
- Modrof, J., Becker, S. and Muhlberger, E. (2003). Ebola virus transcription activator VP30 is a zinc-binding protein. *Journal of Virology*; 77(5): 3334-3338.
- Mohan, G.S., Li, W., Ye, L., Compans, R.W. and Yang, C. (2012). Antigenic Subversion: A Novel Mechanism of Host Immune Evasion by Ebola Virus. *PLoS Pathology*; 8(12): e1003065.
- Muhlberger, E., Weik, M., Volchkov, V.E., Klenk, H.D. and Becker, S. (1999). Comparison of the transcription and replication strategies of Marburg virus and Ebola virus by using artificial replication systems. *Journal of Virology*; 73(3): 2333–2342.
- Muyembe-Tamfumet, J.J., Mulangu, S., Masumu, J., Kayembe, J.M., Kemp, A. and Paweska, J.T. (2012). Ebola virus outbreaks in Africa: past and present. *Onderstepoort Journal of Veterinary Research*; 79(2): 451.
- Nanbo, A., Watanabe, S., Halfmann, P. and Kawaoka, Y. (2013). The spatio-temporal distribution dynamics of Ebola virus proteins and RNA in infected cells. *Scientific Reports*; 3: 1206.
- Noda, T., Ebihara, H., Muramoto, Y., Fujii, K., Takada, A., Sagara, H., Kim, J.H., Kida, J., Feldmann, H. and Kawaoka, Y. (2006). Assembly and budding of Ebolavirus. *PLoS Pathology*; 2(9): e99.
- Noda, T., Kolesnikova, L., Becker, S. and Kawaoka, Y. (2011). The importance of the NP: VP35 ratio in Ebola virus nucleocapsid formation. *Journal of Infectious Disease*; 204(S3): 878-883.
- Osterholm, M.T., Moore, K.A., Kelley, N.S., Brosseau, L.M., Wong, G., Murphy, M.A., Peters, C.J., LeDuc, J.W., Russell, P.K., Herp, M.V., Kapetshi, J., Muyembe, J.T., Ilunga, B.K., Strong, J.E., Grolla, A., Wolz, A., Kargbo, B., Kargbo, D.K., Formenty, P., Sanders, D.A. and Kobinger G.P. (2015). Transmission of Ebola viruses: What we know and what we do not know. *MBio*; 6(2): e00137.
- Oswald, W.B., Geisbert, T.W., Davis, K.J., Geisbert, J.B., Sullivan, N.J., Jahrling, P.B., Parren, P.W. and Burton, D.R. (2007). Neutralizing Antibody Fails to Impact the Course of Ebola Virus Infection in Monkeys. *PLoS Pathology*; 3(1): e9.
- Park, D.J., Dudas, G., Wohl, S., Goba, A., Whitmer, S.L., Andersen, K.G., Sealfon, R.S., Ladner, J.T., Kugelman, J.R., Matranga, C.B., Winnicki, S.M., Qu, J., Gire, S.K., Gladden-Young, A., Jalloh, S., Nosamiefan, D., Yozwiak, N.L., Moses, L.M., Jiang, P.P., Lin, A.E., Schaffner, S.F., Bird, B., Towner, J., Mamoh, M., Gbakie, M., Kanneh, L., Kargbo, D., Massally, J.L., Kamara, F.K., Konuwa, E., Sellu, J., Jalloh, A.A., Mustapha, I., Foday, M., Yillah, M., Erickson, B.R., Sealy, T., Blau, D., Paddock, C., Brault, A., Amman, B.,

- Basile, J., Bearden, S., Belser, J., Bergeron, E., Campbell, S., Chakrabarti, A., Dodd, K., Flint, M., Gibbons, A., Goodman, C., Klena, J., McMullan, L., Morgan, L., Russell, B., Salzer, J., Sanchez, A., Wang, D., Jungreis, I., Tomkins-Tinch, C., Kislyuk, A., Lin, M.F., Chapman, S., MacInnis, B., Matthews, A., Bochicchio, J., Hensley, L.E., Kuhn, J.H., Nusbaum, C., Schieffelin, J.S., Birren, B.W., Forget, M., Nichol, S.T., Palacios, G.F., Ndiaye, D., Happi, C., Gevao, S.M., Vandi, M.A., Kargbo, B., Holmes, E.C., Bedford, T., Gnirke, A., Ströher, U., Rambaut, A., Garry, R.F. and Sabeti, P.C. (2015). Ebola Virus Epidemiology, Transmission, and Evolution during Seven Months in Sierra Leone. *Cell*; 161(7): 1516-1526.
- Parrino, J., Hotchkiss, R.S. and Bray, M. (2007). Prevention of immune cell apoptosis as potential therapeutic strategy for severe infections. *Emerging Infectious Disease*; 13(2): 191-198.
- Qiu, X., Wong, G., Audet, J., Bello, A., Fernando, L., Alimonti, J.B., Fausther-Bovendo, H., Wei, H., Aviles, J., Hiatt, E., Johnson, A., Morton, J., Swope, K., Bohorov, O., Bohorova, N., Goodman, C., Kim, D., Pauly, M.H., Velasco, J., Pettitt, J., Olinger, G.G., Whaley, K., Xu, B., Strong, J.E., Zeitlin, L., Kobinger, G.P. (2014). Reversion of advanced Ebola virus disease in nonhuman primates with ZMapp. *Nature*; 514(7520): 47-53.
- Rollin, P.E., Bausch, D.G., Sanchez, A. (2007). Blood chemistry measurements and D-Dimer levels associated with fatal and nonfatal outcomes in humans infected with Sudan Ebola virus. *Journal of Infectious Disease*; 196(2): S364-371.
- Saeed, M.F., Kolokoltssov, A.A., Albrecht, T., Davey, R.A. (2010). Cellular Entry of Ebola Virus Involves Uptake by a Macropinocytosis-Like Mechanism and Subsequent Trafficking through Early and Late Endosomes. *PLoS Pathology*; 6: e1001110.
- Sanchez, A., Trappier, S.G., Mahy, B.W., Peters, C.J. and Nichol, S.T. (1996). The virion glycoproteins of Ebola viruses are encoded in two reading frames and expressed through transcriptional editing. *Proceedings of National Academy of Science USA*; 93(8): 3602-3607.
- Silva, L.P., Vanzile, M., Bavari, S., Javad Aman, J. M. and Schriemer, D.C. (2012). Assembly of Ebola virus matrix protein VP40 is regulated by Latch-like properties of N and C terminal tails. *PLoS One*; 7: e39978.
- Taylor, D.J., Ballinger, M.J., Zhan, J.J., Hanzly, L.E. and Bruenn, J.A. (2014). Evidence that ebolaviruses and Cueva viruses have been diverging from Marburg viruses since the Miocene. *Peer Journal*; 2: e556.
- Thi, E.P., Mire, C.E., Ursic-Bedoya, R., Geisbert, J.B., Lee, A.C.H., Agans, K.N., Robbins, M., Deer, D.J., Fenton, K.A., MacLachlan, I. and Geisbert, T.W. (2014). Marburg virus infection in nonhuman primates: therapeutic treatment by lipid-encapsulated siRNA. *Science and Translational Medicine*; 6(250): 250ra116.
- Towner, J.S., Rollin, P.E., Bausch, D.G., Sanchez, A., Crary, S.M., Vincent, M., Lee, W.F., Spiropoulou, C.F., Ksiazek, T.G., Lukwiya, M., Kaducu, F., Downing, R. and Nichol, S.T. (2004). Rapid diagnosis of Ebola hemorrhagic fever by reverse transcription-PCR in an outbreak setting and assessment of patient viral load as a predictor of outcome. *Journal of Virology*; 78(8): 4330-4341.
- Tran, E.E.H., Simmons, J.A., Bartesaghi, A., Shoemaker, C.J., Nelson, E., White, J.M., Subramaniam, S. (2014). Spatial Localization of the Ebola Virus Glycoprotein Mucin-Like Domain Determined by Cryo-Electron Tomography. *Journal of Virology*; 88(18): 10958-10962.
- Turone, F. (2014). Doctors trial amiodarone for Ebola in Sierra Leone. *British Medical Journal*; 349: 7198.
- Varkey, J.B., Shantha, J.G., Crozier, I., Kraft, C.S., Lyon, G.M., Mehta, A.K., Kumar, G., Smith, J.R., Kainulainen, M.H., Whitmer, S., Ströher, U., Uyeki, T.M., Ribner, B.S. and Yeh, S. (2015). Persistence of Ebola Virus in Ocular Fluid during Convalescence. *New England Journal of Medicine*; 372(25): 2423-2427.

- Vingolo, E.M., Messano, G.A., Fragiotta, S., (2015). Ocular Manifestations of Ebola Virus Disease: An Ophthalmologist's Guide to Prevent Infection and Panic. *Biomed Research International*; 487073. DOI: 10.1155/2015/487073.
- Volchkov, V.E., Volchkova, V.A., Chepurinov, A.A., Blinov, V.M., Dolnik, O., Netesov, S.V. and Feldmann, H. (1999). Characterization of the L gene and 5' trailer region of Ebola virus. *Journal of General Virology*; 80(2): 355–362.
- Warren, T.K., Wells, J., Panchal, R.G., Stuthman, K.S., Garza, N.L., Van Tongeren, S.A., Dong, L., Retterer, C.J., Eaton, B.P., Pegoraro, G., Honnold, S., Bantia, S., Kotian, P., Chen, X., Taubenheim, B.R., Welch, L.S., Minning, D.M., Babu, Y.S., Sheridan, W.P. and Bavari, S. (2014). Protection against filovirus diseases by a novel broad-spectrum nucleoside analogue BCX4430. *Nature*; 508(7496): 402-405.
- Watanabe, S., Noda, T., Halfmann, P., Jasenosky, L. and Kawaoka, Y. (2007). Ebola Virus (EBOV) VP24 Inhibits Transcription and Replication of the EBOV Genome. *The Journal of Infectious Disease*; 196(2): S284-290.
- Weik, M., Modrof, J., Klenk, H.D., Becker, S. and Mühlberger, E. (2002). Ebola Virus VP30-Mediated Transcription Is Regulated by RNA Secondary Structure Formation. *Journal of Virology*; 76(17): 8532-8539.
- Whelan, S.P.J., Barr, J.N. and Wertz, G.W. (2004). Transcription and replication of non-segmented negative-strand RNA viruses. *Current Topics in Microbiology and Immunology*; 283: 61-119.
- World Health Organization (2014). Potential Ebola therapies and vaccines. http://apps.who.int/iris/bitstream/10665/137590/1/WHO_EVD_HIS_EMP_14.1_en_g.pdf [Accessed 21 August 2015].
- World Health Organization (2014). Update on the WHO Emergency Quality Assessment Mechanism for in-vitro diagnostics for Ebola virus disease. http://www.who.int/diagnostics_laboratory/procurement/141211_evd_idvs_update.pdf [Accessed 19 August 2015].
- Xu, L., Sanchez, A., Yang, Z., Zaki, S.R., Nabel, E.G., Nichol, S.T. and Nabel, G.J. (1998). Immunization for Ebola virus infection. *Nature Medicine*; 4(1): 37-42.
- Ye, L. and Yang, C. (2015). Development of vaccines for prevention of Ebola virus infection. *Microbial Infections*; 17(2): 98–108.
- Zhang, Y., Li, D., Jin, X. and Huang, Z. (2014). Fighting Ebola with ZMapp: spotlight on plant-made antibody. *Science China Life Science*; 57(10): 987-988.

Citation: Rogo, L.D. and Auwal, A.K. Ebola Virus-A Review. *Sokoto Journal of Medical Laboratory Science*; 3(1): 69 - 87

Copyright. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.