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A Historical Perspective and Review of the Evidence to Support Fruit Bats as the Natural Reservoir for Ebola Viruses

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A Historical Perspective and Review of the Evidence to Support Fruit Bats as the Natural Reservoir for Ebola Viruses

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Abstract

The Ebola viruses cause sporadic outbreaks of Ebola hemorrhagic fever (EHF) where origins have been traced to the continent of Africa and the Philippines. Since the initial discovery of *Zaire* and *Sudan ebolavirus* in 1976, the Ebola viruses have been responsible for severe hemorrhagic fever outbreaks in Africa with case fatality rates between 40-90%. The natural reservoir(s) of the Ebola viruses is currently unknown, but there is mounting evidence that fruit bats may play a key role. The goal of the current study is to screen a large variety of bat species from Africa and Asia where Ebola is known to be endemic for the presence of IgG specific antibody to Ebola virus in order to see which bat species may show evidence of past Ebola virus infection. Ebola virus would not be expected to cause lethal disease in its natural reservoir; therefore the presence of IgG antibody would be present. Identifying the species of bats that have been infected will allow researchers to hopefully isolate Ebola virus from bats adding to the evidence that bats are a reservoir species. The knowledge gained may also provide clues to new species of bats yet to be identified as possible natural reservoir(s) as well as expand the known geographical range of known Ebola virus outbreaks. Knowing which species of bats as well as their geographic range may help prevent future Ebola outbreaks by minimizing human-reservoir contact.

Keywords: Ebola, outbreak, IgG, reservoir, hemorrhagic, bat

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Chapter I: Introduction

Background:

The Ebola viruses cause sporadic outbreaks of Ebola hemorrhagic fever (EHF) where origins have been traced to the continent of Africa and the Philippines. The Ebola viruses belong to the family *Filoviridae*, order *Mononegavirales* which are characterized by a filamentous shape with a nonsegmented, single strand, negative sense RNA genome. The family *Filoviridae* currently includes three genera, *Ebolavirus*, *Marburgvirus*, and "Cuevavirus" (proposed). The genus *Ebolavirus* includes five species: *Zaire ebolavirus* (EBOV), *Sudan ebolavirus* (SUDV), *Reston ebolavirus* (RESTV), *Bundibugyo ebolavirus* (BDBV), and *Taï Forest ebolavirus* (TAFV) formerly *Côte d'Ivoire ebolavirus* (CIEBOV) (International Committee on Taxonomy of Viruses. & King, 2012).

Since the initial discovery of the first two species, *Zaire* and *Sudan ebolavirus* in 1976, the Ebola viruses have been responsible for severe hemorrhagic fever outbreaks in Africa with high case fatality rates between 40-90% (World Health Organization, 2009). *Reston ebolavirus* is the only species that has been found outside the African continent and has not been found to be pathogenic to humans (Miranda et al., 1991). There has only been one non-fatal human case of *Côte d'Ivoire ebolavirus* reported (Le Guenno et al., 1995).

Outbreak investigations of Ebola virus have shown that introductions into the human population result from either a single introduction into the human population followed by human-to-human transmission, or through multiple introductions into the human population from a zoonotic source (Newman & Food and Agriculture Organization of the United Nations., 2011). After introduction into the human population, human-to-human transmission of Ebola viruses occurs through contact with infectious bodily fluids particularly in healthcare settings where improper barrier nursing techniques are used or during traditional African burials that include communal washing of bodies.

The natural reservoir(s) of the Ebola viruses is currently unknown, but there is mounting evidence that fruit bats may play a key role, including the close genetic relation to Marburg virus in

which a fruit bat has been identified (Amman et al., 2012; Leroy et al., 2009; Leroy et al., 2005; Pourrut et al., 2009). The goal of the current study is to screen a large variety of bat species from Africa and Asia where Ebola is known to be endemic for the presence of IgG specific antibody to Ebola virus in order to see which bat species may show evidence of past Ebola virus infection. Ebola virus would not be expected to cause lethal disease in its natural reservoir; therefore the presence of IgG antibody would be present. Identifying the species of bats that have been infected will allow researchers to hopefully isolate Ebola virus from bats adding to the evidence that bats are a reservoir species. The knowledge gained may also provide clues to new species of bats yet to be identified as possible natural reservoir(s) as well as expand the known geographical range of known Ebola virus outbreaks. Knowing which species of bats as well as their geographic range may help prevent future Ebola outbreaks by minimizing human-reservoir contact.

Chapter II: Literature Review

Introduction to *Filoviridae*: *Ebolavirus, Marburgvirus, and* "Cuevavirus" (proposed):

The family *Filoviridae* contains the genera *Ebolavirus, Marburgvirus*, and the recently discovered "Cuevavirus" (proposed) (International Committee on Taxonomy of Viruses. & King, 2012). There are five species within the genus *Ebolavirus*. *Zaire ebolavirus, Sudan ebolavirus, Bundibugyo ebolavirus,* and *Taï Forest ebolavirus* are found on the African continent. *Reston ebolavirus,* originating from the Philippines, is the only one that has been found outside of the African continent. *Marburg marburgvirus* is the only species within the genus *Marburgvirus,* but there are two distinct viruses Marburg and Ravn (International Committee on Taxonomy of Viruses. & King, 2012). Lloviu virus is a new Ebola-like filovirus that was recently discovered in Spain (Negredo et al., 2011).

Transmission Patterns and Epidemiology of Ebola Virus:

With the exception of the recognized outbreaks, the epidemiology of Ebola virus infections in humans is unknown (Sanchez A, 2007). After infection from the initial source, infection is spread by person-to-person contact through infected bodily fluids such as blood, excrement, oral secretions, and tissue. Healthcare facilities can be a major source of virus transmission. Improper barrier nursing techniques as well as the reuse of infected needles help spread the virus into the community and to the healthcare workers. For example, in the 1995 *Zaire ebolavirus* outbreak in Zaire, one-fourth of the cases were among healthcare workers (Sanchez A, 2007).

Clinical Presentation and Symptoms:

The incubation period of EHF is between 2 and 21 days (Hartman, Towner, & Nichol, 2010). Symptoms manifest abruptly and are often non-specific flu-like and may include chills, fever, myalgia, and malaise followed by lethargy, nausea, vomiting, abdominal pain, anorexia, diarrhea, coughing, headache, and hypotension (Hartman, et al., 2010). Hemorrhaging occurs in less than 50 percent of patients.

Ebola Hemorrhagic Fever Outbreaks:

Table 1 summarizes the known Ebola virus outbreaks through September 2012.

Table 1. Chronology of known Ebola virus outbreaks

Year	Location	Human cases/deaths (% case-fatality ratio)	Strain
1976	Zaire	318/280 (88%)	*Zaire ebolavirus*
1976	Sudan	284/151 (53%)	*Sudan ebolavirus*
1976	United Kingdom	1/0 (0%)	*Sudan ebolavirus*
1977	Zaire	1/1 (100%)	*Zaire ebolavirus*
1979	Sudan	34/22 (65%)	*Sudan ebolavirus*
1989-1990	USA	Epizootic, 4-6 human cases (0%)	*Reston ebolavirus*
1992	Itally, Philippines	Epizootic	*Reston ebolavirus*
1994	Côte d'Ivoire	1/0 (0%)	*Côte d'Ivoire ebolavirus*
1994-1995	Gabon	52/31 (60%)	*Zaire ebolavirus*
1995	Zaire	315/250 (81%)	*Zaire ebolavirus*
1996	Gabon	37/21 (57%)	*Zaire ebolavirus*
1996	USA, Philippines	Epizootic	*Reston ebolavirus*
1996	Russia	1/1 (100%)	*Zaire ebolavirus*
1996-1997	Gabon, South Africa	60/45 (74%)	*Zaire ebolavirus*
2000-2001	Uganda	425/224 (53%)	*Sudan ebolavirus*
2001-2002	Gabon, Republic of	122/96 (78%)	*Zaire ebolavirus*
2002	Gabon, Republic of Congo	11/10 (90%)	*Zaire ebolavirus*
2002-2003	Republic of Congo	143/128 (89.5%)	*Zaire ebolavirus*
2003-2004	Republic of Congo	35/29 (82.9%)	*Zaire ebolavirus*
2004	Russia	1/1 (100%)	*Zaire ebolavirus*
2004	Sudan	17/7 (41%)	*Sudan ebolavirus*
2005	Republic of Congo	12/10 (83%)	*Zaire ebolavirus*
2007	Democratic Republic of the Congo	264/186 (71%)	*Zaire ebolavirus*
2007-2008	Uganda	131/42 (37%)	*Bundibugyo ebolavirus*
2008	Philippines	6/0 (0%)	*Reston ebolavirus*
2008- 2009	Democratic Republic of the Congo	32/15 (47%)	*Zaire ebolavirus*
2011	Uganda	1/1 (100%)	*Sudan ebolavirus*
2012	Uganda	24/17 (71%)	*Sudan ebolavirus*
2012	Democratic Republic of the Congo	Unknown	*Bundibugyo ebolavirus*

Zaire ebolavirus:

1976 Zaire

Ebola hemorrhagic fever (EHF) was first recognized in 1976 during two simultaneous but independent outbreaks in Sudan and Zaire. Between September 1, 1976 and October 24, 1976, an outbreak of *Zaire ebolavirus* occurred in Zaire, which centered around Yambuku Missionary Hospital (YMH) in the Bumba Zone of the Equateur Region ("Ebola haemorrhagic fever in Zaire, 1976," 1978). The first known case was a 44-year-old male instructor at the Mission School who reported to the outpatient clinic at YMH on August 26, 1976 with what was thought to be malaria ("Ebola haemorrhagic fever in Zaire, 1976," 1978). The man had been touring the northern Equateur Region and had purchased smoked antelope and monkey meat. The man indicated that he and his family had eaten the antelope, but not the monkey meat. He was admitted to YMH on September 5, 1976 with gastrointestinal bleeding and died September 8 ("Ebola haemorrhagic fever in Zaire, 1976," 1978).

There were 318 cases, 280 deaths and a case fatality ratio of 88 percent ("Ebola haemorrhagic fever in Zaire, 1976," 1978). YMH was the main source of transmission during the outbreak where the reuse of needles and close body contact with infected individuals helped spread the virus ("Ebola haemorrhagic fever in Zaire, 1976," 1978). Of the 288 cases where the means of transmission could be determined, 85 received one or more injections at YMH. Another 149 cases acquired the disease after being in contact with patients, usually in their home village. Forty three cases had a history of both injections at YMH and contact with patients ("Ebola haemorrhagic fever in Zaire, 1976," 1978). Thirteen of the seventeen staff members became infected with EHF of whom eleven died ("Ebola haemorrhagic fever in Zaire, 1976," 1978). The outbreak eventually ended with the closing of YMH and quarantining infected patients in their villages.

1977 Zaire

Approximately one year after the original EBOV outbreak, *Zaire ebolavirus* reemerged in the Tandala region of Zaire in 1977 marking the first time the virus had been seen since its discovery. A nine-year-old girl from the village of Bonduni, presented to Tandala Mission Hospital in June 1977 with a three day fever, abdominal pain, and hematemesis (Heymann et al., 1980). Twenty-eight hours after hospitalization, the child died. Ebola virus was isolated from guinea pigs that had been inoculated with the girl's clinical specimens (Heymann, et al., 1980).

1995 Zaire

In April of 1995 an epidemic of dysentery broke out in Kikwit II Maternity Hospital in Kikwit, Zaire (Khan et al., 1999). Later that month, in Kikwit's other large hospital, Kikwit General Hospital, a similar cluster of dysentery was identified among the operating room staff in which two Italian missionary nurses died after caring for a patient who had a laparotomy performed (Khan, et al., 1999). The epidemic dysentery was originally misdiagnosed and later confirmed as *Zaire ebolavirus* after testing by the Centers for Disease Control and Prevention in Atlanta, Georgia. Fourteen samples were tested, which confirmed that the 2 Italian nurses as well as twelve additional members of the surgical team and their contacts had recent infection. There were a total of 315 cases, 250 deaths and a case fatality ratio of 81 percent (Khan, et al., 1999).

December 1994- January/February 1995 Gabon

There were five independent outbreaks of EBOV between November 1994 and February 1995 in Gabon. The first epidemic involved two waves of patients who were from the gold-panning communities of Mekouka, Andock, and Minkebe (Georges et al., 1999). The first wave of patients consisted of 32 patients who went to the hospital in Makokou for treatment. The second wave originated from a patient who fled the hospital to seek treatment from a traditional healer. Sixteen additional cases

occurred in the second wave (Georges, et al., 1999). There were 52 cases, 31 deaths, and a case fatality ratio of 60 percent (Georges, et al., 1999).

January - April 1996 Gabon

In February 1996, the second epidemic of *Zaire ebolavirus* began in the village of Mayibout 2, Gabon. The epidemic began with 18 people who had skinned a chimpanzee cadaver and became ill with fever, headache, and bloody diarrhea (Georges, et al., 1999). All patients were admitted to Makokou General Hospital where four died within 48 hours. The bodies were returned to their villages for burial where traditional burial practices were performed without proper precautions (Georges, et al., 1999). There were 37 cases, 21 deaths and a case fatality ratio of 57 percent (Georges, et al., 1999).

July 1996 - January 1997 Gabon

The third epidemic in *Zaire ebolavirus* in Gabon occurred in Booue area. Virus was isolated from two of six samples from patients hospitalized at Booue (Georges, et al., 1999). A retrospective study of the epidemic traced the index case back to a 39-year-old hunter in a logging camp near Booue in July who had typical symptoms of viral hemorrhagic fever. Furthermore, dead chimpanzees were found in the area of which one was confirmed to be positive for Ebola (Georges, et al., 1999). At the end of August, a second hunter died at the same logging camp. A third hunter became ill twelve days later, and died in the village of Balimba.

At the end of November 1996, another wave of transmission occurred in three locations: Lolo, SHM, a timber company, and Balimba, a logging camp (Georges, et al., 1999). The epidemic also spread to Libreville, the capital of Gabon, Lastourville, and South Africa. A Gabonese doctor performed an endoscopy in Libreville on an infected patient and went to Johannesburg for treatment (Georges, et al., 1999). A nurse who treated the infected doctor became ill and died. There were 60 cases, 45 deaths and a case fatality ratio of 74 percent (Georges, et al., 1999).

2001-2002 Gabon / Republic of Congo

An outbreak of *Zaire ebolavirus* was reported between October 2001 and July 2002 in Gabon and Republic of Congo. This was the first reported instance of Ebola being in the Republic of Congo ("Outbreak(s) of Ebola haemorrhagic fever, Congo and Gabon, October 2001-July 2002," 2003). In November 2001, medical personnel at Mekambo Medical Centre in La Zadie health district of Gabon reported five deaths to the regional health authorities. At the same time there had been a large number of dead non-human primates found in the rainforest and reported to the authorities ("Outbreak(s) of Ebola haemorrhagic fever, Congo and Gabon, October 2001-July 2002," 2003). On November 30, 2001 blood samples from two suspected cases were sent to Centre International de Recherches Médicales de Franceville (CIRMF) for testing. Ebola virus was confirmed in both samples. In Gabon, there were 65 cases, 53 deaths and a case fatality ratio of 82 percent ("Outbreak(s) of Ebola haemorrhagic fever, Congo and Gabon, October 2001-July 2002," 2003). In the Republic of the Congo, there were 57 cases, 43 deaths and a case fatality ratio of 75 percent ("Outbreak(s) of Ebola haemorrhagic fever, Congo and Gabon, October 2001-July 2002," 2003).

2002-2003 *Republic of Congo*

On June 28, 2003, ten deaths and five hospitalized cases were reported by the Kéllé Health Center in Kéllé District, Cuvette-Ouest Region of the Republic of Congo (Kuhn, 2008). This was preceded by a die off chimpanzees, gorillas, and duikers in which *Zaire ebolavirus* had been isolated from their dead carcasses. Human infections were also recorded in workers of a gold mine in Mbomo District (Formenty et al., 2003). There were a total of 143 cases, 128 deaths and a case fatality ratio of 89 percent (Formenty, et al., 2003).

2003-2004 Republic of Congo

An outbreak of *Zaire ebolavirus* occurred in the Mbomo district, Cuvette Ouest Department during November and December of 2003 in the Republic of the Congo. Testing performed by the Institut de Recherche pour le Développement (IRD) and CIRMF confirmed Ebola virus in 16 samples

(World Health Organization, 2004). There were 35 cases, 29 deaths, and a case fatality ratio of 83 percent (World Health Organization, 2004).

2005 Republic of Congo

Between April 25 and June 16, 2005, there were twelve cases of *Zaire ebolavirus* in Etoumbi District, Cuvette Ouest Region, Republic of the Congo. The outbreak began with two men who were poaching for elephants in Parc d'Odzala forest (Nkoghe, Kone, Yada, & Leroy, 2011). They returned to the village of Etoumbi to get help with the elephant and was hospitalized with fever and hemorrhaging. They died shortly after hospitalization. There were ten contacts epidemiologically linked to the index cases in which eight died (Nkoghe, et al., 2011). The case fatality ratio was 83 percent (Nkoghe, et al., 2011).

2007 Democratic Republic of the Congo

A large outbreak of *Zaire ebolavirus* occurred in Luebo, Democratic Republic of the Congo in 2007 that may have started from the direct exposure to fruit bats (Leroy, et al., 2009). The Kampungu agglomeration of villages where the index case, a 55-year-old woman, lived was once located in a forest/savanna transition zone along the Lulua River. Bats were reported to have migrated to the islands of Ndongo and Koumulele to feed on the fruit trees (Leroy, et al., 2009). Villagers routinely hunted the bats for food and sold them at the village market (Leroy, et al., 2009). There were 264 cases and 186 deaths with a case fatality ratio of 71 percent (Leroy, et al., 2009).

2008-2009 Democratic Republic of the Congo

The last known outbreak of *Zaire ebolavirus* occurred in 2008-2009 in the Mweka and Luebo health zones of the Democratic Republic of the Congo. The outbreak was confirmed by laboratory tests at the Institut National de Recherches Biologiques (INRB) in Kinshasa, the CIRMF in Gabon, and the National Institute for Communicable Diseases (NICD), South Africa (World Health Organization,

2009). There were 32 cases reported, 15 fatalities with a case fatality ratio of 47 percent (World Health Organization, 2009).

Sudan ebolavirus:

1976 Sudan

Between June and November of 1976, an outbreak of *Sudan ebolavirus* occurred simultaneously in Sudan as the *Zaire ebolavirus* outbreak was occurring in Yambuku, Zaire. Three employees of a cotton factory in Nzara township became ill with a severe febrile illness with profuse bleeding ("Ebola haemorrhagic fever in Sudan, 1976. Report of a WHO/International Study Team," 1978). The outbreak later spread to Maridi 128km from Nzara, where it was able to amplify in Maridi hospital. There were 284 cases, 151 fatalities with a case fatality ratio of 53 percent ("Ebola haemorrhagic fever in Sudan, 1976. Report of a WHO/International Study Team," 1978).

1979 Sudan

In 1979, thirty four cases of *Sudan ebolavirus* occurred in Nzara, Sudan (Baron, McCormick, & Zubeir, 1983). The outbreak began in August with a 45-year-old man entering Nzara hospital with 3 days of fever, a recent onset of vomiting and diarrhea, and eventually developing gastrointestinal bleeding (Baron, et al., 1983). He passed away three days later. Neither the hospital staff nor his family taking care of him practiced barrier-nursing procedures which led to the outbreak. There were 34 cases, 22 deaths, and a case fatality ratio of 65 percent (Baron, et al., 1983).

August 2000- January 2001 Uganda

The largest outbreak of Ebola hemorrhagic fever to occur was an outbreak of *Sudan ebolavirus* in Gulu, Masindi, and Mbarara districts of Uganda. On October 8, 2000, an unusual febrile illness with hemorrhage and significant mortality was reported to the Ministry of Health in Kampala, Uganda (Centers for Disease Control, 2001). There were 425 presumptive cases of EHF with 224 deaths with a case fatality ratio of 53 percent (Centers for Disease Control, 2001). There were 393 cases in Gulu district, 27 in Masindi, and 5 were from Mbarara. It is unclear where the original case(s) originated but

attending funerals of Ebola hemorrhagic fever case-patients, having contact with case-patients in one's family, and providing medical care to Ebola case-patients without using adequate personal protective measures were all identified as the most important means of transmission during the outbreak (Centers for Disease Control, 2001).

2004 Sudan

In April 2004 *Sudan ebolavirus* reemerged in Yambio, Sudan. The medical staff at Yambio County Health Department and the coordinator of the South Sudan Early Warning and Response Network (EWARN) reported 7 suspected cases of hemorrhagic fever, which included two deaths to the EWARN leader in Lokichoggio, Kenya (Onyango et al., 2007; "Outbreak of Ebola haemorrhagic fever in Yambio, south Sudan, April - June 2004," 2005). Five of the suspected cases were from the same family and two were hospital staff. Symptoms manifested over a three-week period and included fever, vomiting, and bloody diarrhea (Onyango, et al., 2007). Overall, there were 17 confirmed cases, seven deaths and case fatality ratio of 41 percent (Onyango, et al., 2007). The outbreak coincided with an outbreak of measles, which made cases of Ebola difficult to diagnose.

2011 Uganda

In 2011, a single case of *Sudan ebolavirus* occurred in Luwero District, Uganda. On May 6, 2011, a 12 year-old girl presented to Bombo Military Hospital with fever, jaundice, and hemorrhagic signs that included vaginal bleeding (Shoemaker et al., 2012). Her condition worsened and she died three hours after admission. She was isolated from the general hospital population and the hospital staff used proper protective equipment, which limited the outbreak to the one case.

2012 Uganda

An outbreak of *Sudan ebolavirus* was confirmed on July 28, 2012 in Kibaale District, Uganda. A team of officials from the Centers for Disease Control and Prevention in Atlanta was sent to assist in the

outbreak. The outbreak was declared over on October 4, 2012 with 24 cases, 17 deaths, and a case fatality ratio of 71 percent.

***Bundibugyo ebolavirus*:**

2007-2008 Uganda

The most recently discovered species of Ebola virus, *Bundibugyo ebolavirus,* was identified five years ago in Budibugyo District, Uganda. In late November of 2007, 29 blood samples were sent to the Centers for Disease Control and Prevention in Atlanta, Georgia for filovirus testing (Towner et al., 2008). Eight of the samples were acutely positive using an antigen capture ELISA assay and IgM capture assay for *Zaire ebolavirus* (Towner, et al., 2008). Once an outbreak of Ebola virus was identified, an international team of scientists was deployed to Uganda to assist with the outbreak. There were a total of 131 suspect, probable, and confirmed cases of identified (Chowell, Hengartner, Castillo-Chavez, Fenimore, & Hyman, 2004). There were 56 laboratory confirmed cases, 43 of which were acute phase specimens (MacNeil et al., 2010). Out of the 43 acute specimens, there were 17 deaths with a case fatality ratio of 40 percent (MacNeil, et al., 2010).

Bundibugyo ebolavirus is thought to have first appeared in August 2007 in the village of Kabango in Bundibugyo District ("Outbreak news. Ebola virus haemorrhagic fever, Democratic Republic of the Congo," 2007). A 26-year-old woman developed fever and weakness and was hospitalized. She was pregnant and gave birth to a preterm infant that later died and the mother died on August 4th ("Outbreak news. Ebola virus haemorrhagic fever, Democratic Republic of the Congo," 2007). The mother and sister of the index patient were involved in handling her remains and did not use proper barrier nursing techniques. A total of nine cases and 6 deaths from the cluster were reported in which two of the survivors later tested positive for *Bundibugyo ebolavirus* specific IgG ("Outbreak news. Ebola virus haemorrhagic fever, Democratic Republic of the Congo," 2007).

2012 Democratic Republic of the Congo

On August 17, 2012 the Ministry of Health reported 10 suspected cases and 6 deaths of Ebola hemorrhagic fever to the World Health Organization ("Outbreak news. Ebola haemorrhagic fever, Democratic Republic of the Congo," 2012). The outbreak is currently ongoing in Isiro and Dungu Health Zones of Province Orientale. As of October 24, 2012 there have been 52 reported cases and 25 deaths ("Outbreak news. Ebola, Democratic Republic of Congo - update," 2012).

***Cote d'Ivoire ebolavirus*:**

1994 *Cote d'Ivoire*

The only known case of *Cote-d'Ivoire ebolavirus* occurred in the Taï National Forrest of Cote-d'Ivoire. A troop of chimpanzees was being studied in which several members were found dead with hemorrhages (Le Guenno, et al., 1995). In November 1994, a field autopsy was performed on a dead chimpanzee to collect samples in order to find a cause of death. A 34-year old female who autopsied the chimpanzee developed dengue-like symptoms on the 24th of November and was hospitalized on the 26th of November (Le Guenno, et al., 1995). Sera was drawn from the patient and virus isolation attempts were made using Vero E6 monkey kidney cells and AP61 *Aedes pseudosutellaris* mosquito cells. Electron microscopy was used to identify Ebola virus in the Vero E6 cells (Le Guenno, et al., 1995). The patient eventually recovered from the infection.

Reston ebolavirus:

1989-1990 USA

The third species of Ebola to be identified was *Reston ebolavirus* in 1989 and is the only non-African species identified. In October 1989, 100 cynomolgus monkeys (*Macaca fascicularis*), were flown from Manila, Philippine, to New York and taken by truck to Hazleton Research Products in Reston, Virginia (Jahrling et al., 1990). They were placed into quarantine where two animals died and two others became ill. Necropsy findings were consistent with simian hemorrhagic fever (SHF) (Jahrling, et al., 1990). Monkeys continued to die in other rooms, which was not consistent with SHF. A

filovirus was isolated from four monkeys, which was later named *Reston ebolavirus*. There were five confirmed cased of *Reston ebolavirus* in monkeys and no reports of disease in humans (Jahrling, et al., 1990).

1989-1990 Philippines

After the initial importation of infected cynomalgus monkeys into the United States from the Philippines in 1989, studies were conducted at the export facilities in the Philippines in order to document transmission of the virus. In March of 1990, dead monkeys were tested from the two export facilities in the Philippines where the shipments to the United States had originated (Hayes et al., 1992). In facility A, 85 of 161 dead monkeys tested positive for *Reston ebolavirus* antigen (Hayes, et al., 1992). A serosurvey showed that 89 out of 343 monkeys had antibody to the virus. The second facility, facility B, had no dead monkeys that were positive for *Reston ebolavirus* but 53 of 958 were antibody positive.

1992 Italy

In March of 1992, 55 cynomologus monkeys were imported into Siena, Italy from the same monkey import facility in the Philippines implicated in the original 1989-1990 Reston, Virginia *Reston ebolavirus* outbreak. Eight monkeys died from the shipment and *Reston ebolavirus* was isolated and identified by electron microscopy ("Viral haemorrhagic fever in imported monkeys," 1992). There were 16 human contacts with the monkeys but none of them showed signs of infection or seroconverted ("Viral haemorrhagic fever in imported monkeys," 1992).

1996 USA/ Philippines

Reston ebolavirus was once again imported into the United States in April 1996. One hundred cynomologus monkeys arrived from the Philippines to a quarantine facility in Alice, Texas where they were split into two cohorts of 50 animals. One monkey died three days after arrival after exhibiting anorexia and lethargy and was tested for *Reston ebolavirus* antigen (Rollin et al., 1999). Federal regulations require mandatory testing for *Reston ebolavirus* after the death of an imported monkey in

quarantine (Centers for Disease Control, 1990; Pourrut, et al., 2009). A second monkey had similar signs in the same cohort and was euthanized, tested and found positive for *Reston ebolavirus* (Rollin, et al., 1999). The remaining 48 monkeys were euthanized and tested in which two additional monkeys were found positive for *Reston ebolavirus*. Simian hemorrhagic fever was also isolated from some of the animals (Rollin, et al., 1999). The monkeys were exported from the same facility that exported monkeys infected with *Reston ebolavirus* to Reston Virginia in 1989-1990, and to Italy in 1992 (Miranda et al., 1999; "Viral haemorrhagic fever in imported monkeys," 1992).

2008 Philippines

Although *Reston Reston ebolavirus* had been shown to be in the Philippines since 1989, it had only been found in Cynomolgus monkeys. In 2008, *Reston ebolavirus* was discovered in swine further expanding the host range of the virus. In July 2008, assistance from the Foreign Animal Disease Diagnostic Laboratory (FADDL) of the United States Department of Agriculture (USDA) was requested in investigating outbreaks of a respiratory and abortion disease syndrome that had been spreading through Asia (Barrette et al., 2009). Sera and tissue samples were tested for the presence of Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) as well as other common swine viruses. PRRSV was discovered to be infecting the swine simultaneously with *Reston ebolavirus* (Barrette, et al., 2009). Interestingly, *Reston ebolavirus* was only found in samples that were also infected with PRRSV.

Laboratory Accidents:

1976 England, Sudan ebolavirus

Specimens from the 1976 *Sudan ebolavirus* outbreak were sent to investigators at the Microbiological Research Establishment, Porton Down, England to assist in the identification of the new agent responsible for the outbreak (Emond, Evans, Bowen, & Lloyd, 1977). On November 5, 1976, an investigator accidently pricked his thumb through a protective rubber glove while transferring a homogenized guinea pig liver infected with *Sudan ebolavirus*. He was kept under surveillance and became ill on the 6th day (Emond, et al., 1977). On November 11th his temperature was 38°C and

complained of anorexia, nausea, and constant central abdominal pain. He recovered after treatment with interferon and convalescent serum from patients of the recent African outbreak and recovered. Blood collected 14 hours after the patient became feverish was confirmed to have virus particles similar to those of Ebola (Emond, et al., 1977).

2004 Russia, Zaire ebolavirus

On May 5, 2004, a Russian scientist working at the State Research Center for Virology and Biotechnology (VECTOR) in Siberia, accidently punctured her double-gloved hand and pricked her left palm with blood from a guinea pig infected with *Zaire ebolavirus* ("Fatal Laboratory Accident, Siberia," 2004). She was isolated at the hospital of VECTOR's department of dangerous infections where she was under medical observation. She died 14 days later.

Fruit Bats as the Natural Reservoir of *Filoviridae:*

Isolation of *Marburgvirus* from *Rousettus aegyptiacus* Fruit Bats:

The first recognition of Marburg hemorrhagic fever was in 1967 when monkeys from Uganda were imported into Marburg, Germany. Following the original discovery of *marburgvirus* the natural reservoir had been unknown until recently. In 2007, following an outbreak of Marburg hemorrhagic fever involving miners in Kitaka Cave, Uganda, an ecological investigation was launched to try and identify the natural reservoir of Marburg virus (Towner et al., 2009). Of the 611 *R. aegyptiacus* bats collected from Kitaka mine in August 2007 and April-May of 2008, 32 were positive for Marburg virus by Q-RT-PCR and there were five virus isolates obtained (Towner, et al., 2009).

Experimental Infection of Fruit Bats with *Zaire ebolavirus*:

Following the *Zaire ebolavirus* outbreak in Kikwit in 1995, pathogenicity experiments were undertaken at the National Institute for Virology in South Africa in representative plants and vertebrates that were collected from a previous visit. Among the animals infected with Ebola virus were *Epomophorous wahlbergi* fruit bats in which the virus was able to replicate (Swanepoel et al., 1996). Furthermore, virus was recovered 21 days postinoculation from fecal samples.

Serological Evidence for Fruit Bats as the Natural Reservoir of *Ebolavirus:*

In Gabon and the Republic of Congo where known *Zaire ebolavirus* (ZEBOV) outbreaks have occurred, screening of 1,030 animals collected between 2001 and 2005 including 679 bats, were screened for the presence of IgG antibody specific to ZEBOV (Leroy, et al., 2005). IgG antibody to ZEBOV was detected by ELISA in 8/117 *Epomops franqueti* bats, 4/17 *Hypsignathus monstrosus*, and 4/58 *Myonycteris torquata* (Leroy, et al., 2005). Furthermore, 13 bats of these three species had RNA sequences that matched ZEBOV, but none of the bats that were IgG positive for ZEBOV had RNA sequences. The authors suggested that these species of fruit bats are possible reservoirs of ZEBOV (Leroy, et al., 2005).

Since the discovery of ZEBOV in fruit bats in Gabon and Republic of Gabon a larger serological survey was performed between 2003 and 2008 that also included the Democratic Republic of Congo. There were 2,147 bats belonging to nine species tested for the presence of Marburg virus and ZEBOV (Pourrut, et al., 2009). In addition to confirming the presence of ZEBOV in *E. franqueti, H. monstrosus,* and *M. torquata*, antibody was found in 4/197 *Micropteropus pusillus*, 24/307 *R. aegyptiacus,* and 3/24 *Microchiroptera* (Pourrut, et al., 2009).

Fruit bats of the Greater Accra Region have also been found to be positive for IgG antibody to ZEBOV (Hayman et al., 2010; Hayman et al., 2012). In 2008, 1/262 *Eidolon helvum* bats screened using indirect fluorescent tests was positive for IgG antibody to ZEBOV (Hayman, et al., 2010). In order to see if Ebola virus is circulating in the Greater Accra Region, 88 bats from five species were screened following the initial finding of the IgG positive *E. helvum* (Hayman, et al., 2012). Of those, 32 were found IgG positive for Ebola virus. IgG antibody specific to Ebola virus was found in 10/27 *E. franqueti*, 14/37 *Epomophorus gambianus*, 7/16 *H. monstrosus*, and 1/4 *Nanonycteris veldkampii* (Hayman, et al., 2012). Some of the bats reacted with ZEBOV specific antigen while others reacted with REBOV specific antigen. The data suggests that using Taï Forrest EBOV (TEBOV) antigen may

increase the ability to detect Ebola virus in Ghana if *Taï Forest ebolavirus* is the species of Ebola virus circulating in the area (Hayman, et al., 2012).

Reston ebolavirus (REBOV) epidemics have occurred in the Philippines since 1989, but the discovery of REBOV in domestic swine in 2008 put more attention on finding the natural reservoir. During 2008 and 2009, 141 wild bats were caught and tested for REBOV specific IgG antibody using ELISA (Taniguchi et al., 2011). Only one species, *Rousettus amplexicaudatus,* had positive results for IgG antibody to REBOV indicating that this species may be a potential natural reservoir for REBOV.

Chapter III: Materials and Methods

Study Description:

The CDC's Viral Special Pathogens Branch (VSPB) in Atlanta, Georgia investigated many of the Ebola outbreaks detailed in the previous section. Additionally, bat specimens from Nipah virus outbreaks from Southeast Asia were available to screen for REBOV that VSPB had participated.

Characteristics of the Bat Sera Collection:

The collection of bat specimens screened for antibody evidence of Ebola virus consisted of 3,013 sera or blood specimens. The bat sera or blood was collected in the years 1995 through 2012 from seven African countries and eight Asian countries. Some of the bats screened were originally collected during the *Zaire ebolavirus* outbreak in 1995, *Sudan ebolavirus* outbreak in 2001, and the *Sudan ebolavirus* outbreak in 2011. Fifty genera of bats are represented in the collection. Table 2 shows the distribution of the bat specimens screened by country.

Table 2. Characteristics of the Bat Sera Catalog

Country	**Number**
American Samoa	44
Bangladesh	216
Cambodia	296
Fiji	12
Gabon	17
Indonesia	19
Kenya	106
Malawi	152
Philippines	9
Singapore	3
Sudan	203
Tanzania	16
Thailand	57
Uganda	1755
Zaire	108
Total	3013

Table 3 on the following pages gives a description of the bat sera collection screened by taxonomic name, collection location(s), and the number screened.

Table 3. Distribution by Species of the Bat sera Collection

Genus and Species	Sera Collection Location(s)	Number Screened
Casinycteris argynnis	Zaire	2
Chaerephon plicata	Cambodia	108
Chaerephon species unknown	Sudan	4
*Epomops franqueti**	Gabon, Zaire, Uganda	10
*Eidolon helvum**	Kenya, Malawi, Uganda	179
Epomophorus labiatus	Kenya, Sudan, Uganda	590
Eonycteris spelaea	Bangladesh, Indonesia, Philippines, Thailand	77
Epomophorus wahlbergi	Kenya	14
Emballonuridae	Sudan	1
Epomophorus species unknown	Malawi, Sudan, Uganda	108
Epomops species unknown	Gabon, Malawi,	40
Glauconycteris species unknown	Sudan	1
*Hypsignathus monstrosus**	Gabon	1
Hipposideros cafer	Uganda	596
Hipposideros species unknown	Bangladesh, Sudan, Uganda	28
Laephotis species unknown	Tanzania	1
Megaderma lyra	Bangladesh	10
Myotis mystacinus	Cambodia	5
*Micropteropus pusillus**	Sudan, Zaire	83
Myonycteris torquata	Gabon	1
Megaloglossus woermanni	Zaire	26
Megaderma species unknown	Bangladesh	1
Micropteropus species unknown	Sudan	6
Molossidae	Sudan	21
Notopteris macdonaldi	Fiji	7
Neoromicia species unknown	Tanzania	10
Nycteris species unknown	Sudan	6
Pteropus giganteus	Bangladesh	153
Pteropus lylei	Cambodia, Thailand	95
Pteropus samoensis	American Samoa, Fiji	10
Pteropus tonganus	American Samoa, Fiji	39
Pipistrellus species unknown	Malawi, Uganda	2
Pteropodidae	Zaire	1
Pteropus species unknown	Cambodia	8
*Rousettus amplexicaudatus**	Philippines	1
Rousettus angolensis	Gabon	6
Rousettus lanosus	Sudan, Tanzania	3
Rousettus leschenaulti	Bangladesh	49
*Rousettus aegyptiacus**	Uganda	549
Rhinolophus species unknown	Sudan, Tanzania, Uganda	26
Scotoecus albofuscus	Sudan	1
Scotophilus kuhlii	Cambodia, Philippines,	36
Scotoecus species unknown	Sudan	11
Scotophilus species unknown	Sudan, Tanzania	26
Stenonycteris species unknown	Malawi	3
Taphozous melanopogon	Cambodia	36
Taphozous theobaldi	Cambodia	11
Tadarida species unknown	Cambodia	6
Taphozous species unknown	Uganda	2
Unknown	Singapore	3
*Species reported to carry Ebola virus antibody	**TOTAL**	3013

Laboratory Methods:

Enzyme-Linked Immunoassay for the Detection of Ebola Virus IgG:

Using an established protocol adapted to bats, 3,013 bats were screened for the presence of Ebola virus specific immunoglobulin G (IgG) antibody (Ksiazek, West, Rollin, Jahrling, & Peters, 1999). Briefly, Ebola virus antigen and negative control antigen was diluted 1:1,000 in 0.01M, PBS, pH 7.2 and 100 ul per well was absorbed to 96-well plates at 4°C overnight (BD Falcon Cat No. 353910). The plates were then washed three times with 200 ul of a 0.01M PBS and 0.1% Tween-20 wash buffer solution and 100 ul of serum diluent (0.01M PBS pH 7.4, 0.5% skim milk, and 0.1% Tween-20) added to each well. The bat serum was added to the plates in 4-fold serial dilutions beginning with an initial dilution of 1:100. The sera was incubated at 37°C for 1 hr and allowed to bind to the antigen. The plates were again washed three times with 200 ul of wash buffer and 100 ul of anti-bat IgG conjugate (Bethyl laboratories) diluted 1:2,000 in serum diluent was applied to each well and incubated at 37°C for 1 hr. After the incubation, the plates were washed, and 100 ul per well of ABTS substrate (Kirkgard and Perry Laboratories) was added and incubated at 37°C for 30 minutes. The plates were read at 405 nm and 495 nm absorbance on a Biotek platereader. A positive sample was defined as having a titer greater than or equal to 400 and having a sum optical density (OD) greater than 0.95.

Virus Isolation Techniques:

A pool of spleen and liver tissue from 50 *Epomophorus labiatus* bats and 14 insectivorous bats from the 2011 *Sudan ebolavirus* outbreak were setup for virus isolation to screen for the presence of Ebola virus. Approximately 100 mg of tissue was ground using a Genogrinder in 2 mls of Hanks Balanced Salt Solution (HBSS). The resulting suspension was centrifuged and the supernatant was used to inoculate Vero E6 cells in 25 cm^2 flasks and incubated at 37°C for 1 hr. After the 1 hr incubation, flasks were re-fed with Eagles Minimum Essential Medium (EMEM) with 2% fetal bovine serum and incubated at 37°C. Cells were scraped from the flasks on day 7 post inoculation, irradiated with

2x10^6 rads of gamma radiation, and fixed to slides with acetone in order to examine for Ebola virus infection by indirect fluorescent assay (IFA). The process was repeated at day 14 post inoculation.

Chapter IV: Results

Ebola Virus Seroprevalence Patterns in the Bat Sera Collection:

Overall, 3 of the 3,014 (0.09%) bats were positive for Ebola virus specific IgG. There was 1 *Chaerephon plicata* from the October 2001 Cambodia collection that reacted with all five species of Ebola virus. There were two *Epomophorus labiatus* bats that were positive for *Sudan ebolavirus* IgG only. One was collected during August 2010 in Sudan and one was collected in April of 2012 in Uganda.

Limitations of the Study:

One potential limitation of this study is that many of the bat specimens used to screen for Ebola virus specific IgG were collected as early as 1995 during the *Zaire ebolavirus* outbreak in Kikwit. The handling and storage conditions over the years are unknown. The integrity of the specimens may have been compromised which could lead to the inability to detect Ebola virus IgG antibody.

Chapter V: Discussion and Conclusion

Interpretation of the Ebola Virus Seroprevalence in the Bat Sera Collection:

The discovery of an Ebola virus IgG positive *Chaerephon plicata* bat from Cambodia expands the currently known geographical range of Ebola virus in Southeast Asia as well as indicates another possible reservoir. Currently, *Reston ebolavirus* is known to be in the Philippines. Recent research has indicated that *Rousettus amplexicaudatus* from the Philippines may be a possible reservoir (Taniguchi, et al., 2011). It is possible that *Reston ebolavirus* is also in Cambodia and *R. amplexicaudatus* is a possible reservoir species in that country.

There have been 3 Ebola outbreaks in Sudan. Although finding 1 *Epomophorus labiatus* bat from Sudan IgG positive to *Sudan ebolavirus* is not surprising, it is novel in that this species of bat has not been implicated as a possible reservoir species in the literature. The other positive *E. labiatus* bat was collected in Uganda in close proximity to the 2011 Sudan virus outbreak. Since each of these bats only reacted with *Sudan ebolavirus* and were collected where known outbreaks of *Sudan ebolavirus* have occurred, it makes this bat species a prime candidate for further exploration into the natural reservoir of *Sudan ebolavirus*.

The Impact of Ebola Virus Outbreaks on African Communities

The impact of Ebola outbreaks on African communities has varied in the past in size and severity depending on the strain of virus and how quick the detection and response can be put forth by the international community. This is in part due to a poor understanding of what Ebola virus is and how it is spread. For example, the *Zaire ebolavirus* outbreak in the Republic of the Congo in 2002-2003 was thought to be caused by sorcery by certain ethnic groups while other ethnic groups contributed it to the pygmy population who were thought to be “dirty disease spreaders” (Hewlett, Epelboin, Hewlett, & Formenty, 2005). Efforts have been made by public health officials to educate the affected African populations on how Ebola virus is spread and how to protect oneself in part by distributing literature in

the native language of the people and in English. Furthermore, enlisting village elders to cooperate in helping isolate ill patients and calming fears have led to less severe outbreaks.

The countries in Africa where previous Ebola outbreaks have occurred are among some of the poorest in the world. The healthcare facilities in countries such as the Democratic Republic of the Congo may not have a sufficient supply of sterile needles, which leads to the reuse of needles and amplification of the outbreak in the healthcare setting. In many of the hospitals it is expected that family members provided much of the basic supplies such as bed linens and provide supportive care while in the hospital. The close contact between patients and family members taking care of them gives the virus an opportunity to spread between members of an entire family, thus leading to many family members becoming victims of Ebola virus. Additionally, if family members are able to leave the hospital freely after providing care to a relative suspected of having Ebola virus, they may unknowingly spread the virus throughout the community making it more difficult to contain.

An Ebola outbreak can have a devastating effect on an already fragile healthcare system especially in a small village where there may be only one hospital. For example in 1995, during the *Zaire ebolavirus* outbreak, there were 80 healthcare workers who were infected and Kikwit General Hospital had to be closed in order to end the outbreak (Khan, et al., 1999). The loss of healthcare workers and closure of an entire hospital may have devastating effects on an African community especially when it comes to treating other endemic diseases.

Ebola outbreaks have damaging psychological effects throughout the affected communities. Ebola victims are often stigmatized or face rejection from their local communities (De Roo et al., 1998; MacNeil & Rollin, 2012). During the 1995 *Zaire ebolavirus* outbreak in Kikwit, patients who were convalescing felt rejected by society including their family members. Furthermore, Ebola virus survivors develop sequelae and experience long term health problems (MacNeil & Rollin, 2012).

Uganda's Efforts to Enhance Filovirus Surveillance

After the discovery of Marburg virus in *R. aegyptiacus* fruit bats and the discovery of *Bundibugyo ebolavirus* in 2007, Uganda has strengthened their filovirus surveillance activity to better detect and respond to outbreaks. During the *Bundibugyo ebolavirus* outbreak in 2007, a BSL-4 field laboratory was setup at the Uganda Virus Research Institute in Entebbe in order to quickly test specimens without having to send them to the Centers for Disease Control and Prevention in Atlanta. This allowed infectious patients to be isolated from the general population which was critical in stopping the chain of transmission. Since the outbreak, the laboratory in Uganda has been renovated with enhanced security and more storage capacity for handling future outbreaks.

The Centers for Disease Control and Prevention's, Viral Special Pathogens Branch currently has an epidemiologist and a laboratorian stationed at the Ugandan Virus Research Institute. Having personnel stationed in Uganda makes it possible for suspected viral hemorrhagic fever cases to be quickly responded to and tested before an outbreak can spread. In 2011, the rapid recognition and isolation of one case of *Sudan ebolavirus* limited the outbreak to the single patient. Since 2011, the personnel in Uganda have detected two Ebola virus outbreaks and a Marburg virus outbreak in 2012. The ability to test specimens in country allowed for a quick diagnosis and information to be disseminated before the outbreaks could intensify.

Biosafety and Biosecurity Risks of Working with Ebola Virus

The Ebola viruses are among the most dangerous pathogens in the world and classified in the United States as biosafety level 4 (BSL-4) pathogens (Chosewood, Wilson, Centers for Disease Control and Prevention (U.S.), & National Institutes of Health (U.S.), 2009). This designation is given to agents that are frequently fatal, have no treatment, are easily transmitted through aerosols, and pose a high risk to the individual working with the agent (Chosewood, et al., 2009). The Ebola viruses have the ability to cause high case fatality rates, severe disease, human-to-human transmission, and pose a high risk to

public health and the environment if they were to be released from the laboratory. All of these characteristics with the fact there is no licensed vaccine or therapy puts the Ebola viruses in the highest biosafety category. In addition to the biosafety risks of working with Ebola virus in the laboratory there is the potential for their use as agents of bioterrorism. The Ebola viruses are classified as Category A select agents with the United States Department of Health and Human Services (DHHS). Category A select agents are those that are rarely seen in the United States and pose a security risk because they can be easily disseminated, have high mortality rates, cause public panic, and require special action for public health preparedness (Centers for Disease Control and Prevention).

After the bombing of the Alfred E. Murrah Federal Building in Oklahoma City in 1995, Congress passed the *Antiterrorism and Effective Death Penalty Act of 1996* which gives DHHS the authority to register laboratories and track select agents by the Centers for Disease Control and Prevention. Following the terrorist attacks of September 11, 2001, Congress passed the *Uniting and Strengthening America by Providing Appropriate Tools Required to Intercept and Obstruct Terrorism Act Of 2001* (USA PATRIOT Act) and the *Public Health Security and Bioterrorism Preparedness and Response Act of 2002* which set forth the provisions for the possession, use, and transfer of select agents (Centers for Disease Control and Prevention, 2010). The final rules for possession, use and transfer of select agents were published in the Federal Register in 2005 by the Departments of Health and Human Services and Agriculture (42 C.F.R. Part 73, 7 C.F.R. Part 331, and 9 C.F.R. Part 121) (Centers for Disease Control and Prevention Office of Inspector General Department of Health Human Services, 2005).

In October 2012, the Ebola viruses were classified as Tier 1 select agents (Centers for Disease Control and Prevention (CDC) Department of Health and Human Services (HHS), 2012). This designation further enhances the security surrounding who has possession of and the ability to work with Ebola virus. The details surrounding the implications of the new regulations will be worked out as the

new regulations are implemented. Ebola virus will be stored in a separate location from other non-tier 1 select agents in the BSL-4 laboratory. Furthermore, researchers working with Ebola virus will undergo suitability assessments to ensure their mental competency.

Conclusion

Ebola virus outbreaks continue to emerge in rural settings where public health surveillance is lacking or non-existent. Despite popular belief from the media, people living in these communities are the ones who are most at risk. In order to better protect these communities from future outbreaks, and rule out other endemic infections, surveillance and rapid diagnostic testing needs to be implemented in order to ensure an early public health response. Additionally, the importance of preventing healthcare associated Ebola infections by using proper barrier protection and not reusing contaminated medical supplies must be championed through educational material and providing appropriate protective equipment for healthcare staff when possible.

In many of these outbreaks, a zoonotic source has been implemented in the form of hunting for and eating bushmeat (Leroy et al., 2004; Nkoghe, et al., 2011). For example, in 2005 in the Republic of Congo the initial cases of Ebola were identified in men who had been poaching elephants and in the 2007 outbreak in the Democratic Republic of the Congo fruit bats were hunted and eaten as a source of protein which coincided with the outbreak (Leroy, et al., 2009; Nkoghe, et al., 2011). Educational campaigns about the dangers of contracting Ebola virus from infected wildlife should be undertaken to prevent possible future outbreaks. Specifically, bats have been implicated as a possible reservoir for Ebola virus. Efforts should be made to encourage people to be aware of the potential that bats carry Ebola virus and to avoid roosting areas where bats are known to inhabit particularly mines and caves.

The threat of an Ebola outbreak in endemic areas will always exist. The extent and severity of future outbreaks will depend on the ability of public health officials to rapidly diagnose and respond. Equipping Ebola virus endemic countries with a national viral hemorrhagic fever surveillance system

such as the one in Uganda could limit the size and severity of future outbreaks as well as detect novel Ebola strains. Ecological studies into potential reservoirs play an important role in preventing future outbreaks. Once the virus can be definitively linked to a zoonotic source, efforts can be made to prevent infection from the source. A concerted effort between international governments, healthcare officials, and the communities affected by Ebola will have to be made in preventing future outbreaks.

References

Amman, B. R., Carroll, S. A., Reed, Z. D., Sealy, T. K., Balinandi, S., Swanepoel, R., et al. (2012). Seasonal Pulses of Marburg Virus Circulation in Juvenile Rousettus aegyptiacus Bats Coincide with Periods of Increased Risk of Human Infection. *PLoS Pathog, 8*(10), e1002877.

Baron, R. C., McCormick, J. B., & Zubeir, O. A. (1983). Ebola virus disease in southern Sudan: hospital dissemination and intrafamilial spread. *Bull World Health Organ, 61*(6), 997-1003.

Barrette, R. W., Metwally, S. A., Rowland, J. M., Xu, L., Zaki, S. R., Nichol, S. T., et al. (2009). Discovery of swine as a host for the Reston ebolavirus. *Science, 325*(5937), 204-206.

Centers for Disease Control. (1990). Update: Ebola-related filovirus infection in nonhuman primates and interim guidelines for handling nonhuman primates during transit and quarantine. *MMWR Morb Mortal Wkly Rep, 39*(2), 22-24, 29-30.

Centers for Disease Control. (2001). Outbreak of Ebola hemorrhagic fever Uganda, August 2000-January 2001. *MMWR Morb Mortal Wkly Rep, 50*(5), 73-77.

Centers for Disease Control and Prevention. Bioterrorism Agents/Diseases. Retrieved October 26, 2012, from http://www.bt.cdc.gov/agent/agentlist-category.asp#a

Centers for Disease Control and Prevention. (2010). About Us. . Retrieved October 26, 2012, from http://www.selectagents.gov/AboutUS.html

Centers for Disease Control and Prevention (CDC) Department of Health and Human Services (HHS). (2012). Possession, use, and transfer of select agents and toxins; biennial review. Final rule. *Fed Regist, 77*(194), 61083-61115.

Centers for Disease Control and Prevention Office of Inspector General Department of Health Human Services. (2005). Possession, use, and transfer of select agents and toxins. Final rule. *Fed Regist, 70*(52), 13293-13325.

Chosewood, L. C., Wilson, D. E., Centers for Disease Control and Prevention (U.S.), & National Institutes of Health (U.S.). (2009). *Biosafety in microbiological and biomedical laboratories* (5th

ed.). Washington, D.C.: U.S. Dept. of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, National Institutes of Health.

Chowell, G., Hengartner, N. W., Castillo-Chavez, C., Fenimore, P. W., & Hyman, J. M. (2004). The basic reproductive number of Ebola and the effects of public health measures: the cases of Congo and Uganda. *J Theor Biol, 229*(1), 119-126.

De Roo, A., Ado, B., Rose, B., Guimard, Y., Fonck, K., & Colebunders, R. (1998). Survey among survivors of the 1995 Ebola epidemic in Kikwit, Democratic Republic of Congo: their feelings and experiences. *Trop Med Int Health, 3*(11), 883-885.

Ebola haemorrhagic fever in Sudan, 1976. Report of a WHO/International Study Team. (1978). *Bull World Health Organ, 56*(2), 247-270.

Ebola haemorrhagic fever in Zaire, 1976. (1978). *Bull World Health Organ, 56*(2), 271-293.

Emond, R. T., Evans, B., Bowen, E. T., & Lloyd, G. (1977). A case of Ebola virus infection. [Case Reports]. *Br Med J, 2*(6086), 541-544.

Fatal Laboratory Accident, Siberia. (2004). *International Journal of Infectious Diseases, 8*(4), 199-200.

Formenty, P., Libama, F., Epelboin, A., Allarangar, Y., Leroy, E., Moudzeo, H., et al. (2003). [Outbreak of Ebola hemorrhagic fever in the Republic of the Congo, 2003: a new strategy?]. *Med Trop (Mars), 63*(3), 291-295.

Georges, A. J., Leroy, E. M., Renaut, A. A., Benissan, C. T., Nabias, R. J., Ngoc, M. T., et al. (1999). Ebola hemorrhagic fever outbreaks in Gabon, 1994-1997: epidemiologic and health control issues. *J Infect Dis, 179 Suppl 1*, S65-75.

Hartman, A. L., Towner, J. S., & Nichol, S. T. (2010). Ebola and marburg hemorrhagic fever. *Clin Lab Med, 30*(1), 161-177.

Hayes, C. G., Burans, J. P., Ksiazek, T. G., Del Rosario, R. A., Miranda, M. E., Manaloto, C. R., et al. (1992). Outbreak of fatal illness among captive macaques in the Philippines caused by an Ebola-related filovirus. *Am J Trop Med Hyg, 46*(6), 664-671.

Hayman, D. T., Emmerich, P., Yu, M., Wang, L. F., Suu-Ire, R., Fooks, A. R., et al. (2010). Long-term survival of an urban fruit bat seropositive for Ebola and Lagos bat viruses. *PLoS One, 5*(8), e11978.

Hayman, D. T., Yu, M., Crameri, G., Wang, L. F., Suu-Ire, R., Wood, J. L., et al. (2012). Ebola virus antibodies in fruit bats, Ghana, West Africa. *Emerg Infect Dis, 18*(7), 1207-1209.

Hewlett, B. S., Epelboin, A., Hewlett, B. L., & Formenty, P. (2005). Medical anthropology and Ebola in Congo: cultural models and humanistic care. *Bull Soc Pathol Exot, 98*(3), 230-236.

Heymann, D. L., Weisfeld, J. S., Webb, P. A., Johnson, K. M., Cairns, T., & Berquist, H. (1980). Ebola hemorrhagic fever: Tandala, Zaire, 1977-1978. *J Infect Dis, 142*(3), 372-376.

International Committee on Taxonomy of Viruses., & King, A. M. Q. (2012). *Virus taxonomy : classification and nomenclature of viruses : ninth report of the International Committee on Taxonomy of Viruses*. London ; Waltham, MA: Academic Press.

Jahrling, P. B., Geisbert, T. W., Dalgard, D. W., Johnson, E. D., Ksiazek, T. G., Hall, W. C., et al. (1990). Preliminary report: isolation of Ebola virus from monkeys imported to USA. *Lancet, 335*(8688), 502-505.

Khan, A. S., Tshioko, F. K., Heymann, D. L., Le Guenno, B., Nabeth, P., Kerstiens, B., et al. (1999). The reemergence of Ebola hemorrhagic fever, Democratic Republic of the Congo, 1995. Commission de Lutte contre les Epidemies a Kikwit. *J Infect Dis, 179 Suppl 1*, S76-86.

Ksiazek, T. G., West, C. P., Rollin, P. E., Jahrling, P. B., & Peters, C. J. (1999). ELISA for the detection of antibodies to Ebola viruses. *J Infect Dis, 179 Suppl 1*, S192-198.

Kuhn, J. H. (2008). *Filoviruses : a compendium of 40 years of epidemiological, clinical, and laboratory studies*. Vienna: Springer.

Le Guenno, B., Formenty, P., Wyers, M., Gounon, P., Walker, F., & Boesch, C. (1995). Isolation and partial characterisation of a new strain of Ebola virus. *Lancet, 345*(8960), 1271-1274.

Leroy, E. M., Epelboin, A., Mondonge, V., Pourrut, X., Gonzalez, J. P., Muyembe-Tamfum, J. J., et al. (2009). Human Ebola outbreak resulting from direct exposure to fruit bats in Luebo, Democratic Republic of Congo, 2007. *Vector Borne Zoonotic Dis, 9*(6), 723-728.

Leroy, E. M., Kumulungui, B., Pourrut, X., Rouquet, P., Hassanin, A., Yaba, P., et al. (2005). Fruit bats as reservoirs of Ebola virus. *Nature, 438*(7068), 575-576.

Leroy, E. M., Rouquet, P., Formenty, P., Souquiere, S., Kilbourne, A., Froment, J. M., et al. (2004). Multiple Ebola virus transmission events and rapid decline of central African wildlife. *Science, 303*(5656), 387-390.

MacNeil, A., Farnon, E. C., Wamala, J., Okware, S., Cannon, D. L., Reed, Z., et al. (2010). Proportion of deaths and clinical features in Bundibugyo Ebola virus infection, Uganda. *Emerg Infect Dis, 16*(12), 1969-1972.

MacNeil, A., & Rollin, P. E. (2012). Ebola and Marburg hemorrhagic fevers: neglected tropical diseases? *PLoS Negl Trop Dis, 6*(6), e1546.

Miranda, M. E., Ksiazek, T. G., Retuya, T. J., Khan, A. S., Sanchez, A., Fulhorst, C. F., et al. (1999). Epidemiology of Ebola (subtype Reston) virus in the Philippines, 1996. *J Infect Dis, 179 Suppl 1*, S115-119.

Miranda, M. E., White, M. E., Dayrit, M. M., Hayes, C. G., Ksiazek, T. G., & Burans, J. P. (1991). Seroepidemiological study of filovirus related to Ebola in the Philippines. *Lancet, 337*(8738), 425-426.

Negredo, A., Palacios, G., Vazquez-Moron, S., Gonzalez, F., Dopazo, H., Molero, F., et al. (2011). Discovery of an ebolavirus-like filovirus in europe. *PLoS Pathog, 7*(10), e1002304.

Newman, S. H., & Food and Agriculture Organization of the United Nations. (2011). *Investigating the role of bats in emerging zoonoses : balancing ecology, conservation and public health interest*. Rome: Food and Agriculture Organization of the United Nations.

Nkoghe, D., Kone, M. L., Yada, A., & Leroy, E. (2011). A limited outbreak of Ebola haemorrhagic fever in Etoumbi, Republic of Congo, 2005. *Trans R Soc Trop Med Hyg, 105*(8), 466-472.

Onyango, C. O., Opoka, M. L., Ksiazek, T. G., Formenty, P., Ahmed, A., Tukei, P. M., et al. (2007). Laboratory diagnosis of Ebola hemorrhagic fever during an outbreak in Yambio, Sudan, 2004. *J Infect Dis, 196 Suppl 2*, S193-198.

Outbreak news. Ebola haemorrhagic fever, Democratic Republic of the Congo. (2012). *Wkly Epidemiol Rec, 87*(36), 338-339.

Outbreak news. Ebola virus haemorrhagic fever, Democratic Republic of the Congo. (2007). *Wkly Epidemiol Rec, 82*(38), 329.

Outbreak news. Ebola, Democratic Republic of Congo - update. (2012). *Wkly Epidemiol Rec, 87*(38), 357.

Outbreak of Ebola haemorrhagic fever in Yambio, south Sudan, April - June 2004. (2005). *Wkly Epidemiol Rec, 80*(43), 370-375.

Outbreak(s) of Ebola haemorrhagic fever, Congo and Gabon, October 2001-July 2002. (2003). *Wkly Epidemiol Rec, 78*(26), 223-228.

Pourrut, X., Souris, M., Towner, J. S., Rollin, P. E., Nichol, S. T., Gonzalez, J. P., et al. (2009). Large serological survey showing cocirculation of Ebola and Marburg viruses in Gabonese bat populations, and a high seroprevalence of both viruses in Rousettus aegyptiacus. *BMC Infect Dis, 9*, 159.

Rollin, P. E., Williams, R. J., Bressler, D. S., Pearson, S., Cottingham, M., Pucak, G., et al. (1999). Ebola (subtype Reston) virus among quarantined nonhuman primates recently imported from the Philippines to the United States. *J Infect Dis, 179 Suppl 1*, S108-114.

Sanchez A, G. T., Feldmann H. (Ed.). (2007). *Filoviridae: Marburg and Ebola Viruses.* (5 ed.). Philadelphia: Lippincott-Raven.

Shoemaker, T., Macneil, A., Balinandi, S., Campbell, S., Wamala, J. F., McMullan, L. K., et al. (2012). Reemerging Sudan ebola virus disease in Uganda, 2011. *Emerg Infect Dis, 18*(9), 1480-1483.

Swanepoel, R., Leman, P. A., Burt, F. J., Zachariades, N. A., Braack, L. E., Ksiazek, T. G., et al. (1996). Experimental inoculation of plants and animals with Ebola virus. *Emerg Infect Dis, 2*(4), 321-325.

Taniguchi, S., Watanabe, S., Masangkay, J. S., Omatsu, T., Ikegami, T., Alviola, P., et al. (2011). Reston Ebolavirus antibodies in bats, the Philippines. *Emerg Infect Dis, 17*(8), 1559-1560.

Towner, J. S., Amman, B. R., Sealy, T. K., Carroll, S. A., Comer, J. A., Kemp, A., et al. (2009). Isolation of genetically diverse Marburg viruses from Egyptian fruit bats. *PLoS Pathog, 5*(7), e1000536.

Towner, J. S., Sealy, T. K., Khristova, M. L., Albarino, C. G., Conlan, S., Reeder, S. A., et al. (2008). Newly discovered ebola virus associated with hemorrhagic fever outbreak in Uganda. *PLoS Pathog, 4*(11), e1000212.

Viral haemorrhagic fever in imported monkeys. (1992). *Wkly Epidemiol Rec, 67*(19), 142-143.

World Health Organization. (2004, January 6). Ebola haemorrhagic fever in the Republic of the Congo-update 6. from www.who.int/csr/don/2004_01_06/en/

World Health Organization. (2009, February 17, 2009). End of the Ebola Outbreak in the Democratic Republic of the Congo. *Global Alert and Response*