Spring 4-28-2017

A Comparative Analysis of the West African Hemorrhagic Fevers Caused by the Lassa and Ebola Viruses

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A COMPARATIVE ANALYSIS OF THE WEST AFRICAN HEMORRHAGIC FEVERS CAUSED BY THE LASSA AND EBOLA VIRUSES

By

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Submitted to the Honors Program Committee

in partial fulfillment

of the requirements for University Honors Scholars

Southeastern University

2017

Abstract

Lassa fever (LF) and Ebola Hemorrhagic Fever (EHF) are viral diseases endemic to West Africa.The etiological agent of Lassa fever is an enveloped virus from the *Arenaviridae* family and was first discovered in 1969 when two missionary nurses died of a mysterious illness in the town of Lassa in Borno state, Nigeria.[1] This virus is animal-borne (zoonotic) and is carried by the animal vector *Mastomys natalensis* (multimammate rat). The Ebola virus is also zoonotic originating from fruit bats belonging to the *Pteropodidae* family.[2] The first reported case of Ebola Virus Disease (EVD) was a principal who was believed to have visited the Ebola river on his journey through the Democratic Republic of Congo.

Annually, there are about 100,000 to 300,000 reported cases of Lassa fever, with about 5,000 deaths.[1] Concurrently, the Ebola virus has raked in 11,310 reported deaths from about 28,616 suspected cases, 10% of which were health professionals.[3] It is noteworthy, however, that the death toll associated with EVD is more sporadic and widespread than that of LF. Most infections can be attributed to person-person transmission in which healthy individuals get infected by coming in contact with the body fluids (urine, saliva, and semen) of the sick.[1]

Both the Lassa and Ebola viruses are pertinent in current public health discussions especially for their potential as bioweapons.[1,2] Consequently, this paper seeks to discuss the structure and pathogenic mechanisms of both viruses, address current treatments and complications, and propose areas for further research and preventive measures.

Keywords: Lassa virus (LASV), Ebola Virus (EBOV), Lassa Fever (LF), Ebola Virus Disease (EVD), Ebola Hemorrhagic Fever (EHF), Sensorineural Hearing Loss (SNHL), Ribavirin

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Part I: The Lassa Virus

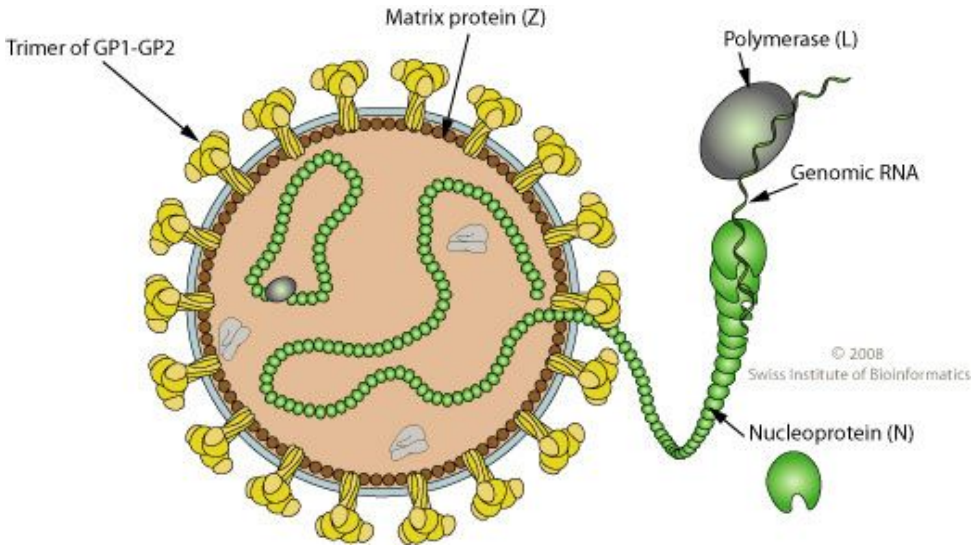

Figure 1: Lassa Virus (LASV)
https://ask.naij.com/health/what-is-lassa-fever-i23734.html

The Lassa virus (LASV) is an enveloped virus belonging to the genus Arenavirus and the family *Arenaviridae*.[5,6] Like other viruses, the LASV infects a host organism (typically humans) and hijacks cell machinery thereby causing the cells to create more viruses that go on to infect other cells.[6]

The genome of LASV consists of two single-stranded RNA species designated small (S) and large (L) which is characteristic of all arenaviruses (see Figure 1).[7,8,9] Arenaviruses are divided into two groups based on their rodent hosts: the New World arenaviruses and the Old World arenaviruses.[9,10] The *Mastomys natalensis* (commonly called the multimammate rat) is the primary host of the LASV (see Figure 2).[11] Its habitats range from savannas and forests to farm lands and urban areas.[11] Consequently, Lassa fever

Figure 2: *Mastomys natalensis*, the primary host of the LASV
http://www.planet-mammiferes.org/drupal/en/node/63?photo=21&zone=5

(LF), which is a hemorrhagic fever caused by LASV is endemic in West African countries like Nigeria, Sierra Leone, Liberia, and Guinea where these rats and their habitats are prevalent (see Figure 3).[12] It was first discovered in 1969 when two missionary nurses died in Nigeria and received its name from the town called Lassa in Northern Nigeria where these first cases occurred.[13] Humans presumably become infected through contact with infected rodent excreta, tissues, or blood.[8] Person-to-person transmission of Lassa fever can also occur.[9] Additionally, pregnant women in the third-trimester are most susceptible to contracting the virus. Between January 1996 and March 1997, over 1,000 cases of Lassa fever with 148 deaths were reported from eastern Sierra Leone.[14,15]

Figure 3: Map of West Africa
https://saylordotorg.github.io/text_world-regional-geography-people-places-and-globalization/s10-03-west-africa.html

Additionally, many of the countries affected by this disease are also endemic regions for malaria epidemics.[16,17] Unfortunately, malaria presents with similar symptoms as Lassa fever does.[17] Some of the symptoms include fatigue, general weakness, fever, headache, and a sore throat which can also be referred to as general malaise.[18, 10] Some of the other symptoms associated

with LF like vomiting, diarrhea, face swelling, low blood pressure, and nose bleeding can also be misdiagnosed as Dengue or Yellow Fever.[19] Thus, the only sure-fire diagnostic method is by using enzyme-linked immunosorbent serologic assays (ELISA), which detect IgM and IgG antibodies as well as the Lassa antigen.[5] It was discovered that these antigens have minute variations depending on the origins of the viral isolates.[20] Using genomic sequencing they were able to categorize these strains into three groups, namely the prototype LP strain from northeastern Nigeria,[11] the GA 391 strain from central Nigeria,[12] and the Josiah strain from Sierra Leone.[10] The LP strain is also called the native LASV strain because it is predominantly found in the town of Lassa, Borno State where the virus was first discovered.[20]

Regardless of the viral strain, however, there is currently only one way to treat a LASV infection.[18] The disease has no cure or vaccine, but experimentation continues to be done on monkeys like *Cynomolgus macaques* because their response to the virus has been most similar to humans. Researchers have avoided mice models because they are too close to the host species *Mastomys natalensis* and thus do not have an immunogenic response to the virus.[15]

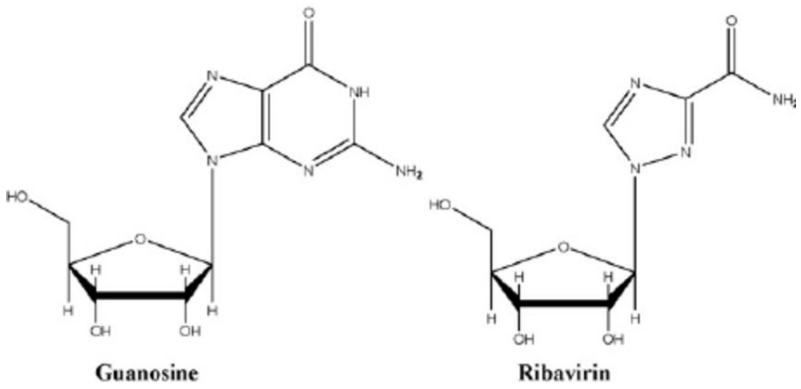

Figure 4: Ribavirin: an analogue of Guanosine
https://www.researchgate.net/figure/24365388_fig1_Fig-1-Chemical-structures-of-guanosine-and-its-nucleoside-analog-ribavirin

Additionally, Ribavirin which was initially approved as a treatment for severe respiratory syncytial virus (RSV) in children has been found to alleviate the symptoms of LF.[20] This drug is an analogue to guanosine (a purine nucleoside comprised of guanine attached to a ribose

sugar which is involved in a variety of cellular function including the synthesis of nucleic acids and proteins, as an ATP substitute, and in intracellular signal transduction).[20] Twenty years after its initial discovery, in the early 1990s, Ribavirin began to be used as a monotherapy treatment for Hepatitis C Virus (HCV).[20] At least half of the patients treated with Ribavirin had improvements in their serum aminotransferase levels, but the viral levels of HCV in these patients did not relent even after prolonged treatment.[21]

Apart from direct antiviral effects Ribavirin has been found to have immunomodulatory ones as well. In patients, Ribavirin induced a switch in T-helper (T_H) cells from T_H2 to T_H1 (see Figure 5 mechanism a).[22] Helper T cells are important players in the adaptive immune system. T_H1 cells specifically are pro-inflammatory which means they secrete cytokines that increase the inflammatory response.[22] These cytokines, *interferon-γ (IFN-γ)* and *tumor necrosis factor-α* (*TNF*-α), activate macrophages to kill microbes and cytotoxic T lymphocytes (CTLs) to kill infected cells.

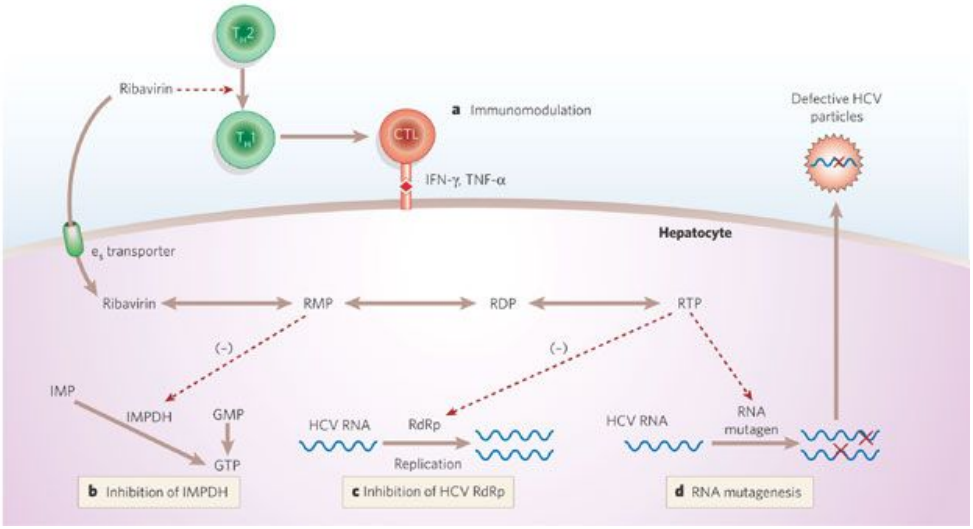

Figure 5: Proposed mechanisms of Ribavirin action on HCV
http://www.nature.com/nature/journal/v436/n7053/fig_tab/nature04082_F3.html

Once inside the cell, Ribavirin is phosphorylated to its GMP, GDP, and GTP analogues which are RMP, RDP, and RTP respectively.[22,23] Research by Maag et al. showed that a misincorporation of RTP by RNA polymerase in place of GTP can block RNA elongation and lead to premature termination which would inhibit viral spread (see Figure 5 mechanism c.).[22] This only occurs at high concentrations of the drug, however, so it was assumed that this was not the main mechanism of action in HCV infections.[22] Another possible candidate for the Ribavirin mechanism is its action as a competitive inhibitor of inosine monophosphate dehydrogenase (IMPDH) (Figure 5 mechanism b).[22] IMPDH is an enzyme that catalyzes the rate-limiting step of GTP and ATP synthesis:inosine-5-phosphate (IMP) to xanthosine monophosphate (XMP) which is subsequently converted to GMP/AMP by GMP/AMP synthetase.[22] Consequently, Ribavirin leads to the depletion of GTP which is necessary for viral RNA synthesis.[23]

Unlike human polymerases that check and recheck RNA/DNA to ensure the fidelity of the transcripts, HCV RNA polymerase is less concerned about mutations because they are usually beneficial to the virus.[23] Crotty et al. proposed that ribavirin might act as a viral mutagen and increase the frequency of mutations in the virus (Figure 5 mechanism d).[24] At some point, these mutations become detrimental to the virus and lead to "error catastrophe".[24,25] Unfortunately, studies have shown that these mechanisms are not effective enough to completely stem a viral load.[23,24] Thus current treatments with Ribavirin are supplemented with interferon alpha (IFN-α).[26] IFN-α induces IFN-stimulating genes which establish a non-virus-specific antiviral state with the infected cell which eventually lead to protein degradation, inflammatory cell responses and apoptosis.[26] When administered together, Ribavirin seems to amplify or stabilize the activity of IFN-α.[26]

All things considered, scientists are still unsure how the drug works on the LASV, but it is suspected that the drug might be acting on the viral RNA and blocking its translation to proteins in the host cells either by mechanisms b or c pictured in Figure 5.[27] Additionally, the drug only seems to have this effect if administered early in the infection which could be due to the fact that the virus has not had much time to replicate or spread systemically allowing Ribavirin to effectively depress viral activity. [27]

Part II: Ebola Virus

Ebola, also known as the Ebola Virus Disease (EVD) or Ebola Hemorrhagic Fever (EHF) is a severe and often fatal disease in humans.[28] The Ebola virus (EBOV) was first discovered in 1976 when there were two simultaneous outbreaks in the towns of Nzara, South Sudan and Yambuku, Democratic Republic of Congo as shown in Figure 6.[28] The disease actually got its name from the Ebola river in Congo.[28] The Ebola virus belongs to the Filoviridae family in which there are five different variants or species: Zaire (EBOV), Bundibugyo (BDBV), Sudan (SUDV), Reston (RESTV), and Tai Forest (TAFV).[28] The first three have been associated with large outbreaks in Africa, the most recent of which being the Zaire outbreak in West Africa in 2014.[28]

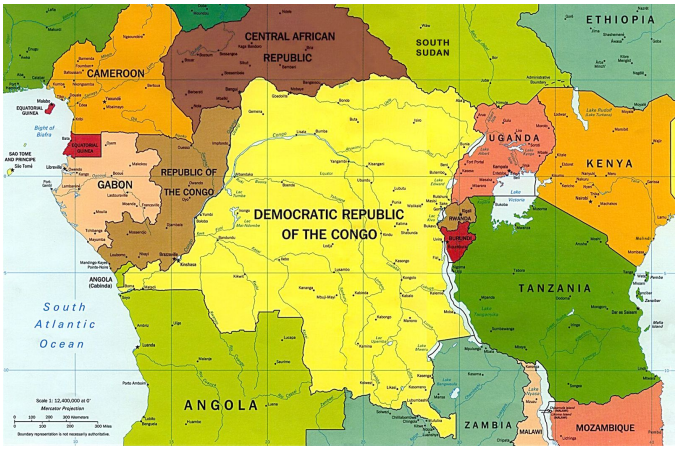

Figure 6: Map of Central Africa
http://www.geographicguide.com/africa-maps/central-map.htm

EBOV is a filamentous virion containing linear negative-stranded RNA.[29] The virus encodes for seven proteins which direct the synthesis of eight proteins.[29] GP proteins on the

surface of the virus mediate the attachment of the virus to host cells and the subsequent fusion of the viral and host membranes.[29] EBOV is believed to enter the cell via receptor-mediated endocytosis. Previously, it was believed that the VP24 and matrix VP40 proteins on the virus were minor structural components solely involved in viral nucleocapsid formation, viral budding or assembly, and host range determination.[28] Current research, however, suggests that VP24 might be involved in blocking interferon signaling. Interferons are signaling proteins released by infected host cells in response to the presence of viruses, bacteria, parasites, or tumor cells.[29] These interferons act as warning signals to neighbouring cells alerting them of a potential infection.[30] It is assumed that the other components of the virion structure including its nucleoprotein(N), VP35,VP30, and L proteins are involved in stabilizing the RNA in its helical conformation.[28]

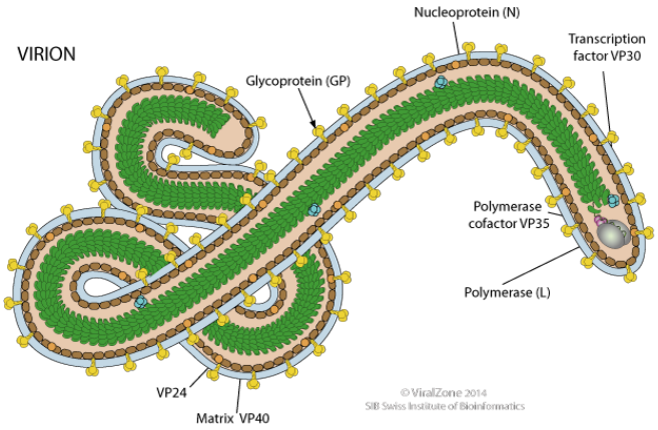

Figure 7: Ebola Virus (EBOV)
http://viralzone.expasy.org/all_by_species/207.html

Much like the Lassa virus, the Ebola virus is transmitted from animals to humans and through the human population by contact with contaminated surfaces or materials like bedding or clothing that have been exposed to body fluids of infected individuals.[11,28] All strains of the virus except for RESTV are pathogenic to humans.[31] It is believed that the original (natural) hosts of the virus were fruit bats of the Pteropodidae family (Figure 8). It was introduced into the human population by contact with the blood, organs, and other body fluids of infected chimps, gorillas, monkeys, antelopes, and porcupines.[31]

The incubation period for the virus is between 2 and 21 days and humans are not infectious until they present symptoms.[31] The first symptoms of an infection are very similar to those of Lassa fever, malaria, and other endemic illnesses and can only be effectively diagnosed using an ELISA; however, a major difference is the fact that the major complication of Ebola impair kidney and liver functions.[32] On average the fatality rate for an EVD infection is about 78% and the virus can survive in semen for between 3 and 12 months even after recovery. There is currently no cure or proven treatment for EVD, but supportive care including rehydration with oral or intravenous fluids has been the most common way of maintaining patients.[32,33]

Figure 8: Fruit bat (primary host of EBOV)
http://phenomena.nationalgeographic.com/2014/12/04/fruit-bats-have-sonar-too-but-its-not-very-good/

After the most recent outbreak of EVD in August 2014, research began on the recombinant vesicular stomatitis virus (rVSV). Vesicular stomatitis is a viral disease that primarily affects horses and cattle, but can also be harmful to swine, sheep, goats, and llamas.[34] Humans are rarely affected by this disease even when they come into contact with infected animals, however, the recombinant form of VSV has been mutated to express the filovirus glycoprotein (GP): rVSV-ZEBOV. Upon administration to monkeys it was found that it protected them from Ebola and Marburg virus infections both as a prophylactic and postexposure.[35] As of April 2016, phase trials for the rVSV-ZEBOV vaccine had begun in Gabon, Kenya, Germany, and Switzerland.[36] The vaccine was given intramuscularly into the

deltoid at different concentrations and it was noted that 11 of the 51 participants in Geneva complained of arthralgia and developed skin lesions.[36] Nonetheless, they discovered high levels of neutralizing antibodies in the blood plasma which lasted in the blood for about 180 days.[36] This research holds promise because it suggests that the recombinant virus is immunogenic in humans and the next step would be to test how effectively these antibodies bind to and neutralize the EBOV virus both *in vitro* and *in vivo*.[36]

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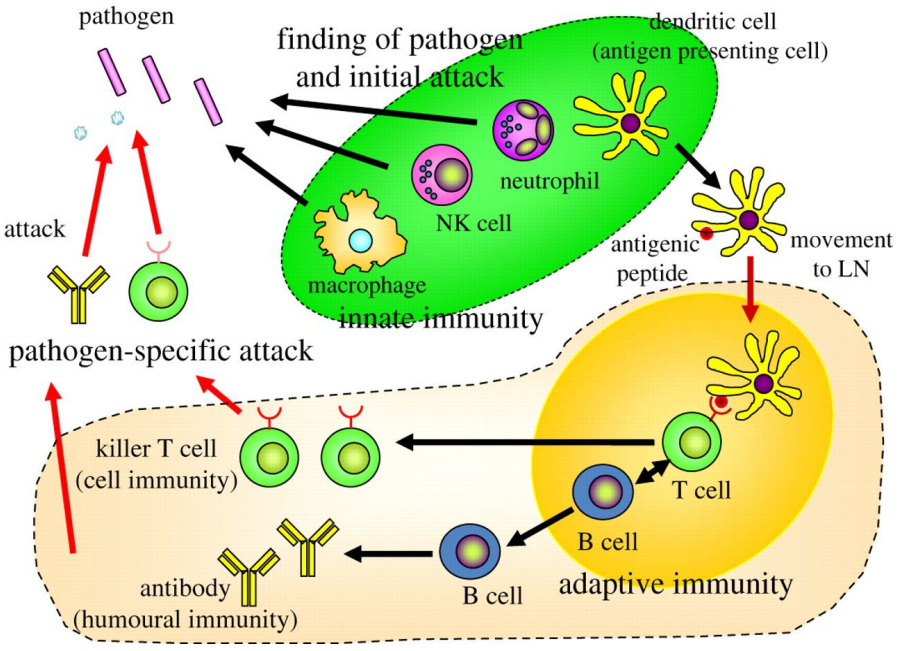
CHAPTER TWO: LITERATURE REVIEW

Part I: What Do We Know?

Viral Mechanisms and Pathogenesis

While the Lassa and Ebola viruses belong to different virus families they share similarities in their mechanisms of pathogenesis.[37] *In vitro* experiments suggest that both viruses infect macrophages and endothelial cells at the onset of the infection.[37] Macrophages and endothelial cells play an important role in the innate immune system. The innate immune system is the body's general response to a pathogen.[38] It is considered the next line of defense after a pathogen or foreign substance has already passed through the skin or the mucosa lining the body's orifices.[38] The innate response is nonspecific and involves a variety of proteins (interferons, antimicrobial proteins, and complement) and phagocytic cells (like neutrophils and macrophages) which recognize foreign antigens and quickly activate the inflammatory response (Figure 9). The inflammatory response is activated when these phagocytic cells as well as other cells involved in the nonspecific innate response begin to secrete cytokines/chemokines

Figure 9: Immune response
http://rstb.royalsocietypublishing.org/content/366/1579/2748



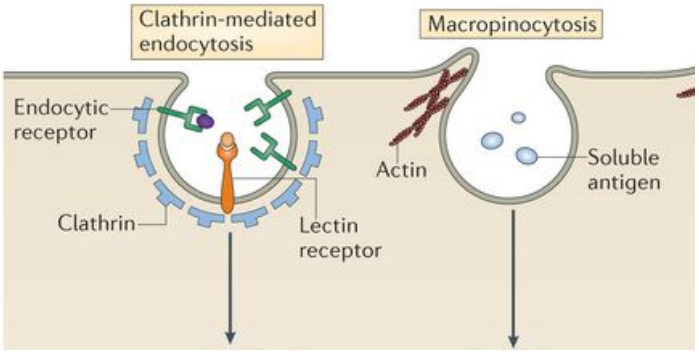
that attract other important immune cells (like B and T Lymphocytes) to the infected area (Figure 9).[39] It is believed that both the Lassa and Ebola viruses activate similar innate response pathways and trigger the rapid and uncontrolled release of cytokines, or inflammatory mediators.[40] Dendritic cells (DCs) are also key players of the innate immune system because they act as antigen presenting cells to B and T lymphocytes.[40] Current research show that both viruses have contradicting effects on these different cell populations ie. they activate macrophages and endothelial cells to release cytokines and activate an immune response, while they use DCs to replicate and inhibit them from releasing cytokines or synthesizing the costimulatory proteins necessary for antigen presentation.[40] This also serves to slow down or possibly inhibit the adaptive immune response to the infection.

The mechanism for the Lassa Virus is the least understood of the two, however more recent research showed that T-cells play a dual role of not only protecting the infected organism from LASV, but also in enhancing LF pathogenesis.[35] In this experiment the scientists used genetically engineered (HHD) mice which were susceptible to LF because they had human T cells.[41] This theory is supported by the fact that macrophages also act as antigen presenting cells and the costimulatory effects of the T cells they bind to can further activate them to secrete inflammatory mediators, thereby increasing the immune response.[41] In the case of Lassa fever, it seems that it is postulated that with increasing viremia, T cells continue to encounter LASV epitopes on the macrophages and thus continue to stimulate them as well.[41] This overstimulation of the macrophages leads to severe hepatic and pulmonary damage, in addition to the shock syndrome associated with Lassa Fever. Mammalian studies have further supported this theory by showing the seemingly paradoxical observation that high doses of LASV tend to be less lethal

than low ones.[42] This might seem to contradict the previous experiment, but in reality it follows the kinetic model of proteins.[42] In the sense that there is a concentration at which the virus reaches its maximum virility. At that point all the T cells are "exhausted" and are no longer available to overstimulate the macrophages.[42] This attenuates the disease making it less fatal. Thus, on the one hand it seems that the adaptive immune system is a necessary part of the battle against the virus and on the other it might increase its adverse effects.[42]

Similar to the LASV, the Ebola Virus initially infects macrophages and dendritic cells.[43] One characteristic difference between the two, however, is the fact that Ebola presents with a cytokine storm, which is typical of many hemorrhagic diseases.[43] A cytokine storm is a secretion of numerous pro-inflammatory cytokines and induces contradictory signals that adversely affect immune cells and other tissues of the body.[43] The Ebola virus seems to primarily affect the spleen and kidneys in addition to the immune system. This is important to note because these two organs are involved in maintaining the body's fluid and chemical balance as well as the blood supply of coagulation factors.[44] Additionally, the EBOV is able to invade almost all human cells by using attachment mechanisms like receptor-mediated endocytosis and macropinocytosis (Figure 10). Viral activation leads to a steady increase in the production of proinflammatory cytokines and chemokines including IL-1β, IL-1RA, IL-6, IL-8, IL-15, IL-16, MIP-1α, MIP-1β, MCP-1, MIF, IP-10 GRO-α and eotaxin (Figure 11).[45] Disease

Figure 10: EBOV mechanisms of entry into host cell
http://www.nature.com/nri/journal/v15/n4/full/nri3818.html?WT.feed_name=subjects_antigen-presenting-cells



progression also leads to the nitric oxide production which dilates and increases the permeability of blood vessels.[45] These changes trigger the coagulation cascade which leads to widespread elevated thrombomodulin and ferritin levels. Thrombomodulin is a protein cofactor expressed on endothelial cell surfaces.[46] Its function is to modify thrombin which is an essential component of the coagulation pathway. Thrombin is the enzyme that converts fibrinogen to fibrin which eventually forms the fibrous mesh associated with a clot.[46] Ferritin is an intracellular protein that stores iron.[46] Excess amounts of these proteins play a role in the formation of blood clots (Disseminated Intravascular Coagulation; DIC) that could possibly inhibit the flow of blood to vital organs like the liver, brain, or kidneys which could lead to the tissue damage associated with EVD.[46]

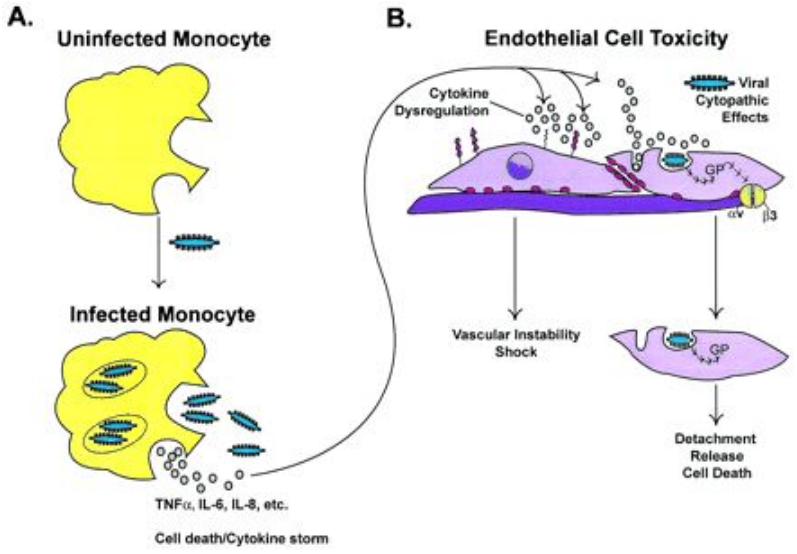


Figure 11: Cytokine storm associated with infected macrophages and its effects on endothelial cells
http://rstb.royalsocietypublishing.org/content/366/1579/2748

Given the fact that the Ebola virus is on average more lethal than the Lassa virus, the major complication associated with this disease is sepsis.[31] Sepsis can be defined as the systemic inflammatory response to an infection.[31] It can cause a variety of responses from shock, and multiple organ failure to death.[31] EBOV activates classical sepsis in which pattern recognition receptors like toll-like receptors (TLRs) or nucleotide-binding oligomerization domain (NOD)-like receptors initiate systemic inflammation.[31] At the same time, the can lead to

immunosuppression which would make the body more susceptible to infection by other microorganisms.[31]

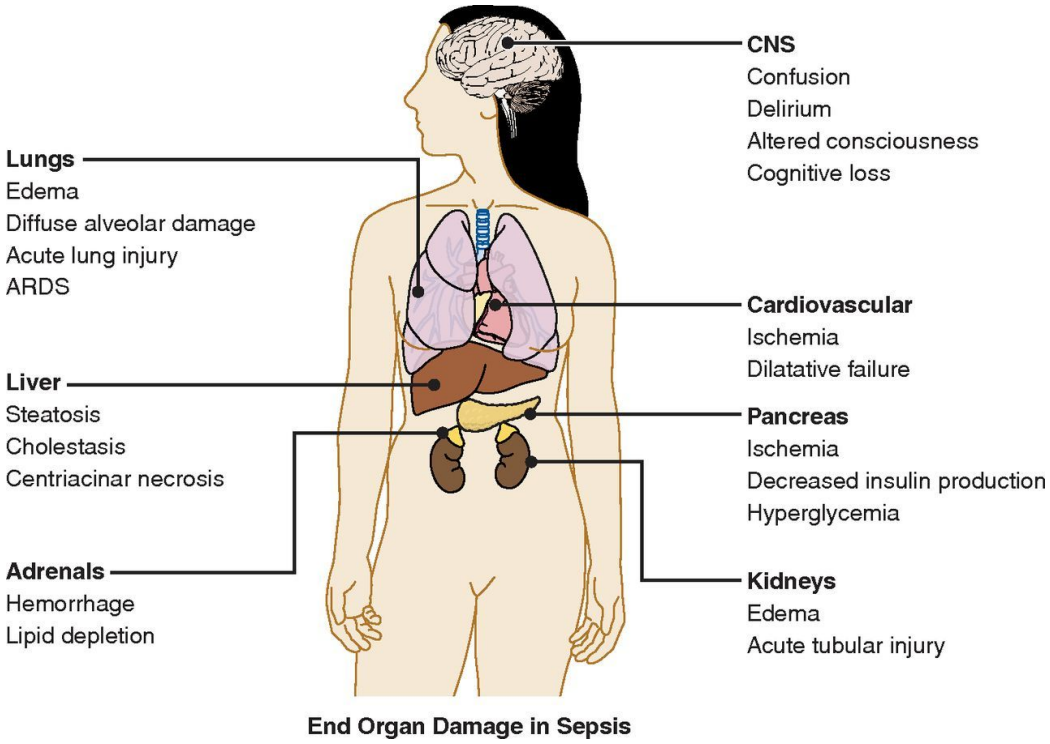


Figure 12: Systemic organ damage as a result of sepsis
http://rstb.royalsocietypublishing.org/content/366/1579/2748

Part II: Where Can We Go?

Future Studies and Research Proposal

Of the two viruses Ebola has received the most recognition with global research efforts focusing on vaccines and treatment regimes for a virus that could potentially be a pandemic someday. The Lassa virus which is more prevalent in third world countries does not receive the same amount of interest either due to lack of funding from the failing health sectors of West Africa, or a general disinterest in something that is not as "imminently" threatening as Boko Haram terrorist, famine, malaria, or drought. For this reason, it is important that West Africa begins a conversation on the subject of Lassa fever and how to eradicate it from the society.

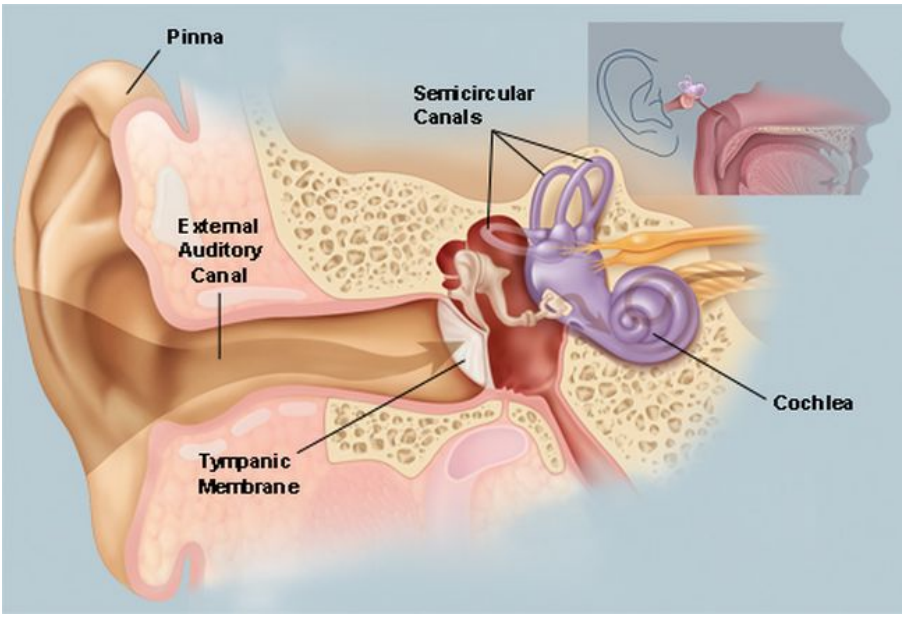
One of the biggest complications of Lassa fever is hearing loss. Statistics show that of the people infected with the LASV, 25% will develop hearing loss.[10] Unlike the other symptoms/complications of the disease like hypovolemic shock or hemmoraghing which can be accounted for by the viral mechanism of action *in vivo*, no one really understands what happens in the progression of the disease to cause hearing loss.[10]

Virus-associated hearing loss is not a new concept in virology. Many viruses including the West Nile Virus, the mumps virus, the Varicella Zoster virus, and the measles virus have all been found to cause some degree of sensorineural hearing loss in infected patients.[47-50] In these cases, the hearing loss is as a result of an acute infection and occurs as soon as the subjects are exposed to the virus.[50] Additionally, the patient spontaneously recovered his or her ability to hear once the infection has passed. Some LASV associated hearing loss has been reported to behave in this manner as a result of an acute infection, but sometimes patients have been found to develop hearing loss during the convalescence phase of their illness as well. Researchers believe

that this could be a result of an autoimmune response resulting from the virus activating immune cells in the infected patients.[51] In these cases, even though the viral titer was low, the activated immune cells were primed and ready to attack the ear leading to sensorineural hearing loss.[51]

The dynamics of the immune system and the role it plays in the propagation of the disease has been identified as a potential factor in the occurrence of the most common complication of LF: Sensorineural Hearing Loss (SNHL). Hearing, or auditory perception, is the ability to perceive sound by detecting vibrations in the surrounding air.[50] The pinna directs sound waves through the external auditory canal to the tympanic membrane all of which are part of the outer ear (see Figure 13).[50] At the tympanic membrane sound vibrations are transmitted to the ossicles of the middle ear which amplify the sound before it gets to the cochlea.[50-51] Hair cells in the cochlea (the inner ear) are involved in transducing that sound wave into electrical impulses that can be transmitted through the vestibulocochlear nerve to the temporal lobe of the brain where it is perceived as sound.[52]

Figure 13: The macroscopic anatomy of the human ear
http://www.webmd.com/brain/picture-of-the-ear#1



Sound waves can be classified based on their frequencies and intensities.[52] The frequency of a sound is measured in hertz (Hz) and dictates its pitch. The range for typical human hearing is 20-20000Hz, with conversation frequencies in the 300-3000 Hz range,

clapping between 2,200 and 2,800Hz, and the sound from crickets within the 4000-5000 Hz range.[53] The intensity of a sound is measured in decibels (dB) and it dictates how loud a sound is. A whisper is about 15dB, a normal conversation about 60 dB, and a car horn is typically 110dB.[53]

Sometimes things can go wrong with the hearing process and hearing loss occurs. There are three types of hearing loss namely conductive, sensorineural, and mixed.[50] Conductive hearing loss affects the outer and middle ear and is characterized by foreign bodies or malformations that impede the sound waves from arriving at their destination.[52] Sensorineural hearing loss (SNHL), on the other hand, affects the inner ear and is also known as nerve-related hearing loss. This type of hearing loss is usually caused by excessive exposure to loud noises, head trauma, viruses or diseases. SNHL also tends to be irreversible.[53,54] Finally, mixed hearing loss is a combination of both conductive and sensorineural hearing loss. Hearing loss can be measured as either an absolute inability to hear a certain sound frequency or as a degree of loss that affects only sounds of certain intensities/loudness.

Due to the fact that hearing loss is the most common and least understood complication of this disease and the goal of this proposal is to identify the presence or lack thereof of a correlative relationship between certain viral strains and the incidence of hearing loss.[8]

Specific Aims

1. To determine what strains of the Lassa virus cause sensorineural hearing loss (SNHL) in *Cynomolgus macaques* with functional and depressed immune systems.

Hypothesis: *The combination of a heightened immune system and the most native strain of the Lassa virus (the NP strain) would increase the likelihood of hearing loss in the subjects.*

2. To test the effects of Ribavirin treatment on hearing loss in the affected group from the first specific aim.

Hypothesis: *Ribavirin will have little to no effect on hearing loss unless administered early.*

Research Design and Methods

The subject model for this experiment is the *Cynomolgus macaque*. *Cynomolgus macaques* are a species of monkeys that have previously been used in studies with LASV because they have been found to respond to the virus with symptoms similar to humans.[15] Researchers have avoided mice models because they are too close to the host species *Mastomys natalensis* and thus do not have an immunogenic response to the virus.[15]

I. The first specific aim is to determine what strains of the Lassa virus cause sensorineural hearing loss (SNHL) in *Cynomolgus macaques* with functional and depressed immune systems.

A. The subject group would be 16 *Cynomolgus macaques*

B. These *macaques* would all be initially exposed to the Brain Auditory Evoked Response (BAER) test in which they would have nodes put in their ears and a sound would be released at a certain frequency (see Figure 14). The movement of the sound wave would be tracked to the brain with peaks being identified at the auditory nerve, pons, and

midbrain. Once it is ensured that the subjects have functional hearing, they would be split into two groups with 8 *macaques* in each.

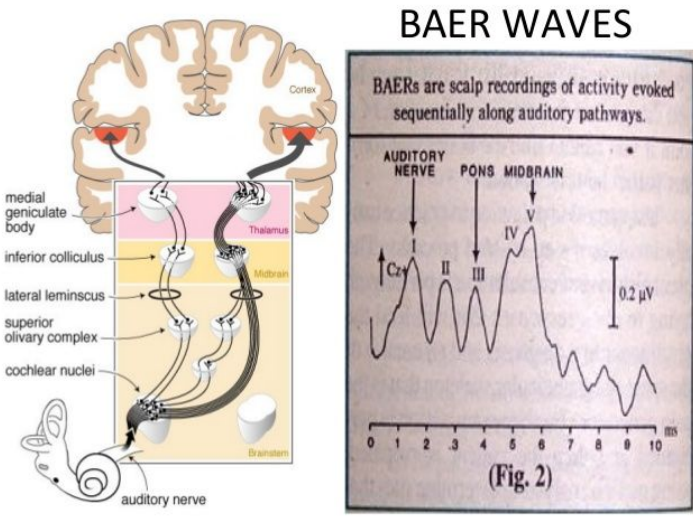


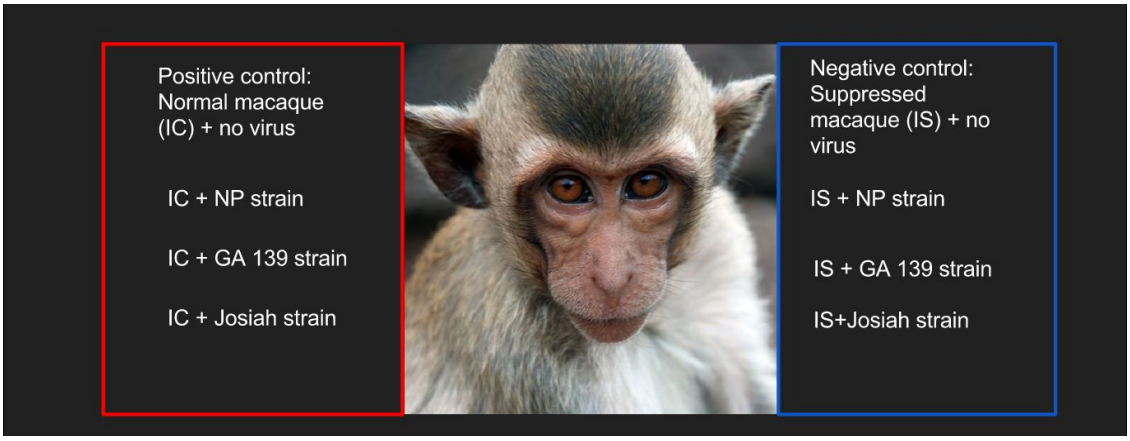
Figure 14: BAER test showing normal hearing
http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0102-09352014000200016

C. One group of *macaques* would be given pure drinking water, while the other group will be given nicotine-spiked drinking water for a period of 2 weeks. Prior studies have shown that nicotine depresses the immune system, thus these *macaques* would constitute the immunosuppressed (IS) subject group with the other macaques being the immunocompetent (IC) group.

D. The next step would be to divide the subjects once again into four groups: control, LP strain, GA391 strain, and the Josiah strain groups. The groups receiving the viral strains would be given 1 mL injections intravenously (see Figure 15).

E. Subjects would be allowed to run the course of the illness for about 3

Figure 15: Visual Representation of Specific Aim I



weeks, and the hearing test would be performed weekly to determine if there is any hearing loss.

Expected Results:According to research conducted by Okokhere[12], the majority of the documented hearing loss cases occurred in northeastern Nigeria. This area is where the NP or native strain of the LASV is prevalent. Thus, I would expect that the *macaques* exposed to this strain would be most likely to develop hearing loss. Additionally, based on previous research which suggest that the immune system might play a role in the incidence of hearing loss.[18] I would expect that the immunocompetent (IC)+NP strain would be even more likely to have hearing loss, or a greater degree of hearing loss than the immunosuppressed (IS)+NP strain. The presence of hearing loss would be identified by a BAER test similar to the right side result in Figure 14 in which many or all the previously seen peaks have tapered out.

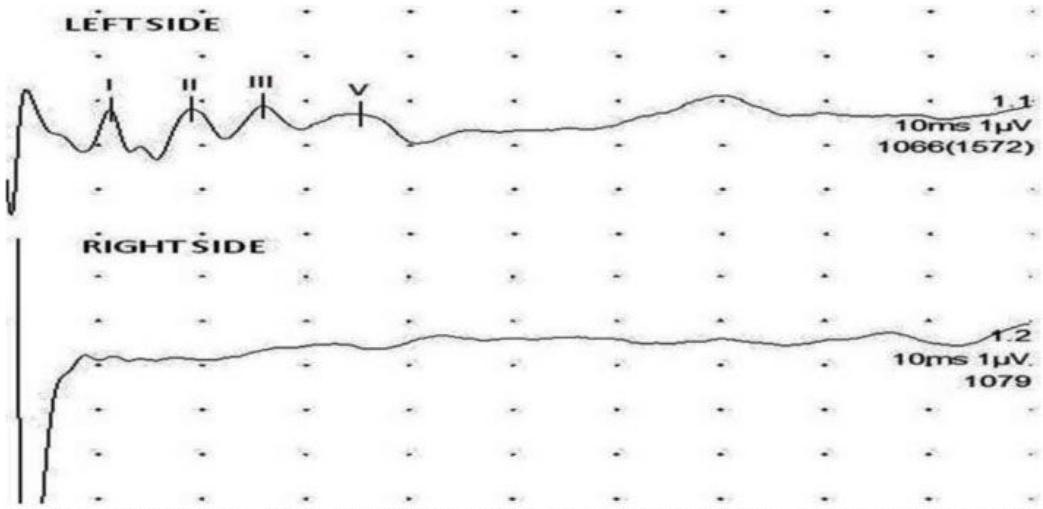


Figure 16: Sample of results expected from BAER test for Specific Aim I

II. **The second specific aim is to test the effects of Ribavirin treatment on hearing loss in the affected group from the Specific Aim I.**

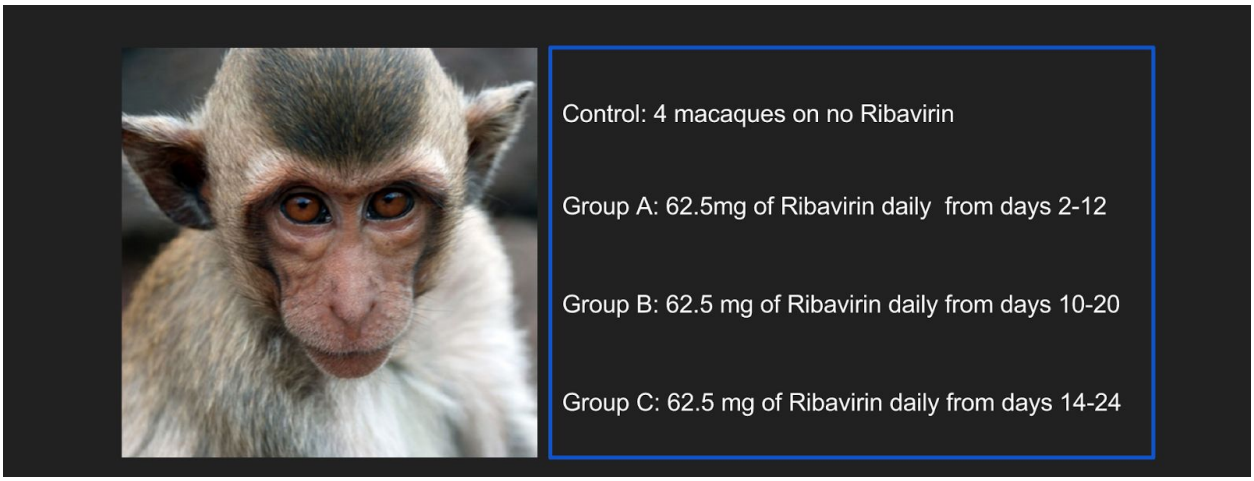
A. In this experiment, the subject group would be 16 *macaques* exposed to the same conditions as the subjects from the group in Specific Aim I that acquired hearing loss.

B. These subjects would be split into groups of 4 .

C. The groups would then be treated with 62.5 mg of Ribavirin daily for 10 days beginning at days 2,10, and 14 of the infection. The remaining 4 *macaques* would be the control with no Ribavirin treatment (see Figure 17).

D. All subjects would undergo the BAER test weekly. In this experiment, they would be exposed to the same frequency at different decibel levels in order to determine the degree of hearing loss developed.

Figure 17: Visual representation of Specific Aim II



Expected Results: The treatment regimen of 62.5 mg was calculated based on the treatment for humans infected with the Lassa virus.[18] Currently, the drug is only effective in curbing the disease's symptoms if administered early, thus I would expect that the group receiving the treatment at day two would have less severe hearing loss than those beginning the treatment at day 14 (see Figure 18).

One of the biggest shortcomings of this experiment is the small sample size. Both specific aims use *Cynomolgus macaques* as subjects and while these organisms are ideal models because they respond to the virus with human-like symptoms, they are highly expensive to maintain. Thus, the results from this project might be a little skewed because of the small data size. In order to address this, both specific aims could be attempted using knockout or transgenic mice. I would personally recommend transgenic mice over knockout mice because completely knocking out the immune system of these mice might allow them respond to the virus, but they might not live long enough to be valuable to the experiment. However, transgenic mice with human or macaque immune cell genes would still have functional immune systems that could manipulated as needed. This would allow for a larger study sample and more data results.

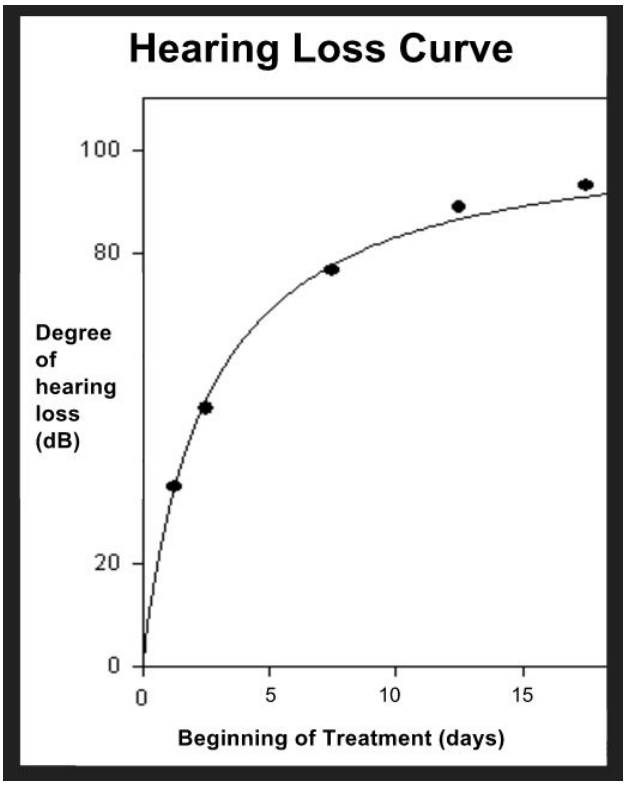


Figure 18: Expected results for Specific Aim II

CHAPTER THREE: CONCLUSION

While Lassa fever and Ebola showed up in African history within the last century, 1969 and 1979 respectively, they were both essentially forgotten until recent times.[55] Within the last couple of years there have been barely controlled epidemics of both viruses in West Africa. According to the World Health Organization (WHO), between August 2015 and May 2016 there were about 273 reported cases of Lassa Fever and 149 deaths in over 23 of the 36 states in Nigeria.[55] Ebola took more lives in 2014 when there was a worldwide scare relating to an outbreak ravaging Guinea, Liberia, and Sierra Leone. A total of 28,616 cases were reported and about 11,310 deaths.[55] While the Lassa fever epidemic was restricted to African countries where the host rat is prevalent, a total of four individuals were infected in America during the 2014 Ebola epidemic and of those four only one man died. This variation in fatalities leaves much to be desired in the public health and epidemic sector of the affected West African countries and begs the question: What are West African countries doing wrong?

In answering this question one must consider first of all that West Africans are intrinsically superstitious. These epidemics originate in rural areas where most people still believe that a lot of disease are the manifestations of the wrath of the gods. In the cities where one might expect to find the learned and more knowledgeable members of the society, things are only slightly better. As the tales of a disease that is ravaging the people of Liberia, Guinea, and Senegal begin to spread, panic ensues and stories begin to spring up about ways to protect yourself from the disease.[56] During the last Ebola epidemic (2014) a lot of people tried everything from bathing with salt water twice a day to drinking an assortment of herbal concoctions. None of which have any scientific basis whatsoever. In March-June 2016, when the

rumblings began of a Lassa fever epidemic in Nigeria, people stopped eating “bushmeat”.[57] Bush meat, which is the West African term for wild game, includes a variety of animals from mammals to reptiles. These animals are hunted, roasted, and sold to highest bidder.[57] No one thinks to check if the animal was sick before it was killed, or if the exposure to heat was long and intense enough to kill all living microbes in the meat.[57] Unfortunately, many of these microbes could have variations of heat shock proteins that might be able to withstand such temperature extremes, in which case the meat is still dangerous even after it's been roasted.[57] But the people cannot be faulted because they do not know any better. Their response is one of ignorance because the public health infrastructure of many of these countries are subpar and lack awareness schemes.[58] One way to increase awareness is to create fact sheets that can be distributed in schools, work places, and general social gatherings that give key details about these diseases and how to respond to and prevent the spread of infection*.

To further put things in perspective, about 100,000 to 300,000 reported cases and 5,000 deaths from Lassa Fever might not seem significant in the large scheme of things considering there are over 7 billion people in the world today.[4] However, of those 7 billion people, 245 million of them live in West Africa, and of those West Africans 5,000 people die annually from this disease.[6] A recent study has estimated 22 million people distributed in areas of Central and West Africa to be at risk of Ebola. It is also important to understand the fact that these death toll numbers are not a completely accurate representation because many of the people affected by the disease do not have access to medical care and thus cannot be counted among the reported cases. Yet, nothing tangible is being done to address the situation. Hopefully, the research presented in this paper will lay the framework for more investigations and begin a much-needed conversation.

*An example of a fact sheet from the World Health Organization can be found at the end of this document.[59]

REFERENCES

1. Amorosa V, MacNeil A, McConnell R, et al. Imported Lassa Fever, Pennsylvania, USA, 2010. Emerging Infectious Diseases. 2010;16(10):1598-600.

2. Asogun DA, Adomeh DI, Ehimuan J, et al. Molecular diagnostics for Lassa Fever at Irrua specialist teaching hospital, Nigeria: lessons learnt from two years of laboratory operation. PLoS Neglected Tropical Diseases. 2012;6(9):e1839.

3."Ebola data and statistics". World Health Organisation. Retrieved 9 June 2016.

4. Baize S, Marianneau P, Georges-Courbot M-C, et al. Recent advances in vaccines against viral haemorrhagic fevers. Current Opinion in Infectious Diseases. 2001;14(5):513-8.

5.Bausch DG, Demby AH, Coulibaly M, et al. Lassa fever in Guinea, West Africa. I. Epidemiology of human disease and clinical observations. Vector Borne and Zoonotic Diseases. 2001;1:269-82.

6. McCormick JB, Fisher-Hoch SP. Lassa fever. Curr Top Microbiol Immunol. 2002;262:75–109. http://dx.doi.org/10.1007/ 978-3-642-56029-3_4

7. Fichet-Calvet E, Rogers DJ. Risk maps of Lassa fever in West Africa. PLoS Negl Trop Dis. 2009;3:e388.

8. Pavloskii EN. Natural nidality of transmissible diseases, with special reference to the landscape epidemiology of zooanthroponoses. Urbana (IL): University of Illinois Press; 1966.

9. Salazar-Bravo J, Dragoo JW, Bowen MD, Peters CJ, Ksiazek TG, Yates TL. Natural nidality in Bolivian hemorrhagic fever and the systematics of the reservoir species. Infect Genet Evol. 2002; 1:191–9. http://dx.doi.org/10.1016/ S1567-1348(02)00026-6

10. Monath TP, Newhouse VF, Kemp GE, Setzer HW, Cacciapuoti A. Lassa virus isolation from Mastomys natalensis rodents during an epidemic in Sierra Leone. Science. 1974;185:263–5. http://dx.doi.org/10.1126/science.185.4147.263

11. Wulff H, McIntosh BM, Hamner DB, Johnson KM. Isolation of an arenavirus closely related to Lassa virus from Mastomys natalensis in south-east Africa. Bull World Health Organ. 1977; 55:441–4.

12. Günther S, Hoofd G, Charrel R, Röser C, Becker-Ziaja B, Lloyd G, et al. Mopeia virus–related arenavirus in natal multimammate mice, Morogoro, Tanzania. Emerg Infect Dis. 2009;15:2008–12. http://dx.doi.org/10.3201/eid1512.090864

13. Gryseels S, Rieger T, Oestereich L, Cuypers B, Borremans B, Makundi R, et al. Gairo virus, a novel arenavirus of the widespread Mastomys natalensis: genetically divergent, but ecologically similar to Lassa and Morogoro viruses. Virology. 2015;476:249– 56. http://dx.doi.org/10.1016/j.virol.2014.12.011

14. Ishii A, Thomas Y, Moonga L, Nakamura I, Ohnuma A, Hangombe B, et al. Novel arenavirus, Zambia. Emerg Infect Dis. 2011;17:1921-4. http://dx.doi.org/10.3201/eid1710.10452

15. Colangelo P, Verheyen E, Leirs H, Tatard C, Denys C, Dobigny G, et al. A mitochondrial phylogeographic scenario for the most widespread African rodent, Mastomys natalensis. Biol J Linn Soc Lond. 2013;108:901–16. http://dx.doi.org/10.1111/bij.12013

16. Mills JN, Yates TL, Childs J, Parmenter RR, Ksiazek TG, Rollin PE, et al. Guidelines for working with rodents potentially infected with hantavirus. J Mammal. 1995;76:716–22. http://dx.doi.org/10.2307/1382742

17. Vieth S, Drosten C, Lenz O, Vincent M, Omilabu S, Hass M, et al. RT-PCR assay for detection of Lassa virus and related Old World arenaviruses targeting the L gene. Trans R Soc Trop Med Hyg. 2007;101:1253–64. http://dx.doi.org/10.1016/ j.trstmh.2005.03.018

18. Ölschläger S, Lelke M, Emmerich P, Panning M, Drosten C, Hass M, et al. Improved detection of Lassa virus by reverse transcription-PCR targeting the 5′ region of S RNA. J Clin Microbiol. 2010;48:2009–13. http://dx.doi.org/10.1128/JCM.02351-09

19. Ehichioya DU, Hass M, Becker-Ziaja B, Ehimuan J, Asogun DA, Fichet-Calvet E, et al. Current molecular epidemiology of Lassa virus in Nigeria. J Clin Microbiol. 2011;49:1157–61. http://dx.doi.org/10.1128/JCM.01891-10

20.Flatz L, Rieger T, Merkler D, Bergthaler A, Regen T, Schedensack M, et al. (2010) T Cell-Dependence of Lassa Fever Pathogenesis. PLoS Pathog 6(3): e1000836. doi:10.1371/journal.ppat.1000836.

21.Hoofnagle, J. H., Lau, D., Conjeevaram, H., Kleiner, D. & Di Bisceglie, A. M. Prolonged therapy of chronic hepatitis C with ribavirin. *J. Viral Hepat.***3**, 247−252 (1996).

22.Maag, D., Castro, C., Hong, Z. & Cameron, C. E. Hepatitis C virus RNA-dependent RNA polymerase (NS5B) as a mediator of the antiviral activity of ribavirin. *J. Biol. Chem.* **276**, 46094−46098 (2001).

23.Hedstrom L. IMP Dehydrogenase: Structure, Mechanism and Inhibition. Chemical reviews. 2009;109(7):2903-2928. doi:10.1021/cr900021w.

24.Crotty, S. *et al*. The broad-spectrum antiviral ribonucleoside ribavirin is an RNA virus mutagen. *Nature Med.* **6**, 1375−1379 (2000).

25.Zhou, S., Liu, R., Baroudy, B. M., Malcolm, B. A. & Reyes, G. R. The effect of ribavirin and IMPDH inhibitors on hepatitis C virus subgenomic replicon RNA. *Virology* **310**, 333−342 (2003).

26.de Veer, M. J. *et al*. Functional classification of interferon-stimulated genes identified using microarrays. *J. Leukocyte Biol.* **69**, 912−920 (2001).

27.Hadi CM, Goba A, Khan SH, et al. Ribavirin for Lassa Fever Postexposure Prophylaxis. Emerging Infectious Diseases. 2010;16(12):2009-2011. doi:10.3201/eid1612.100994

28.Leroy E, Kumulungui B, Pourrut X et al. Fruit bats as reservoirs of Ebola virus. Nature. 2005;438(7068):575-576. doi:10.1038/438575a.

29.Feldmann H, Geisbert T. Ebola haemorrhagic fever. 2017.

30.Pigott DM, Golding N, Mylne A, Huang Z, Henry AJ, Weiss DJ, Brady OJ, Kraemer MU, Smith DL, Moyes CL, Bhatt S, Gething PW, Horby PW, Bogoch II, Brownstein JS, Mekaru SR, Tatem AJ, Khan K, Hay SI: Mapping the zoonotic niche of Ebola virus disease in Africa.*Elife* 2014, 3. doi:10.7554/eLife.04395..

31.Lee, Jeffrey E; Saphire, Erica Ollmann (1 January 2009). "Ebolavirus glycoprotein structure and mechanism of entry". *Future virology*. **4** (6): 621–635. doi:10.2217/fvl.09.56.

32.Reid S, Leung L, Hartmann A et al. Ebola Virus VP24 Binds Karyopherin 1 and Blocks STAT1 Nuclear Accumulation. Journal of Virology. 2006;80(11):5156-5167. doi:10.1128/jvi.02349-05.

33.Bwaka MA, Bonnet MJ, Calain P, Colebunders R, De Roo A, Guimard Y, Katwiki KR, Kibadi K, Kipasa MA, Kuvula KJ, Mapanda BB, Massamba M, Mupapa KD, Muyembe-Tamfum JJ, Ndaberey E, Peters CJ, Rollin PE, Van den Enden E, Van den Enden E: Ebola hemorrhagic fever in Kikwit, Democratic Republic of the Congo: clinical observations in 103 patients. J Infect Dis. 1999, 179: S1-S7. 10.1086/514308.

34.Timoney P. Vesicular stomatitis. Veterinary Record. 2016;179(5):119-120. doi:10.1136/vr.i4075.

35.Marzi A, Engelmann F, Feldmann F et al. Antibodies are necessary for rVSV/ZEBOV-GP-mediated protection against lethal Ebola virus challenge in nonhuman primates. Proceedings of the National Academy of Sciences. 2013;110(5):1893-1898. doi:10.1073/pnas.1209591110.

36.Agnandji S, Huttner A, Zinser M et al. Phase 1 Trials of rVSV Ebola Vaccine in Africa and Europe. New England Journal of Medicine. 2016;374(17):1647-1660. doi:10.1056/nejmoa1502924.

37. Crowcroft N. Management of Lassa Fever in European countries. Euro Surveillance. 2002;7(3):50-2.

38. Cummins D, Bennett D, Fisher-Hoch SP, et al. Lassa fever encephalopathy: clinical and laboratory findings. Journal of Tropical Medicine and Hygiene. 1992;95:197-201.

39.Clegg, J. C. S., M. D. Bowen, M. J. Buchmeier, J.-P. Gonzalez, I. S. Lukashevich, C. J. Peters, R. Rico-Hesse, and V. Romanowski.Family Arenaviridae, virus taxonomy: seventh report of the International Committee on Taxonomy of Viruses, in press. Academic Press, San Diego, Calif.

40.McCormick J. B. (1987) Epidemiology and control of Lassa fever. Curr. Top. Microbiol. Immunol. 134:69–78.

41.Anonymous (1997) Lassa fever. Wkly. Epidemiol. Rec. 20:145–146.

42.Ruo S. L., Mitchell S. W., Kiley M. P., Roumillat L. F., Fisher-Hoch S. P., McCormick J. B. (1991) Antigenic relatedness between arenaviruses defined at the epitope level by monoclonal antibodies. J. Gen. Virol. 72:549–555.

43. Bowen M. D., Peters C. J., Nichol S. T. (1997) Phylogenetic analysis of the Arenaviridae: patterns of virus evolution and evidence for cospeciation between arenaviruses and their rodent hosts. Mol. Phylogenet. Evol.8:301–316.

44.Clegg J. C., Wilson S. M., Oram J. D. (1991) Nucleotide sequence of the S RNA of Lassa virus (Nigerian strain) and comparative analysis of arenavirus gene products. Virus Res. 18:151–164.

45.Kuntzen T, Kuhn S, Kuntzen D, Seifert B, Müllhaupt B, Geier A. Influence of Ribavirin Serum Levels on Outcome of Antiviral Treatment and Anemia in Hepatitis C Virus Infection. *Plos ONE* [serial online]. July 7, 2016;11(7):1-13. Available from: Academic Search Complete, Ipswich, MA. Accessed December 9, 2016.

46.Waldenström J, Westin J, Lagging M, et al. Randomized Trial Evaluating the Impact of Ribavirin Monotherapy and Double Dosing on Viral Kinetics, Ribavirin Pharmacokinetics and Anemia in Hepatitis C Virus Genotype 1 Infection. *Plos ONE* [serial online]. May 11, 2016;11(5):1-18. Available from: Academic Search Complete, Ipswich, MA. Accessed December 9, 2016.

47.Tong W, Zhu J, Jiang Z, et al. Response to Pegylated Interferon Plus Ribavirin in Patients with Hepatitis C Virus Genotype 6a Infection from Guangdong and Guangxi Province of China. *Gastroenterology Research & Practice* [serial online]. February 29, 2016;:1-6. Available from: Academic Search Complete, Ipswich, MA. Accessed December 9, 2016.

48. National Academies of Sciences, Engineering, and Medicine. Hearing Health Care: Priorities for Improving Access and Affordability. Washington, DC: National Academies Press; 2016.

49. President ’s Council of Advisors on Science and Technology. Hearing technology report. Available at: https://www.whitehouse.gov/sites/default/ files/ microsites/ostp/PCAST/pcast_hearing_tech_ letterreport_ final.pdf. Accessed June 7, 2016.

50. Lin FR, Yaffe K, Xia J, et al. Hearing loss and cognitive decline in older adults. JAMA Intern Med. 2013;173(4): 293 –299.

51. Lopez D, McCaul KA, Hankey GJ, et al. Falls, injuries from falls, health related quality of life and mortality in older adults with vision and hearing impairment —is there a gender difference? Maturitas. 2011;69(4):359 –364.

52. Lin FR, Niparko JK, Ferrucci L. Hearing loss prevalence in the United States. Arch Intern Med. 2011;171(20): 1851 –1852.

53. US Census Bureau. Current Population Survey. Available at: https://www.census.gov/cps/data/ cpstablecreator.html. Accessed June 7, 2016.

54. Johnson CL, Paulose-Ram R, Ogden CL, et al. National Health and Nutrition Examination Survey: analytic guidelines, 1999 –2010. Available at: http://www.cdc. gov/nchs/data/series/sr_02/sr02_161.pdf. Accessed June 7, 2016.

55.Ibekwe T. Lassa fever: Lessons from the West African sub-region...10th European Federation of Audiology Societies (EFAS) Congress. *Journal Of Hearing Science* [serial online]. June 2011;1(1):32. Available from: CINAHL Plus with Full Text, Ipswich, MA. Accessed December 9, 2016.

56.Lasisi O, Ayodele J, Ijaduola G. Challenges in management of childhood sensorineural hearing loss in sub-Saharan Africa, Nigeria. *International Journal Of Pediatric Otorhinolaryngology* [serial online]. April 2006;70(4):625-629. Available from: Academic Search Complete, Ipswich, MA. Accessed December 9, 2016.

57.Lassa Fever – Nigeria. World Health Organization. 2017. Available at: http://www.who.int/csr/don/27-may-2016-lassa-fever-nigeria/en/. Accessed April 1, 2017.

58.Aidam J, Sombié I. The West African Health Organization's experience in improving the health research environment in the ECOWAS region. *Health Research Policy and Systems*. 2016;14:30. doi:10.1186/s12961-016-0102-7.

59. http://www.who.int/csr/disease/lassafever/en/