

Research Article

Therapeutic Measures against the Current Virulent Endemicity of Ebola Virus

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Abstract - Given the current situation of the third and the worst outbreak of Ebola Virus disease in Africa and of course in the world, there have been rising concerns on the evolution of different strains of Ebola virus that have been discovered and isolated during these outbreaks. This enough is a compelling warning of impending more virulent strains future outbreaks with devastating consequences. In events that culminate in the loss of vascular integrity, release of cyclooxygenase II (COX II) which results in pain, diarrhea and even shock, Ebola virus 'engineers' the host cells to synthesize the virus' proteins into the body instead of the normal host proteins. Ebola virus attaches itself to the cell membrane and the viral RNA is released into the cytoplasm where it directs the synthesis of new viral proteins and genetic material. New viral genomes are rapidly coated in protein to create cores. Ebola virus RNA polymerase binds to a single promoter located at the 3' end of the genome. Genes close to the 3' end of the genome are transcribed in 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.

Keywords: Ebola virus; Vaccine; Antigen-antibody immune responses; Inoculation; Interferons; Gene

Introduction

A wise saying I once heard from Chief AfeBabalola (SAN) says "if you want to keep anything from a black man you just hide it in a book." Why? It is because we do not have a good disposition towards empirical application of theoretical principles in solving societal maladies and challenges. In the light of recent events surrounding the bioterrorism facing several West African countries including Sierra Leone, Guinea, Liberia and now spreading to Nigeria my country, I have developed a suggestive and an insightful quest into answering the insuperable challenge posed to us by the ravaging Ebola hemorrhagic viral disease.

I got livid by the way Africans seem helpless and are now turning to entreat the United States to salvage the situation which started in December 2013 in Guinea and has continued to spread for several months in which thousands have been infected and hundreds have died.

I hereby write to give my own little contribution on the possible way out of our current predicament. Nevertheless, the role of developed countries in salvaging the situation cannot be overemphasized in putting an end to this seemingly threat to humanity. Collective efforts are needed in providing funds to the affected countries in order to put stringent measures in place in curbing the spread.

In order to make this research work self-sufficient on the subject of matter, certain information have been extracted from reliable sources with necessary references given in the bibliography section provided at the end to credit others' work and contribution in order to avoid plagiarism.

Overview Of Ebola Hemorrhagic Viral Disease

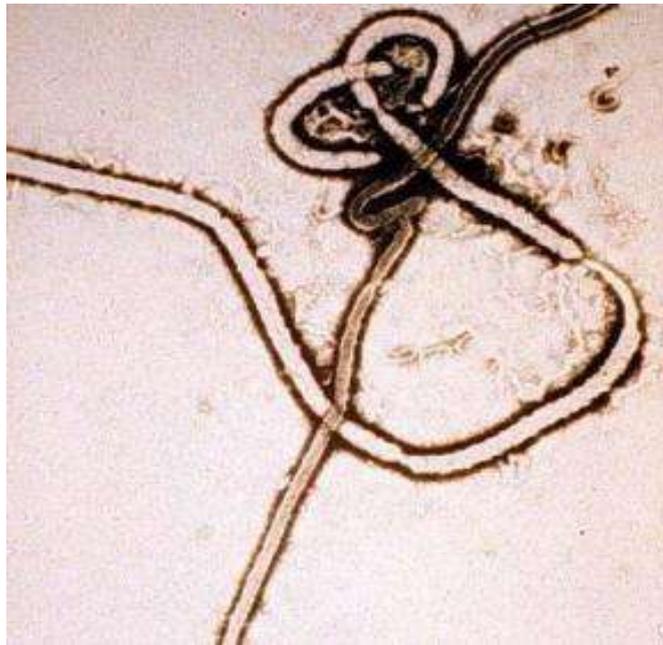
Ebola virus disease (EVD) is a severe viral disease (which is often fatal) that affects mammals, including humans. According to Baltimore classification system of viruses which is based on the mode of replication and genome type, Ebola virus can be placed in group 5 together with the negative-sense single-stranded RNA viruses.

- GENOME: Single stranded negative sense RNA
- Order: Mononegavirales
- Family: Filoviridae
- Genus: Ebola like viruses
- Species: Ebola

Genus Ebolavirus is 1 of 3 members of the *Filoviridae* family (filovirus), along with genus Marburgvirus and genus Cytomegalovirus.

Genus Ebolavirus comprises 5 distinct species:

1. Bundibugyoebolavirus (BDBV)
2. Zaire ebolavirus (EBOV)
3. Reston ebolavirus (RESTV)
4. Sudan ebolavirus (SUDV)
5. Tai Forest ebolavirus (TAFV).



Ebola Virus As Seen Under the Microscope

Bundibugyo Ebola virus, Zaire Ebola virus, and Sudan Ebola virus have been associated with large Ebola virus disease outbreaks in Africa, whereas Reston Ebola virus and Taï Forest Ebola virus have not. The Reston Ebola virus 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.

Epidemiology

The first documented case of Ebola virus was in 1976 in two simultaneous outbreaks at Nzara, and also at Yambuku in Sudan and the Democratic Republic of Congo respectively. The latter was in a village situated near the Ebola River, from which the disease takes its name.

Year	Country	Ebolavirus species	Cases	Deaths	Case Fatality
2012	Democratic Republic of Congo	Bundibugyo	57	29	51%
2012	Uganda	Sudan	7	4	57%
2012	Uganda	Sudan	24	17	71%
2011	Uganda	Sudan	1	1	100%
2008	Democratic Republic of Congo	Zaire	32	14	44%
2007	Uganda	Bundibugyo	149	37	25%
2007	Democratic Republic of Congo	Zaire	264	187	71%
2005	Congo	Zaire	12	10	83%
2004	Sudan	Sudan	17	7	41%
2003 (Nov-Dec)	Congo	Zaire	35	29	83%
2003 (Jan-Apr)	Congo	Zaire	143	128	90%
2001-2002	Congo	Zaire	59	44	75%
2001-2002	Gabon	Zaire	65	53	82%
2000	Uganda	Sudan	425	224	53%
1996	South Africa (ex-Gabon)	Zaire	1	1	100%
1996 (Jul-Dec)	Gabon	Zaire	60	45	75%
1996 (Jan-Apr)	Gabon	Zaire	31	21	68%
1995	Democratic Republic of Congo	Zaire	315	254	81%
1994	Cote d'Ivoire	Taï Forest	1	0	0%
1994	Gabon	Zaire	52	31	60%
1979	Sudan	Sudan	34	22	65%
1977	Democratic Republic of Congo	Zaire	1	1	100%
1976	Sudan	Sudan	284	151	53%
1976	Democratic Republic of Congo	Zaire	318	280	88%

Extracted from: <http://www.who.int/mediacentre/factsheets/fs103/en/>

The first known case, a 44-year old male instructor at the Yambuku Mission School, came to the Mission hospital on 26 August 1976 with a febrile illness felt due to malaria. He was given an injection of chloroquine at the dispensary. The fever dropped and remained normal over the next four days but rose to 39.2° on 1 September. The typical syndrome evolved from that day and he died on 8 September with severe haemorrhage. From the 1st of September to 24th of October, there were 318 cases resulting in 280 deaths, the epidemic peaked during the fourth week and then receded somewhat more gradually over the next 5 weeks. Date of symptom onset was not available for about 10% of cases.

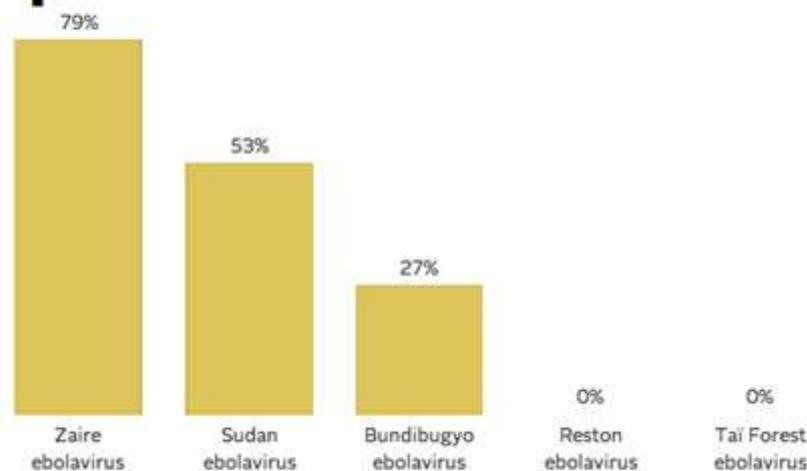
Fifty-five villages of less than 5,000 persons had cases. All infected villages in the epidemic area were within 60 km of Yambuku. This area includes about 100 villages. The larger towns of Abumombazi and Bumba, about 100 km to the north and south, respectively, had imported cases, as did Kinshasa, 1 100 km to the south-west. The large majority of affected villages were along roads running east and west of Yambuku, along which were located more villages than the north-south road. About 43 of 73 villages

in the Yandongi collectivity were affected. This collectivity had an attack rate of 8.0 cases per 1,000 persons.

The epidemic spread was relatively slow in the epidemic area during the initial outbreak. Within the first two weeks after the onset, the epidemic cases were occurring no further than 30 km from Yambuku. Almost another two weeks passed before a sick nursing sister was evacuated to Kinshasa. It was over a month until cases were imported into Abumombazi and Bumba. The mean duration of active disease was 26 days per locality and ranged from 1 to 55 days. At the Yambuku Mission Hospital, where all staff members contacted patients or instruments used for treating patients, 13 of 17 hospital employees acquired the disease and 11 died.

In Nigeria so far, about 21 cases have been recorded with 8 deaths, most of whom had primary contact with Patrick Sawyer (the Liberian that introduced the virus to Nigeria). The painful death of DrAmeyoAdadevoh and others left an imprint on the nation, forcing her to spring up fast and rise to the situation.

Death rates of the 5 Ebola virus species



However, the fact that the health sector in Africa generally is relatively poor and not yet at its best is consequent of the rapid spread of the disease from the origin in Democratic Republic of Congo to other west African countries without being contained till date. This has also led to the frequent infection of health workers owing to lack or inadequacy of preventive clothings.

Incubation Period

This is the interval between invasion of the body by an infecting organism and the appearance of the first sign or symptom it causes. Symptoms typically start two days to

three weeks after contracting the virus, with a fever, sore throat, muscle pains, and headaches. Male survivors may still be able to transmit the disease via semen for nearly two months. Hence, it is possible to have been infected with the disease and not even know specifically during this period of incubation within which the virus weakens and even destroys the immune system.

Mode of Transmission

Ebola is introduced into the human population through close contact with the blood, secretions, organs or other body fluids of infected animals. In Africa, infection has

been documented through unprotected and indiscriminate handling of infected chimpanzees, gorillas, fruit bats, monkeys, forest antelopes and porcupines found ill or dead 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, sweat, semen, breast milk, salivary secretions, organs or other body fluids of infected people. Burial ceremonies in which mourners and embalmers have direct contact with the body of the deceased person can also play a crucial role in the transmission of Ebola Virus. Also, indirect contact with environments contaminated with such fluids (blood, sweat, semen, breast milk, salivary secretions).

Health-care workers have frequently been infected while treating patients with suspected or confirmed Ebola Virus Disease (EVD). This has occurred through close contacts with patients when infection control precautions are not strictly practiced or may occur as a result of mistakes in the line of caring for an infected patient (one of the health hazards in medical practices).

Among workers in contact with monkeys or pigs infected with Reston Ebola virus, several infections have been documented in people who were clinically asymptomatic. Thus, Reston Ebola virus appears less capable of causing disease in humans compared to other strains of the Ebola virus.^[2]

Airborne transmission has not been documented during previous EVD outbreaks. They are, however, infectious as breathable 0.8–1.2 micrometer laboratory generated droplets; because of this potential route of infection, these viruses have been classified as a Category A biological weapon (organisms that can be used purposefully weapons in bioterrorism or biological warfare [BW] and result in high mortality, cause major negative influence on public health, may cause public panic).

Thus, I state that Ebola Virus, from this laboratory micropipette experiment is very likely to become airborne as it mutates. Also, if an Ebola positive patient sneezes (vigorously), those who are close to such person at that point should be quarantined and closely monitored.

Recently the virus has been shown to travel without contact from pigs to non-human primates. However, it is not certain for how long this virus can stay outside the host environment and still be able to cause infection.

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 and human populations.

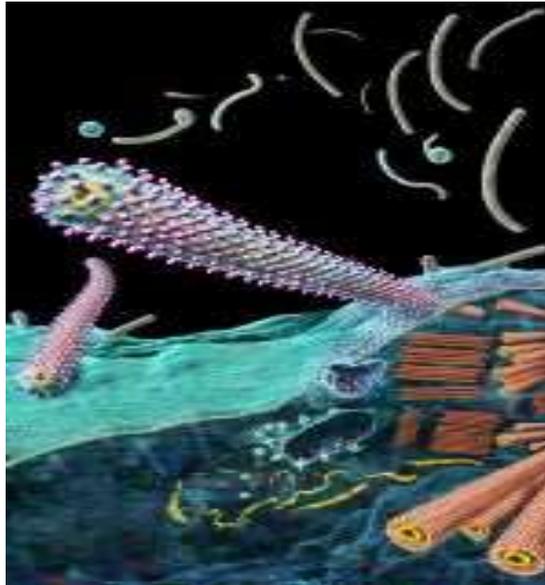
Reservoir

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 observed, and they have also been implicated in Marburg virus infections in 1975 and 1980. Of the 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*, *Epomopsfranqueti*, and *Myonycteristorquata*) have been identified as being in contact with EBOV. They are now suspected to represent the EBOV reservoir hosts.

Cycle of Events Inside Human Hosts

A good understanding of this is needed if a meaningful vaccine will be developed. The Ebolavirus life cycle begins with virion attachment to specific cell-surface receptors. This is 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. Ebolavirus RNA polymerase 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.



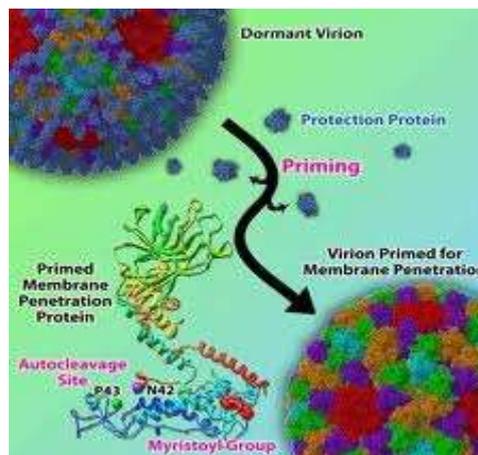
*Ebola virus 'engineers' the host cells to synthesize the virus' proteins into the body instead of the normal host proteins. Ebola virus attaches itself to the cell membrane at middle left. Viral RNA (yellow) is released into the cytoplasm where it directs the synthesis of new viral proteins and genetic material. New viral genomes are rapidly coated in protein to create cores. These viral cores pile up in the cell and migrate to the cell surface. Transmembrane proteins (purple) are produced which are conveyed to the cell surface. The cores push their way through the cell membrane, becoming enveloped in cell membrane and collecting their transmembrane proteins (spikes) as they do so. Courtesy **Russell Kightley Media**.*

The most abundant protein produced is the nucleoprotein, whose concentration in the cell determines when the virus' RNA polymerase switches from gene transcription to genome replication. Replication results into 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.

Once infected by the virus, it becomes active inside the human host and the endothelial cells, mononuclear phagocytes, and hepatocytes are the main targets of infection. After infection, a secreted glycoprotein (sGP) known as the Ebola virus glycoprotein (EGP) is synthesized. The EGP forms a trimeric complex, which binds the virus to the endothelial cells lining the interior surface of blood vessels.

The cytopathic effect, from infection in the endothelial cells, results in a loss of vascular integrity, hence, internal bleeding results, first from the tiny capillaries and then progresses to larger vessels which culminate in external bleeding. At this stage the clotting factors become affected due to decreased synthesis of the high molecular weight kinogen which initiates the intrinsic pathway in the coagulation cascade so blood does not clot inside (this is a possibility). This loss in vascular integrity continues with synthesis of EGP, which reduces specific integrins responsible for cell adhesion to the inter-cellular structure, and damage to the liver, which leads to coagulopathy.



Ebola replication overwhelms protein synthesis in infected cells of host immune defenses. The sGP forms a dimeric protein that interferes with the signaling of neutrophils (a type of the white blood cells) which allows the virus to evade the immune system by inhibiting the early stages 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. 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.

Clinical Manifestation

Early symptoms of Ebola virus disease include sudden onset of fever, weakness, muscle pain, headaches and a sore throat. These symptoms can appear two to 21 days after infection. These nonspecific and atypical early symptoms can be mistaken for signs of diseases such as malaria, typhoid fever, meningitis or even the plague.

The early symptoms progress to vomiting, diarrhea, impaired kidney and liver function and sometimes internal and external bleeding. Laboratory findings include low white blood cell and platelet counts and elevated liver enzymes.

Protective Guidelines That Should Be Strictly Observed

- **Gloves** - gloves should be worn when handling infected materials and sites. Conventional disposable non-sterile plastic gloves may be adequate. Long sleeved disposable gloves can be used for protection of the arms when treating infected patients and when necessary (during sports of fluid from patients).
- **Masks** - masks are necessary but when used they should be of the high efficiency filter type, which should provide protection for atleast 10-15 minutes.
- **Hands** - hand washing before and after contact with the patients is perhaps the most important measure in preventing the spread of infection. Either a non-medicated soap or a detergent antiseptic preparation should be adequate. 70% alcohol is more effective in removing transient as well as residual flora and should be used in such a high risk situation.
- **Bedpans and urinals** - gloves should be worn when handling bedpans and urinals. The contents should be disposed of directly into the sluice or bedpan disinfectant. The bedpan or urinal should then be heat disinfected and dried. A bedpan washer/disinfectant and a high temperature washing-up machine should be available in all isolation units.
- **Wastes** - all clinical wastes should be disposed in colour-coded bags for incineration.
- **Equipment** - disposable or autoclavable equipments should be used whenever possible. Essential items of patient care such as sphygmomanometers and stethoscopes should be left in the room and disinfected when the patient is discharged or before being used on another patient. Hard surfaces may be disinfected by wiping with a phenolic or hypochlorite solution. Other equipment may be disinfected by wiping with 70% alcohol. Sphygmomanometer
- **Gowns and aprons**- disposable aprons are recommended. Cotton gowns provide limited protection but are acceptable in lack of better ones. Gowns made of water-repellent material give better protection. The gown or apron should be left hanging in the room and changed daily or earlier when soiled. Although disposable aprons are preferable, non-disposable plastic aprons may be used and can be disinfected by formalin or alcohol due to financial and economic challenges in the affected countries or even burnt.

cuffs may be disinfected by low temperature steam.

- **Needles and syringes** - these should be disposable and placed in a hardened container which is sealed before disposal.
- **Linen** - Vigorous bed-making should be avoided. Linen from infected patients should be placed in a colour-coded linen bag for transfer to the laundry. Linen which may present a hazard to the laundry staff and so should first be sealed in labeled bags.
- **Crockery and cutlery** - disposable items may be used when a dishwasher heating the items to over 80°C is not available. Food should be placed in polythene bags and discarded with other wastes from the isolation units.
- **Laboratory specimens** - Containers and used materials should be placed in a biohazard bags.
- **Transporting patients** - patients should be sent to other departments only if it is essential to do so even in the isolation units. The department should be notified in advance so that they may take suitable measures to prevent the spread of infection. This is sometimes necessary for further investigations in the course of treatment.

Note: Under the Public Health Act 1936, the removal of the bodies believed to have died from highly infectious diseases such as Ebola Virus Disease from the hospital is prohibited, except for the purpose of being taken directly to a mortuary or being buried or cremated. **Cremation** is considered ideal for safe disposal of bodies of persons who have died from Ebola Virus Disease in order to minimize further transmission of infection. It involves the application of high temperature to reduce body organic components (cellular structures, tissues and organs) to basic chemical components (ashes).

Developing the Vaccine

There are relatively a lot of measures that can be taken against the Ebola virus outbreak some of which are mentioned in this work.

- One way is to render it harmless by interrupting the process which culminates in the synthesis of Ebola virus glycoprotein (GP) that forms a trimeric complex (which a drug can do), which then binds the virus to the endothelial cells lining the interior surface of blood vessels causing hemorrhage to occur.
- Interfering with the virus RNA polymerase chain reaction which causes the release of the virus nucleocapsid into the cytosol, hence partially uncoating the nucleocapsids and transcribing the genes into positive-

strand mRNAs, which are then translated into structural and nonstructural proteins of the virus.

- Termination of its life cycle at any possible point including prion removal before it acquires its full force in combating the host (which some drugs can do)
- Performing genome study and karyotyping to detect the gene responsible for rapid viral replication and developing a vaccine with that knowledge solely to trigger the antigen-antibody immune responses. However, going through this route may rather seem too ambiguous to comprehend and execute in certain instances.

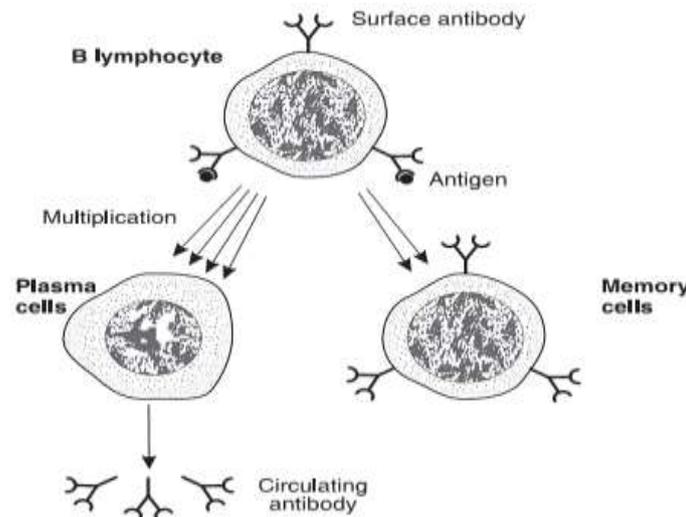
Symptomatic Treatment

I am of a strong opinion that Ebola virus, looking at the cycle of events inside human hosts and being a viral disease can be best tackled with a vaccine. The effectiveness of a potential vaccine with the recent massive outbreak should be reinforced with a well monitored symptomatic treatment involving minimizing invasive procedures (due to hemorrhage and coagulopathy), balancing fluids and electrolytes to counter dehydration, monitored administration of Vitamin K supplements, prevention of disseminated intravascular coagulation (in which there is uncontrolled activation of clotting factors and fibrinolytic enzymes throughout small blood vessels; fibrin is deposited, platelets and clotting factors are consumed, and fibrin degradation products inhibit fibrin polymerization, resulting in tissue necrosis and bleeding), administration of pro-coagulants late in infection to control bleeding, maintaining oxygen levels, pain management, and the use of medications to treat bacterial or fungal secondary (superimposed) infections. I also will advocate a calculated but notwithstanding low dose of Phylloquinone (Vitamin K) to keep the coagulopathy and hemorrhage in check during the period of illness because we are dealing with a hemorrhagic disease. Aspirin, which acts as anti-prostaglandin also produces anti-inflammatory effects in its overall anti-pyretic action (*though the fact that Acetylsalicylic acid [Aspirin] may worsen hemorrhage cannot be ruled out because it works as an anti-platelet at low dose*). Early treatment may increase the patient's chance of survival; however, it is also subject to a number of factors such as the amount of exposure to contaminated body fluids, etc. Since most patients die due to hypovolemic shock, it is better to control the level of body fluids and also give anti-shock promptly when the need arises at 20mls per kg in children over 30 to 60 minutes and constantly reviewing vital signs.

However it is an increasingly interesting fact to discover that the reservoir and natural hosts of Ebola virus (the fruit bats, (*Hypsignathus Monstrosus*, *Epomopsfranqueti*, and *Myonycteristorquata*) themselves do not get infected; the questions thence to ask is how do these creatures

protect themselves? A forensic or critical (laboratory) analysis of their serum could help identify the specific antibodies that are of crucial importance to their survival. Also, it should be noted that since some humans have survived the disease upon sensitive and specific

laboratory tests, thorough investigation to discover how the immune system was able to overcome will as well be of vital importance in overhauling the overwhelming outbreak.



B-lymphocyte activation by antigen binding leads to formation of antibody-secreting plasma cells and antigen-sensitive memory cells.

Furthermore, a very interesting fact that should be looked into is how the fruit bats are able to survive as carriers and yet not dying from this deadly disease. Also, owing to the results of the studies done on the 1995 outbreak of Ebola Virus Disease in Democratic Republic of Congo which showed that seven out of 8 infected people survived after being given the blood of survivors of the disease, I would suggest the use the blood of long time survivors for the time being who would have developed antibodies that would combat the antigenic components in infected persons. However, using this therapy as well requires adequate screening and strict precautions in the area of blood donations in ensuring compatibility with cross matching (donor cells are mixed with recipient plasma, and recipient cells also are mixed with donor plasma; there should be no agglutination in both cases).

NOTE: Administration of antibiotics indiscriminately without a convincing need should be avoided as they may cause more harm than good for patients infected with Ebola virus as they are not suffering from bacterial infection but a viral infection instead).

The Potential Vaccine

Since viruses cannot be grown in microbiological broths or on agar gels, they have to be cultured using suitable tissue culture techniques and cell lines such as the immortal HeLa cell line for isolation of viruses – obtained from cancer of the cervix from the late Henrietta Lacks or Vero cell line for the isolation of viruses – from African green monkey kidney. The embryo of fowls may also yield

expected positive results but it has higher risks of being contaminated.

The virus can be isolated or obtained and conveyed from the serum of infected persons or other mammals to a specially prepared research laboratory.

Processes Involved In the Isolation of Ebola Virus

- HeLa cells preparation in laboratory flasks or test-tubes.
- The HeLa cells are adherent cells which spread out across culture flasks. They are signalled to stop growing when adjacent cells become apposed together or touch each other.
- When fully grown and separated into the different containment bottles, the cells are then infected with the virus in the process of inoculation.
- I advocate the use of Zaire strain of Ebola virus as it seems to pose the most deadly threat since discovery in 1976 and of course in the recent massive outbreak across West Africa.
- The set-up apparatus after inoculation is then incubated at 37°C
- Examine the cultures for evidence of infection such as cytopathogenic effect (CPE), interference,

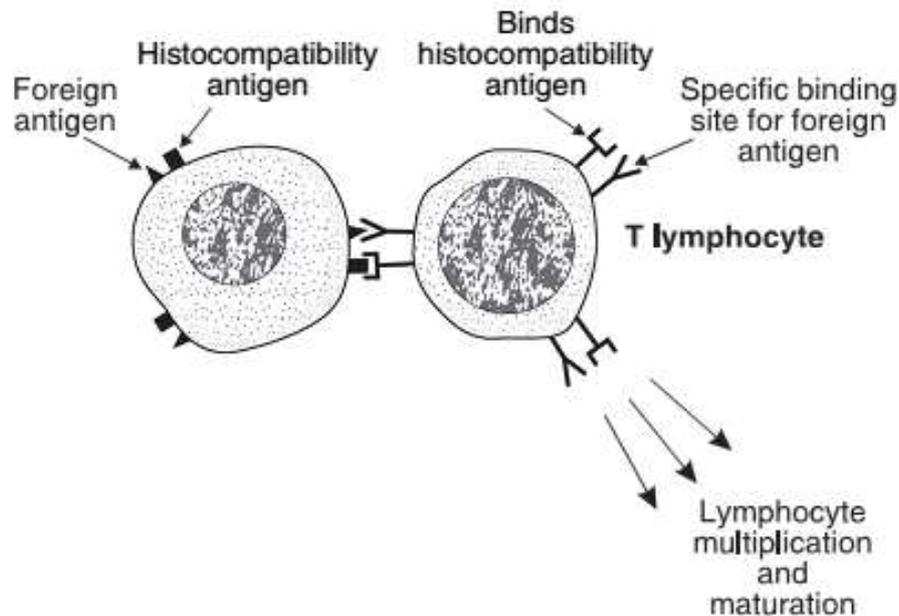
haemadsorption, and also for the presence of viral antigens.

- Harvesting of the virus from positive cultures by freeze-thawing and low-speed centrifugation
- The supernatant is then used as antigen (in the vaccine to trigger antigen-antibody immune responses at standardized doses coupled with certain adjuvants.
- Virus identification by testing the antigen against a battery of antisera with complement fixation, neutralization, haemagglutination inhibition tests, Enzyme-linked immunosorbent assay (ELISA) and immunofluorescence.

NOTE: *In order not to alter the chemical constituents in the course of processing the vaccine, it will be best to first inactivate the virus then proceed with inoculation and the*

following steps in the bid to making the vaccine as effective as possible.

To choose from a wide variety of methods to make this vaccine, I will further suggest the use of an inactivated vaccine and not an attenuated one. The live or attenuated vaccine has a higher risk of making Ebola virus turn out to give a more deadly and more virulent strain than the one we are currently contending inside (human) hosts upon administration of even the first shot of the vaccine. In respect of this factor, I will give an outline on how to go about inactivating the virus bearing in mind that it is easily killed at high temperature. The virus can be denatured or inactivated to weaken the replicating power of the virus. Its effectiveness can be confirmed at inoculation and weakened the more if the virus remains hyper-reactive following the first inactivating process. The success of these techniques can be detected by examination of the cytopathogenic effects of the attenuated virus on the culture cell lines. The virus can be inactivated using the following techniques;



T-lymphocyte activation by cell surface antigen. Binding requires the presence of both foreign and normal histocompatibility antigens. The activating cell may be any body cell carrying an abnormal antigen or a specialized antigen presenting cell which has phagocytosed antigenic material.

Pasteurization - Pasteurization involves increasing the temperature of solution to a value that will sufficiently denature Ebola Virus over a period of time as it does not matter whether the virus has an envelope or not because the envelope alone cannot protect the virus from such high temperatures. However, there are some proteins which have been found to act as thermal stabilizers for viruses. Of course, if the target protein is not heat-resistant, using this technique could denature that target protein as well as the viral impurity. Typical incubation lasts for 10 hours and is performed at about 70°C. The aim of this technique

is to minimize number of times the virus will replicate when introduced into the human body.

Ultraviolet (UV) Radiation - UV rays can damage the DNA of living organisms by creating nucleic acid dimers. However, the damages are usually not important due to low penetration of UVs through living tissues. UV rays can be used, however, to inactivate Ebola virus since the virus particles are small and the UV rays can reach the genetic material, inducing the dimerization of nucleic acids. Once the DNA dimerized, the virus particles cannot replicate

their genetic material which prevent them from spreading. Also, UV light in combination with riboflavin has been shown to be effective in reducing pathogens in blood transfusion products. Riboflavin and UV light damages the nucleic acids in viruses, bacteria, parasites, and donor white blood cells rendering them unable to replicate and cause disease. I suppose interference with ultraviolet rays is actually of high importance to salvage any future occurrence of the vaccine forming cancerous and adherent cells in humans following immunization (because of the risk in the use of the immortal Hela cells earlier mentioned).

The use of **Formalin** may also yield expected positive results in the inactivation of Ebola virus.

The effectiveness of these techniques can be evaluated by culture with embryonated eggs in that the more powerful its replicating power is in the embryo the less powerful it will replicate inside human hosts so enough to overwhelm the immune system but will be active enough to sensitize the immune system to produce antibodies against the virus at first shot. This can be reinforced with subsequent shots. Embryonated eggs brought in on a daily basis from biosecureflocks are best to be used for Ebola virus. A seed ampoule is used to inoculate the chick eggs during the inoculation phase. This is followed by a mandatory three-day incubation period during which the virus grows to ensure that sufficient quantities can support further manufacturing. After three days, all of the eggs are candled to make sure there are no cracks or contamination; the eggs are then chilled to 2°C to 8°C to constrict vessels and make harvesting easier. The allantoic fluid is then harvested.

Hence, the virus particles are destroyed and cannot replicate, but the virus capsid proteins are intact enough to be recognized by the immune system to evoke a response.

Types of Specimens

Specimens that may be tested for the presence of viral agents in infected persons are the following:

Autopsy or Biopsy Specimens

Autopsy specimens for Ebola virus isolation should be collected within 24 hours after death. Samples (1.0-2.5 cm Tubes of tissue) from sites of pathology are collected using separate, sterile instruments and separate sterile containers for each specimen to avoid cross contamination. Tissues are transported to the laboratory on wet ice or cold pack. If they cannot be tested within 48 hours they should be frozen.

Blood Specimens

Although blood is not the optimal specimen for isolation of most viruses, it may be used for the recovery of some of the vector-borne viruses such as Enteroviruses, and *Cytomegalovirus (CMV)* and possibly Ebola virus as there is

no scientific proof of non-transmittance through air. Specimens for Ebola virus isolation should be collected as soon as a viral etiology is suspected, otherwise early neutralizing antibody may prevent recovery of the virus from the blood in some cases. Either serum or leukocyte preparations may be used for viral isolation. For isolation of the virus from leukocytes, 8 ml of blood is collected into a tube containing an anticoagulant. Although heparin is often used, it is reported to inactivate herpes simplex virus, and therefore EDTA bottle may be preferable as an anticoagulant. For isolation of Ebola virus from the serum or blood clot, 8 ml of blood is collected aseptically without an anticoagulant. Blood specimens should be transported to the laboratory on wet ice or a cold pack, but not frozen.

Others include;

- Cerebrospinal Fluids (CSF)
- Cervical Specimens
- Rectal Swabs
- Saliva
- Semen Specimens
- Stool and Urine Specimens

NOTE: *If a medium specifically formulated for collection of viral specimens is not available, a sterile, well-buffered bacteriological broth, such as tryptose phosphate broth, may be used. If no other media is available, sterile water may be used; however, viral recovery will be enhanced by the use of a protein containing media. Cotton-or Dacron-tipped swabs are preferred for collection of specimens; prolonged contact with calcium alginate swabs has been reported to inactivate herpes simplex virus.*

The medium used for collection and holding of swab specimens should contain protein to stabilize the more labile viruses, and the use of media containing charcoal should be avoided as this may reduce viral recovery rates.

The next sequence of events in making this vaccine effective and to help trigger a more vigorous immune response against the deadly virus will be the addition of excipients (pharmacologically inert substances used to bind contents of drugs) and necessary adjuvants (agents added to drugs to enhance their medical effectiveness) to the vaccine for an excellent result to be obtained even with storage of this vaccine.

Aluminium Hydroxide Hydrated Gels – to promote an earlier, more potent and more persistent immune response to the active antigenic 'ingredients' or the virus itself (less than 2 milligrams of the salts, and less than a milligram of actual aluminium). Although vaccination does result in a temporary increase in the amount of aluminium in the body of infants, this is not a lasting effect, since the body gets rid of most of the aluminium in just a few days. Two studies from 2002 and 2011 compared the impact of aluminium from diet and vaccines in infants. Both of these found that the total amount of aluminium absorbed from

both sources is significantly less than the recommended safe maximum amount. The 2011 study concluded that 'the benefits of using vaccines containing aluminium adjuvant outweigh any theoretical concerns'.

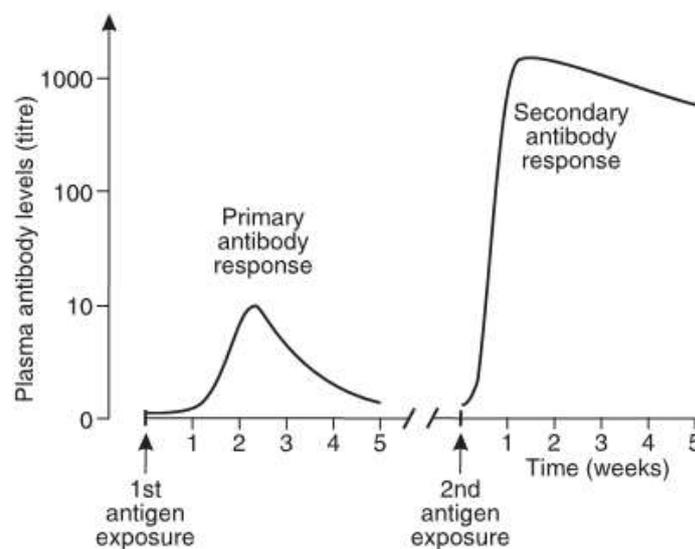
Neomycin – antibiotics used to prevent growth of bacteria during production and storage of vaccine. However this is to be used only in trace amount. **Antibiotics which have been associated with allergic reactions (such as penicillins, cephalosporins and sulphonamides) are not used in vaccines.**

Human Serum Albumin- as a stabilizing agent (NOT MORE THAN 0.3mg/dose)

Preservatives can also be used to prevent the serious adverse effects associated with infections or contamination of the vaccine especially upon storage. A good example in trace amount is formaldehyde or phenoxyethanol.

At the end of further laboratory studies in the employment and application of this research work, I suggest that three doses of the vaccine should be given over a calculated

period of time to provide primary and secondary immunity, allowing the body to produce antibodies and also for the risk of not loading the system with the virus from the first two doses and later reinforced with the extant dose to provide the maximally possible immunity. The first shot should be strong enough to sensitize the memory B cells to store the information and prepare the body in case of subsequent attacks. I aim to couple this vaccine or reinforce the effectiveness the vaccine with immune markers like interferon alpha-n3 or Interferon alpha-2b proteins (which have been seen to be effective against viruses) so as to make it effective in people with weakened immunity such as people with Cancer or AIDS and even Diabetes. Interferons are proteins produced by virus-infected cells and they have the ability to prevent viral replication in other cells. According to Dereck Gatherer, a bioinformatics at Lancaster University in the United Kingdom, who studies viral genetics and evolution, immune markers such as CD4 and CD8 T lymphocytes which are crucial to the function of the immune system depleted in persons with Ebola virus, our bet in the development of this vaccine will be to reinforce such markers and possibly couple them in very minute amount to the vaccine to help the immune system regulate itself.



Plasma antibody levels following first and second exposures to a given antigen.

Another marker to be coupled to the vaccine to maximize the fight against the deadly Ebola virus is a 'gene called human leukocyte antigen-B which makes a protein that is important to the immune system. A 2007 study found that people with certain versions of this gene, called B*07 and B*14 are more likely to survive Ebola.'

In the final analysis, some people may be resistant to Ebola infection entirely, if they have a mutation in the gene called NPC₁. Studies show that, when researchers take cells from people with NPC₁ mutation and try to infect them with Ebola virus in a laboratory plate, these cells are resistant to the virus. Hence isolation of this marker and

adding it in a slightly significant amount can as well aid in overhauling the deadly disease and restoring life to our society.

I also want to use this medium to enjoin scientists all over the world, most especially Africa scientists (Pharmacists and other researchers) who seem not to be doing enough for the whole world to see even in previous outbreaks to join in this quest of finding a lasting solution to the perilous viral diseases affecting mankind, animals, and plants. This is much more needed at this time of endemicity of Ebola Virus Disease because of the impact it has conferred on economy of the nations, discrimination of

affected persons, and the fear of eating bush meats by Africans who are used to hunting them for food; shaking of hands, the loss of loved-ones to the dreaded virus (emotionally, psychologically, and of course physically) and most importantly the fear of saving people dying of other ailments not related to Ebola across the regions affected. Though, several medications such as ZMAPP (by the MAP *Pharmaceuticals*), the GSK EBOV (by *GlaxoSmithKline* Pharmaceuticals) vaccines among others have been undergoing clinical trials in different phases. I am of a daunting opinion that the strain of Ebola virus we are dealing with currently in the outbreak in West Africa is likely to be different in some forms in its DNA structure compared with that of 1976 owing to the fact that this is seeming to be more virulent, leading to more deaths as it has been termed the worst outbreak of Ebola in history. Thus, we must all be at our bests in order to salvage any dreadful future re-occurrence as we are still battling with the third and worst Ebola outbreak in history.

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