



Pathogenesis of Ebola virus: A deadly virion hosted by bats

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ABSTRACT

Ebola Virus Infection is a rare but deadly virus that causes bleeding inside and outside the body. As the virus spreads through the body, it damages the immune system and organs. Ultimately, it causes levels of blood-clotting cells to drop. This leads to severe, uncontrollable bleeding. The disease, also known as Ebola hemorrhagic fever or Ebola virus, kills up to 90% of people who are infected. Although movies and books describe major outbreaks of Ebola-like disease in the U.S., they're just fiction. So far serious Ebola cases have only shown up in Central and West Africa. It has strong safety measures in place for people who have Ebola. Ebola can spread from country to country when people travel. So it is possible for it to reach the U.S. if an infected person travels here. But there are ways to prevent people from coming to U.S. airports with the disease. Airline crews are trained to spot the symptoms of Ebola in passengers flying from places where the virus is found. Crews are told to quarantine anyone who looks infected. Ebola isn't as contagious as more common viruses like colds, influenza, or measles. It spreads to people by contact with the skin or bodily fluids of an infected animal, like a monkey, chimp, or fruit bat. Then it moves from person to person the same way. Those who care for a sick person or bury someone who has died from the disease often get it. Other ways to get Ebola include touching contaminated needles or surfaces. Infection of Ebola can be spread from air, water, or food. A person who has Ebola but has no symptoms can't spread the disease, either. Early on, Ebola can feel like the flu or other illnesses. Symptoms show up 2 to 21 days after infection and usually include: High fever, Headache, Joint and muscle aches, Sore throat, Weakness, Stomach pain, Lack of appetite, Blood vomiting. As the disease gets worse, it causes bleeding inside the body, as well as from the eyes, ears, and nose. Some people will vomit or cough up blood, have bloody diarrhea and get a rash. Sometimes it's hard to tell if a person has Ebola from the symptoms alone. Doctors may test to rule out other diseases like cholera or malaria. Tests of blood and tissues also can diagnose Ebola.

Keywords: Bats (*Pteropus vampyrus*), Ebola Virus (*Zaire Ebolavirus*), Virions, Genomes, ELISA, PCR, Vaccines



Ebola virus (Zaire Ebolavirus) is a virus that causes Ebola virus disease. It is a virological taxon species included in the genus *Ebolavirus*, family *Filoviridae*, members are called *Filovirus*, the order is *Mononegavirales*. The Zaire Ebola virus is the most dangerous of the five species of Ebola viruses of the *Ebola virus* genus. The virus causes an extremely severe hemorrhagic fever in humans and other primates. EBOV is a select agent, World Health Organization Risk Group 4 Pathogen, a U.S. National Institutes of Health/National Institute

of Allergy and Infectious Diseases Category A Priority Pathogen, U.S. CDC Centers for Disease Control and Prevention Category A Bioterrorism Agent and listed as a Biological Agent for Export Control by the Australia Group.¹



Figure-1: Carrier of Ebola Virus (Bats)

The name *Zaire Ebolavirus* is derived from *Zaire*, the country (now the Democratic Republic of Congo) in which the Ebola virus was first discovered, and the taxonomic suffix *Ebolavirus*. The EBOV genome is approximately 19 kb in length. It encodes seven structural proteins:

nucleoprotein (NP), polymerase cofactor (VP35), (VP40), GP, transcription activator (VP30), VP24 and RNA polymerase (L). The Ebola Virus genetics is difficult to study due to the virulent nature of the virus.²

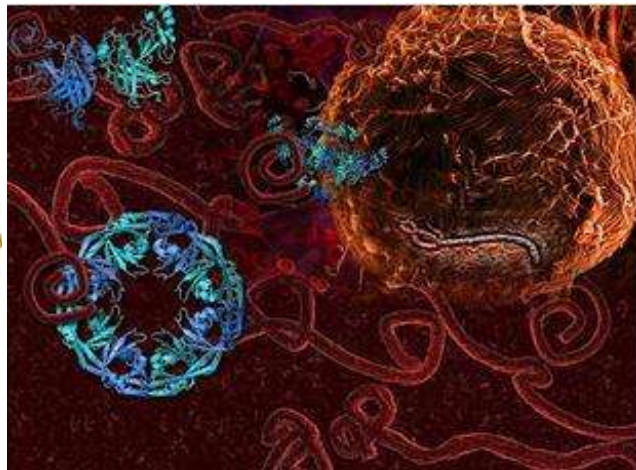
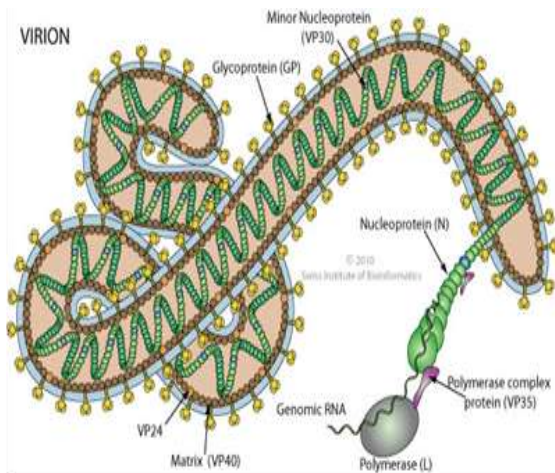


Figure-2: Ebola Virion

Structure: EBOV carries a negative-sense RNA genome in virions that are cylindrical/tubular and contain viral envelope, matrix and nucleocapsid components. The overall cylinders are generally approx. 80 nm in diameter, and having a virally encoded glycoprotein (GP) projecting as 7-10 nm long spikes from its lipid bilayer surface. The cylinders are of variable length, typically 800 nm, but sometimes up to 1000 nm long. The outer viral envelope of the virion is derived by budding from domains of host cell membrane into which the GP spikes have been inserted during their biosynthesis.

Individual GP molecules appear with spacings of about 10 nm. Viral proteins VP40 and VP24 are located between the envelope and the nucleocapsid, in the *matrix space*. At the center of the virion structure is the nucleocapsid, which is composed of a series of viral proteins attached to a 18–19 kb linear, negative-sense RNA without 3'-polyadenylation or 5'-capping, the RNA is helically wound and complexed with the NP, VP35, VP30 and L proteins; this helix has a diameter of 80 nm and contains a central channel of 20–30 nm in diameter.³

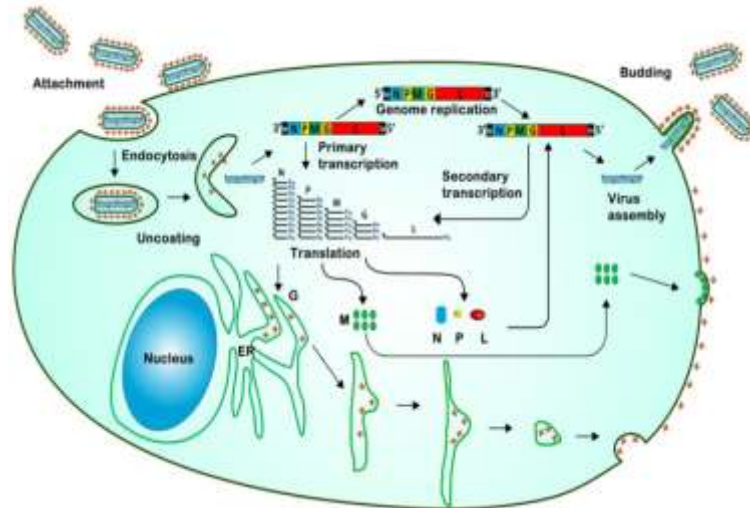


Figure-3: Genome of Ebola Virus

The overall shape of the virions after purification and visualization (e.g., by ultracentrifugation and electron microscopy, respectively) varies considerably; simple cylinders are far less prevalent than structures showing reversed direction, branches and loops (i.e., U-, shepherd's crook-, 9- or eye bolt-shapes, or other

or circular/coiled appearances), the origin of which may be in the laboratory techniques applied. The characteristic "threadlike" structure is, however, a more general morphologic characteristic of filoviruses (alongside their GP-decorated viral envelope, RNA nucleocapsid, etc.).⁴



Figure-4: Scanning Electron Micrograph of Ebola Virus

Genome

Each virion contains one molecule of linear, single-stranded, negative-sense RNA, 18,959 to 18,961 nucleotides in length. The 3' terminus is not polyadenylated and the 5' end is not capped. It was found that 472 nucleotides from the 3' end and 731 nucleotides from the 5' end are sufficient for replication. It codes for seven structural proteins and one non-structural protein. The gene order is 3' – leader – NP – VP35 – VP40 – GP/sGP – VP30 – VP24 – L – trailer – 5'; with the leader and trailer

being non-transcribed regions, which carry important signals to control transcription, replication and packaging of the viral genomes into new virions. The genomic material by itself is not infectious, because viral proteins, among them the RNA-dependent RNA polymerase, are necessary to transcribe the viral genome into mRNAs because it is a negative sense RNA virus, as well as for replication of the viral genome. Sections of the NP and the L genes from filoviruses have been identified as endogenous in the genomes of several groups of small mammals. Host-encoded Niemann–

Pick C1 (NPC1), a cholesterol transporter protein, appears to be essential for entry of Ebola virions into the host cell and for its ultimate replication. In one study, mice that were heterozygous for NPC1 were shown to be protected from lethal challenge with mouse-adapted Ebola virus. In another study, small molecules were shown to inhibit Ebola virus infection by preventing viral envelope glycoprotein (GP) from binding to NPC1. Hence, NPC1 was shown to be critical to entry of this filovirus, because it mediates infection by binding directly to viral GP.⁵

Entry

When cells from Niemann Pick Type C patients lacking this transporter were exposed to Ebola virus in the laboratory, the cells survived and appeared impervious to the virus, further indicating that Ebola relies on NPC1 to enter cells; mutations in the NPC1 gene in humans were conjectured as a possible mode to make some individuals resistant to this deadly viral disease. The same studies described similar results regarding NPC1's role in virus entry for Marburg virus, a related filovirus. A further study has also presented evidence that NPC1 is critical receptor mediating Ebola infection via its direct binding to the viral GP and that it is the second "lysosomal" domain of NPC1 that mediates this binding. Together, these studies suggest NPC1 may be potential therapeutic target for an Ebola anti-viral drug.⁶

Replication

Being a cellular, viruses such as Ebola do not replicate through any type of cell division; rather, they use a combination of host- and virally encoded enzymes, alongside host cell structures, to produce

multiple copies of themselves; these then self-assemble into viral macromolecular structures in the host cell. Specific steps for Ebola virus include:

- The virus attaches to host receptors through the glycoprotein (GP) surface peplomer and is endocytosed into macropinosomes in the host cell.
- Viral membrane fuses with vesicle membrane, nucleocapsid is released into the cytoplasm.
- Encapsidated, negative-sense genomic ssRNA is used as a template for the synthesis (3'-5') of poly-adenylated, monocistronic mRNAs.
- Using the host cell's ribosomes, tRNA molecules, etc., the mRNA is translated into individual viral proteins.
- Viral proteins are processed, glycoprotein precursor (GP0) is cleaved to GP1 and GP2, which are then heavily glycosylated using cellular enzymes and substrates. These two molecules assemble, first into hetero-dimers and then into trimers to give the surface peplomers. Secreted glycoprotein (sGP) precursor is cleaved to sGP and delta peptide, both of which are released from the cell.⁷
- As viral protein levels rise, a switch occurs from translation to replication. Using the negative-sense genomic RNA as a template, a complementary +ss RNA is synthesized; this is then used as a template for the synthesis of new genomic (-) ssRNA, which is rapidly encapsidated.
- The newly formed nucleocapsids and envelope proteins associate at the host cell's plasma membrane; budding occurs, destroying the cell.⁸

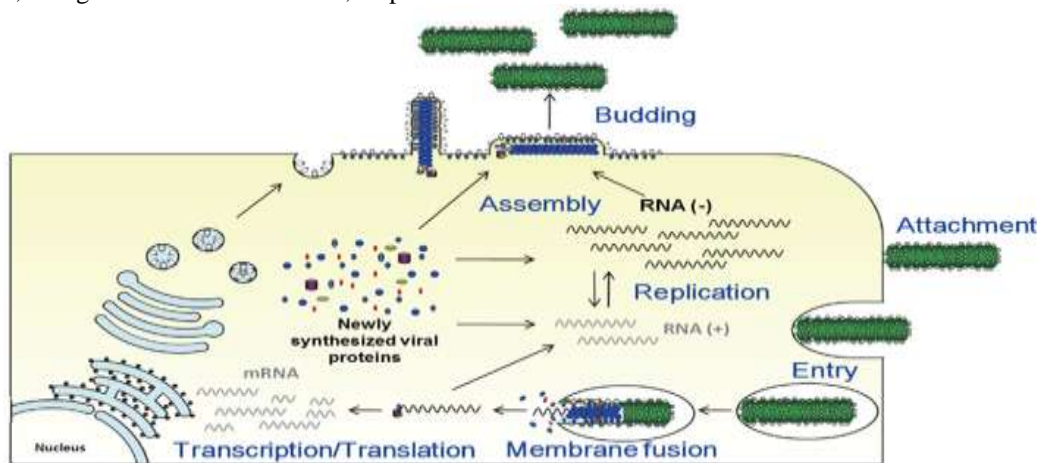


Figure-5: Replication of Ebola Virus

Types

The five characterised Ebola species are:

Zaire Ebolavirus: Also known simply as the *Zaire virus*, ZEBOV has the highest case-fatality rate of

the Ebolaviruses, up to 90% in some epidemics, with an average case fatality rate of approximately 83% over 27 years. There have been more outbreaks of *Zaire Ebola virus* than of any other species. The first outbreak occurred on 26 August

1976 in Yambuku. The first recorded case was Mabalo Lokela, a 44-year-old schoolteacher. The symptoms resembled malaria and subsequent patients received quinine. Transmission has been attributed to reuse of unsterilized needles and close personal contact.⁹

Sudan Ebolavirus: Like the *Zaire virus*, SEBOV emerged in 1976; it was at first assumed identical with the Zaire species. SEBOV is believed to have broken out first among cotton factory workers in Nzara, Sudan with the first case reported as a worker exposed to a potential natural reservoir. The virus was not found in any of the local animals and insects that were tested in response. The carrier is still unknown. The lack of barrier nursing facilitated the spread of the disease. The most recent outbreak occurred in May, 2004. Twenty confirmed cases were reported in Yambio County, Sudan with five deaths resulting. The average fatality rates for SEBOV were 54% in 1976, 68% in 1979, and 53% in 2000 and 2001.¹⁰

Reston Ebolavirus: Discovered during an outbreak of simian hemorrhagic fever virus (SHFV) in crab-eating acaques from Hazleton Laboratories in 1989. Since the initial outbreak in Reston, Virginia, it has since been found in non-human primates in Pennsylvania, Texas and Siena, Italy. In each case, the affected animals had been imported from a facility in the Philippines, where the virus has also infected pigs. Despite having a Biosafety status of Level-4 and its apparent pathogenicity in monkeys, REBOV did not cause disease in exposed human laboratory workers.¹¹

Côte d'Ivoire Ebolavirus: Also referred to as *Tai Forest Ebolavirus* and by the English place name, "Ivory Coast", it was first discovered among chimpanzees from the Taï Forest in Côte d'Ivoire, Africa, in 1994. Necropsies showed blood within the heart was brown, no obvious marks were seen on the organs, and one necropsy showed lungs filled with blood. Studies of tissue taken from the chimpanzees showed results similar to human cases during the 1976 Ebola outbreaks in Zaire and Sudan. As more dead chimpanzees were discovered, many tested positive for Ebola using molecular techniques. Experts believed the source of the virus was the meat of infected Western Red Colobus monkeys, upon which the chimpanzees preyed. One of the scientists performing the necropsies on the infected chimpanzees contracted Ebola. She developed symptoms similar to those of dengue fever approximately a week after the necropsy and was transported to Switzerland for treatment. She was discharged from the hospital

after two weeks and had fully recovered six weeks after the infection.¹²

Bundibugyo Ebolavirus: On 24 November 2007, the Uganda Ministry of Health confirmed an outbreak of Ebolavirus in the Bundibugyo District. After confirmation of samples tested by the United States National Reference Laboratories and the CDC, the World Health Organization confirmed the presence of the new species. On 20 February 2008, the Uganda Ministry officially announced the end of the epidemic in Bundibugyo, with the last infected person discharged on 8 January 2008. An epidemiological study conducted by WHO and Uganda Ministry of Health scientists determined there were 116 confirmed and probable cases of the new Ebola species, and that the outbreak had a mortality rate of 34% (39 deaths). In 2012, there was an outbreak of Bundibugyo Ebolavirus in a northeastern province of the Democratic Republic of the Congo. There were 15 confirmed cases and 10 fatalities.¹³

Ebola virus disease or Ebola hemorrhagic fever is the human disease caused by the Ebola virus. Symptoms typically start two days to three weeks after contracting the virus, with a fever, sore throat, muscle pains and headaches. Typically nausea, vomiting, and diarrhea follow, along with decreased functioning of the liver and kidneys. At this point, some people begin to have bleeding problems. The virus may be acquired upon contact with blood or bodily fluids of an infected animal (commonly monkeys or fruit bats). Spread through the air has not been documented in the natural environment. Fruit bats are believed to carry and spread the virus without being affected. Once human infection occurs, the disease may spread between people as well. Male survivors may be able to transmit the disease via semen for nearly two months. In order to make the diagnosis, typically other diseases with similar symptoms such as malaria, cholera and other viral hemorrhagic fevers are first excluded. To confirm the diagnosis blood samples are tested for viral antibodies, viral RNA or the virus itself. Prevention includes decreasing the spread of disease from infected monkeys and pigs to humans. This may be done by checking such animals for infection and killing and properly disposing of the bodies if the disease is discovered. Properly cooking meat and wearing protective clothing when handling meat may also be helpful, as are wearing protective clothing and washing hands when around a person with the disease. Samples of bodily fluids and tissues from people with the disease should be handled with special caution. There is no specific treatment for the disease; efforts to help persons who are infected include

giving either oral rehydration therapy (slightly sweet and salty water to drink) or intravenous fluids. The disease has high mortality rate: often killing between 50% and 90% of those infected with the virus. This was first identified in Sudan and the Democratic Republic of the Congo. The disease typically occurs in outbreaks in tropical regions of Sub-Saharan Africa. From 1976 through 2013, fewer than 1,000 people per year have been infected. The largest outbreak to date is the ongoing 2014 West Africa Ebola outbreak, which is affecting Guinea, Sierra Leone, Liberia and likely Nigeria. As of August 2014 more than 1600 cases have been identified. Efforts are ongoing to develop a vaccine; however, none yet exists.¹⁴

Symptoms of Ebola

Signs and symptoms of Ebola usually begin suddenly with a flu-like stage characterized by fatigue, fever, headaches and joint, muscle and abdominal pain. Vomiting, diarrhea and loss of appetite are also common. Less common symptoms include the following: sore throat, chest pain, hiccups, shortness of breath and trouble swallowing. The average time between contracting the infection and the start of symptoms is 8 to 10 days, but it can vary between 2 and 21 days. Skin manifestations may include a maculopapular rash. Early symptoms of Ebola virus may be similar to those of malaria, dengue fever or other tropical fevers, before the disease progresses to the bleeding phase.¹⁵

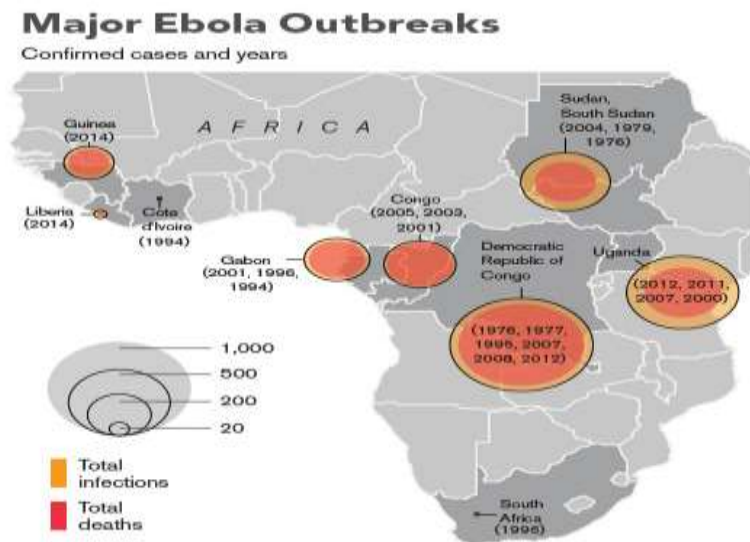


Figure-6: Ebola Virus Outbreaks

Bleeding: In the bleeding phase, internal and subcutaneous bleeding may present itself through reddening of the eyes and bloody vomit. Bleeding into the skin may create petechiae, purpura, ecchymoses and hematomas (especially around needle injection sites). All people infected show some symptoms of circulatory system involvement, including impaired blood clotting. Bleeding from puncture sites and mucous membranes (e.g. gastrointestinal tract, nose, vagina and gums) is reported in 40–50% of cases. Types of bleeding known to occur with Ebola virus disease include vomiting blood, coughing it up or blood in the stool. Heavy bleeding is rare and is usually confined to the gastrointestinal tract. In general, the development of bleeding symptoms often indicates a worse prognosis and this blood loss can result in death.¹⁶

Life cycles of the Ebolavirus: EVD is caused by four of five viruses classified in the genus *Ebola virus*, family *Filoviridae*, order *Mononegavirales*. These four viruses are Bundibugyo virus (BDBV), Ebola virus (EBOV), Sudan virus (SUDV), Tai Forest virus (TAFV). The fifth virus, Reston virus (RESTV), is not thought to be disease-causing in humans. During an outbreak, those at highest risk are health care workers and close contacts of those with the infection.

Transmission: It is not entirely clear how Ebola is spread. EVD is believed to occur after an Ebola virus is transmitted to an initial human by contact with an infected animal's body fluids. Human-to-human transmission can occur via direct contact with blood or bodily fluids from an infected person (including embalming of an infected dead person) or by contact with contaminated medical equipment, particularly needles and syringes. Semen is infectious in survivors for up to

50 days. Transmission through oral exposure and through conjunctiva exposure is likely and has been confirmed in non-human primates. The potential for widespread EVD infections is considered low as the disease is only spread by direct contact with the secretions from someone who is showing signs of infection. The quick onset of symptoms makes it easier to identify sick individuals and limits a person's ability to spread the disease by traveling. Because dead bodies are still infectious, some doctors disposed of them in a safe manner, despite

local traditional burial rituals. Bats drop partially eaten fruits and pulp, then land mammals such as gorillas and duikers feed on these fallen fruits. This chain of events forms a possible indirect means of transmission from the natural host to animal populations, which has led to research towards viral shedding in the saliva of bats. Fruit production, animal behavior and other factors vary at different times and places that may trigger outbreaks among animal populations.¹⁷

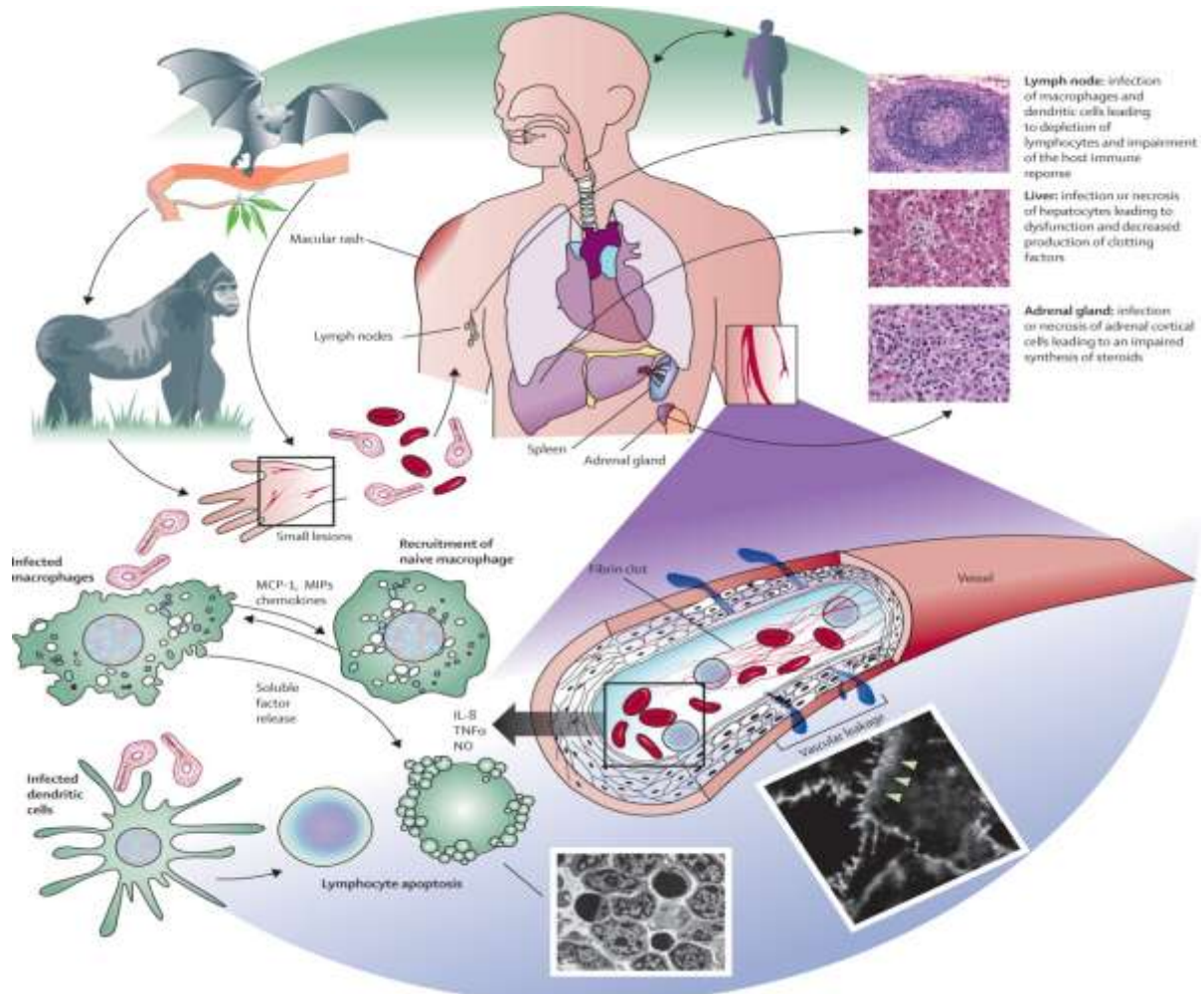


Figure-7: Life cycle of Ebola Virus

Reservoir: Bushmeat being prepared for cooking in Ghana, 2013. Human consumption of equatorial animals in Africa in the form of bushmeat has been linked to the transmission of diseases to people, including Ebola. Bats are considered the most likely natural reservoir of the Ebola virus (EBOV); plants, arthropods and birds have also been considered. Bats were known to reside in the cotton factory in which the first cases for the 1976 and 1979 outbreaks were employed and they have also been implicated in Marburg virus infections in 1975 and 1980. Of 24 plant species and 19 vertebrate species experimentally inoculated with

EBOV, only bats became infected. The absence of clinical signs in these bats is characteristic of a reservoir species. In a 2002–2003 survey of 1,030 animals including 679 bats from Gabon and the Republic of the Congo, 13 fruit bats were found to contain EBOV RNA fragments. As of 2005, three types of fruit bats (*Hypsignathus monstrosus*, *Epomops franqueti* and *Myonycteris torquata*) have been identified as being in contact with EBOV. They are now suspected to represent the EBOV reservoir hosts. Antibodies against Ebola Zaire and Reston viruses have been found in fruit bats in Bangladesh, thus identifying potential virus hosts and signs of the filoviruses in

Asia.¹⁸ Between 1976 and 1998, in 30,000 mammals, birds, reptiles, amphibians, and arthropods sampled from outbreak regions, no *Ebolavirus* was detected apart from some genetic traces found in six rodents (*Mus setulosus* and *Praomys*) and one shrew (*Sylvisorex ollula*) collected from the Central African Republic. Traces of EBOV were detected in the carcasses of gorillas and chimpanzees during outbreaks in 2001 and 2003, which later became the source of human infections. However, the high lethality from infection in these species makes them unlikely as a natural reservoir. Transmission between natural reservoir and humans is rare and outbreaks are usually traceable to a single case where an individual has handled the carcass of gorilla, chimpanzee, or duiker. Fruit bats are also eaten by people in parts of West Africa where they are smoked, grilled or made into a spicy soup. Like all mono negaviruses, Ebolavirions contain linear nonsegmented, single-strand, non-infectious RNA genomes of negative polarity that

possesses inverse-complementary 3' and 5' termini, do not possess a 5' cap, are not polyadenylated, and are not covalently linked to a protein. Ebola virus genomes are approximately 19 kilobase pairs long and contain seven genes in the order 3'-UTR-NP-VP35-VP40-GP-VP30-VP24-L-5'-UTR. The genomes of the five different Ebolaviruses (BDBV, EBOV, RESTV, SUDV and TAFV) differ in sequence and the number and location of gene overlaps.¹⁹

Structure: Like all filo viruses, Ebola virions are filamentous particles that may appear in the shape of a shepherd's crook or in the shape of a "U" or a "6" and they may be coiled, toroid or branched. In general, Ebola virions are 80 nm in width, but vary somewhat in length. In general, the median particle length of Ebola viruses ranges from 974 to 1,086 nm (in contrast to marburgvirions, whose median particle length was measured at 795–828 nm), but particles as long as 14,000 nm have been detected in tissue culture.²⁰



Figure-8: Hazards by Ebola Virus

Pathogenesis: Endothelial cells, mononuclear phagocytes and hepatocytes are the main targets of infection. After infection, a secreted glycoprotein (sGP) known as the Ebola virus glycoprotein (GP) is synthesized. Ebola replication overwhelms protein synthesis of infected cells and

host immune defenses. The GP forms a trimeric complex, which binds the virus to the endothelial cells lining the interior surface of blood vessels. The sGP forms a dimeric protein that interferes with the signaling of neutrophils, a type of white blood cell, which allows the virus to evade the

immune system by inhibiting early steps of neutrophil activation. These white blood cells also serve as carriers to transport the virus throughout the entire body to places such as the lymph nodes, liver, lungs and spleen.²¹ The presence of viral particles and cell damage resulting from budding causes the release of cytokines (to be specific, TNF- α , IL-6, IL-8, etc.), which are the signaling molecules for fever and inflammation. The cytopathic effect, from infection in the endothelial cells, results in a loss of vascular integrity. This loss in vascular integrity is furthered with synthesis of GP, which reduces specific integrins responsible for cell adhesion to the inter-cellular structure, and damage to the liver, which leads to coagulopathy.

Replication: The Ebola virus life cycle begins with virion attachment to specific cell-surface receptors, followed by fusion of the virion envelope with cellular membranes and the concomitant release of the virus nucleocapsid into the cytosol. The viral RNA polymerase, encoded by the L gene, partially uncoats the nucleocapsid and transcribes the genes into positive-strand mRNAs, which are then translated into structural

and nonstructural proteins. Ebola virus RNA polymerase (L) binds to a single promoter located at the 3' end of the genome. Transcription either terminates after a gene or continues to the next gene downstream. This means that genes close to the 3' end of the genome are transcribed in the greatest abundance, whereas those toward the 5' end are least likely to be transcribed. The gene order is, therefore, a simple but effective form of transcriptional regulation. The most abundant protein produced is the nucleoprotein, whose concentration in the cell determines when L switches from gene transcription to genome replication. Replication results in full-length, positive-strand antigenomes that are, in turn, transcribed into negative-strand virus progeny genome copy. Newly synthesized structural proteins and genomes self-assemble and accumulate near the inside of the cell membrane. Virions bud off from the cell, gaining their envelopes from the cellular membrane they bud from. The mature progeny particles then infect other cells to repeat the cycle. The Ebola Virus genetics are difficult to study due to its virulent nature.²²

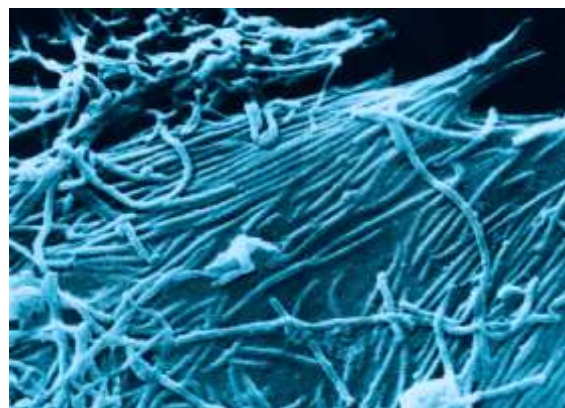


Figure-9: Ebola Virus Virion

Diagnosis: The medical history, especially travel and work history along with exposure to wildlife are important to suspect the diagnosis of EVD. The diagnosis is confirmed by isolating the virus, detecting its RNA or proteins, or detecting antibodies against the virus in a person's blood. Isolating the virus by cell culture, detecting the viral RNA by polymerase chain reaction (PCR) and detecting proteins by enzyme-linked immunosorbent assay (ELISA) is effective early and in those who have died from the disease. Detecting antibodies against the virus is effective late in the disease and in those who recover. During an outbreak, virus isolation is often not feasible. The most common diagnostic methods are therefore real time PCR and ELISA detection of proteins, which can be performed in field or mobile

hospitals. Filovirions can be seen and identified in cell culture by electron microscopy due to their unique filamentous shapes, but electron microscopy cannot tell the difference between the various filoviruses despite there being some length differences. Phylogenetic tree comparing the Ebolavirus and Marburgvirus. Numbers indicate percent confidence of branches.²³ The genera *Ebolavirus* and *Marburgvirus* were originally classified as the species of the now-obsolete *Filovirus* genus. In March 1998, the Vertebrate Virus Subcommittee proposed in the International Committee on Taxonomy of Viruses (ICTV) to change the *Filovirus* genus to the *Filoviridae* family with two specific genera: *Ebola-like viruses* and *Marburg-like viruses*. This proposal was implemented in

Washington, DC, on April 2001 and in Paris on July 2002. In 2000, another proposal was made in Washington, D.C., to change the "-like viruses" to "-virus" resulting in today's *Ebolavirus* and *Marburgvirus*. Rates of genetic change are 100 times slower than influenza A in humans, but on the same magnitude as those of hepatitis B. Extrapolating backwards using these rates indicates that *Ebolavirus* and *Marburgvirus* diverged several thousand years ago. However, paleoviruses (genomic fossils) of filoviruses (Filoviridae) found in mammals indicate that the family itself is at least tens of millions of years old. Fossilized viruses that are closely related to *Ebolaviruses* have been found in the genome of the Chinese hamster.²⁴

Differential diagnosis: The symptoms of EVD are similar to those of Marburg virus disease. It can

also easily be confused with many other diseases common in Equatorial Africa such as other viral hemorrhagic fevers, falciparum malaria, typhoid fever, shigellosis, rickettsial diseases such as typhus, cholera, gram-negative septicemia, borreliosis such as relapsing fever or EHEC enteritis. Other infectious diseases that should be included in the differential diagnosis include the following: leptospirosis, scrub typhus, plague, Q fever, candidiasis, histoplasmosis, trypanosomiasis, visceral leishmaniasis, hemorrhagic smallpox, measles, and fulminant viral hepatitis. Non-infectious diseases that can be confused with EVD are acute promyelocytic leukemia, hemolytic uremic syndrome, snake envenomation, clotting factor deficiencies/platelet disorders, thrombotic thrombocytopenic purpura, hereditary hemorrhagic telangiectasia, Kawasaki disease, and even warfarin poisoning.²⁵

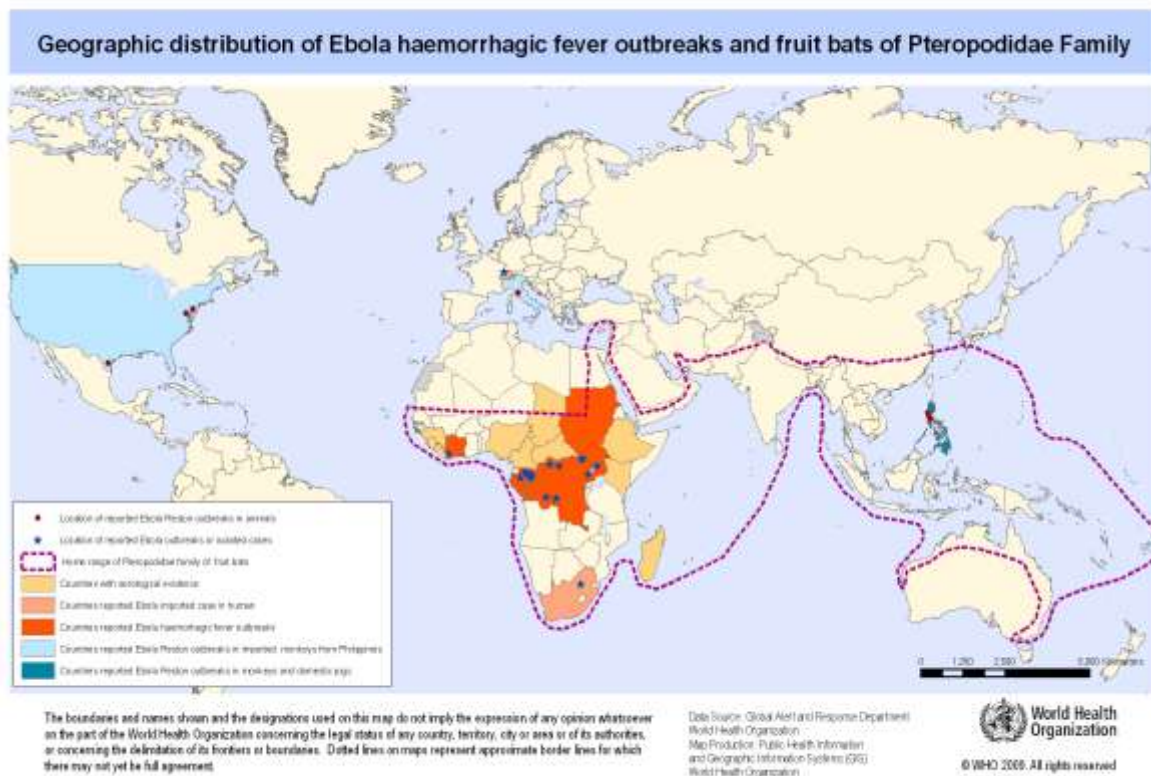


Figure-10: Ebola Virus prone regions

Prevention: A researcher working with the Ebola virus while wearing a BSL-4 positive pressure suit to avoid infection Behavioral changes. Ebola viruses are contagious, with prevention predominantly involving behavior changes, proper full-body personal protective equipment, and disinfection. Techniques to avoid infection involve not contacting infected blood or secretions, including from those who are dead. This involves suspecting and diagnosing the disease early and using standard precautions for all patients in the

healthcare setting. Recommended measures when caring for those who are infected include isolating them, sterilizing equipment, and wearing protective clothing including masks, gloves, gowns and goggles. Hand washing is important but can be difficult in areas where there is not even enough water for drinking.

Due to lack of proper equipment and hygienic practices, large-scale epidemics have occurred mostly in poor, isolated areas without modern hospitals or well-educated medical staff.

Traditional burial rituals, especially those requiring embalming of bodies, should be discouraged or modified. Airline crews who fly to these areas of the world are taught to identify Ebola and isolate anyone who has symptoms.²⁶

Quarantine: Quarantine, also known as enforced isolation, is usually effective in decreasing

spread. Governments often quarantine areas where the disease is occurring or individuals who may be infected. In the United States the law allows quarantine of those infected with Ebola. The lack of roads and transportation may help slow the disease in Africa. During the 2014 outbreak Liberia closed schools.²⁷



Figure-11: Vaccine of Ebola Virus

Vaccine: No vaccine is currently available for humans. The most promising candidates are DNA vaccines or vaccines derived from adenoviruses, vesicular stomatitis Indiana virus (VSIV) or filovirus-like particles

(VLPs) because these candidates could protect nonhuman primates from Ebolavirus-induced disease. DNA vaccines, adenovirus-based vaccines, and VSIV-based vaccines have entered clinical trials.²⁸

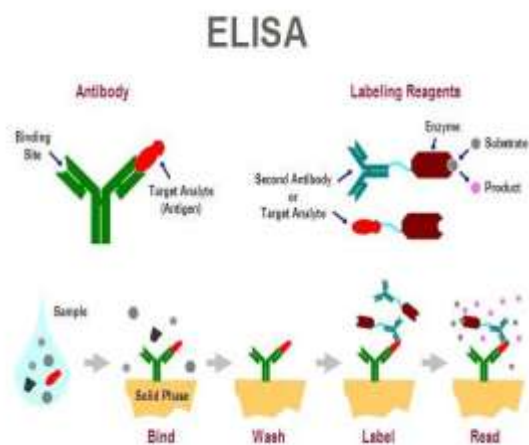


Figure-12: Culture of Ebola Virus

Vaccines have protected nonhuman primates. Immunization takes six months, which impedes the counter-epidemic use of the vaccines. Searching for a quicker onset of effectiveness, in 2003 a vaccine using an adenoviral (ADV) vector carrying the Ebola spike protein was tested on crab-eating macaques. Twenty-eight days later they were challenged with the virus and remained resistant. A vaccine based on attenuated recombinant vesicular stomatitis virus (VSV) vector carrying either the Ebola glycoprotein or the Marburg glycoprotein in

2005 protected nonhuman primates, opening clinical trials in humans. The study by October completed the first human trial, over three months giving three vaccinations safely inducing an immune response. Individuals for a year were followed, and, in 2006, a study testing a faster-acting, single-shot vaccine began; this new study was completed in 2008. Trying the vaccine on a strain of Ebola that more resembles one that infects humans is the next step. On 6 December 2011, the development of a successful vaccine against Ebola

for mice was reported. Unlike the predecessors, it can be freeze-dried and thus stored for long periods in wait for an outbreak. An experimental vaccine made by researchers at Canada's national laboratory in Winnipeg was used in 2009 to preemptively treat a German scientist who might have been infected during a lab accident. However,

actual EBOV infection could never be demonstrated without a doubt. Experimentally, recombinant vesicular stomatitis Indiana virus (VSIV) expressing the glycoprotein of EBOV or SUDV has been used successfully in nonhuman primate models as post-exposure prophylaxis.²⁹



Figure-13: Serology of Ebola Virus

Laboratory: Ebola viruses are World Health Organization Risk Group 4 pathogens, requiring biosafety level 4-equivalent containment. Laboratory researchers must be properly trained in BSL-4 practices and wear proper personal protective equipment.³⁰

Treatment: A hospital isolation ward in Gulu, Uganda, during the October 2000 outbreak. No Ebolavirus-specific treatment exists. Treatment is primarily supportive in nature and includes

minimizing invasive procedures, balancing fluids and electrolytes to counter dehydration, administration of anticoagulants early in infection to prevent or control disseminated intravascular coagulation, administration of procoagulants late in infection to control bleeding, maintaining oxygen levels, pain management, and the use of medications to treat bacterial or fungal secondary infections. Early treatment may increase the chance of survival. A number of experimental treatments are being studied.³¹



Figure-14: Medical Emergency of Viral attack

Prognosis: The disease has a high mortality rate: often between 50 percent and 90 percent. If an infected person survives, recovery may be quick and complete. Prolonged cases are often complicated by the occurrence of long-term problems, such as inflammation of the testicles, joint pains, muscle pains, skin peeling,

or hair loss. Eye symptoms, such as light sensitivity, excess tearing, iritis, iridocyclitis, choroiditis and blindness have also been described. EBOV and SUDV may be able to persist in the semen of some survivors for up to seven weeks, which could give rise to infections and disease via sexual intercourse.³²

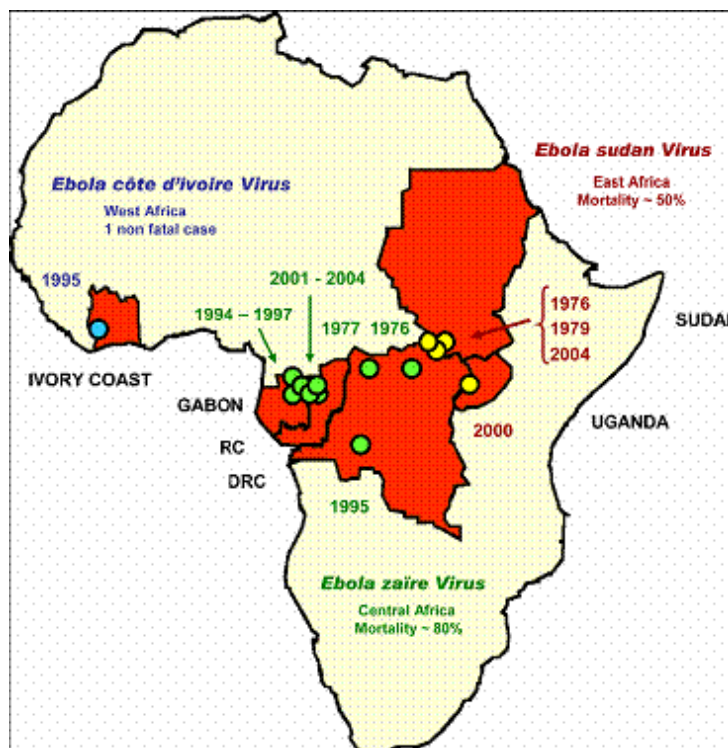


Figure-15: Epidemiology of Ebola Virus

Epidemiology: CDC worker incinerates medical waste from Ebola patients in Zaire in 1976. The disease typically occurs in outbreaks in tropical regions of Sub-Saharan Africa. From 1976 (when it was first identified) through 2013, fewer than 1,000 people per year have been infected. The largest outbreak to date is the ongoing 2014 West Africa Ebola outbreak, which is affecting Guinea, Sierra Leone and Liberia. As of August 2014 it is also affecting Nigeria. As of the end of July 2014 more than 1320 cases have been identified.³³

2007 to 2011: As of 30 August 2007, 103 people (100 adults and three children) were infected by a suspected hemorrhagic fever outbreak in the village of Kumpungu, Democratic Republic of the Congo. The outbreak started after the funerals of two village chiefs, and 217 people in four villages fell ill. The World Health Organization sent a team to take blood samples for analysis and confirmed that many of the cases were the result of *Ebolavirus*. The Congo's last major Ebola epidemic killed 245 people in 1995 in Kikwit,

about 200 miles (320 km) from the source of the August 2007 outbreak.³⁴

2014 outbreak: Increase over time in the cases and deaths during the 2014 outbreak. In March 2014, an outbreak of the Ebola virus occurred in the Western African nation of Guinea. This is the first Ebola virus outbreak registered in the region. As of 10 April, 157 suspected and confirmed cases and 101 deaths had been reported in Guinea, 22 suspected cases in Liberia including 14 deaths, 8 suspected cases in Sierra Leone including 6 deaths, and 1 suspected case in Mali. By late June 2014, the death toll had reached 390 with over 600 cases reported. By 23 July 2014, the World Health Organization had reported 1201 confirmed cases including 672 deaths since the epidemic began in March. On 31 July 2014, WHO reports the death toll has reached 826 from 1440 cases. Emory University Hospital was the first US hospital to care for people exposed to Ebola. Two American medical providers, Kent Brantly and Nancy Writebol, were exposed while treating infected patients in Liberia. Arrangements were made for them to be transported to Emory via speciality

aircraft. Emory Hospital has a specially built isolation unit set up in collaboration with the CDC to treat people exposed to certain serious infectious diseases. On 2 August 2014, Brantly was flown in to Dobbins Air Force Base in Marietta, Georgia, and transferred to Emory Hospital. On 8th August 2014, The World Health Organization (WHO) declared the Ebola outbreak in West Africa to be an international public health emergency that requires an extraordinary response

to stop its spread. WHO announced the Ebola outbreak the largest and longest in history is worrying enough to merit being declared an international health emergency. WHO declared similar emergencies for the swine flu pandemic in 2009 and for polio in May 2014. Ebola virus can be transmitted to dogs and pigs. While dogs may be asymptomatic, pigs tend to develop symptomatic disease.³⁵



Figure-16: Research on Ebola Virus

Research

Medications: Favipiravir looks like it may be useful in a mouse model of the disease. Estrogen receptor drugs used to treat infertility and breast cancer (clomiphene and toremifene) inhibit the progress of Ebola virus in infected mice. Ninety percent of the mice treated with clomiphene and fifty percent of those treated with toremifene survived the tests. Given their oral availability and history of human use, these drugs would be candidates for treating Ebola virus infection in remote geographical locations, either on their own or together with other antiviral drugs.³⁶

Antibodies: Researchers looking at slides of cultures of cells that make monoclonal antibodies.

These are grown in a lab and the researchers are analyzing the products to select the most promising of them.³⁷ During an outbreak 1999 in the Democratic Republic of the Congo, seven of eight people who received blood transfusions from individuals who had previously survived the infection survived themselves. However, this potential treatment is considered controversial. Intravenous antibodies appear to be protective in non-human primates who have been exposed to large doses of Ebola. On July 31, 2014, an experimental drug, ZMapp, was first tested on humans. It was administered to two Americans who had been infected with Ebola. Both people appeared to have had positive results.³⁸

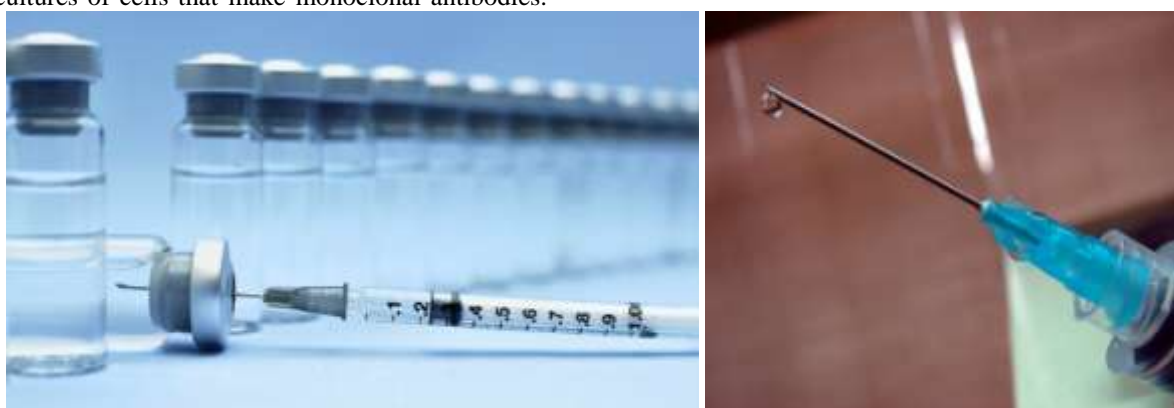


Figure-17: Ebola Virus Vaccine

Other treatments: Other promising treatments rely on antisense technology. Both small interfering

RNAs (siRNAs) and phosphorodiamidate morpholino oligomers (PMOs) targeting the Zaire

Ebola virus (ZEBOV) RNA polymerase L protein could prevent disease in nonhuman primates. TKM-Ebola is a small-interfering RNA compound, currently tested in a phase I clinical trial in people.^{39,40}

Conclusion

Ebola virus disease (EVD), formerly known as Ebola haemorrhagic fever, is a severe, often fatal illness in humans. EVD outbreaks have a case fatality rate of up to 90%. EVD outbreaks occur primarily in remote villages in Central and West Africa, near tropical rainforests. The virus is transmitted to people from wild animals and spreads in the human population through human-to-human transmission. Fruit bats of the *Pteropodidae* family are considered to be the natural host of the Ebola virus. Severely ill patients require intensive supportive care. No licensed specific treatment or vaccine is available for use in people or animals. Ebola first appeared in 1976 in 2 simultaneous outbreaks, in Nzara, Sudan, and in Yambuku, Democratic Republic of Congo. The latter was in a village situated near the Ebola River, from which the disease takes its name. Genus Ebolavirus is 1 of 3 members of the *Filoviridae* family (filovirus), along with genus Marburgvirus and genus Cuevavirus. Genus Ebolavirus comprises 5 distinct species:

Sudan Ebolavirus, Reston Ebolavirus, Tai Forest ebolavirus, Bundibugyo Ebolavirus, Reston ebolavirus BDBV, EBOV, and SUDV have been associated with large EVD outbreaks in Africa, whereas RESTV and TAFV have not. The RESTV species, found in Philippines and the People's Republic of China, can infect humans, but no illness or death in humans from this species has been reported to date. Ebola is introduced into the human population through close contact with the blood, secretions, organs or other bodily fluids of infected animals. In Africa, infection has been documented through the handling of infected chimpanzees, gorillas, fruit bats, monkeys, forest antelope and porcupines found ill or dead or in the rainforest. Ebola then spreads in the community through human-to-human transmission, with infection resulting from direct contact (through broken skin or mucous membranes) with the blood, secretions, organs or other bodily fluids of infected people, and indirect contact with environments contaminated with such 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. Men who have recovered from the disease can still transmit the virus through their semen for up to 7 weeks after recovery from illness. 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. Among workers in contact with monkeys or pigs infected with Reston ebolavirus, several infections have been documented in people who were clinically asymptomatic. Thus, RESTV appears less capable of causing disease in humans than other Ebola species. However, the only available evidence available comes from healthy adult males. It would be premature to extrapolate the health effects of the virus to all population groups, such as immuno-compromised persons, persons with underlying medical conditions, pregnant women and children. More studies of RESTV are needed before definitive conclusions can be drawn about the pathogenicity and virulence of this virus in humans.

Signs and symptoms: EVD is a severe acute viral illness often characterized by the sudden onset of fever, intense weakness, muscle pain, headache and sore throat. This is followed by vomiting, diarrhoea, rash, impaired kidney and liver function, and in some cases, both internal and external bleeding. Laboratory findings include low white blood cell and platelet counts and elevated liver enzymes. People are infectious as long as their blood and secretions contain the virus. Ebola virus was isolated from semen 61 days after onset of illness in a man who was infected in a laboratory. The incubation period, that is, the time interval from infection with the virus to onset of symptoms, is 2 to 21 days.

Diagnosis: Other diseases that should be ruled out before a diagnosis of EVD can be made include: malaria, typhoid fever, shigellosis, cholera, leptospirosis, plague, rickettsiosis, relapsing fever, meningitis, hepatitis and other viral haemorrhagic fevers. Ebola virus infections can be diagnosed definitively in a laboratory through several types of tests:

1. antibody-capture enzyme-linked immunosorbent assay (ELISA)
2. antigen detection tests
3. serum neutralization test
4. reverse transcriptase polymerase chain reaction (RT-PCR) assay
5. electron microscopy
6. virus isolation by cell culture.

Samples from patients are an extreme biohazard risk; testing should be conducted under maximum biological containment conditions.

Vaccine and treatment: No licensed vaccine for EVD is available. Several vaccines are being tested, but none are available for clinical use. Severely ill patients require intensive supportive care. Patients are frequently dehydrated and require oral rehydration with solutions containing electrolytes or intravenous fluids. No specific

treatment is available. New drug therapies are being evaluated.

Natural host of Ebola virus: In Africa, fruit bats, particularly species of the genera *Hypsignathus monstrosus*, *Epomops franqueti* and *Myonycteris torquata*, are considered possible natural hosts for Ebola virus. As a result, the geographic distribution of Ebolaviruses may overlap with the range of the fruit bats.

Ebola virus in animals: Although non-human primates have been a source of infection for humans, they are not thought to be the reservoir but rather an accidental host like human beings. Since 1994, Ebola outbreaks from the EBOV and TAFV species have been observed in chimpanzees and gorillas. RESTV has caused severe EVD outbreaks in macaque monkeys (*Macaca fascicularis*) farmed in Philippines and detected in monkeys imported into the USA in 1989, 1990 and 1996, and in monkeys imported to Italy from Philippines in 1992. Since 2008, RESTV viruses have been detected during several outbreaks of a deadly disease in pigs in People's Republic of China and Philippines. Asymptomatic infection in pigs has been reported and experimental inoculations have shown that RESTV cannot cause disease in pigs.

Prevention and control: No animal vaccine against RESTV is available. Routine cleaning and disinfection of pig or monkey farms (with sodium hypochlorite or other detergents) should be effective in inactivating the virus. If an outbreak is suspected, the premises should be quarantined immediately. Culling of infected animals, with close supervision of burial or incineration of carcasses, may be necessary to reduce the risk of animal-to-human transmission. Restricting or banning the movement of animals from infected farms to other areas can reduce the spread of the disease. As RESTV outbreaks in pigs and monkeys have preceded human infections, the establishment of an active animal health surveillance system to detect new cases is essential in providing early warning for veterinary and human public health authorities.

Reducing the risk of Ebola infection in people: In the absence of effective treatment and a human vaccine, raising awareness of the risk factors for Ebola infection and the protective measures individuals can take is the only way to reduce human infection and death. In Africa, during EVD outbreaks, educational public health messages for risk reduction should focus on several factors: 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 in the community arising from direct or close contact with infected patients, particularly with their bodily fluids. Close physical contact with Ebola patients should be avoided. 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. Communities affected by Ebola should inform the population about the nature of the disease and about outbreak containment measures, including burial of the dead. People who have died from Ebola should be promptly and safely buried. Pig farms in Africa can play a role in the amplification of infection because of the presence of fruit bats on these farms. Appropriate biosecurity measures should be in place to limit transmission. For RESTV, educational public health messages should focus on reducing the risk of pig-to-human transmission as a result of unsafe animal husbandry and slaughtering practices, and unsafe consumption of fresh blood, raw milk or animal tissue. Gloves and other appropriate protective clothing should be worn when handling sick animals or their tissues and when slaughtering animals. In regions where RESTV has been reported in pigs, all animal products (blood, meat and milk) should be thoroughly cooked before eating.

Controlling infection in health-care settings: Human-to-human transmission of the Ebola virus is primarily associated with direct or indirect contact with blood and body fluids. Transmission to health-care workers has been reported when appropriate infection control measures have not been observed. It is not always possible to identify patients with EBV early because initial symptoms may be non-specific. For this reason, it is important that health-care workers apply standard precautions consistently with all patients – regardless of their diagnosis – in all work practices at all times. These include basic hand hygiene, respiratory hygiene, the use of personal protective equipment (according to the risk of 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, in addition to standard precautions, other infection control measures to avoid any exposure to the patient's blood and body fluids and direct unprotected contact with the possibly contaminated environment. When in close contact (within 1 metre) 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). Laboratory workers are also at risk. Samples taken from

suspected human and animal Ebola cases for diagnosis should be handled by trained staff and processed in suitably equipped laboratories.

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