WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.074
Volume 7, Issue 19, 120-168. Review Article ISSN 2277– 7105

EBOLA VIRUS DISEASE OUTBREAKS AS GLOBAL DISASTERS

***Musarrat Sharif, Afsheen Ali and Nageen Iqbal**

Institute of Molecular Biology and Biotechnology, University of Lahore, Lahore, Pakistan.

Article Received on
03 October 2018,

Revised on 23 October 2018,
Accepted on 13 Nov. 2018

DOI: 10.20959/wjpr201819-13771

***Corresponding Author**
Prof. Musarrat Sharif
Institute of Molecular Biology and Biotechnology, University of Lahore, Lahore, Pakistan.

ABSTRACT

Ebola virus disease (EVD) is caused by Ebola viruses (EBOV), members of the group of hemorrhagic fevers and it is one of the most dangerous infection diseases with mortality rates up to 90%. Ebola was firstly described in 1976 and since then occurred sporadically in Central Africa. Till 2014, twenty four outbreaks were described, but the number of deaths not exceeding 300 per outbreak. As of June 20, 2017 the cumulative number, suspected, and laboratory-confirmed cases attributed to Ebola virus was 26, 969, including 11,135 deaths. Pathogenesis: Ebola Viruses do not replicate through cell division, but instead insert their own genetic sequencing into the deoxyribonucleic acid (DNA) of the host cell and subsequently hijack all cellular processes, including transcription and translation. In essence, the host cell becomes a factory of viral proteins. As new viral capsules are formed, they bud from the host cell, taking a part of the host cell's outer membrane, thus cloaking themselves against detection by the host's immune system. In some cases, the patient's immune system can produce enough antibodies to defeat the infection. With EVD, the virus can often reproduce so rapidly that the immune system never catches up. Transmission: The natural reservoir of EBOV is believed to be bats, particularly fruit bats, and it is primarily transmitted between humans and from animals to humans through body fluids. Clinical presentation: Symptoms of EVD include abrupt onset of fever, myalgia's, and headache in the early phase, followed by vomiting, diarrhea and possible progression to hemorrhagic rash, life-threatening bleeding, and multi organ failure in the later phase. Treatment: There are no approved treatments or vaccines available for EVD until today; the mainstay of therapy is supportive care. However, there are a bunch of therapeutic approaches on the track which could have the real impact on control and prevention of this global threat. High fatality, combined with the absence of treatment and vaccination options, makes Ebola virus an important public health pathogen.

KEYWORDS: Ebola virus, pathogen, disease, death, humans and nonhuman primates.

INTRODUCTION

The Ebola Virus genera belong to the *Filoviradae* family of viruses in the *Mononegavirales* order. All Filoviruses encode their genome in a single stranded negative sense RNA and are known to cause disease in humans and nonhuman primates. In addition to Ebolavirus, the Filoviridae family also includes the Marburgvirus and Cuevavirus genera as members. While Marburg and *Cuevaviruses* only have one species or strain, Ebolavirus has five: Bundibugyo, Reston, Sudan, Tai Forest and Zaire. The Reston strain is non-pathogenic in humans, but does cause disease in monkeys and pigs. As Ebola virus spreads through the world, Pakistan is also at high risk of getting affected by this deadly virus that has no cure, till date. Ebola hemorrhagic fever has caused more than 7,000 deaths so far and the count is rising by every passing day. Ebola is perhaps the most mysterious virus of this age, formerly known as Ebola hemorrhagic fever, is a worm like virus that is transmitted from wild-animals to humans and other humans exposed to the risk of the virus if in company of an infected patient. Ebola virus is spread through direct blood contact with a person who has been infected. This threat turns into a worry when one knows that no proper treatment has been discovered yet to fight against Ebola. The virus causes internal bleeding in a person which can only be controlled is special and adequate medical health services are provided. However, during in the initial outbreak of the virus, people generally believed the virus to be cholera or Lassa fever which are lesser lethal forms of hemorrhage fever (Feldmann *et al.*, 2010).

The worst Ebola virus outbreak that the world witnessed started with the death of a little African boy, Emile Ouamouno**.** It was the end of last year when this boy who resided in *Meliandou*, a small village isolated in the suburbs of Africa, used to travel back & forth to Liberia to sell his goods. It was in December 2013 that the virus infested him leading to his death after a few weeks of experiencing high fever, nausea, vomiting ultimately leading to the failure of his organs due to internal bleeding. His unfortunate death was followed by the death of his sister a few days later and his pregnant mother who lost her life against this disease. Ever since, the outbreak has resulted in over 5,500 deaths in West Africa alone. Medical authorities have been highly working to develop vaccines for the virus that could prevent future outbursts the disease however, nothing has been announced yet. However, two vaccines are currently being tested with hopes to put an end to this fatal threat. According to a recent report, the first experimental Ebola vaccine has shown success in the first human

trials. Marburg virus was the first genera of the Filoviridae family to be discovered (Slenczka *et al.*, 2014).

In 1967, there were simultaneous outbreaks in Marburg, Germany; Frankfurt, Germany; and Belgrade, Yugoslavia. The epidemic began when laboratory workers in all three cities were initially exposed to the Marburg infected tissues and blood from African green monkeys that had been imported from Uganda. The virus spread via nosocomial transmission and to household contacts of lab workers. By the end of the outbreak, 31 people were infected and seven people died. Since 1967, there have been 12 cases and/or isolated outbreaks of the Marburgvirus, most of which have occurred in the central region of Africa, namely the nations of Gabon, Uganda, the DRC and South Sudan. The Zaire and Sudan Ebolavirus strains were discovered in 1976 in Central Africa during two simultaneous outbreaks, both of which will be described in detail below. A third Ebola Strain, Cote d'Ivoire (now known as the Tai Forest strain) was discovered in 1994 when an ethnologist was infected while performing a necropsy on a diseased chimpanzee. There was only one patient involved, the ethnologist, and she survived the infection. There have been no known cases involving this strain since (Chippaux *et al.*, 2014).

The fifth subtype (Reston) was discovered in 1989 in Reston, Virginia when scientists were investigating an outbreak of what was thought to be Simian hemorrhagic fever in cynomolgus macaques. Instead, they found that the monkeys were infected with a novel strain of Ebolavirus. It was suspected that the monkeys were exposed to this new strain of Ebolavirus while in transit to the United States from the Philippines. This strain has only been shown to cause disease in non-human primates; it has not exhibited pathogenic tendencies in humans (Olival *et al.*, 2014). The *Cuevaviridae* genera, and its sole member, *Lloviuvirus* was discovered in 2002 when a team of scientists investigated a massive bat die-off in French, Spanish and 14 Portuguese caves. Initially suspecting pneumonia, the team instead discovered sequences of a unique member of the Filoviridae family in the spleen, liver and lung samples of dead *M. schreibersii* bats. They placed the novel virus in new genera *Cuevaviridae*, which is named for cueva, the Spanish word for cave. The strain itself, *Lloviuvirus*, was named after the Lloviu cave, where the virus was found. Interestingly, the virus sequences that were isolated in dead bats were missing in live bats of the same species, suggesting that *Lloviuvirus* infection may be pathogenic in bats. This would separate *Lluoviuvirus* from other Filoviridae species. Bats are thought to be the natural reservoir for

the Ebolavirus and Marburgvirus strains and by definition they do not succumb to the disease even though they are infected and shed virus. While a bat natural reservoir had definitively been determined for Marburg virus, proving that bats are also the natural reservoir for Ebolavirus has proved elusive (Negredo *et al.*, 2011). The Bundibugyo strain was discovered in 2007 in the Bundibugyo district in Uganda. While the details involving the primary patient and the primary spillover event are scant, it is known that secondary transmissions occurred via close human contact. 149 people were infected in the outbreak and 37 people died, resulting in a Case Fatality Rate of 25%. There has only been one other outbreak of the Bundibugyo recorded since, which occurred in 2012 in the Democratic Republic of Congo when 77 people were infected and 36 died (CFR of 47%) (MacNeil *et al.*, 2010).

HISTORY OF EBOLA

The Ebola virus causes an acute, serious illness which is often fatal if untreated. Ebola virus disease (EVD) first appeared in 1976 in 2 simultaneous outbreaks, one in Nzara, Sudan, and the other in Yambuku, Democratic Republic of Congo. The latter occurred in a village near the Ebola River, from which the disease takes its name. The current outbreak in West Africa, (first cases notified in March 2014), is the largest and most complex Ebola outbreak since the Ebola virus was first discovered in 1976. Between 1976 and 2013, the World Health Organization reports a total of 24 outbreaks involving 1,716 cases. The largest outbreak to date was the epidemic in West Africa, which occurred from December 2013 to January 2016 with 28,638 cases and 11,315 deaths (Bausch, 2014). The most severely affected countries, Guinea, Liberia and Sierra Leone, have very weak health systems, lack human and infrastructural resources, and have only recently emerged from long periods of conflict and instability. According to info available at WHO website:

- Ebola virus disease (EVD), formerly known as Ebola hemorrhagic fever, is a severe, often fatal illness in humans.
- The virus is transmitted to people from wild animals and spreads in the human population through human-to-human transmission.
- The average EVD case fatality rate is around 50%. Case fatality rates have varied from 25% to 90% in past outbreaks.
- The first EVD outbreaks occurred in remote villages in Central Africa, near tropical rainforests, but the most recent outbreak in West Africa has involved major urban as well as rural areas.

- Community engagement is key to successfully controlling outbreaks. Good outbreak control relies on applying a package of interventions, namely case management, surveillance and contact tracing, a good laboratory service, safe burials and social mobilization.
- Early supportive care with rehydration, symptomatic treatment improves survival. There is as yet no licensed treatment proven to neutralize the virus but a range of blood, immunological and drug therapies are under development.
- There are currently no licensed Ebola vaccines but 2 potential candidates are undergoing evaluation.

EBOLA VIRUS MOLECULAR BIOLOGY

Ebolavirus is an enveloped, non-segmented virus that encodes its genome in a single stranded, negative sense RNA. It appears long and filamentous under the electron microscope, which helped give rise to the Filoviridae name. Ebola has a uniform diameter of 80 nm its length can reach 14000 nanometers. Its seven genes encode eight proteins, which are arranged in a linear order. Short non-transcribed regions are located at the extreme 3' and 5' ends, called the leader and the trailer, respectively. The structural proteins are arranged as follows: nucleoprotein (NP), virion protein (VP) 35, VP 40, Glycoprotein (GP), VP 30, VP 24, and an RNA dependent polymerase (L). All encode for one structural protein, with the exception of GP, which also produces a soluble protein, sGP, which is secreted into the bloodstream from infected cells (Feldmann *et al.*, 2010).

This soluble glycoprotein is distinctive to the Ebolavirus genera, as no other virus in the *Mononegavirales* order produces a soluble protein from its genome. Each of the proteins in the Ebola genome multi task, making Ebola incredibly efficient. VP24, VP35 and NP work together to form nucleocapsid structures (Lee JE *et al.*, 2008); NP and VP30 are responsible for capsid assembly and binding viral RNAVP30, VP35, NP and L are involved in transcription and replication of the genome, L, VP40 and VP35 mediate assembly of new VP40 and VP24 direct virus budding (Misasi *et al.*, 2014). The genes also functional individually: VP35 and VP24 act as interferon antagonists (Feldmann *et al.*, 2010), which create an environment conducive for unrestricted viral multiplication; V4P40 is the main protein involved in particle formation and maintaining structural integrity of the virion and GP mediates entry into host cells (Konstantinov *et al.*, 2013). The role of sGP is unknown but it is thought that it might act as a decoy for antibody surveillance in the blood, distracting the

immune system while the real virus infects cells and replicates (Ansari, 2014). The genome of the virus and its RNA are encapsulated inside the lipid bilayer envelope, which originates from the host cell membrane that protects the viral genome and facilitates entry into host cells (Sullivan *et al.*, 2011).

GP, the trimeric-spiked main surface protein embedded in the bilayer of the virus, is responsible for binding to host cells and mediating fusion between the viral envelope and the host cell membrane (Ansari, 2014). It is formed by two parts: GP1 and GP2 (Feldmann *et al.*, 2010). GP1 forms the trimeric spikes that are visible on the exterior of an Ebolavirus and are heavily glycosylated. The receptor-binding site on GP1 that is imperative for attachment and entry into host cells, and the actual target of neutralizing. Antibody is hidden underneath the glycan caps. These binding sites are only revealed when GP1 is cleaved after it enters the endosomal compartment of the host cell (Misasi *et al.*, 2014). GP2 is tasked with fusing with host cell membranes and initiating entry into the cytoplasm of the cell. The structure of GP, and the sequestering of the antibody binding site, ensures that antibody surveillance of the host immune system is not the same as antibody receptor binding, a trait that is thought to contribute to immune system evasion (Lee *et al.*, 2008).

EBOLA PATHOGENESIS

Scientists have not yet determined the essential surface protein on host cells that Ebolavirus targets and attaches to, but it is known that dendritic cells are one of the first cells to be attacked in an Ebola infection (Ying *et al.*, 2014). It has been speculated that Ebola gains entry into cells by producing an increased amount of phosphatidylserine, a lipid that is exposed when cells are primed for apoptosis (Misasi *et al.*, 2014). By expressing phosphatidylserine, Ebola tricks dendritic cells into thinking that the virus is in fact debris that needs to be engulfed and destroyed, thereby essentially inviting itself into the dendritic cell endosome. Vaccinia virus also uses this mode of apoptotic mimicry; it is thought to be a survival mechanism for larger viruses, such as Ebola and Vaccinia, because it would be difficult for them to enter cells via more traditional routes (Mercer, 2011).Once a cell is targeted by Ebolavirus, the GP spikes bind to the surface of that cell and the virus gets taken up into the endosome by micropinocytosis (Misasi *et al.*, 2014). However, Ebola needs to gain access to the cytoplasm before it can replicate. In order for the virus to escape the endosomal compartment, two cysteine proteases: cathepsin B and cathepsin L cleave GP into two separate molecules: GP1, a receptor binding subunit and GP2, a membrane fusion

subunit (Ansari *et al.,* 2014). Although viruses normally avoid cysteine proteases because they are known to break down viral particles, Ebola needs these enzymes to jettison the heavily glycosylated caps that form the GP trimer. Once cleaved, the receptor-binding fragment (18kD N-Terminal Fragment) that resides on the underside of GP1 is exposed. This fragment binds to Niemann Pick C1 (NPC1), a protein that dwells in endosomal membranes (NPC1 normally traffics intracellular cholesterol) (Kawaoka *et al.,* 2015).

NPC1 acts as a receptor for Ebola virus and expedites viral entry. In fact, without this receptor, Ebola is stuck in the endosome and is rendered non-functional, and for this reason is currently being studied as a possible anti-viral drug target (Miller *et al.,* 2012).

NPC1-GP1 binding is imperative for viral escape from the endosome and entry into the cytoplasm of the cell. While GP1 binds to NPC1, GP2 is responsible for mediating the fusion of the virus to the endosomal membrane (Lee *et al.,* 2008). The virus is then taken up into the cytoplasm of the cell where the genome is uncoated, transcribed into mRNA, replicated and assembled. New viruses are formed, released and available to target new cells. Ebola's early and preferred sites of viral replication are dendritic cells, the primary antigen presenting cells of the innate immune system. One of the main functions of any antigen presenting cell is to alert the adaptive immune system of viral invasion by capturing antigen and presenting it to naive T Cells (in the context of Class I MHC) that reside in the lymph nodes. Dendritic cells also are responsible for secreting pro-stimulatory cytokines that induce cellular and humoral adaptive immune responses that can clear an infection. Ebola-infected dendritic cells never mature or secrete the cytokines necessary to upregulate MHC Class I to the cell surface and antigen can't be presented to T Cells in the lymph node. As a result, neither cellular nor humoral responses will be activated. By infecting and disabling dendritic cells, Ebola eliminates the critical link between innate and adaptive immune systems. Infected dendritic cells are also tasked with releasing interferon, a type of cytokine that slows down viral replication, which gives the immune system time to mount an adaptive immune response (Lichtman, 2011). However, one of the hallmarks of an Ebola infection is its ability to prevent the activation of interferon. Specifically, VP24 and VP35 serve as interferon antagonists that leave the immune system highly compromised and unable to control viral replication. The second target cells of Ebola are monocytes and macrophages, also cells of the innate immune system (Ying *et al.,* 2006). Their main responsibilities are to phagocytize cellular debris and present antigen to naïve T Cells, much like dendritic cells. Under normal

circumstances, infected macrophages and monocytes release inflammatory cytokines upon encountering an antigen, such as TNF, IL1, IL2, IL6, IL15, IL8, and MIP1 and nitric oxide (Zampieri *et al.*, 2007). TNF, IL6, IL8 and IL12 are inflammatory cytokines that are responsible for many of the symptoms that are associated with an illness, such as fever and lethargy. They are also responsible for vascular permeability, which allows lymphocytes access to the site of infection. MIP1 and MCP1 are tasked with recruiting more monocytes and macrophages to fight off pathogens. Towards the tail end of an infection a regulatory process is activated by IL10, an anti-inflammatory cytokine. IL10 suppresses the production of inflammatory cytokines and allows the body to return to its normal state (Lichtman, 2011).

However, during an Ebola infection, the inflammatory process is never regulated and inflammation operates in a continuous loop. Infected macrophages travel to the lymph nodes; they express cytokines that increase inflammation and growth stimulation chemokines that attract more monocytes and macrophages to the site of infection; these new monocytes and macrophages become infected themselves; they in turn express more cytokines, which increases inflammation (Bradfute *et al.*, 2011). While IL10 has been detected in patients who have fatal Ebola infections, it could be that not enough is produced to overcome cytokine storm that causes much of the pathogenesis associated with the disease. Unregulated inflammation and viral replication are responsible for coagulation abnormalities; hypotension (the result of hepatocellular damage and high nitric oxide levels); increased vascular permeability, including damage to endothelial cells (generally late in an infection), vascular collapse, severe vomiting, diarrhea, hypovolemic shock and organ failure. Ebola patients also experience large loss of lymphocytes, known as bystander lymphocytes apoptosis. While lymphoid tissues are targets for viral invasion, the lymphocytes themselves are not. The apoptosis is correlated with an increase of Fas/FasL and TNF-induced TRAIL ligands, but the mechanisms underlying the cause of the apoptosis are not well known. It is thought that dendritic cell dysfunction or an overproduction of nitric oxide from infected macrophages might play a role in stimulating the large die-off of lymphocytes. Necrosis of the liver, while not fatal in and of itself, results in decreased synthesis of coagulation factors, which contribute to hemorrhagic symptoms and are consistent with disseminated intravascular coagulation (Fletcher *et al.*, 2014). It also has been speculated that tissue factors released from infected macrophages and monocytes may be responsible for coagulation disorders (Feldmann *et al.*, 2010).

IMMUNE RESPONSE TO EBOLA INFECTIONS

There is considerable debate in the scientific community regarding the adaptive immune response in Ebola patients. In particular, a consensus has not been reached about which arm of the adaptive system is more essential T cell activation or antibody production. Part of the problem in coming to an agreement is the dearth of Ebola studies on human subjects. Ebola outbreaks occur unexpectedly and infrequently and when they do, patients oftentimes die too quickly to obtain measurable samples. Furthermore, the lethality of the virus leaves very few options for cellular experiments or autopsy because Ebola must be tested and examined in a BSL4 laboratory. There are fewer than 50 of these labs in the world and none that exist in Ebola endemic regions. As a result, most of the understanding with respect to how the adaptive immune system functions during an Ebola infection is extrapolated from mouse, guinea pig and non-human primate models. Most of these experiments are done in the context of measuring immune responses in a variety of vaccine platforms and the results are at times contradictory. For example, some mouse models were able to show that protection from lethal Ebola challenge was defined by a robust CD8+ T Cell response and that CD4+ T Cells were not required for eVLP-mediated protection while other studies in non-human primates showed the opposite effect. Likewise, the research on the necessity of a humoral response in mouse and non-human primate models is contradictory as well (Bradfute *et al.,* 2011).

Despite the pathogenicity of the Ebola virus, there have been studies done on human blood samples that have elucidated the differences in the immune responses between patients who die from an Ebola infection and those who live. One study was done on a subset of patients from the Mayibout and Booue outbreaks in the Gabon in 1996. A longitudinal analysis was performed on blood samples that were taken from a group of fatal cases and survivors over the course of their illness and during the recovery period of survivors. A control group of healthy people who were not infected with the virus was also included in the analysis. The results indicated that fatal outcomes were associated with a suboptimal humoral response defined by indiscernible IgM and no detection of IgG production whatsoever. The release of Interferon-gamma in the early days of infection in fatal cases indicates that the immune system attempts to mount an T Cell response but these early efforts are followed by the complete disappearance of T Cell activity (as measured by T Cell related mRNA) and extensive lymphocyte apoptosis during the last five days infection. Although viral titers were relatively equal between fatal cases and survivors on Day 2 of the disease, by Day 4 fatal cases had viral titers that were up to 200% higher than survivors (Baize *et al.,* 1999).

Patients who survived an Ebola infection mounted early IgM antibodies and Ebola specific IgG directed at VP 25, VP40 and NP. While survivors did not mount a CD8+ response during the symptomatic phase of the disease, increasing levels of Fas Fas L and perforin during the recovery phase indicate that a cytotoxic T cell response corresponds with viral clearance from the blood. While both humoral and cellular responses are important for clearing an infection, the severely impaired and non26 functional antibody responses in fatal cases support the theory that the humoral activity is imperative to control virus replication. A cross sectional study was done using blood samples taken from 42 non survivors and 14 survivors of the Gabon and Democratic Republic of Congo outbreaks from 1996 and 2003. Those who cleared the infection were able to regulate the inflammatory cytokine storm brought on by infected macrophages and monocytes; did not experience bystander lymphocyte apoptosis; and mounted early humoral and cellular responses, including tightly regulated activation of cytotoxic T Cells. Fatal cases experienced cytokine levels that were five – 1000 times greater than those found in healthy people and peaked two days before death (Wauquier *et al.,* 2010).

Immune events that occur early in the infection can determine whether or not a person will be able to control viral replication or if the infection will result in death (Zampieri *et al.*, 2007). A fatal case of Ebola will successfully suppress the innate and adaptive immune responses by infecting and disabling dendritic cells. Infected macrophages secret inflammatory cytokines that are never regulated and results in prolonged cytokine secretion. VP35 and VP24 suppress interferon activation, allowing the virus to proliferate and spread throughout the body via the circulatory system. Although T cell activation is attempted, it is quickly disabled by down-regulation of Type One interferon (Wauquier *et al.,* 2008). Increased lymphocyte apoptosis results in increasing viral loads; patients who fail to recover also have virtually no viral antigen specific antibodies. By the end of a fatal infection, patients suffer from chronic inflammation and high viral titers that manifest in systemic organ failure, impairment of the vascular system, hypovolemic shock and death (Zampieri *et al.,* 2007).

Although there are clear immune response disparities between survivors and fatalities, it has been difficult for scientists to understand why exactly some people can clear the infection and some cannot. It has been suggested that the Ebolavirus is not able to replicate in people who are deficient in the NPC1 gene that is imperative for viral entry into the cell cytoplasm (NPC1 deficiency results in Niemen-Pick disease, a recessive disorder that causes accumulation of cholesterol in the endosome of cells) (Miller, 2012).

There may be confounding factors as well such as underlying/pre-existing health conditions, co-infections and general overall health prior to exposure to Ebola. Also, individuals who seek and obtain proper care as soon as symptoms present themselves, and before viral load and inflammation become uncontrolled, invariably have more positive outcomes. The mode of infection may be a factor. In the inaugural Zaire Ebolavirus outbreak, those who were infected through the use of contaminated needles had a 100% fatality rate, while the overall case fatality rate for the infections transmitted by close contact is around 70% - 90% (Bradfute *et al.*, 2011).

EBOLA IN PAKISTAN

Since August-September, the threat of Ebola to hit Pakistan has been making headlines. Ever since the WHO representative in Pakistan, Dr Michel Thieren made a statement that Ebola is spreading across the globe like a forest fire, Pakistan is also at a high risk of being struck by the disease. Therefore, the government should take precautionary measures in order to prevent the virus from hitting the country. Since his statement, awareness campaigns and fight against Ebola drives have been making rounds in different cities of Pakistan. The situation was under control until a supposed case of Ebola virus took the headlines. 40 year old Zulfiqar Ahmed from Chiniot was admitted into a hospital in Faisalabad raging with uncontrolled high fever. Majority of the people including the towns' doctors believed him to have been caught with the disease. His death in the past week wreaked an uproar of fear in the citizens of Pakistan. However, on further research of the case, Parliamentary Secretary for Cabinet Secretariat Raja Javed Akhlas declared that, "The person, who died in Chiniot, has expired due to Hepatitis C and not Ebola virus," Zulfiqar had recently come back from a trip to West Africa hence became a victim of the misapprehension. However, no further cases have been reported as the campaigning continues amongst the general public. Since then, there are two more suspected cases reported, one in Karachi and one in Islamabad (Khan, 2015).

So far government of Pakistan has not announced its policy to track and contain Ebola virus, if it arrives in Pakistan. We do not know if there are any quarantine zones set up at airports and hospitals and if there are any, how well equipped them. The need of time is to have an effective policy in place and get ready to meet the deadly virus if and when it arrives in Pakistan Ebola virus disease (EVD; also Ebola hemorrhagic fever, or EHF), or simply Ebola, is a viral hemorrhagic fever of humans and other primates caused by ebolaviruses. Signs and

symptoms typically start between two days and three weeks after contracting the virus with a fever, sore throat, muscular pain, and headaches. The later symptoms include vomiting, diarrhea and rash, along with decreased function of the liver and kidneys. At this time some people begin to bleed both internally and externally. The disease has a high risk of death, killing between 25 and 90 percent of those infected, with an average of about 50 percent. This is often due to low blood pressure from fluid loss, and typically follows six to sixteen days after symptoms appear. The virus spreads by direct contact with body fluids such as blood of an infected human or other animals. This may also occur through contact with an item recently contaminated with bodily fluids. Spread of the disease through the air between primates, including humans, has not been documented in either laboratory or natural conditions. Semen or breast milk of a person after recovery from EVD may carry the virus for several weeks to months. Fruit bats are believed to be the normal carrier in nature, able to spread the virus without being affected by it (Yazdani, 2015).

EBOLA VIRUS TRANSMISSION, CLINICAL PRESENTATION, AND ASYMPTOMATIC CASES

TRANSMISSION

Ebola spreads through human-to-human transmission via direct contact (through broken skin or mucous membranes) with the blood, secretions, organs or other bodily fluids of infected people, and with surfaces and materials (e.g. bedding, clothing) contaminated with these fluids. Burial ceremonies in which mourners have direct contact with the body of the deceased person can also play a role in the transmission of Ebola. People remain infectious as long as their blood contains the virus (Chippaux *et al.*, 2014).

SEXUAL TRANSMISSION

More surveillance data and research are needed on the risks of sexual transmission and particularly on the prevalence of viable and transmissible virus in semen over time. The Ebola virus can only be transmitted when a susceptible individual comes into direct contact with the bodily fluids of an infected and symptomatic Ebola patient (Judson *et al.*, 2015). These bodily fluids include: vomitus, feces, blood, saliva, tears, breast milk, and semen. Breast milk and semen were found to be culture positive after Ebolavirus had already cleared from the blood and up to 40 days post disease onset. The virus contained in these fluids enters the uninfected host through breaks in mucosal surfaces or abrasions in the epidermis. The route of transmission that carries the most risk is household transmission, which generally

occurs when a family member or friend cares for an Ebola patient and is exposed to the virus through close personal contact. Data from the first Sudan Ebolavirus outbreak in 1976 indicated that persons who provided nursing care to sick family members had a 5.1 fold increased risk of infection (Baron *et al.,* 1983). A study of patients in the Kikwit outbreak in 1996 showed that direct physical contact with an infected person during the clinically apparent phase of illness was the most important risk factor for household transmission (Khan *et al.,* 2015).

Nosocomial transmission can occur when health care workers are exposed to the bodily fluids of infected patients. Ideally, doctors and nurses should protect themselves by wearing personal protective equipment (PPE) with full body coverage. However if the strict donning, doffing and decontamination protocols are not followed, there is a risk of viral transmission and self-contamination. Of particular concern is the removal of PPE after caring for patients. Even if the protective gear is put on properly and no exposure to virally infected bodily fluids occurs during the course of care, a health care worker can still become infected if there is a breach in doffing protocols. Ebola viruses can live on inanimate objects and fomites for up to a few hours (or longer in ideal conditions), (Bausch *et al.,* 2007) therefore in order to prevent exposure to the virus it is imperative that the removal of PPE and proper decontamination procedures are followed in a systematic way and under supervision. The lack of PPE and the unfamiliarity with its proper use is not a problem that is peculiar to West Africa. Hospitals in the developed world also ran into nosocomial challenges while treating Ebola patients. A nurse in Spain also contracted the disease when she touched her face with the gloves she wore while treating a Spanish missionary priest (Minder *et al.,* 2014).

After Thomas Eric Duncan was admitted into Dallas Presbyterian Hospital with Ebola in the September 2014, two nurses who cared for him were infected with the virus. It is thought the lack of appropriate protective gear; improper training; and lax disinfecting procedures were the reasons behind the transmission of the virus (Hunt *et al.,* 2014). In past outbreaks, nosocomial transmission has accounted for a significant proportion of cases. In the first outbreak in Zaire, the single greatest risk factor for contracting Ebola, especially during the early part of the epidemic, was receiving an injection at the Yambuku Mission Hospital (which was the focal point of the outbreak). Furthermore, 11 out of 17 of the Mission Hospital staff died and the hospital had to close. During the 1976 Sudanese outbreak, Maridi Hospital was the amplifying source of the epidemic where 46% of the cases hospitalized. A

quarter of the 315 cases in the 1995 Ebola outbreak in Kikwit were among doctors and nurses, all of whom cared for Ebola patients without protective gear. During the 2007 Ebola outbreak in Bundibugyo, Uganda, 14 healthcare workers were infected before implementation of Standard barrier practices. After implementation of the precautions there were not any nosocomial cases reported (Baron *et al.*, 1983). People in Africa are also at risk of exposure to the virus when they participate in traditional funeral practices where is it common for mourners to wash, kiss and touch corpses. Unlike other viruses, Ebola remains pathogenic in the blood and bodily fluids of a deceased patient and those who handle or touch the bodies are at risk of being exposed to the virus. The funeral rite can last for weeks as the deceased are often transported back to their home communities for the burial. In addition, family members and friends travel significant distances to attend funerals and then return home, enabling the virus to spread across borders (Chippaux *et al.*, 2014). In the case of Ebola, the target cells of the Ebolavirus do not reside in the epithelial cells of the bronchial tubes, respiratory tract or lung, but rather in the cells of the innate immune system, such as dendritic cells, macrophages, monocytes, and eventually the vascular system. Research that has been conducted thus far on the pathogenesis of Ebola has not shown that the cells of the respiratory tract become infected and very few patients show respiratory symptoms such as coughing and wheezing (Bausch *et al.*, 2007).

CAUSES

Ebola virus has been found in African monkeys, chimps and other nonhuman primates. A milder strain of Ebola has been discovered in monkeys and pigs in the Philippines. Marburg virus has been found in monkeys, chimps and fruit bats in Africa (Georges, 1999).

TRANSMISSION FROM ANIMALS TO HUMANS (Smith, 2006)

Experts suspect that both viruses are transmitted to humans through an infected animal's bodily fluids. Examples include:

- **Blood:** Butchering or eating infected animals can spread the viruses. Scientists who have operated on infected animals as part of their research have also contracted the virus.
- **Waste products:** Tourists in certain African caves and some underground mine workers have been infected with the Marburg virus, possibly through contact with the feces or urine of infected bats.

TRANSMISSION FROM PERSON TO PERSON

Infected people typically don't become contagious until they develop symptoms. Family members are often infected as they care for sick relatives or prepare the dead for burial. Medical personnel can be infected if they don't use protective gear, such as surgical masks and gloves. There's no evidence that Ebola virus or Marburg virus can be spread via insect bites.

RISK FACTORS (Jhonsons, 2015)

For most people, the risk of getting Ebola hemorrhagic fever or Marburg hemorrhagic fever is low. The risk increases if people:

- **Travel to Africa:** You're at increased risk if you visit or work in areas where Ebola virus or Marburg virus outbreaks have occurred.
- **Conduct animal research:** People are more likely to contract the Ebola or Marburg virus if they conduct animal research with monkeys imported from Africa or the Philippines.
- **Provide medical or personal care:** Family members are often infected as they care for sick relatives. Medical personnel also can be infected if they don't use protective gear, such as surgical masks and gloves.
- **Prepare people for burial:** The bodies of people who have died of Ebola or Marburg hemorrhagic fever are still contagious. Helping prepare these bodies for burial can increase your risk of developing the disease.

SIGNS AND SYMPTOMS OF EBOLA VIRUS

The most critical aspect of the disease is the fact that the signs and symptoms are no special than a regular cold and fever. With temperatures running high, the human body is destined to experience muscle fatigue, lethargy, chills headaches. However, one may never find out that they have been infected with this deadly virus until the severity of symptoms increase. However, below are the officially stated symptoms of the virus (Sanchez, 2006).

- Fever
- Headache
- Muscle Pain & Fatigue
- Nausea
- Vomiting
- Weakness
- Abdominal Pain.

Over time, symptoms become increasingly severe and may include:

- Nausea and vomiting
- Diarrhea (may be bloody)
- Red eyes
- Raised rash
- Chest pain and cough
- Sore throat
- Stomach pain
- Severe weight loss
- Bruising
- Bleeding, usually from the eyes, and when close to death, possible bleeding from the ears, nose and rectum
- Internal bleeding.

These symptoms may surface within 2-20 days since the penetration of the virus in a human body. If a patient survives the attack, his body develops antibodies that protect him from Ebola infestation for the next 10 years of his life. The incubation period, that is, the time interval from infection with the virus to onset of symptoms is 2 to 21 days. Humans are not infectious until they develop symptoms. First symptoms are the sudden onset of fever fatigue, muscle pain, headache and sore throat. This is followed by vomiting, diarrhea, rash, symptoms of impaired kidney and liver function, and in some cases, both internal and external bleeding (e.g. oozing from the gums, blood in the stools). Laboratory findings include low white blood cell and platelet counts and elevated liver enzymes (Feldmann, 2010).

EBOLA DIAGNOSIS

Other diseases such as malaria, cholera, typhoid fever, meningitis and other viral hemorrhagic fevers may resemble EVD. But the actual disease can be detected by taking blood samples for testing the presence of viral RNA, viral antibodies or for the virus itself to confirm the diagnosis. Confirmation that symptoms are caused by Ebola virus infection are made using the following investigations:

- Antibody-capture Enzyme-Linked Immunosorbent Assay (ELISA)
- Antigen-capture detection tests
- Serum neutralization test

- Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) assay
- Electron microscopy
- Virus isolation by cell culture.

Samples of body fluids and tissues from people with the disease should be handled with special caution. Samples from patients are an extreme biohazard risk; laboratory testing on non-inactivated samples should be conducted under maximum biological containment conditions. The medical services include rapid detection of cases of disease, contact tracing of those who have come into contact with infected individuals, quick access to laboratory services, proper healthcare for those who are infected, and proper disposal of the dead through cremation or burial (Feldmann *et al.*, 2010).

CLINICAL FEATURES

The onset of the disease is abrupt after an incubation period of 2 to 21 days. The clinical features can be divided into four main phases as follows: **Phase 1**: Influenza-like syndrome: The onset is abrupt with nonspecific symptoms or signs such as high fever, headache, arthralgia, nausea, sore throat, and myalgia. **Phase 2**: Acute (days 1-6): Persistent fever not responding to antimalarial drugs or to antibiotics, headache and intense fatigue followed by diarrhea and abdominal pain and vomiting**. Phase 3**: Pseudo-remission (days 7-8): During this phase the patient feels better and seeks food. The health situation presents with some improvement. Some patients may recover during this phase and survive from the disease. **Phase 4**: Aggravation (day 9): In many if not most cases, the health status gets worse. The following symptoms may be observed: Skin manifestations: petaechiae (not so obvious on black skin), purpura (morbiliform skin rash). Respiratory disorders: dyspnea, cough, hiccups, throat and chest pain. Cardiovascular distress and hypovolemic shock. Based on these clinical manifestations, it is obvious that at the start of EHF, the disease can mimic many other tropical diseases such as malaria or typhoid fever. In most outbreaks, recognition of EHF is delayed because physicians are not accustomed to seeing this illness and its symptoms are generally nonspecific (McElroy, 2014).

CLINICAL PRESENTATION OF DISEASE

The clinical symptoms of an Ebola infection follow a common pattern. After initial virus exposure, there is a two-21 day incubation period (mean of five-seven days) during which a patient has a pre-clinical infection and is not considered contagious and cannot transmit the virus to a non-infected person. After the incubation period is over and Ebola viral load

reaches a certain threshold, there is abrupt onset of symptoms after which point a person is contagious and able to transmit the virus. Symptom manifestation can be divided into three distinct groups. In the early febrile phase, zero-three days post symptom onset, patients experience fever, malaise, fatigue, body aches and anorexia; all non-specific symptoms that can be misdiagnosed or confused with malaria, typhoid, cholera or influenza. In the second gastrointestinal phase, generally three to 10 days post symptom onset, fever persists as gastrointestinal symptoms begin to manifest with patients experiencing nausea, vomiting and diarrhea. In some patients, the fluid loss is of the magnitude of cholera patients: up to five (possibly 10) liters a day. It is during this phase that hemorrhagic symptoms will appear which includes petechiae, ecchymoses, oozing and bleeding from venipuncture sites, mucosal hemorrhages and macropapular rashes (Feldmann *et al.,* 2010).

In the third phase, seven to 16 days after symptom onset, patients diverge into one of two categories: those who succumb to the infection and those who will survive. In those cases that will be fatal, there is profound fluid loss due to chronic diarrhea and vomiting that results in electrolyte depletion. The body goes into circulatory collapse, hypovolemic shock, and systematic organ failure, most notably metabolic acidosis and a failure to produce urine. During this phase patients can also suffer from hyponatremia, which can cause brain swelling with raised intracranial pressure. Patients also tend to lose consciousness or fall into a coma and experience rapid breathing before death. In patients who will survive, symptoms begin to improve during this phase, and most patients who survive to day 13 ultimately live (Feldmann *et al.,* 2010).

ASYMPTOMATIC CASES OF EBOLA

While most patients infected with Ebolavirus exhibit severe symptoms, there have been documented cases of patients who are clearly infected with the virus (as measured by circulating anti-Ebola antibodies and a positive RT-PCR for Ebola RNA fragments), yet remain asymptomatic throughout the course of the disease. It is assumed that these individuals are most likely not infectious, and it is likewise assumed that their exposure confers protective immunity, despite the fact that they did not present symptoms (Bellanemail *et al.,* 2014).

Retrospective serological surveys done after the inaugural Ebola outbreak in Sudan in 1976 showed that 19% of contacts of persons with the disease had anti-Ebola antibodies even though they never became ill themselves. During the 1976 Zaire Ebolavirus outbreak, less

than 2.5% of people who had contact with fatal cases experienced subclinical infections. Furthermore, serological surveys conducted in 1977 in the Tandala region of Zaire showed that 79 out of 1096 people (7%) tested were found to have antibodies to Ebola although they gave no history of severe disease (Heymann *et al.*, 1980).

More recently, after the Kikwit outbreak in the Democratic Republic of Congo in 1996, samples taken from 152 contacts of confirmed Ebola patients showed that five of them (3.2%) were IgM and IgG positive even though there was not morbidity associated with their infection (Rowe *et al.*, 1999).

In Northeastern Gabon there were three Zaire Ebola outbreaks between 1994 and 1996 (Heffernan *et al.*, 2005). Nine hundred and seventy nine (979) people from the region were serologically tested (taken from a study population of 2533) in an attempt to ascertain the level asymptomatic cases from those outbreaks. The results showed that 14 of the 979 were seropositive for IgM and IgG anti-Ebola antibodies. Of these 14, four were listed as previous cases in official documents from the February 1996 outbreak and each one of them had clinical disease and typical Ebola-like symptoms. The remaining ten seropositive people were not listed as patients in any outbreak and did not report having had Ebola-like symptoms. In another study of asymptomatic patients from the Gabon in 1996, investigators sampled 24 contacts of diagnosed Ebola patients (Leroy *et al.*, 2007) (it is unclear how investigators came to study these particular 24 people or why they were chosen in the first place. It is likewise unknown what the underlying health conditions were of the 24 people). Each contact was prospectively studied to measure immune responses and disease progression. They all shared a household with laboratory confirmed Ebola patients and administered care to them without any protective equipment, even rudimentary prophylaxis such as gloves. Although they were directly exposed to the feces, vomit, sweat and blood of nonfatal and fatal Ebola patients, they did not develop symptoms. Samples from the 24 individuals were taken on four occasions over a one-month period, starting with one week after initial exposure to the virus. The first samples that were taken did not detect antibody, indicating that they were Ebola naïve and had not previously mounted an immune response. Two to three weeks after exposure, 11 of the 24 patients produced measureable Ebola specific IgM and IgG response to Ebola antigens. IgM antibody was detected 15 – 18 days post viral exposure, followed by Ebolavirus specific IgG2 and IgG3 antibody reactive to NP and VP40 approximately one

week after that antibody production in asymptomatic patients was delayed in comparison to antibody production in symptomatic patients (Leroy *et al.*, 2001).

Although circulating Ebola antigen was never detected, at day seven today 21 post exposures, Ebola RNA was detected in seven out of the 11 antibody positive asymptomatic individuals via nested RT-PCR. Furthermore, the RNA fragments were only found in the peripheral blood mononuclear cells, indicating a low viral load, which is consistent with the lack of circulating antigen (Leroy *et al.*, 2000).

All told, 46% of close contacts of Ebola patients in this study had replicative Ebola infection and did not present any symptoms of the disease. While the number of asymptomatic patients uncovered in the studies reviewed here are very low, they nonetheless establish that it is possible for people to have exposure to the Ebolavirus, develop an infection that provokes an immune response, and yet never present symptoms.

The authors do not hypothesize that this percentage could be applied to any or all Ebola outbreaks, rather they only reported on what was found in this individual investigation. In absence of further studies, it is difficult, if not impossible, to assume that the same percentage of asymptomatic individuals can be found in any given Ebola outbreak. There have also been known cases of Ebola infected patients who do have symptoms, but they are very mild. In the 1976 outbreaks, WHO teams noted that there was a continuum of symptoms that ranged from mild to rapidly fatal. In the 2007 outbreak in the Democratic Republic of Congo, it is thought that the index patient, a 42- year-old man, contracted the virus after handling bats he purchased in a market. His only symptoms were a low-grade fever and headache, and never transmitted the virus to his wife, with whom he is assumed to have had close contact. However, his daughter became ill and died during a time frame that was consistent with the Ebola incubation period, leading investigators to conclude that her father infected her. An investigation into the lifestyle patterns of that family suggested that while the father walked from town to his home village, a trip of three to four hours, he carried his daughter on his back. It was speculated that the daughter could have become infected through exposure to her father's sweat (Rowe *et al.*, 1999).

COMPLICATIONS

Both Ebola and Marburg hemorrhagic fevers lead to death for a high percentage of people who are affected. As the illness progresses, it can cause:

- Multiple organ failure
- Severe bleeding
- Jaundice
- Delirium
- Seizures
- Coma
- Shock.

One reason the viruses are so deadly is that they interfere with the immune system's ability to mount a defense. But scientists don't understand why some people recover from Ebola and Marburg and others don't (Sanchez, 2006).

For people who survive, recovery is slow. It may take months to regain weight and strength, and the viruses remain in the body for weeks. People may experience:

- Hair loss
- Sensory changes
- Liver inflammation (hepatitis)
- Weakness
- Fatigue
- Headaches
- Eye inflammation
- Testicular inflammation.

DIAGNOSIS

Early laboratory confirmation of suspected clinical hemorrhagic fever cases is essential to implement appropriate control measures. Definitive diagnosis of suspected cases of EHF is usually made by polymerase chain reaction detection and virus isolation on Vero cells. As a class-4 pathogen, Ebola virus culture requires a maximum containment facility. Additional laboratory diagnostic tests include enzyme-linked immunosorbent assays (ELISAs) for the detection of Ebola immunoglobulin (Ig_G- and IgM specific antibodies and virus antigens; more specialized molecular testing is also available but is not readily available in the usual clinical setting. In Africa, laboratory confirmation of Ebola cases has been challenging and early recognition of the first outbreaks was severely hampered as a result. Because the disease was poorly known or rare, laboratory investigations were oriented toward the more common, endemic pathogens in the area. Since 1994, the incidence of Ebola outbreaks

increased and, as a consequence, the awareness of the disease has improved and facilities capable of diagnosing EHV were established in Africa. National Public Health laboratories in endemic countries like Uganda (UVRI), Kenya (KEMRI), and Gabon (CIRMF) have already developed capacities to diagnose EHF by ELISA and reverse transcriptase (RT)-PCR. South Africa is the only African country with a maximum-containment. Ebola virus enclosed-suit laboratory where all class-4 viral pathogens can be handled safely. After the 2008-2009 Ebola outbreak in Kaluamba, DRC, the Ebola diagnostic technologies of ELISAs for the detection of antigens and IgM antibody, and RT-PCR have been transferred to the National Institute of Biomedical Research in Kinshasa (Kelly, 2014).

TREATMENT OF EBOLA VIRUS DISEASE

Ebola survivors have a very different immune response to the virus than those who die from the disease. Survivors mount an early innate response that is marked by cytokine regulation as well as an adaptive response that is defined by anti- Ebola antibody production and cytotoxic T Cell activity. Although the scientific community has been able to establish that there are indeed differences in immune responses, the reasons why there are differences have remained elusive. Potential confounders and genetic differences have been discussed above. Disparities in the qualitative and quantitative measures of care also have been considered a defining factor in determining patient outcomes several reports from the field of the West African outbreak have provided more details as to what kind of treatment in particular is thought to contribute to patient survival. Specifically, patients who are treated with aggressive rehydration therapies under adequate medical supervision have a greater chance of survival (Roberts *et al.*, 2014).

(Bah *et al.*, 2015). Although oral and intravenous rehydration therapies have been used in past outbreaks: intravenous fluids were first used in the 1976 outbreak in Zaire to treat three nuns. In Kikwit in 1995, rehydration therapies were used in the last few weeks of the outbreak in approximately 25 patients (Clark *et al.*, 2012). However, their effectiveness has not been rigorously evaluated (Lamontagne *et al.*, 2015).

Aggressive rehydration therapy was thought to have been a factor in the treatment of two Ebola patients who were treated at Emory University Hospital in July – August 2014 (Lyon *et al.*, 2014). Patient One began treatment in Atlanta on Day 10 of his illness; Patient Two began her treatment on Day 15. Both patients were hypovolemic upon presentation; had low potassium, calcium and sodium measurements; and both showed signs of liver dysfunction,

manifestations that are all known significant risk factors for death. Patient One had more severe symptoms: persistent rash, vomiting of blood and diarrheal output of 2-4 liters a day, while Patient Two didn't experience nausea or vomiting and remained afebrile throughout the course of her illness. Upon admission to Emory, both patients received aggressive fluid and electrolyte replacement with an emphasis on potassium and calcium replacement. At the beginning of his illness in Africa, Patient One received intravenous Ringers Solution and was able to drink Tang and Gatorade, despite anorexia. Patient Two was well enough in Africa to drink oral rehydration fluids from the onset of her symptoms. Both patients received three doses of ZMAPP in the context of their treatment and Patient One also received one unit of convalescent whole blood from a patient who recovered from Ebola. Because both patients received different layers of treatment throughout their illness, it is difficult to isolate which treatment in particular may have had the largest impact on recovery. However, doctors who treated both patients, and investigators who reported on the protocols used at Emory, believe that rehydration therapies, with an emphasis on calcium and potassium replacement, had significant value in the recovery of these two patients. Under normal circumstances, intravenous fluid therapy and electrolyte replacement could assuage dehydration, but it is difficult, if not impossible, to extend this type of care in an Ebola hot zone considering the number of patients, the dearth of health care workers and the limited time in, and the cumbersome nature of, personal protective equipment (Lamontagne *et al.*, 2015).

It is important to consider that the Emory patients were treated under ideal conditions: they were in an internationally known hospital with a 24 hour staff dedicated exclusively to monitoring their progress and maintaining fluid and electrolyte levels. While this type of intense care does not exist in West Africa, rehydration therapies have nonetheless improved outcomes in Ebola patients there. In Nigeria, only 40% of patients in the recent outbreak died, and intense rehydration therapy was credited with the high survival rates. Some patients in Nigeria were drinking up to five or six liters of Oral Rehydration Solution a day, at times forcing themselves to do so despite overwhelming nausea, weakness and lethargy. One patient reported that it was difficult for her to even wrap her fingers around her cup, let alone lift it to her mouth to drink. Although this data is anecdotal in nature, and cannot be proven scientifically, Nigerian doctors believe that focusing on rehydration as the main component of patient management made a difference in patient outcomes. A more systematic study was conducted on 37 laboratory confirmed Ebola cases in Conakry, Guinea from March 25 – April 26 (Bah *et al.*, 2015).

All 37 patients were treated in an MSF-run Ebola Treatment Center and were admitted, on average, five days after symptom onset. Most presented with symptoms typical of an Ebola infection: fever, vomiting, diarrhea, lack of appetite and lethargy. Very few of the patients had known coinfections or conditions, and a regression analysis revealed that age was the only statistically significant predictor of death. Investigators did not include the type of treatment received into the model, so it is impossible to know how significant the different regimes were in preventing death. However, 99% of patients (N=36) received. By way of comparison, the Case Fatality Rate at the very beginning of the outbreak in Guinea was 86% among confirmed cases and 71% among clinically suspected cases. It is suspected that most of those early cases were misdiagnosed and/or did not receive medical care specific for Ebola, if they received care at all. Therefore comparing Case Fatality Rates from the beginning of the outbreak to the Case Fatality Rates reported in this study of 37 patients at an MSF-run Ebola Treatment Center might not be a fair comparison. At best, it might be a comparison between Case Fatality Rates of those who received no medical treatment at all to those who received any kind of medical treatment (Baize *et al.,* 2015).

Another Ebola Treatment Center in West Africa likewise concluded that rehydration therapy ameliorated the consequences of hypovolemic shock and contributed to the survival of Ebola patients (Chertow *et al.,* 2015). Doctors from the ELWA-3 Ebola treatment center in Monrovia, Liberia put a priority on rehydration therapies and implemented a systematic way of categorizing patients according to their rehydration needs upon presentation:

1. Hypovolemic, not in shock, and able to provide self-care;
2. Hypovolemic, not in shock, but unable to provide self-care
3. In shock with evidence of organ failure whose outcome would not be altered by any available medical intervention. It was observed that early rehydration interventions, whether administered orally or intravenously, controlled symptoms, mitigated massive gastrointestinal losses (up to five – ten liters a day), limited the life-threatening consequences of hypovolemic shock and increased the chance of recovery (Lamontagne *et al.,* 2015).

However, the success of these treatments is only as good as a doctor's ability to implement them. Routine use of intravenous therapy in ELWA-3 was hindered by the number of doctors and nurses available to care for patients (this study cited a ratio of 1 doctor for 30-50 patients) and the limited time that health care staff was able to be in personal protective equipment (each doctor was limited to 60 minutes three times a day in protective gear, which equaled

one – two minutes per patient per day). Indeed, this gap in quantitative measures of care seems to be the primary deterrent to including aggressive hydration therapies in the treatment of patients. It has not been proven statistically that the use of rehydration therapies, oral or intravenously, have an impact on reducing case fatality rates and improving patient outcomes. All that we have are narratives from previous outbreaks and from the field in West Africa. Nonetheless, it is believed that many Ebola patients are dying without adequate fluid resuscitation and that this 'unmet standard of care' (Perner *et al.*, 2015).

Doctors in the field in West Africa and those who have been studying this outbreak endorse a focus on immediate and consistent rehydration as a significant way to save the lives of Ebola patients (Lamontagne *et al.*, 2015).

EXPERIMENTAL TREATMENTS FOR EBOLA: VACCINES AND ANTI-VIRAL THERAPIES

Although there are no vaccines and anti-viral medications approved to treat Ebola patients, there are several new drugs under development, some of which are currently in clinical trials.

BLOOD PRODUCT FROM CONVALESCENT PATIENTS

This type of therapy involves transfusing blood products from Ebola survivors into currently infected Ebola patients (Cohen, 2014). The hope is that neutralizing antibodies contained in survivor blood will prime the immune system of the infected patient and enhance the ability of the recipient to clear the virus This treatment has had mixed results in non-human primate experiments. A 2007 study showed that neutralizing human monoclonal antibody, KZ52, an antibody that was derived from a survivor of the Kikwit outbreak, not only failed to protect macaques against challenge with Ebola virus but also had a minimal effect on the explosive viral replication following infection. However, it did show potential neutralizing activity in cell culture and small animal models (Li *et al.*, 2015).

On the other hand, during the 1995 outbreak in Kikwit, eight patients were given whole blood transfusions from Ebola survivors of that same outbreak. Seven of the patients survived, however they were given the treatment late in the progression of their disease and it is thought that they most likely would have survived anyway (Mupapa *et al.*, 1999). In the Yambuku outbreak in 1976, 13 Ebola survivors donated plasma, and a laboratory technician received one of the units and survived (Heymann *et al.*, 2014). Likewise, in the 2014 outbreak, The Bill and Melinda Gates Foundation also earmarked $5.7 million to fund

another clinical trial in Liberia that is being run by Clinical RM, a US medical research organization. The trial protocol delineates that 70 participants will receive plasma from survivors at the ELWA2 hospital in Monrovia in three 200ml doses over the course of four days. The control group, comprised of patients who are not eligible to receive the plasma due to incompatibilities with the donor pool, will receive the same standard of care regime as the treatment group, which is defined as intravenous fluid treatment and the consistent monitoring of vital signs, electrolyte levels and blood pressure. Post treatment measurements of viral load will be tracked pre and post transfer and both groups will be followed until either recovery or death. A concern in this trial in February 2015 was the dearth of patients (less than five cases a week); the fact that some patients are ineligible; and the refusal of some to receive a donation. As a result, a third trial has begun in Sierra Leone, where as of February 2015, up to 60-80 people were being infected, using the plasma donated for the Liberia trial (Carroll *et al.*, 2015).

ZMapp –It is antiviral monoclonal antibody cocktail that binds to GP and neutralizes the virus and prevents it from entering cells. There are three different epitopes on GP1 that the drug targets: one on the glycan cap and two at the base of GP1 (Murin *et al.*, 2014). Mouse models and studies using non-human primates that were given ZMapp showed a decreased death rate in the treated animals. ZMapp was administered to seven patients in the current Ebola outbreak as part of their treatment protocol. Five survived and two died, but the drug was given in the context of a comprehensive treatment plan so it is impossible to isolate the effectiveness of the drug. The red and blue antibodies bind near the base of virus, preventing the virus from entering cells. A blue antibody binds to the glycan cap, signaling the immune system to the site of infection (O'Carroll *et al.*, 2015). One of the major drawbacks with ZMapp is that the drug is manufactured using tobacco plants and the process is laborious and time-consuming (Cohen, 2014).

ZMapp Biopharmaceuticals, the developer of the drug, is working with the US Biomedical Advanced Research. Development Authority (BARDA) as well as Genetech and Regeneron, two biotech companies, to develop ideas that will increase production. One potential idea is to switch from the current model that uses tobacco plants in Kentucky to manufacturing the drug in China using hamster ovaries. Mapp Biopharmaceuticals also recently signed a $24.9 million contract with BARDA to acquire US Food and Drug Administration approval for the drug and the drug went to clinical trial in February 2015. It is being run by the NIAID at the

ELWA2 Ebola Treatment Center in Monrovia, and at the NIH Clinical Research Center in Bethesda. Eligible participants are adults and children who have been diagnosed with Ebola. All patients will be randomized to the treatment group, all of whom will receive three infusions of ZMAPP on sequential days as well as optimized care for an Ebola infection, which includes intravenous fluids, balancing electrolytes, and maintaining blood oxygen and pressure. The control group will receive optimized care only. Starting the trial been hampered by the lack of product; all available doses of ZMAPP were exhausted in 2014 (McCarthy *et al.*, 2014).

Brincidofovir – Produced by Chimerix in Durham, North Carolina, Brincidofovir was originally manufactured to treat DNA viruses such as adenoviruses, poxviruses, and herpesviruses. In the process of testing the drug for its initial purpose, Brincidofovir was discovered to limit Ebolavirus replication in cell culture. The orally administered drug was being used in a clinical trial in the MSF-run ELWA-3 Treatment Center in Monrovia, Liberia. The trial was being run by scientists from the University of Oxford and uses the following metric to determine success: if under 50% of the patients treated survive the infection, then the drug will be deemed no better than current supportive care. If more than 80% of patients survive, the drug will be considered effective. 50% – 80% survival will warrant further testing. Due to ethical concerns, there is no control group in this study. However, as February 2015, the study was halted because there were not enough patients to conduct a statistically significant trial. Furthermore, Chimerix declared that the drug has been deprioritized and they would not be participating in any development of the drug in the future (Loftus *et al.*, 2015).

Faviporavir – A product of Fujifilm in Japan, *Favriporavir* is an RNA polymerase inhibitor, meaning that the drug thwarts the virus ability to assemble. The drug was initially developed to treat novel or drug resistant influenza strains in Japan. With respect to treating Ebola patients, *Favriporavir* has shown efficacy in mouse models if it is administered up to six days post exposure, but wasn't as effective in non-human primate models (Lai *et al.*, 2014). Mouse models for Ebola are not ideal because rodents only develop a mild form of the disease. To overcome this, scientists used genetically engineered mice that were more susceptible to lethal doses of the virus, which muddied the ability to apply the results to the human target population. At the time of this writing, the French biomedical company INSERM finished running human clinical trials at the MSF Ebola Treatment Center in Gueckedou, Guinea in which 69 adults and adolescents took the drug for up to 10 days and their outcomes were

compared to patients who were treated at the same Center three months prior to the trial start (Cohen *et al.*, 2015).

Forty-eight percent of the patients died, but it was unclear if the outcome was related to the amount of viral load measured when they presented or if it was due to the drug. The results of the trial were inconclusive with INSERM citing that patient viral load may have been more of a determining factor than the drug efficacy. Patients who had lower viral load had better outcomes than those with a higher viral load at the start of treatment, leaving investigators to speculate that the drug could improve outcomes if administered early in infection (Lai *et al.*, 2014).

PREVENTION

Prevention focuses on avoiding contact with the viruses. The following precautions can help prevent infection and spread of Ebola and Marburg (Ippolito, 2012).

- **Avoid areas of known outbreaks.** Before traveling to Africa, find out about current epidemics by checking the Centers for Disease Control and Prevention website.
- **Wash your hands frequently.** As with other infectious diseases, one of the most important preventive measures is frequent hand-washing. Use soap and water, or use alcohol-based hand rubs containing at least 60 percent alcohol when soap and water aren't available.
- **Avoid bush meat.** In developing countries, avoid buying or eating the wild animals, including nonhuman primates, sold in local markets.
- **Avoid contact with infected people.** In particular, caregivers should avoid contact with an infected person's body fluids and tissues, including blood, semen, vaginal secretions and saliva. People with Ebola or Marburg are most contagious in the later stages of the disease.
- **Follow infection-control procedures.** If you're a health care worker, wear protective clothing, such as gloves, masks, gowns and eye shields. Keep infected people isolated from others. Dispose of needles and sterilize other instruments.
- **Don't handle remains.** The bodies of people who have died of Ebola or Marburg disease are still contagious. Specially organized and trained teams should bury the remains, using appropriate safety equipment.

Prevention includes limiting the spread of disease from infected animals to humans. It also includes wearing proper protective clothing and washing hands when around a person with the disease. No specific treatment or vaccine for the virus is available, although a number of potential treatments are being studied. Supportive efforts, however, improve outcomes. This includes either oral rehydration therapy (drinking slightly sweetened and salty water) or giving intravenous fluids as well as treating symptoms. Good outbreak control relies on applying a package of interventions, namely case management, surveillance and contact tracing, a good laboratory service, safe burials and social mobilisation. Community engagement is key to successfully controlling outbreaks. Raising awareness of risk factors for Ebola infection and protective measures that individuals can take is an effective way to reduce human transmission. Risk reduction messaging should focus on several factors (Ippolito, 2012).

- **Reducing the risk of wildlife-to-human transmission** from contact with infected fruit bats or monkeys/apes and the consumption of their raw meat. Animals should be handled with gloves and other appropriate protective clothing. Animal products (blood and meat) should be thoroughly cooked before consumption.
- **Reducing the risk of human-to-human transmission** from direct or close contact with people with Ebola symptoms, particularly with their bodily fluids. Gloves and appropriate personal protective equipment should be worn when taking care of ill patients at home. Regular hand washing is required after visiting patients in hospital, as well as after taking care of patients at home.
- **Reducing the risk of possible sexual transmission,** because the risk of sexual transmission cannot be ruled out, men and women who have recovered from Ebola should abstain from all types of sex (including anal- and oral sex) for at least three months after onset of symptoms. If sexual abstinence is not possible, male or female condom use is recommended. Contact with body fluids should be avoided and washing with soap and water is recommended. WHO does not recommend isolation of male or female convalescent patients whose blood has been tested negative for Ebola virus?
- **Outbreak containment measures,** including prompt and safe burial of the dead, identifying people who may have been in contact with someone infected with Ebola and monitoring their health for 21 days, the importance of separating the healthy from the sick to prevent further spread, and the importance of good hygiene and maintaining a clean environment (Waheed, 2014).

CONTROLLING INFECTION IN HEALTH-CARE SETTINGS

Health-care workers have frequently been infected while treating patients with suspected or confirmed EVD. This has occurred through close contact with patients when infection control precautions are not strictly practiced. Health-care workers should always take standard precautions when caring for patients, regardless of their presumed diagnosis. These include basic hand hygiene, respiratory hygiene, use of personal protective equipment (to block splashes or other contact with infected materials), safe injection practices and safe burial practices. Health-care workers caring for patients with suspected or confirmed Ebola virus should apply extra infection control measures to prevent contact with the patient's blood and body fluids and contaminated surfaces or materials such as clothing and bedding. When in close contact (within 1 meter) of patients with EBV, health-care workers should wear face protection (a face shield or a medical mask and goggles), a clean, non-sterile long-sleeved gown, and gloves (sterile gloves for some procedures (Dixon, 2014).

HISTORY OF EBOLA VIRUS OUTBREAKS

TWO INAUGURAL OUTBREAKS

The second outbreak occurred at almost the exact same time (September to November 1976) in neighboring Yambuku, Zaire (now known as the Democratic Republic of Congo). This outbreak began when the index case, a 44-year-old male schoolteacher, presented at the Yambuku Mission Hospital on August 26 with symptoms that were thought to be malaria. He was given a shot of anti-malarial medication after which his fever abated. On September 1, he developed fever again along with symptoms that were consistent with Ebola. He was admitted to the hospital on September 5 and died on September 8. There were nine additional cases in early September, all of which 53 appeared to be unrelated with the exception that all had received treatment at the Hospital. It was later determined that 85 cases (out of 288 cases where transmission could be identified) could trace back acquisition of the disease to receiving injections at Yambuku Mission Hospital, 149 of the 288 contracted the disease through close contact with an infected person; 43 of 288 had both contact with an ill person and a history of receiving injections at the hospital. The disease hit hospital staff particularly hard: 11 out of 17 staff members died and the hospital closed after the medical director and three Belgian missionaries also died.

By the end of the outbreak there were a total of 318 documented cases and 280 deaths with a Case Fatality Rate of 88%. However, the Case Fatality Rate among those cases who became

exposed to Ebola via injection was 100%: no one who had exclusive contact with the disease from a contaminated needle survived. The primary zoonotic event was never identified, and it is unclear if the index case was infected prior to seeking care and brought Ebola into the hospital or if the virus was already in the hospital and he himself was infected there. Be that as it may, the means by which the virus appeared in Zaire has not been identified. It was speculated at the time of the outbreak that the virus was brought directly from Sudan. It is also worth mentioning that the index case had been on a tour in rural areas surrounding Yambuku with mission workers prior to becoming sick. It was reported that he purchased and handled monkey and antelope meat on August 22, and that his family later ate the antelope, but not the monkey. The timing of this bush meat contact with respect to the onset of his symptoms may fit with the now-known pathogenesis and incubation period of Ebola, however no animal has been implicated as the source of the zoonotic event.

Ebola Hemorrhagic Fever, now known as Ebola Virus Disease, was first discovered during two simultaneous epidemics of the then-unknown causative agent in Central Africa in 1976 Sudan, 1976 (Feldmann *et al.*, 2010). The first outbreak was in Nzara and Maridi, Sudan from June–November 1976. The outbreak began when three men who worked in a cotton factory in Nzara became ill and died. Although all three men worked together, they did not live close to one another and had no known contact outside of the factory. Two of the three original cases, known as YB and BZ, are believed to only have transmitted the virus to members of their family who cared for them while they were sick. YG developed symptoms on June 27, 1976 was admitted into the Nzara Hospital on June 30 and died on July 6. BZ was admitted into the hospital on July 12 and died on July 14.

The third case, known as PG developed symptoms on July 18, 1976, was admitted into the hospital on July 24 and died on July 27. PG was active and sociable in his community and is thought to have been the original source of 48 cases and 27 deaths. PG's contacts spread the disease throughout the community of Nzara as well as into the city of Maridi in late July (128 km away) where at least three people were admitted to Maridi Hospital with the Ebola-like symptoms. The virus then spread throughout the hospital to staff who worked there, to other patients and into the community at large. In the beginning of September, there was an additional cluster of six cases and 25 contacts in Nzara. Although these six new cases worked at the cotton factory, they were unrelated 52 to the three original cases (or any of their contacts) and they reported having no previous contact with anyone who had symptoms

consistent with Ebola. Patients in both cities presented with symptoms typical of Ebola: they initially had fever and headache that quickly progressed to diarrhea, vomiting, chest pain and rash (in about half of the cases). While the cases in Nzara and Maridi had similar clinical manifestations, the transmission patterns in the two cities were very different. In Nzara, Ebola was spread mainly through the contacts of the original three factory workers, while nosocomial transmission was the driver behind the spread of the disease in Maridi. Of the 213 cases in Maridi, 93 of them acquired the disease in the hospital and of those, 72 were staff members who contracted the disease while they were working. By the time outbreak in Sudan was over, there were 67 cases and 31 deaths in Nzara; and 213 cases and 116 deaths in Maridi for an overall Case Fatality Rate of 51%. The spillover event or zoonotic reservoir was never definitively identified for this outbreak, however the cotton factory was implicated as the possible source of the infection.

CAUSATIVE AGENT AND CONTROL IN THE TWO INAUGURAL OUTBREAKS

Ebola was identified as the causative agent in the first two outbreaks when the virus was isolated from patients from Sudan and Zaire. Analysis showed that two distinct subtypes, the Sudan Ebolavirus strain and Zaire Ebolavirus strain, caused the Sudan and Zaire outbreaks respectively. After the identification of this new pathogen, a team from the Center for Disease Control joined a group of international scientists to investigate and control the outbreak in Zaire. The team was known as International Commission for the Investigation and Control of Ebola Hemorrhagic Fever in Zaire. It was this group that gave the virus the name Ebola, a namesake of the Ebola River, which runs in Northwestern DRC, close to where the outbreak occurred (Breman *et al.,* 2014).

CONTAINMENT OF THE OUTBREAKS

There were several factors that contributed to the successful termination of the first outbreak in Zaire. All Commission activities and logistics were coordinated with the sole intention of ending the outbreak. They maintained open channels of communication with the Minister of Health of Zaire: they met with him daily to share information, to update him on progress, and to delineate upcoming action plans. Team members also worked closely with local leaders, explaining what they knew and promised to remain in the area until the outbreak was over. Teams went into the field immediately to find and isolate active cases and trace contacts. They also made recommendations and advised on abbreviated funeral rites that limited transmission of the virus while preserving the cultural context of traditional burial practices.

However, it was reported by the team that the most effective mechanism to control and end the outbreak was house-to-house visits. While the ostensible purpose of these visits was to trace contacts and find new cases, they were also imperative to establish trust with local communities. Many infected patients and their contacts fled out of fear of the disease and out of suspicion of Western medicine, opting instead to seek treatment from traditional healers. An outbreak cannot be ended if patients are transmitting the disease out of the reach of infection control and in an attempt to assuage uneasiness and distrust; clinicians from the local university hospital were included as an integral part of the international teams. By the time the Commission was disbanded at the end of the outbreak, they had visited 550 villages at least twice over a 2-month period and a third visit was made in the villages where Ebola was found (Breman *et al.*, 2014).

EVIDENCE OF ENDEMIC EBOLA IN THE TWO INAUGURAL OUTBREAKS

Although the outbreaks in 1976 were the first Ebola outbreaks on record, it is possible that there had been earlier Ebola occurrences in remote regions of Africa and outside epidemiologic surveillance. A retrospective investigation from a single, isolated Zaire Ebola case in 1977 led researchers to believe that there was a possibility that Ebola Virus Disease was endemic, but sporadic, in the Northern part of the Democratic Republic of Congo since 1972. The investigation began when a single Ebola case presented in Mission Hospital in Tandala, Zaire in June 1977. The patient was a nine-year-old girl who lived with her family in Bonduni Village, 20 km from Tandala, on the border of Zaire and the Central Africa Republic. She was admitted to the hospital after she developed fever, abdominal pain and hematemesis. She was clinically diagnosed with Zaire Ebolavirus, immediately isolated, and standard barrier care methods were implemented. Her family did not report that they had traveled outside of the village before she became ill; no one else in her family or in her village suffered from a similar illness for up to four weeks before the onset of the little girl's symptoms; and there were no secondary cases identified among her contacts. Her family members were tested after the little girl's death and blood and serum analysis revealed that none of them had anti-Ebola antibodies. A retrospective investigation of hospital records revealed that there was one other patient, a 12-year-old girl from Bowabili, 30 km south of Tandala, who had been treated for febrile hemorrhagic disease five months later in November 1977. This child's little sister had also been ill at the same and serological testing on the little sister revealed that she had Ebola antibodies, although no other family members did. Furthermore, it was also found that a physician from Tandala Hospital, where both girls were

treated, also had Ebolavirus antibodies. It is suspected that he contracted the disease when he lacerated a finger while performing an autopsy in 1972 on a patient who died of hemorrhagic illness. The doctor became sick 12 days after the autopsy with symptoms consistent with Ebola, but he recovered approximately 10 days after fever onset (Pourrut *et al.*, 2005)

After 1977, the virus went 'silent' for 15 years, with no outbreaks recorded until the Zaire strain emerged in Kikwit, Democratic Republic of Congo from January – July 1995. The index patient was thought to be a 42-year-old man who came into contact with the virus unknown natural reservoir while working in a charcoal pit. He became ill on January 6, 1995 and was admitted into Kikwit General Hospital on January 13. He transmitted the virus directly to three immediate family members; ten of his extended family members were identified as secondary cases over the next nine weeks.

These initial cases and their contacts spread the virus throughout the community via person-to-person and funeral transmission. The chain of infection eventually led to Kikwit Maternity Hospital in mid-March when there was a small nosocomial outbreak, initially diagnosed as dysentery, among nine employees. Towards the end of April, there were cases of Ebola reported among the surgical staff at Kikwit General Hospital, all of which were traced back to a surgery that was performed on a lab technician who was employed at the Maternity Hospital.

The virus was also introduced into Kikwit General by two other sources. The first was through a nurse from the Maternity Hospital who was admitted into Kikwit General as a patient after she was nosocomial exposed by an obstetric patient; the second introduction into Kikwit General was through an obstetric nurse who was exposed while caring for a cesarean patient. By the end of the outbreak, there were 315 cases and 256 deaths, 25% of whom were health care providers.

It is thought that the lag time between the presentation of the first cases and investigation of the disease fueled the growth of this outbreak. A local ad-hoc committee responsible for investigating the outbreak wasn't formed until May 1, 1995, five months after the index case, and it was tasked to investigate an epidemic of dysentery deaths, not Ebola. It was only after the committee consulted with a member of the Ministry of Health of the Democratic Republic of Congo, J.J. Muyembe-Tamfum, who had worked on the 1976 outbreak, that Ebola was even considered as a possible causative agent. Samples from 14 patients were sent to the

Center for Disease Control in early May; Ebola was confirmed as the cause of the outbreak on May 9, 1995. At that point, international teams were brought in to manage, control and end the outbreak. Identifying cases and tracing contacts proved to be challenging considering that no public health surveillance infrastructure in Kikwit existed. There were other obstacles as well: there were no telephones and very few transportation options. There was a propensity for patients to hide, deny or otherwise conceal their illness for fear of stigma.

Health education was hampered by the lack of mass media, so information campaigns were rolled out using flyers, posters, banners and broadcast messages via megaphone in the streets. As rudimentary as the methods implemented may have been, they worked. On July 16, the last Ebola patient died, only a few months after the CDC and international health community were called in. Their quick success reaffirmed that education, surveillance and the use of proper barrier-nursing practices can interrupt Ebola transmission rapidly and effectively.

There were three other Zaire Ebola outbreaks in the mid 1990's, all of which occurred in northeastern Gabon. The first occurred in two separate waves in December 1994 and January 1995 in and around Mekouka, Gabon, a town located close to the border of Cameroon. The first wave of Ebola cases originated in three gold mining camps located at the edge of a rainforest when 32 miners became ill.

They traveled 100 km by river to the hospital in Makoku to seek treatment where they were initially diagnosed with Yellow Fever and immediately vaccinated. Retrospective testing of samples from those patients revealed that Zaire Ebolavirus was the causative agent. Against medical advice, one of the miners checked himself out of the hospital to seek care from a local traditional healer (a ganga) in the nearby village of Mayela. The escaped patient and the nganga were responsible for the second wave of 16 cases. Each one of the 16 could trace the transmission event back to caring for a relative in the hospital, sleeping at the nganga's home or close contact with individuals who were employed at the hospital. By the time the outbreak burned itself out, there were 51 cases and 31 deaths.

While the zoonotic reservoir for this outbreak was not identified, there were reports of a large number of deaths in a local population of gorillas and an anecdotal tale from one of the patients regarding a bizarrely behaved chimpanzee that was later killed. Neither story could be verified with gorilla cadavers or skeletons in the forest in question. No animals collected in the area surrounding the gold mines revealed Ebola infection, but mines are a known

habitat for bats. The second Zaire Ebolavirus epidemic in Gabon occurred in early February 1996 in the village of Mayibout 2, Gabon, which lies in between Mékouka and Andock (the 61 location of the gold mines where the first epidemic broke out) and Makokou (where the patients from the gold mines were treated). The outbreak is thought to have begun when 18 people carried and helped butcher a chimpanzee carcass that they found in the forest.

It was said that the meat was rotted and the chimpanzee appeared to be ill before it died. After handling the meat, the patients and their contacts became ill with fever, headache, and bloody diarrhea and were sent to Makokou Hospital, despite governmental instructions to the contrary. The bodies of the 4 patients were returned by river to Mayibout2; a fifth patient, who escaped from the hospital while symptomatic, died when he returned home to Mayibout 2. The bodies of the initial patients were buried according to traditional burial ceremonies and without any special precautions to avoid viral transmission. The disease eventually spread to the neighboring villages of Mayibout I and Mvadi before it ended. By the end of the outbreak, 31 cases were identified and 21 of them died.

Apparently unaware that he had contracted Ebola, he flew to Johannesburg, South Africa for treatment, where he infected a nurse who cared for him. There were no other known cases associated with the Johannesburg nurse or the doctor. The epidemic was declared over in Gabon in March 1997, with a total of 60 cases and 45 deaths (Pourrut *et al.*, 2005).

The period of 2000– 2004 saw several outbreaks of Zaire Ebola virus. The first outbreak was in the area surrounding the city of Mekambo, Gabon, which is located where Gab on and the Republic of Congo share a border. Rather than one large epidemic of human to human transmission events that can be traced back to one zoonotic spillover event, the cases of Ebola that occurred from October 2001 – May 2002 in Mekambo area were a series of independent outbreaks stemming from six different spillover events, each of which were related to hunting.

The first spillover occurred in the village of Mendemba in Gabon on October 21, 2001 when a hunter handled an antelope carcass that he found. At the time of his presentation to the hospital with febrile symptoms, the index case did not draw the attention of health authorities as a possible Ebola patient and was not diagnosed as such. It is unclear what his diagnosis was, but he was retrospectively diagnosed with Ebola. This initial case generated several secondary infections, but the disease did not draw the notice of regional health authorities

until six members of the same family fell ill and died over a three-week period. On November 30, samples from this family were sent to France for analysis; Zaire Ebolavirus was identified as the causative agent on December 8, at which point the WHO was notified. In the following days and weeks there were several more suspected Ebola patients being admitted to Mekambo Hospital as well as to Mekouka Hospital, most likely the result of community based and nosocomial transmission additionally, there were reports of 20 dead gorillas and four chimpanzees in the rainforest of the same district.

The second transmission event occurred on November 28, 2001 in the village of Ekata in Gabon when hunters manipulated an antelope; the third transmission event occurred on December 1 in Olloba, Democratic Republic of Congo when hunters butchered a gorilla carcass. Three weeks later the fourth transmission event happened in Ekata on December 22 from an unknown source; the fifth on December 29 in Etakangaye, Gabon when hunters handling a chimpanzee carcass became exposed. The sixth identified zoonotic transmission event occurred on March 27, 2002 when hunters from Grand-Etoumbi butchered and ate a gorilla carcass they found in the forest. By the end of these sequential outbreaks that took place from October 2001 – March 2002 there were 65 cases and 53 dead in Gabon and 57 cases and 43 dead in the Democratic Republic of Congo. All but two cases were epidemiologically linked to an official chain of transmission. Two gorillas that were butchered by one of the index cases in this series of spillover events were found positive for Ebola, but these were the only animals positively identified as being infected.

The second of the Zaire Ebolavirus outbreaks that occurred from 2000 – 2004 affected the area surrounding Mbomo. There were two zoonotic events, one in Yembelengoye and another in a gold-mining camp in Mvoula following the handling of animal carcasses. From December 2002 to May 2003, there were 143 cases and 128 deaths associated with this outbreak. The third outbreak to occur during this period was in Mbanza, Democratic Republic of Congo when cases from an unknown source were reported between October and December 2003. There were 35 cases and 29 deaths. Figure Ten Map of the border region of Gabon and the Democratic Republic of Congo; the site of six independent Ebola spillover events from October 2001 – May 2002 (Pourrut *et al.*, 2005)

January - March 2014 The outbreak in West Africa began slowly in the remote regions of Guinea in December 2013, but it didn't take long for it to escape the confines of Guinea and fan out into Sierra Leone, Liberia, Mali and Nigeria (Bah *et al.*, 2015). Retrospective analysis

revealed that the putative index case was a two-year-old boy in Meliandou, a village in the city of Gueckedou, Guinea. It is unclear how he became infected, but it is thought that he was exposed to the virus through the handling of bats. The manner in which the virus was transmitted to the index notwithstanding the chain of human-to-human transmission has not been broken since the primary spillover event in December. As of March 35, 2015 there have been 24,907 suspected and probable cases and 10, 329 confirmed deaths, making this outbreak the largest Ebola epidemic ever recorded (Pourrut *et al.*, 2005).

While the outbreak was in its embryonic stages in the early months of 2014, cases began to mount in hospitals in Gueckedou, Macenta and Kissidougou. Physicians initially suspected that cholera was the causative agent and while there were several patients who did test positive for the disease. Befuddled doctors soon became suspicious that the illness in question was not cholera (Tam, 2014) but rather some kind of 'mystery disease, and The Ministry of Health of Guinea was notified about a circulating disease that was characterized by fever, diarrhea, vomiting and high mortality (Sack *et al.*, 2014). On March 14, 2014, a Ministry of Health team was sent to Gueckendou to investigate and two weeks later, Zaire Ebolavirus was identified as the causative agent. On March 23, 2014, the World Health Organization in Geneva was officially notified of the outbreak (Baize *et al.*, 2015). At that point in time there were 86 cases and 59 deaths in the Gueckedou, Macenta, Nzerekore and Kissidougo districts of Guinea (unless otherwise noted, case and death tallies include confirmed, suspected and probable).

By the end of March, Ebola left the confines of rural Guinea and crossed borders. On March 27, 2014, Ebola spread to Conkary, the capital of Guinea when four men who had attended their brother's funeral in the central Guinean town of Dabola returned to city and began to exhibit symptoms consistent with Ebola. While all four tested positive for the virus, it was not confirmed if the dead brother had Ebola himself, although he did exhibit symptoms of hemorrhagic fever before he died. On March 29, 2014, Ebola was first recorded in Liberia when seven suspected cases were detected in the Foya District of Lofa County. Two of these cases tested positive for Ebola, one of whom, a 35-year-old woman, became ill after she returned from a trip to Guinea, where she presumably contracted the virus. Before she died on March 31, 2014 she was cared for by her sister, who in turn became sick herself. It is unknown if the sister also traveled to Guinea and contracted the virus there or if she was exposed through direct contact with her sister in Liberia.

Around the same time, a second woman brought Ebola into Lofa County after she visited a Guinean market. She developed symptoms consistent with Ebola while she was in Guinea, at which point her sister traveled from Liberia to pick her up and brought her home. Eventually, the sick sister was admitted into Foya-Borma Hospital where she died on March 20, 2014. Soon after, the sister became symptomatic, and concerned about her condition, took a taxi to see her husband who was migrant worker at the Firestone rubber plant located outside of Monrovia, the capital of Liberia (population one million people). The sister was symptomatic during the 12-hour taxicab ride (a 360 km journey) from Lofa County to Monrovia and exposed the driver as well as five other people along the way, all of whom later died of the virus. After she arrived in Monrovia, she caught a ride by motorcycle to Firestone (the fate of the bike driver remains unknown) where she was hospitalized. On March 30, 2014, Firestone alerted the Liberian Ministry of Health that there was an active case of Ebola on the 120,000-acre plantation. On April 1, 2014 the woman's husband and children were put under quarantine, and although one of the children developed Ebola-like symptoms, no one in the family ever tested positive for the virus. After Ebola was officially identified, the WHO headquarters in Geneva sent thirty-eight epidemiologists, logisticians and data managers to support the search and management of cases across the entirety of the southeastern region of Guinea. The WHO West African Regional Office in Brazzaville, Congo primary task was to deploy personnel to affected regions to support the efforts of Ministries of Health and to guide control efforts on the ground. To that end, together they began making needs assessments and implementing a coordinated response to the outbreak. The Ministry of Health of Guinea established an isolation facility in Gueckedou, and Rapid Response Teams in Conkary conducted contact tracing and 'sensitized' health care workers and affected villagers about Ebola and how to reduce transmission. After cases were detected in Liberia, a National Task force was established to lead the response that included members of the WHO, the International Red Cross, Samaritan's Purse, Pentecostal Mission Unlimited and UNICEF. The team worked together to distribute Personal Protective Equipment to 41 health care facilities; to strengthen infection prevention and control protocols in Foya Hospital; and to train health care workers in Montserrado County on how to treat and isolate Ebola patients. Personal protective equipment and medical supplies were also sent to Bong and Nimba counties. However, rather than appointing Dr. Pierre Formety, the WHO's top Ebola authority as the coordinator of the regional response, the West African office chose an official from the Guinea WHO Office who had never before been involved in an Ebola outbreak (Sack *et al.*, 2014).

On June 23, 2014, MSF released a statement declaring that the epidemic was out of control. The organization had reached their human capital limits and was unable to send more teams to new outbreak sites. They accused the WHO, civil, political and religious leaders of 'failing to acknowledge the scale of the epidemic' and shirking their responsibilities to curb the spread of the disease. By this point in time, MSF had treated 470 patients in Ebola Treatment Centers in Conkary, Telimele and Gueckedou (Guinea); Koidu, Daru, Buedu and Kaiahun (Sierra Leone). On September 18, 2014, the United Nations Security Council declared the Ebola outbreak in the West Africa "threat to international peace and security" and advised UN member states to provide more resources to fight the outbreak, which marked the first time the Security Council exercised its powers to intervene in a public health crisis. On September 19, 2014 the UN Mission for Ebola Emergency Response (UNMEER) was formed to address the epidemic and was tasked with coordinating the United Nations vast resources to combat the epidemic under the leadership of Dr. David Nabarro (Sack *et al.*, 2014).

In the 2014-2015 Ebola outbreak in West Africa, healthcare workers represented only 3.9% of all confirmed and probable cases of EVD in Sierra Leone, Liberia, and Guinea combined (WHO, 2015). In comparison, healthcare workers accounted for 25% of all infections during the 1995 outbreak in Kikwit (Khan *et al*., 1999). During the 2014-2015 West Africa outbreak, the majority of transmission events were between family members (74%). Direct contact with the bodies of those who died from EVD proved to be one of the most dangerous – and effective – methods of transmission. Changes in behaviors related to mourning and burial, along with the adoption of safe burial practices, were critical in controlling that epidemic (Baseler *et al*., 2017).

ZOONOTIC RESERVOIR OUTBREAK

The zoonotic reservoir for the Ebolavirus has never been definitively identified and it was thought that perhaps non-human primates that tested positive for Ebola RNA might be the reservoir host. Several human Ebola outbreaks have been associated with the handling and consumption of infected mammal carcasses (most frequently chimpanzees) and there have been documented epidemics in non-human primate communities that occurred concurrently with human outbreaks. However, all evidence that has been collected and observed with respect to Ebola pathogenesis in non-human primates suggests that they, like humans,

develop symptomatic and fatal infection. This would point to their role as an intermediary or dead-end host, rather than the reservoir (Olival *et al.*, 2014).

Bats, on the other hand, have stronger evidence to support the hypothesis that they may be the reservoir host. In 1996, lab experiments proved that bats infected with Zaire Ebolavirus were able to replicate virus, mount an adaptive immune response and survive without showing signs of overt infection (Olival *et al.*, 2014). In 2002, during a post-outbreak investigation, Ebolavirus RNA was detected in three different fruit bats that lived in the Gabonese forest (Pigott *et al.*, 2014). In 2005, viral RNA sequences were found in the liver and the spleen of three different kinds of fruit bats, none of which exhibited symptoms: Hypisignathus Monstrosus, Epomops Franqueti and Myonycetris Torquata. While IgG antibodies to Zaire Ebola bvirus were found in these species of bats as well, neither RNA nor IgG antibodies were found at the same time. Although nucleotide sequences were found in the bat specimens, scientists were not able to isolate the virus itself (Olival *et al.*, 2014). The index case in the 2007 outbreak in Luebo, Democratic Republic of Congo was reported to have contact with freshly killed bats in a market just prior to falling ill with mild case of Ebola. All of these findings give weight to the hypothesis that bats are the natural reservoir host of the Ebolavirus; however, none proves it beyond a rea son able doubt. The strongest evidence that has implicated bats as the reservoir host was in 2007 when Marburgvirus was isolated from the fruit bat *Rousettus Aegypticus* (Towner *et al.*, 2009).

The exact mechanisms of bat immunology is not entirely understood, but it is believed that bats can maintain an asymptomatic state while sustaining viral replication and mounting an adaptive immune response (with a corresponding helper T Cell activation) that clears the infection. While some believe that bats only shed virus while under active viral replication, others have hypothesized that bats maintain a chronic, active viral state and that shedding occur in spatial pulses during times of immunologic stress related to food scarcity or pregnancy With respect to the spillover event that started the 2014 outbreak all that was known was that the index patient was a two-year-old boy in Meliandou. No hard data had been collected regarding any exposure he may have had to bats or other intermediate hosts. There was not any evidence that suggested a decline in non-human primate or mammal populations in the forests surrounding Meliandou, that wasn’t explained by hunting or migration, prior to or concurrently with human transmission of the virus in December. Operating under the assumption that the two-year old boy from Guekendou was the index

case and that he was exposed to the virus from either the natural reservoir or intermediate host, a team of scientists spent four weeks in April 2014 in Meliandou looking for data that could shed some light as to the nature of the primary spillover event. The goal for the expedition was to locate the zoonotic reservoir, to ascertain if there had been widespread wildlife decline prior to the outbreak, and to study human behaviors and practices associated with hunting bats and other wildlife. The team did not find that there had been a decline in local wildlife populations either concurrently with or prior to the outbreak in Meliandou, leading researchers to believe that handling of wild game or bushmeat was not the spillover culprit. In this area of Guinea, most large game that is consumed is not hunted locally, rather it is brought in from areas in the northwestern part of the country. While this does leave open the possibility that the imported meat was infected, this is not likely. Even had wild life been infected with Ebola, the index case would have most likely been one of the hunters or a person who bought and prepared the meat for consumption, not a two-year-old child (Mari Saez *et al.*, 2014).

Fruit Bats, on the other hand, are hunted and consumed locally. Local bat hunting is primarily the responsibility of the patriarch of the family, who hunts with a gun, nets or his bare hands. Bats are generally hunted in forest patches or caves surrounding Meliandou and the catch is either sold at market or burned in a fire to be used for meat in sauces. Children also hunt and capture bats with their friends as a form of amusement, but do so in a different manner than adult hunters. They find bats in hollow trees or under thatched rooftops and use a stick or their hands to directly catch them or knock them to the ground. The species of bats that are hunted for food are different than those bats that are hunted for amusement by children; hunters target fruit bats while children aim at insectivorous bats. The investigating team was able to capture and sacrifice 13 species of bats representing six different bat families (they captured 88 bats in Meliandou, 20 in Kagbadou, four in Kelema and 57 in Zaima) in the area including and surrounding Meliandou, three of which (Eidolon Helvum, Hypsignathus Monstrosus and Mops Condylurus) were found serologically or RT-PCR positive for Ebolavirus, although no viral RNA was detected and tests for Ebolavirus IgG antibodies were inconclusive. This does not prove that the bats are the reservoir and it certainly does not prove that bats in the area at the time of the spillover event were infectious or shedding virus. All it means is that at the time of the research, bats were found to have either Ebola antibodies or fragments of virus in their bodies. Through interviews with local villagers and observations of the topography of the land surrounding the village, researchers did discover a

hollow tree located 50 meters from the home of the index patient. This tree was known to be the residence of a colony of bats as well as a playground for children, including the index patient. Researchers were not able to examine the tree or capture any of the bats that resided in it (Plowright *et al.,* 2015). On March 24, 2014 as the epidemic grew and authorities issued warnings about the dangers of consuming bushmeat, the tree was burned down. There are conflicting stories as to the rationale for burning the tree. Some villagers reported that the tree was burned because of the bushmeat ban, others say that the tree was burned during a botched attempt to extract honey and yet other stories indicate that the tree was burned accidentally by children who were playing with fire. Regardless of how or why the tree was burned, there are consistent reports that a rain of bats' emerged from the tree as flames consumed it and a large number of these bats perished in the fire. Those bats were collected by residents of Gueckendou with the intent of consumption, but they were discarded the next day after the official ban on bushmeat was announced. RNA sequencing analysis from ash samples and soil around the base of the t ree indicated that Mops Condylurns bats, also known as the insectivorous Angolan Free Tailed Bat, inhabited the tree (Leroy, 2004).

The results of this investigation into the potential zoonotic reservoirs in Gueckendou did not uncover any evidence that handling of diseased intermediate hosts was the cause of the outbreak. Likewise there was no evidence that implicated adult hunting and consumption of bats as the zoonotic event. No hunters resided in the household of the index patient and had the spillover been tied to consumption, the person who handled the bats during hunting and/or preparation would have been the index patient, which was not the case in this outbreak. The only epidemiologic evidence uncovered in Gueckedou with respect to possible zoonotic reservoirs and the two-year-old child was the hollowed out tree and the bats that resided in it, but nothing discovered in the investigation could implicate bats in that tree as the zoonotic source. Given anecdotal evidence about bat handling behaviors in the village and the elimination of other sources of spillover normally attributed to Ebolavirus outbreaks, it is possible that the zoonotic event could be related to the Mops Condylurns bats residing in a hollow tree where the index patient played, but it is far from conclusive. It seems highly unlikely that a two-year-old child would be old enough or dexterous enough to engage in the hunting of bats, however it is possible that he could have been exposed to the virus while playing in the tree or he might have played with an already-dead infected bat or ingested a small quantity of infected bat droppings (Mari *et al.,* 2014).

CONCLUSIONS

EVD is a painful reminder that an outbreak anywhere can be a risk everywhere. The Global Health Security Agenda seeks to enforce public health systems in most affected countries in order to eliminate the spreads before they become emergencies. Although great improvements have been achieved over the past decade, better surveillance, real-time sharing of data and taking rapid action based on the available information remain necessary. Because Ebola virus is primarily transmitted through contact with the body fluids of symptomatic patients, the infection spread can be stopped by an early diagnosis, contact tracing, patient isolation and care, infection control and safe burial. EHF epidemics constitute a significant public health concern in Africa and an effective vaccine is needed urgently. Such a vaccine would primarily benefit doctors, nurses, and field epidemiologists working in endemic countries. The second target group would be the scientists working with Ebola virus as well as veterinarians and those involved in wildlife conservation in endemic areas. Since its discovery in 1976, much is known about Ebola virology, physiopathology, clinical features, and epidemiology, but the missing link certainly remains the virus reservoir in nature. The current research focused on bats as putative ZEBOV reservoirs has to be reinforced and extended to the reservoirs of other Ebola species. The early detection and isolation of a patient with EVD decreases the risk for transmission in the community.

REFERENCES

1. Ansari AA. Clinical features and pathobiology of Ebola virus infection. J Autoimmun, 2014; 55(0): 1-9.
2. Bah EI, Lamah M, Fletcher T, Jacob ST, Brett MD and Sall AA. Clinical presentation of patients with Ebola virus disease in Conakry, Guinea. N Engl J Med, 2015; 372(1): 40-7.
3. Baize S, Leroy EM, Georges-Courbo TM, Capron M, Lansoud-Soukate J and Debre P. Defective humoral responses and extensive intravascular apoptosis are associated with fatal outcome in Ebola virus-infected patients. Nature Medicine, 1999; 423-426.
4. Baize S, Pannetier D, Oestereich L, Rieger T, Koivogui L and Magassouba N. Emergence of Zaire Ebola virus disease in Guinea. N Engl J Med, 2015; 371(15): 1418-25.
5. Baron RC, McCormick JB and Zubeir OA. Ebola virus disease in southern Sudan: hospital issemination and intrafamilial spread. Bulletin W Health Org, 1983; 61(6): 997-1003.
6. Baseler L., Chertow D, et. Al. The pathogenesis of Ebola virus disease. *Annu. Rev. Pathol. Mech. Dis.*, 2017; 12: 387–418.

7. Bausch DG, Schwarz L. Outbreak of Ebola virus disease in Guinea: where ecology meets economy. PLoS Negl Trop Dis, 2014; 8: 3506.
8. Bausch DG, Towner JS, Dowell SF, Kaducu F, Lukwiya M and Sanchez A. Assessment of the risk of Ebola virus transmission from bodily fluids and fomites. J Infect Dis, 2007; 196: S142-7.
9. Bellanemail S, Pulliam J, Dushoff J, Meyers L. Ebola control: effect of asymptomatic infection and acquired immunity. The Lancet, 2014; 384(9953): 1499-1500.
10. Bradfute SB, Bavari S. Correlates of immunity to Filovirus Infection. Viruses, 2011; 3(7): 982–1000.
11. Breman JG, Johnson KM. Ebola then and now. N Engl J Med, 2014; 371(18): 1663-6.
12. Carroll OL. Trials Using Survivors' blood for treatment to start in Sierra Leone. The Guardian, 2015.
13. Chippaux J. (2014). Outbreaks of Ebola virus disease in Africa: the beginnings of a tragic saga. Journal of Venomous Animals and Toxins including Tropical Diseases. 20(44).
14. Clark DV, PB Jahrling and JV Lawler (2012). Clinical Management of Filovirus-Infected Patients. Viruses. 4(9): 1668-1686.
15. Chertow DS, C Kleine, JK Edwards, R Scaini, R Giuliani and A Sprecher (2015) Ebola Virus Disease in West Africa. Clinical Manifestations and Management. N Engl J Med. 371(22): 2054-7.
16. Cohen J, Kupferschmidt K. A dose of reality. Science, 2014; 346(6212): 908-11.
17. Cohen J. Many caveats on promising Ebola drug trial. Science, 2015.
18. Dixon MG, Schafer IJ. Ebola viral disease outbreak-West Africa. Morb Mortal Wkly Rep, 2014; 63(25): 548-51.
19. Feldmann H, Geisbert T. Ebola haemorrhagic fever. The Lancet, 2010; 377(9768): 849-862.
20. Fletcher T, Fowler A, Beeching R. Understanding organ dysfunction in Ebola virus disease. Intensive Care Medicine, 2014; 40(12): 1936-1939.
21. Georges A, EM Leroy, AA Renaut, CT Benissan, RJ Nabias and MT Ngoc (1999).
22. Heffernan RT, Pambo B, Hatchett RJ, Leman PA, Swanepoel R, Ryder RW. Low Seroprevalence of IgG Antibodies to Ebola Virus in an epidemic zone: Ogooué-Ivindo region, Northeastern Gabon, 1997. J Infecti Dis, 2005; 191(6): 964-8.
23. Heymann DL, Weisfeld JS, Webb PA, Johnson KM, Cairns T, Berquist H. Ebola hemorrhagic fever: Tandala, Zaire, 1977–1978. Journal of Infectious Diseases, 1980; 142(3): 372-6.

24. Heymann DL (2014). Ebola: learn from the past. Nature. 514(7522): 299-300.
25. Hunt D, Jacobson S, Hacker H. Nurses: Hospital's Ebola response put workers, patients at risk. Immunology, 2014; 124(3): 453-460.
26. Ippolito G, Feldmann H, Lanini S, Vairo F, Di Caro A, Capobianchi MR, et al. Viral hemorrhagic fevers: advancing the level of treatment. BMC Med, 2012; 10: 31.
27. Johnson & Johnson joins global Janssen Ebola vaccine effort. January 22, 2015.
28. Judson S, Prescott J, Munste V. Understanding Ebola virus transmission. Viruses, 2015; 7: 511-521.
29. Kawaoka Y. How Ebola Virus infects cells. N Engl J Med, 2015; 352(25): 2645-6.
30. Khan A. et al. The Reemergence of Ebola hemorrhagic fever, democratic republic of the Congo, 1995. *J Infect Dis.*, 1999; 179(Suppl 1): S76-86.
31. Khan H, Ahmad I. Threat of Ebola virus disease for Pakistan. Gomal J Med Sci, 2015; 2: 127–128.
32. Konstantinov I, et al. Poster: The Ebola virus. Science. February 3, 2013; http://visual-science.com/projects/ebola/poster/.
33. Lai KY, Ng WYG, Cheng FF. Human Ebola virus infection in West Africa: a review of available therapeutic agents that target different steps of the life cycle of Ebola virus. Infect Dis Poverty, 2014; 3(1): 43.
34. Lamontagne F, Clément C, Fletcher T, Jacob ST, Fischer WA and Fowler RA. Doing today's work superbly well-treating Ebola with current tools. N Engl J Med, 2015; 371(17): 1565-6.
35. Lee JE. Structure of the Ebola virus glycoprotein bound to an antibody from a human survivor. Nature, 2008; 454: 177-182.
36. Leroy E, S Baize (2000). Human asymptomatic Ebola infection and strong inflammatory response. The Lancet. 355(9222): 2210-2215.
37. Leroy E, Baize S. Human asymptomatic Ebola infection and strong inflammatory response. The Lancet, 2001; 355(9222): 2210-2215.
38. Leroy EM, Kumulungui B, Pourrut X, Rouquet P, Hassanin A and Yaba P. Fruit bats as reservoirs of Ebola virus. Nature, 2004; 438(7068): 575-6.
39. Leroy EM. Ebola Outbreak Resulting from Direct Exposure to Fruit Bats in Luebo, Democratic Republic of Congo. Vector-Borne and Zoo Dis, 2007; 9(6): 723-728.
40. Li H, Ying T, Yu F, Lu L, Jiang S. Development of therapeutics for treatment of Ebola virus infection. 2015; 17(2): 109-17.

41. Lichtman AH, Annas AK. Basic Immunology: Functions and Directions of the Immune System. Third Edition ed. Philadelphia, PA: Saunders Elsevier; 2011.
42. Loftus P, Chimerix. Scraps Testing of Experimental Ebola Drug in Liberia. The Wall Street Journal, 2015.
43. Lyon GM, Mehta AK, Varkey JB, Brantly K, Plyler L, McElroy AK, et al. Clinical Care of Two Patients with Ebola Virus Disease in the United States. N Engl J Med, 2014; 71(25): 2402-9.
44. MacNeil A, Farnon EC, Wamala J, Okware S, Cannon DL and Reed Z. Proportion of deaths and clinical features in Bundibugyo Ebola virus infection, Uganda. Emerg Infect Dis, 2010; 1969-72.
45. Mari Saez A, Weiss S, Nowak K, Lapeyre V, Zimmermann F, Dux A, et al. Investigating the zoonotic origin of the West African Ebola epidemic. EMBO Mol Med, 2014; 30; 7(1): 17-23.
46. McCarthy M. US signs contract with ZMapp maker to accelerate development of the Ebola drug. British Med J, 2014; 349: g5488.
47. McElroy AK, Erickson BR, Flietstra TD, Rollin PE, Nichol ST, Towner JS, et al. Ebola hemorrhagic fever: novel biomarker correlates of clinical outcome. J Infect Dis, 2014; 210(4): 558-66.
48. Mercer J. Viral apoptotic mimicry party: PS Bring your own Gas6. Cell host & microbe, 2011; 9(4): 255-7.
49. Miller EH, Obernosterer G, Raaben M, Herbert AS, Deffieu MS and Krishnan A. Ebola virus entry requires the host-programmed recognition of an intracellular receptor. The EMBO Journal, 2012; 31(8): 1947-1960.
50. Minder R. Officials Cite Error With Gloves in Spanish Case of Ebola. The New York Times. October 8, 2014.
51. Misasi J, Sullivan NJ. Camouflage and Misdirection: The Full-On Assault of Ebola Virus Disease. Cell, 2014; 159(3): 477-486.
52. Mupapa K, Massamba M, Kibadi K, Kuvula K, Bwaka A and Kipasa M. Treatment of Ebola Hemorrhagic Fever with Blood Transfusions from Convalescent Patients. J Infect Dis, 1999; S18-23.
53. Murin CD, Fusco ML, Bornholdt ZA, Qiu X, Olinger GG and Zeitlin L. Structures of protective antibodies reveal sites of vulnerability on Ebola virus. Proceedings of the National Academy of Sciences, 2014; 111(48): 17182-7.

54. Negredo A, Palacios G, Vázquez-Morón S and González FDH. Discovery of an Ebolavirus-Like Filovirus in Europe. PLoS Pathogens, 2011; 7(10).
55. Olival KJ, Hayman DT. Filoviruses in bats: current knowledge and future directions. Viruses, 2014; 6(4): 1759-88.
56. O'Carroll L (2015). Trials Using Survivors' Blood for Treatment to Start in Sierra Leone. The Guardian.
57. Perner A, Fowler RA, Bellomo R and Roberts I. Ebola care and research protocols. Intensive Care Medicine, 2015; 41(1): 111-114.
58. Pigott DM, Golding N, Mylne A, Huang Z, Henry AJ and Weiss DJ. Mapping the zoonotic niche of Ebola virus disease in Africa. Elife, 2014; 3: e04395.
59. Plowright RK, Eby P, Hudson PJ, Smith IL, Westcott D and Bryden WL. Ecological dynamics of emerging bat virus spillover. Proc Biol Sci, 2015; 282(1798): 2124.
60. Pourrut X, Kumulungui B, Wittmann T, Moussavou G, Délicat A and Yaba P. The natural history of Ebola virus in Africa. Microb Infect, 2005; 7(7–8): 1005-14.
61. Roberts I, Perner A. Ebola virus disease: clinical care and patient-centered research. Lancet, 2014; 384(9959): 2001-2.
62. Rowe AK, Bertolli J, Khan AS, Mukunu R, Muyembe-Tamfum JJ and Bressle D. Clinical, Virologic, and Immunologic Follow-Up of Convalescent Ebola Hemorrhagic Fever Patients and Their Household Contacts, Kikwit, Democratic Republic of the Congo. J Infect Dis, 1999; 179(1): S28-35.
63. Sack K, Fink S, Belluck P, Nossiter A. How Ebola Roared Back. New York Times. December 29, 2014.
64. Sanchez A, Geisbert TW, Feldmann H, Knipe DM, Howley PM. *Filoviridae:* Marburg and Ebola viruses. In: Philadelphia: Fieldsvirology. Lippincott Williams & Wilkins, 2006; 1409–1448.
65. Slenczka W, Klenk HD. Forty years of marburg virus. J Infect Dis, 2014; 196(2): S131-5.
66. Smith RD. Responding to global infectious disease outbreaks, Lessons from SARS on the role of risk perception, communication and management. Soc Sci Med, 2006; 63(12): 3113-3123.
67. Sullivan NJ, Martin JE, Graham BS, Nabel GJ. Correlates of protective immunity for Ebola vaccines: implications for regulatory approval by the animal rule. Nat Rev Microbiol, 2011; 7(5): 393-400.
68. Tam R. This is how you get Ebola, as explained by science. PBS News Hour. September 30, 2014.

69. Towner JS, Amman BR, Sealy TK, Carroll SAR, Comer JA and Kemp A. Isolation of genetically diverse Marburg viruses from Egyptian fruit bats. PLoS pathogens, 2009; 5(7): e1000536.
70. Waheed Y. Ebola in West Africa: an international medical emergency. Asian Pac J Trop Biomed, 2014; 4(9): 673-4.
71. Wauquier N, Becquart P, Padilla C, Baize S, Leroy EM. Human Fatal Zaire Ebola Virus Infection Is Associated with an Aberrant Innate Immunity and with Massive Lymphocyte Apoptosis. PLoS Neglected Trop Dis, 2010; 4(10): e837.
72. WHO. Health worker Ebola infections in Guinea, Liberia and Sierra Leone: A Preliminary Report 21 May 2015. Accessed June 20, 2017. http://www.who.int/hrh/documents/21may2015_web_final.pdf.
73. Yazdani N, Abbas A. Pakistan adopts defensive strategies against Ebola contagion. Arch Pharma Pract, 2015; 6: 13.
74. Ying C, Yu L, Jie YH. Ebola Virus Disease: General Characteristics, Thoughts, and Perspectives. Biomedi Environ Sci, 2006; 27(8): 651-653.
75. Ying C, Yu L, Jie YH. Ebola Virus Disease: General Characteristics, Thoughts, and Perspectives. Biomedi Environ Sci, 2014; 27(8): 651-653.
76. Zampieri C, Sullivan N, Nabel G. Immunopathology of highly virulent pathogens: insights from Ebola virus. Nature Immunology, 2007; 8: 1159-1164.