

## REVIEW ON HISTORY AND EVIDENCE OF FRUIT BATS AS THE NATURAL RESERVOIR FOR EBOLA VIRUSES

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### ABSTRACT

The Ebola viruses are negative sense RNA genome they cause sporadic outbreaks of Ebola hemorrhagic fever (EHF) and its genus has five species: *Zaire Ebola virus* (ZEBOV), *Reston Ebola virus* (REBOV), *Sudan Ebola virus* (SEBOV), *Bundibugyo Ebola virus* (BDBV) and *Cote d'Ivoire Ebola virus* (CIEBOV). With the exception of REBOV they all cause severe hemorrhagic fevers in humans with high case

fatality rates, in Africa 40-90% often linked to exposure to wild animal tissues during butchering. Currently, there is no approved treatment or vaccination available due to their high lethality. *Ebola virus* introduction into human population resulted from through single or multiple introductions from a zoonotic source into the human population then followed by human-to-human. The natural reservoirs of *Ebola viruses* is currently unknown, but there is some evidences that fruit bats may play a key role because they have close genetic relation with Marburg virus in which a fruit bat has been identified. There is some evidence that transmission might occur when bats give birth. Pregnant fruit bats are also more likely to be seropositive than non pregnant females. *Ebola virus* would not be expected to cause lethal disease in its natural reservoir. The symptoms progress over the time and the patients suffer from dehydration, stupor, confusion, hypotension, multi-organ failure, leading to fulminant shock and eventually death. Fatal cases tend to develop early clinical signs during the infection and death often occurs between the sixth and sixteen days of illness. Currently, the standard treatment consists of supportive therapy, including maintenance of blood volume and electrolyte balance, as well as analgesics and standard nursing care. Finally, to prevent

infection from animals all sick and dead wild animals should be avoided, good personal hygiene should be used when handling and preparing meat and the meat should be thoroughly cooked and also healthcare workers should use the personal protective equipment currently recommended by experts to prevent exposure to blood and body fluids.

**KEYWORDS:** *Ebola viruses, Fatality rate, Fruit bats, Natural reservoir.*

## INTRODUCTION

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 non segmented, single strand, negative sense RNA genome. The family *Filoviridae* currently includes three genera, *Ebola virus*, Marburg virus, and “Cueva virus” (proposed). The genus *Ebola virus* includes five species: *Zaire Ebola virus* (EBOV), *Sudan Ebola virus* (SUDV), *Reston Ebola virus* (RESTV), *Bundibugyo Ebola virus* (BDBV), and *Tai Forest Ebola virus* (TAFV) formerly *Côte d’Ivoire Ebola virus* (CIEBOV) (ICTV and King, 2012).

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

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 and Agriculture Organization of the United Nation, 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 (Amman *et al.*, 2012; Leroy *et al.*, 2009).

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. *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 (Leroy *et al.*, 2005). Therefore, the aim of this paper was to compile the history of outbreak, evidence of fruit bats as natural reservoir and also to draw attention to transmission, control and prevention of *Ebola virus*.

## LITERATURE REVIEW

### Viral Taxonomy

*Ebola viruses* (EBOV) and the closely related *Marburg viruses* (MARV) make up the family Filoviridae in the order Mononegavirales (Feldmann *et al.*, 2004). The genus *Ebola virus* is divided into five species: *Zaire Ebola virus* (ZEBOV), *Reston Ebola virus* (REBOV), *Sudan Ebola virus* (SEBOV), *Bundibugyo Ebola virus* (BDBV) and *Cote d'Ivoire Ebola virus* (CIEBOV). With the exception of REBOV they all cause severe hemorrhagic fevers in humans with high case fatality rates. Since there is currently no approved treatment or vaccination available, and due to their high lethality, Filoviruses are classified as biosafety level (BSL) 4 agents, as well as category A biothreat agents (Borio *et al.*, 2002).

### Taxonomy

Group V: Negative sense ssRNA viruses

Order: *Mononegavirales*

Species

Family: *Filoviridae*

Type:

*Zaire Ebola virus* (ZEBOV)

Genus: *Ebolavirus*

*Marburgvirus*

Main:

*Reston Ebola virus* (REBOV)

*Cuevavirus*

*Sudan Ebola virus* (SEBOV)

*Bundibugyo Ebola virus* (BDBV)

*Cote d'Ivoire Ebola virus* (CIEBOV)

Source: (Feldmann *et al.*, 2004)

### Virion Morphology

EBOV particles usually form long filamentous rods with a uniform diameter of approximately 80 nm and a mean length of approximately 1250 nm. Virions formed like the number 6 and circular forms also appear, but are comparatively rare. The centre of the particles is made up of the ribo nucleoprotein (RNP) complex, which consists of the nucleoprotein (NP), the virion protein (VP) 35, VP30, the RNA dependent RNA polymerase (L) and the vRNA, and has a diameter of about 50 nm (Geisbert and Jahrling, 1995).

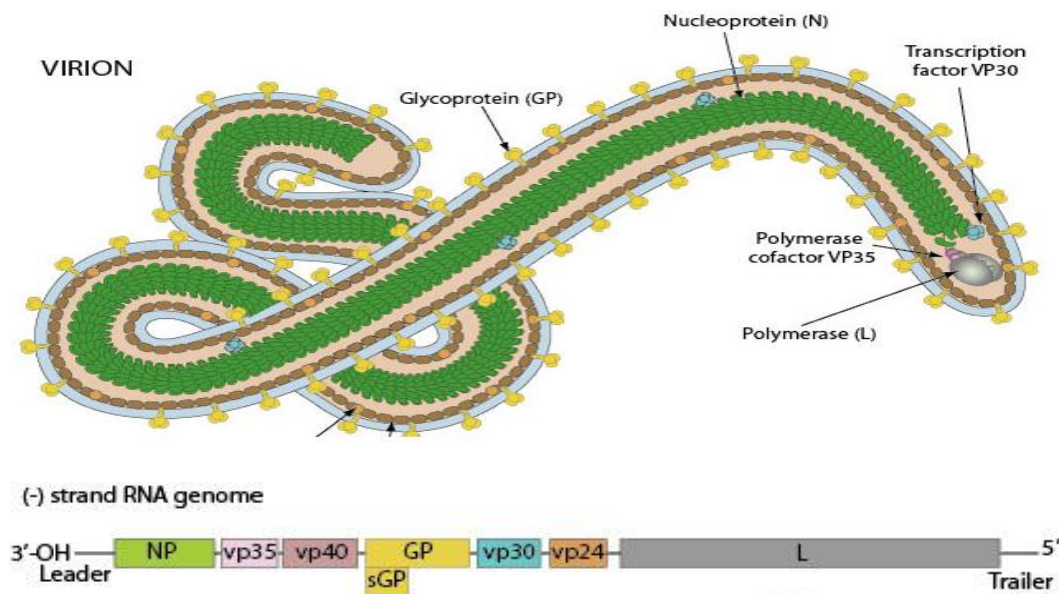


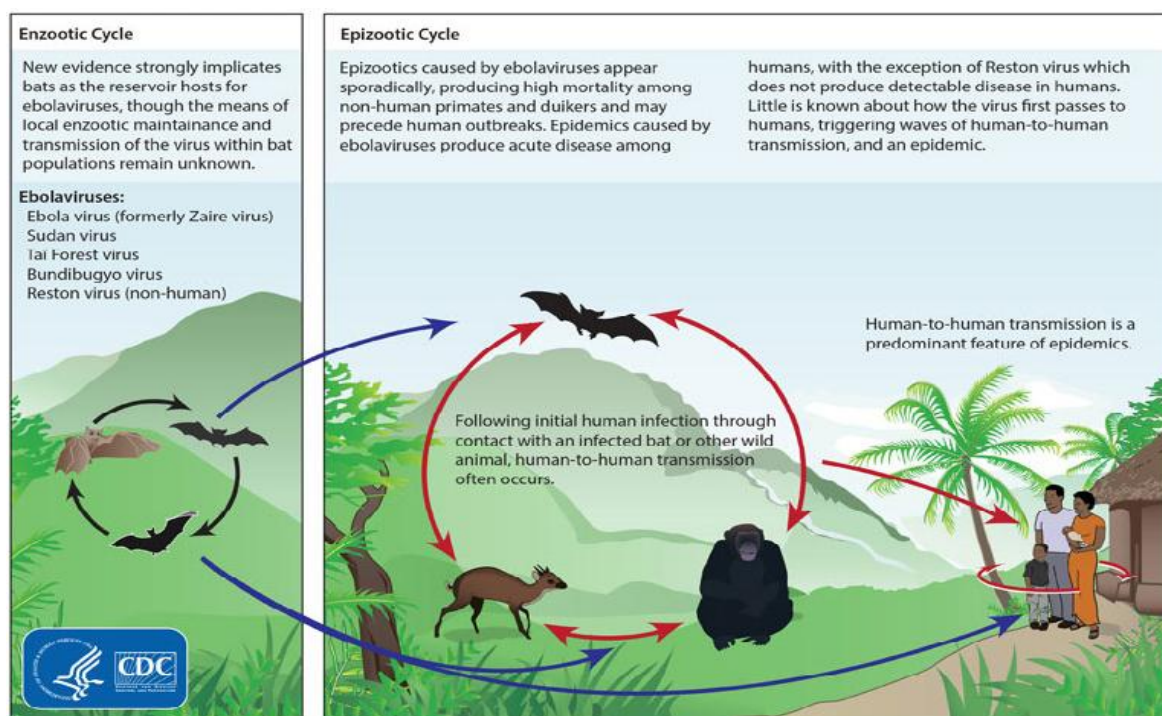
Figure1. Morphology of *Ebola virus* and its genome. Source: (Hulo *et al.*, 2011)

### Geographic Distribution

*Zaire Ebola virus*, *Sudan Ebola virus*, *Tai Forest Ebola virus* and *Bundibugyo Ebola virus* are endemic in parts of Africa south of the Sahara desert. Human illnesses caused by these viruses have been reported mainly in central and western Africa, and have typically been associated with rain forests. While outbreaks have been documented in a limited number of countries, serological surveys, as well as the distribution of bat species known to be infected, suggest that some viruses may be more wide spread. *Reston Ebola virus* occurs in the Philippines. This or other Filo viruses might also exist in other locations. Antibodies to Filo viruses have been detected in several species of fruit bats in China and Bangladesh, and 18% of healthy Bornean orangutans (*Pongo pygmaeus*) in rehabilitation facilities were seropositive on Kalimantan Island, Indonesia.

## Transmission Pattern and Epidemiology of Ebola Virus

How *Ebola virus* are transmitted between bats, or transmitted from bats to other animals, is still uncertain. Although these viruses can be found in bat tissues and blood, they typically seem to be absent from secretions or excretions such as oral fluids, urine and feces (although virus was found in the feces of one experimentally infected bat), and attempts to inoculate bats by exposing respiratory and oral mucus membranes to virus were unsuccessful. It is possible that virus shedding in secretions and excretions occurs intermittently, at very low levels and/or under certain physiological conditions. There is some evidence that transmission might occur when bats give birth. Pregnant fruit bats are also more likely to be seropositive than non pregnant females (Vogel, 2014).



**Figure 2. *Ebola virus* transmission from fruit bats to humans. Source: (CDC, 2014).**

*Ebola virus* emerges periodically in nonhuman primates or people after infection from an outside source. Some *Ebola virus* might be acquired directly from bats; however, humans often become ill after handling the carcasses of animals found in the forest, especially nonhuman primates and duikers. Blood, secretions and excretions, and tissues from these animals may contain infectious virus. *Ebola virus* has been reported to survive for some time in blood and tissues at room temperature, and can be transmitted on fomites, particularly those contaminated by blood. Survival is prolonged when viruses are kept at 4°C. In incidental hosts, Filo viruses are thought to enter the body mainly through mucous membranes and broken skin (Changula *et al.*, 2014).



Once *Ebola virus* has infected humans, they can spread from person to person. Viruses are thought to occur in secretions and excretions only after the onset of fever. Blood can contain large amounts of virus, contaminating the environment if patients hemorrhage. These viruses are also found in many secretions and excretions that are not visibly contaminated with blood, including saliva, tears, breast milk, semen and feces. Urine may be a source of virus, but *Zaire Ebola virus* was absent from patients' urine during one outbreak. Aerosol and/or respiratory droplet transmission between nonhuman primates is still controversial: it has been implicated in some experimentally infected nonhuman primates, but alternative explanations may be possible, and virus did not seem to spread readily between cages in other studies. While people might theoretically become infected by this route, aerosols do not seem to be important during human outbreaks (WHO, 2014).

Filo viruses disappear from blood and most tissues after the acute stage of the disease. They may, however, persist for a time in some "immune privileged" body sites, such as the testes and possibly the anterior chamber of the eye. *Zaire Ebola virus* was isolated from the semen of another convalescent patient up to 82 days after the onset of clinical signs, and detected by RT-PCR for as long as 91 days. This virus was also recovered from the breast milk of a convalescing patient, 15 days after the onset of disease (after the virus had been cleared from the blood), and transmission to a nursing child may be possible. How efficiently Filo viruses can spread by casual contact during the early stages of the illness is still uncertain, but the risk is currently thought to be low except during close contact (Bausch *et al.*, 2007).

### **Clinical Presentation and Symptoms**

Recognizing the signs of EVD is challenging, the incubation period usually lasts 5 to 7 days, although it can be as short as 2 days and as long as 21 days. Approximately 95% of the patients appear signs within 21 days after exposure which is the recommended period for follow-up of contacts (McElroy *et al.*, 2014; WHO, 2014).

Clinical EHF is featured by sudden onset of fever, fatigue, chills, general malaise, headaches, myalgia, anorexia and gastrointestinal distress within 3-13 days following exposure to virus. Many patients develop hemorrhagic manifestations from which the term "hemorrhagic fever" has been derived (McElroy *et al.*, 2014). Hemorrhagic fever occurs in less than half of infected subjects and gross bleeding is relatively rare (Fauci, 2014).

The most common signs reported between symptom appearance and case detection included fever, fatigue, loss of appetite, vomiting, diarrhea, headache and abdominal pain. Certain hemorrhagic features were rarely reported (WHO, 2014). Bleeding takes place most commonly in the gastrointestinal tract and may demonstrate as melena, petechiae, conjunctival hemorrhage, hematuria, easy bruising, or intraperitoneal bleeding. Mucous membrane bleeding, failure of venipuncture sites and excessive clot formation have also been described. These symptoms progress over the time and patients suffer from dehydration, stupor, confusion, hypotension, multi-organ failure, leading to fulminant shock and eventually death. Fatal cases tend to develop early clinical signs during the infection and death often occurs between the sixth and sixteen days of illness (Wiwanitkit, 2014).

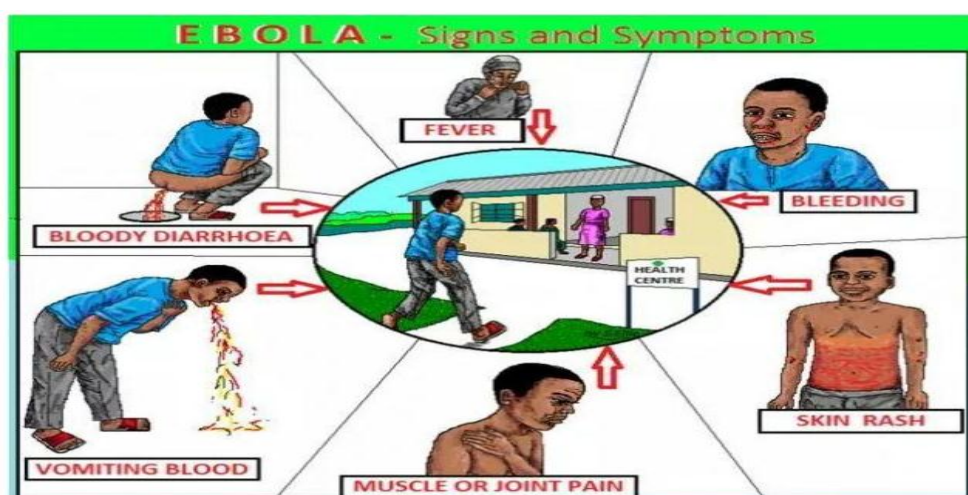


Figure 3. Clinical signs and symptoms of *Ebola virus*. Source: (CDC, 2014)

### Diagnosis

It can be difficult to distinguish EVD from other infectious diseases such as malaria, typhoid fever and meningitis. Confirmation that symptoms are caused by *Ebola virus* infection are made using the following investigations:

- Antibody-capture enzyme-linked immunosorbent assay (ELISA)
- Antigen-capture detection tests
- Serum neutralization test
- Reverse transcriptase polymerase chain reaction (RT-PCR) assay
- Electron microscopy
- Virus isolation by cell culture.

Samples from patients are an extreme biohazard risk; laboratory testing on non-inactivated samples should be conducted under maximum biological containment conditions (WHO, 2014).

### Treatment, Prevention and Control

A wide range of studies in vitro and several animal models have been developed for EBOV however; currently neither a licensed vaccine nor an approved treatment is available. Standard treatment currently consists of supportive therapy, including maintenance of blood volume and electrolyte balance, as well as analgesics and standard nursing care (Okware *et al.*, 2002).

In Africa, *Ebola virus* infections are often linked to exposure to wild animal tissues during butchering. Because the full host range may not be known, all sick and dead wild animals should be avoided (including for use as food). To prevent infection from animals that might be infected but have not yet developed obvious clinical signs, good personal hygiene should be used when handling and preparing meat, and the meat should be thoroughly cooked (Okware *et al.*, 2002).

Human epidemics have been successfully stopped in the past by tracing infected individuals, and isolating patients in facilities with barrier nursing procedures and strict infection control measures. Healthcare workers should use the personal protective equipment currently recommended by experts (e.g., gloves, gowns, masks, eye protection and other equipment) to prevent exposure to blood and body fluids. Burial practices should avoid all contact with the body or fomites. During convalescence, the possibility of exposure during breastfeeding or sexual intercourse should be considered. Ebola virus have been found in milk 15 days after the onset of illness (although the maximum period of shedding is unknown), and in semen for much longer. Sexual abstinence has been recommended for at least three months after recovery. *Reston Ebola virus* is not known to affect humans. As a precaution, tissues from infected animals should not be eaten or handled. Good hygiene and appropriate personal protective equipment should be used if these animals or their tissues must be handled (Gunther *et al.*, 2011).

### EBOLA DISEASE OUT BREAK

The first EVD outbreak was reported in Zaire (now Democratic Republic of Congo (DRC)) in 1976 and the causative agent was named after the nearby Ebola River. In 1976 two outbreaks occurred around the same period one in Eastern Sudan and one in Eastern Zaire resulting in 53% and 89% mortality and the first discoveries of *Sudan* and *Zaire Ebola viruses*, respectively. Subsequently there was one human Ebola virus case in 1977 in DRC, and a cluster of 34 cases in E. Sudan in 1979. No Ebola virus outbreaks occurred again until

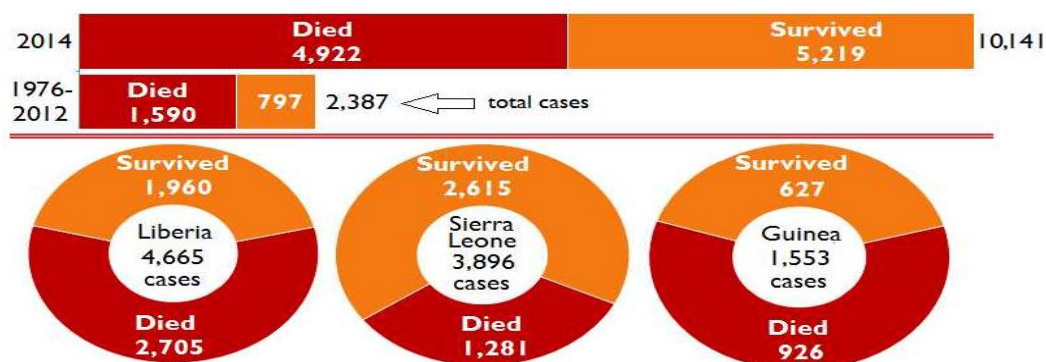


1994, when there were a series of outbreaks between 1994–1997 and more again between 2000–2004. There has only been a single, non-fatal case of *Tai Forest Ebola virus* in humans, a veterinarian who was infected after performing a necropsy on a chimpanzee in 1994 (Pourrut *et al.*, 2005).

*Bundibugyo Ebola virus*: was discovered after human cases of hemorrhagic fever in late 2007 in Western Uganda, but the links to an animal reservoir are not clear. A large *Ebola virus* outbreak occurred in DRC in 2007 (186 deaths out of 260 cases, 71.5% mortality), and the initial human “index case” was later speculated to have been linked to purchasing freshly killed fruit bats for consumption (Albarino *et al.*, 2013a).

*Reston Ebola virus*: was first discovered in 1989 from laboratory macaques exported from the Philippines to the USA. Subsequent detections of the same virus were made in primates in 1992 and 1996. A small percentage of people (1% of 458 exposed individuals) from the 1989 and 1996 events were found to have IgG antibodies to *Reston Ebola virus* but were asymptomatic. Its infection in humans is rare and not known to cause any human disease (Miranda, 2011).

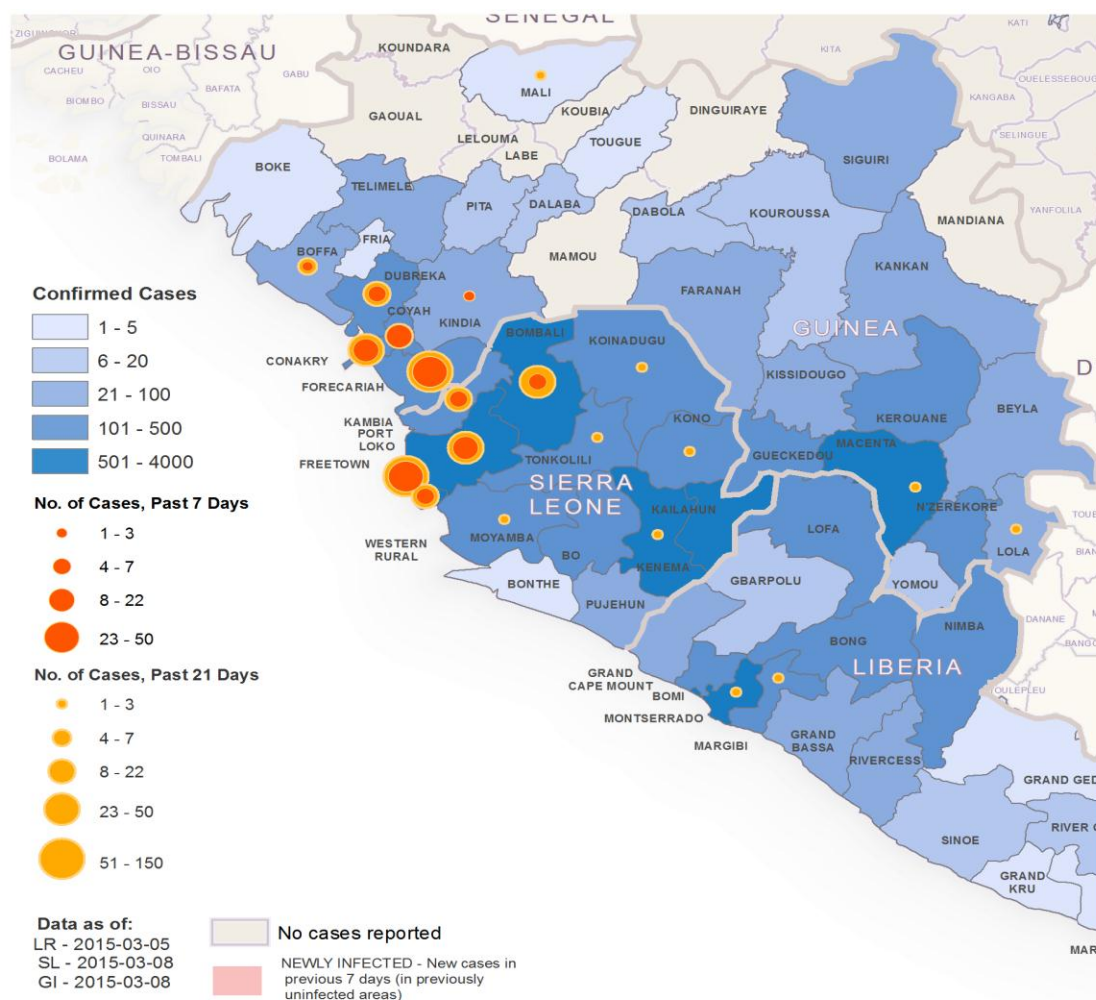
Currently, in March 2014, there is an ongoing outbreak of *Ebola virus* in Guinea, West Africa. The outbreak is the first in West Africa and has become the largest, most persistent ever documented. The outbreak is continuing to spread in Guinea, Sierra Leone, and Liberia (“affected countries”) and has ended in Nigeria and Senegal, after having infected 20 people in Nigeria and one in Senegal. As of late October, nearly 10,000 people have contracted EVD, of whom almost 5,000 have died (Figure 3) (WHO, 2014).



**Figure 4. Ebola Outbreaks: 1976-2014. Source: (WHO, 2014)**

Disease transmission along the shared borders of Guinea, Liberia, and Sierra Leone has been intense and despite efforts to detect the disease at the borders, people infected with Ebola

have imported the disease into other countries. Contact tracing and disease containment have halted the spread in Senegal and Nigeria where the outbreak ended on October 17 and 19, respectively. In Nigeria, an imported case resulted in an outbreak that infected 20 people and killed eight. A single imported case in Senegal was contained to the index case. WHO is investigating a new EVD case in Mali. Until October, no EVD cases outside of Africa resulted in secondary cases. In that month, health workers in the United States and Spain contracted EVD while caring for EVD patients. No additional cases have been reported from the health workers. No other reports have emerged of EVD spreading in other countries where EVD patients have been evacuated. Previous outbreaks were confined to rural and forested areas, whereas the current outbreak is spreading in rural and urban settings alike. Responders are struggling to isolate cases and contain the outbreak in densely populated urban areas that lack sufficient access to clean water and sanitation and face severe shortages of health workers and clinics (WHO, 2014).



**Figure 5: Distribution of EVD cases by affected areas and confirmation status**

**Source: (CDC, 2014)**

## FRUIT BATS AS NATURAL RESERVOIR FOR EBOLA VIRUS

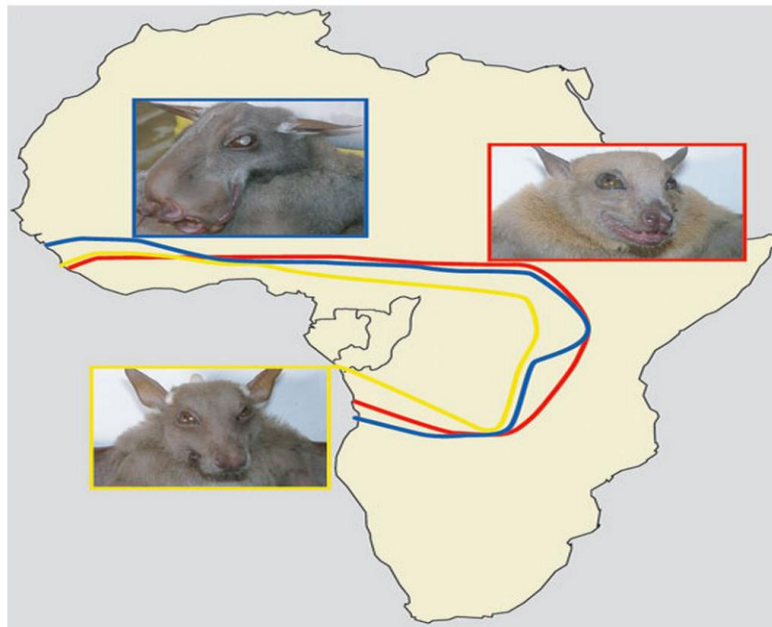
Fruit bats are the second-most species group of mammals, after rodents. The approximately 925 species of living fruit bats make up around 20% of all known living mammal species. In some tropical areas, there are more species of fruit bats than of all other kinds of mammals combined (Vaughan *et al.*, 2000).

Fruit bats are often divided into two major groups, usually given the rank of suborders, Mega chiroptera and Micro chiroptera. Mega chiroptera includes one family (Pteropodidae) and about 166 species. All feed primarily on plant material, either fruit, nectar or pollen. The remaining 16 families (around 759 species) belong to Micro chiroptera. The majority of species are insectivorous, and insectivory is widely distributed through all micro chiropteran families. However, many micro chiropterans have become specialized to eat other kinds of diets. Some bats are carnivorous (feeding on rodents, other bats, reptiles, birds, amphibians, and even fish), many consume fruit, some are specialized for extracting nectar from flowers, and one subfamily (three species in the subfamily Desmodontinae) feeds on nothing but the blood of other vertebrates (Teeling *et al.*, 2005).

Mega chiropterans and micro chiropterans differ in many other ways. Mega chiropterans are found only in the Old World tropics, while micro chiropterans are much more broadly distributed. Micro chiropterans use highly sophisticated echolocation for orientation; mega chiropterans orient primarily using their eyes, although members of one genus, *Rousettus*, are capable of a simple form of echolocation that is not related to echolocation in micro chiropterans. Mega chiropteran species control their body temperature within a tight range of temperatures and none hibernates; many micro chiropterans have labile body temperatures, and some hibernate (Nowak, 1991).

### Evidence of Fruit Bat as Key Reservoir for Ebola Virus

The natural reservoir for Ebola has yet to be confirmed; however, fruit bats are considered to be the most likely candidate species. Three species of bats, *Hypsignathus monstrosus*, *Myonycteris torquata* and *Epomops franqueti*, were identified as the most likely candidates to be reservoir species for Ebola viruses due to high seroprevalence and the isolation of RNA closely related to *Zaire Ebola Virus* (Loupland *et al.*, 2014).



**Figure 6. The most likely candidates of Fruit bats to be reservoir species for Ebola.**

Fruit bats were known to roost in the cotton factory in which the first cases of the 1976 and 1979 outbreaks were observed (Pourrut *et al.*, 2005). Of 24 plant and 19 vertebrate species experimentally inoculated with EBOV, only bats became infected. The bats displayed no clinical signs of disease, which is considered evidence that these bats are a reservoir species of EBOV. In a 2002–2003 survey of 1,030 animals including 679 bats from Gabon and the Republic of the Congo, 13 fruit bats were found to contain EBOV RNA (Leoy *et al.*, 2005). Antibodies against Zaire and Reston viruses have been found in fruit bats in Bangladesh, suggesting that these bats are also potential hosts of the virus and that the Filoviruses are present in Asia (Olival *et al.*, 2013).

Between 1976 and 1998, in 30,000 mammals, birds, reptiles, amphibians and arthropods sampled from regions of EBOV outbreaks, no *Ebola virus* was detected apart from some genetic traces found in six rodents (belonging to the species *Mussetulosus* and *Praomys*) and one shrew (*Sylvisorexollula*) collected from the Central African Republic. However, further research efforts have not confirmed rodents as a reservoir. Traces of EBOV were detected in the carcasses of gorillas and chimpanzees during outbreaks in 2001 and 2003, which later became the source of human infections. However, the high rates of death in these species resulting from EBOV infection make it unlikely that these species represent a natural reservoir for the virus (Pourrut *et al.*, 2005).

### Experimental Research Supporting Fruit Bat as Natural Reservoir

Experimental studies supporting the role of bats as reservoirs are few, but two key studies have investigated the capacity for bats to become infected with Filoviruses and to survive infection. As mentioned above, *Zaire Ebola virus* could replicate and lead to seroconversion without disease in three species of bats infected (*Tadarida condylura*, *T. pumila*, and *Epomophorus wahlbergi*) and that virus could be isolated from feces. Using captive bred *R. aegyptiacus* bats of known serological and infection status Paweska *et al.* (2012) demonstrated that viremia could be induced. Following viremia, IgG antibody could be detected 9 to 21 days post infection. None of the bats showed clinical symptoms, nor was gross pathology seen. However, it is worth noting that these studies in *R. aegyptiacus* could not induce infection following oral or intra-nasal inoculation (the above results were following intra-dermal or intra-peritoneal inoculation), nor could virus be isolated from secretions.

Similarly, the study by Swanepoel *et al.* (2007) inoculated *Tadarida* spp. bats by subcutaneous injection; however, fecal shedding was observed in one individual. Thus, while these results are consistent with *R. aegyptiacus* being a reservoir host, they do not shed light on the potential mechanisms for bat-to-bat transmission.

Additional experiments underway using a captive *Rousettus* colony housed at CDC Atlanta will likely shed more light on some of these unresolved issues. Lastly, Albarino and colleagues point out that the virus used by Paweska *et al.* (2012) was passaged almost 40 times in primate Vero cells prior to infecting bats, and it is not known how this may affect the infectivity or virulence of this virus (Albarino *et al.*, 2013b).

### Serological Evidence for Fruit Bat as Natural Reservoir

In Gabon and the Republic of Congo where known *Zaire Ebola virus* (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. IgG antibody to ZEBOV was detected by ELISA in 8/117 *Epomops franqueti* bats, 4/17 *Hypsignathus monstrosus*, and 4/58 *Myonycteris torquata*. 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 and 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 ZEBOV. 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 *Micro chiroptera* (Pourrut *et al.*, 2009).

*Reston Ebola virus* (REBOV) epidemics have occurred in the Philippines since 1989. During 2008 and 2009, 141 wild bats were caught and tested for REBOV specific IgG antibody using ELISA. 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 (Taniguchi *et al.*, 2011).

### STATUS OF FRUIT BATS IN ETHIOPIA

Ethiopia reflects the situation of fruit bats investigations in the Afro-tropics in general. A full of checklist of Ethiopian fruit bats was first compiled in the 1970s and subsequently supplemented by new findings. Although this list of species seems to be fairly complete, the possibility of discovering species new to the country and/or new to science, persist. The territory of the country is not entirely surveyed especially taking into account its geographical heterogeneity. Most of the territory of the country is covered with mountain massifs separated from each other by lowlands, including the Rift valley, which the main zoogeographical barrier within the country. In accordance with this geomorphological diversity, the Ethiopian fauna is characterized by a high level of endemism. At present, 34 mammalian species are considered to be endemic to Ethiopia and this list probably is not final (Kuskop and Lavrenchenko, 2000).

New records are reported for 30 species of Ethiopian fruit bats collected by joint Ethiopian-Russian Biological Expeditions. *Myonycteris torquata* and *Pipistrellus aero* are recorded for the first time from Ethiopia. Principal new localities are reported for ten species including, *Stenonycteris lanosus*, *Lissonycteris angolensis*, *Hypsinathus monstrosus*, *Micropteropus pusillus*, *Nycteris thebaica*, *Triaenops persicus*, *Myotis scotti*, *M. welwitschii*, *Pipistrellus rutilus* and *Laephotis wintini*. Generally, the local faunas of microchiroptera of Ethiopian mountiane forests are impoverished. Nevertheless, both of the only known endemic species, *Myotis scotti* and *Plecotus balensis*, being putative derivative of palaeartic lineages, inhabit this environment (Lavrenchenko, 2003).

## **EBOLA VIRUS DYNAMICS AND ECOLOGY IN FRUIT BATS**

### **Seasonality of Infection Dynamics in Fruit Bats**

Given that many aspects of fruit bat biology, such as mating, birthing, and migration are seasonal, Amman *et al.* (2012) were the first to test the hypothesis that birthing might be linked to increases in infection prevalence and ultimately spillover for *Ebola virus* in fruit bats.

Their analyses suggested *Ebola virus* infection occurred in distinct pulses in older juvenile bats (approximately 6 months old), coinciding with twice yearly birthing seasons. Relatedly, Pourrut *et al.* (2009) found that pregnant female's bats were statistically more likely to be seropositive for Ebola virus.

Recent theoretical studies using stochastic epidemiological models with a seasonal birth pulse suggest increased synchrony of birthing increases the necessary critical community size necessary for infection persistence (Peel *et al.*, 2014). Thus, seasonal birthing may decrease the probability of pathogens persisting in a colony, but lead to increased periods of infection prevalence following birthing. Whether this is true of all *Ebola viruses* in all locations is unknown and further field studies, integrated with modeling, are necessary to understand the role of host ecology on the persistence and emergence of Ebola viruses in fruit bats (Hayman *et al.*, 2013; Wood *et al.*, 2012).

There however, uncertainties about how strong the effects of seasonal birthing for *Ebola viruses*, and how much coloniality and other factors drive infection dynamics. Further still, it has recently been demonstrated that host population structure may be a useful tool to predict infection presence and this remains to be seen for the potential reservoirs of *Ebola viruses* (Peel *et al.*, 2013).

### **Viral Shedding and Immunity in Fruit Bats**

There is little understood about *Ebola virus* shedding and persistence in fruit bats, though several key studies suggested that the within-host infection dynamics are the classical "susceptible-infected-immune" cycle (Paweska *et al.*, 2012). Swanepoel *et al.* (2007) showed that in experimental infection studies Ebola virus replicated in the three species of fruit bats infected (*Tadarida condylura*, *Tadarida pumila* and *Epomophorus wahlbergi*) with virus isolated from feces 21 day after infection.

The finding of wide spread anti-positive bats suggests that survival following Ebola virus infection is common among fruit bat species. The most compelling evidence for the long term survival of free ranging fruit bats following Ebola virus infection is a study by Hayman *et al.* (2010), in which a seropositive bat was known to be alive 13 months after release with a radio collar.

### **Multi-Host and Multi-Pathogen in Fruit Bats**

Multi-species interactions are critical to understand in order to accurately model viral dynamics in fruit bat populations. To date, there is evidence for *Ebola virus* infection in a total of 17 fruit bat species for (*Zaire Ebola virus* and *Reston Ebola virus*), but no currently known fruit bat hosts for *Bundibugyo*, *Sudan*, or *Tai Forest Ebola virus*. Multiple fruit bat species could potentially act as reservoirs for *Zaire Ebola virus* and *Reston Ebola virus*. Many of these species have overlapping geographic ranges, and have the potential (at a geographic, not necessarily ecological, scale) to interact and share pathogens (Pourrut *et al.*, 2009).

However, while either fragments of virus (PCR) or antibodies were detected in these hosts, their true role as reservoirs versus incidental hosts and the relative contribution of each species to interspecific host dynamics is currently unknown. Multiple circulating pathogens can also change within-host and within-population dynamics and could confer cross-species immunity (Hayman *et al.*, 2013).

Though cross-reactivity is shown among *Ebola viruses*, it is unknown how this translates to immunity within the hosts. Leroy *et al.* (2005), demonstrated numerous fruit bats infected (detected by PCR) with similar *Zaire Ebola virus* species PCR fragments some years apart, but within the species, these short genomic fragments differed between species and collection time.

### **Meta Population and Connectivity**

Another key aspect of ecological theory that must be investigated further is the role that meta-population dynamics may play in the ecology and evolution of Filoviruses. The evidence of direct movement between different caves for *R. aegyptiacus* and have found that there is genetic similarity between viruses detected in geographically distant locations (Amman *et al.*, 2012).

*R. aegyptiacus* exist as a large meta-population with virus circulation over broad geographic ranges. Population genetic studies using mitochondrial and microsatellite markers have confirmed that a congeneric species, *Rousettus leschenaultia*, is highly vagile and panmictic across large areas (e.g., from India throughout China). Further investigations to understand host movement and connectivity of potential Filovirus reservoirs are warranted (Chen *et al.*, 2010).

## **FUTURE DIRECTIONS IN FRUIT BAT EBOLA VIRUS RESEARCH**

### **Unexplored Diversity and Geographic Gaps a More Unified Surveillance Strategy**

There are over 925 fruit bat species globally and only a small fraction (~15%) has been targeted for viral discovery to date. Global surveillance programs like CDC's Global Disease Detection centers or United States Agency for International Development's (USAID) Emerging Pandemic threat Program have established laboratory protocols for screening specimens from a diversity of wild mammal hosts. For example, the USAID predict project uses degenerate PCR primers to screen bats, rodents, and primates across multiple (~10–20) viral families including Filoviruses in 20 countries around the world. Through capacity building in emerging infectious disease “hotspots” globally (Jones *et al.*, 2008), these efforts have the potential to establish a new baseline for the “unknown” zoonotic pool in wildlife and redraw the biogeographic boundaries of pathogen distribution and host range (Anthony *et al.*, 2013).

While it is important to survey wildlife showing clinical signs of disease, most viruses are discovered in fruit bats from asymptomatic animals, and a two-pronged approach of screening both healthy and diseased animals is required (Levinson *et al.*, 2013). Modeling approaches to target fruit bat host species based on life-history traits (Luis *et al.*, 2013) or viral “habitat” suitability using ecological niche models can both be used to refine the taxonomic and geographic scale of surveillance for novel Filoviruses or novel Filovirus host species (Peterson *et al.*, 2006).

### **Develop More Sensitive, Non-invasive tools for Longitudinal Monitoring of Fruit Bat Populations**

As part of a more unified Filovirus surveillance strategy in fruit bats, it will also be necessary to develop non-invasive sampling protocols and better detection methods for viral discovery (Anthony *et al.*, 2013).

Developing more sensitive assays to detect antibodies or virus from small quantities of blood (Smith *et al.*, 2010) or bat excreta, respectively, has two potential benefits. First, bats (of which many species are threatened) do not need to be killed to identify potential *Ebola virus* reservoirs, or study the distribution and the seasonality of viral shedding or infection. Second, for management interventions, it is most important to understand the routes of viral shedding in fruit bats and the seasonality of this shedding, rather than the presence or absence of a virus in a given animal or tissue type. Thus, there may be more value in detecting a virus in bat feces, urine, or saliva than there would be in bat tissue if transmission is occurring indirectly in bat habitat (caves or mines) (Anthony *et al.*, 2013). However, if the risk interface is through bush meat hunting and direct butchering of bats, then understanding prevalence and viral load in tissues and blood would be most relevant (Leroy *et al.*, 2009).

There is also a need for better studies of immunological responses in bats (Baker *et al.*, 2013). Understanding bat immune responses to Filoviruses will help understand the ecology of these viruses within the natural setting because it can be challenging to interpret antibody data in wild species and difficult to use these data to decide whether or not a species is a reservoir. More specific and sensitive assays, such as Luminex technology and pseudo type assays may help resolve some of these issues (Wright *et al.*, 2010). These assays still require positive and negative controls, but Peel *et al.* (2013), have shown how similar data can be analyzed in the absence of validated gold standard assays from the appropriate species and population (and applied these methods to bat sera).

### **Better Understanding Viral Shedding and Transmission in Bats**

While we have a decent understanding of the progression of infection and immunity in individual humans (Feldmann *et al.*, 2011), little is known about antibody persistence and viremia in bats. Experimental infections studies in captive bats and long-term monitoring of bat populations in the field using mark-recapture should help to inform this. As previously mentioned, a large number of outbreaks have been directly linked to mining activities or cave exposure (Timen *et al.*, 2007). While experimental studies with BSL-4 agents such as Filoviruses can be challenging, captive studies can be used to understand infection and antibody dynamics in the absence of experimental challenge. Experimental studies of Filoviruses in primates have been useful to describe Filovirus infection, including the symptoms, inflammatory response, viral shedding and therapeutic potential of immunoglobulin in primates (Jahrling *et al.*, 2007).



The mechanism(s) of transmission to primates, which are epidemiologically linked to several Filovirus outbreaks and are severely affected by infection, remain unknown. Again, these studies are useful for understanding whether transmission to target, novel hosts is possible, but do not necessarily elucidate the mechanisms for transmission of Filoviruses between putative reservoir bat hosts or bats and non-bat species. Studies of transmission mechanisms between and from bats to target species, such as pigs and primates, are a priority for experimental studies. While there are many inherent difficulties with performing such studies for Filoviruses, including extensive field situations, BSL-4 level facilities, and ethical issues, these experiments could greatly improve our understanding of Filovirus ecology (Weingartl *et al.*, 2012).

### **Better Understanding Host Ecology and Spillover Potential to Humans**

While there is evidence to support specific instances of viral spillover, the epidemiological links between bats, *Ebola viruses*, and human and primate infection are not clear. Recent epidemiological surveys following an outbreak reported increased bat activity through bat migration and hunting prior to an outbreak of *Ebola virus* in DRC (Leroy *et al.*, 2009). One recent study found a high prevalence (15%) of IgG antibodies to Zaire Ebola virus in human populations in Gabon, and that populations living in forest areas were at a higher risk to being seropositive as compared to human populations in the grassland, savannah, and lake area. Interestingly, no significant differences in seroprevalence were found between populations that hunted or had contact with animals to those that did not (Leroy *et al.*, 2009).

Bats may drop partially eaten; Ebola virus-contaminated fruits that terrestrial mammals eat and become infected. The role of fruit tree masting in inter-species interactions and Filovirus spillover, e.g., between frugivorous bats, ungulates (duikers), and primates in the forest, is suspected but not known. Video studies to those in Bangladesh have shown how apes in Africa share fruit resources, but it is currently unknown if partially eaten fruits can lead to infection with Filoviruses (Gonzalez *et al.*, 2007).

Models using the SIR structure have been used for human epidemic dynamics but not for wildlife (Lekone *et al.*, 2006). Multi-species SIR models could be developed to describe Filovirus transmission within bats and between bats and other host species (e.g., gorillas) and could be parameterized using data from field and experimental investigations. These epidemiological studies could be used to answer questions regarding the transmission processes, including if the viruses could persist within specific populations or species alone.

These models may also be used to highlight which aspects of host and virus biology may be important and require further study, through the use of sensitivity analyses (Wood *et al.*, 2012).

Collectively, these ecological studies will be critical to inform disease management options. For example, management options that reduce human bat contact during seasonal periods of high risk viral shedding, or at key interfaces, will likely be the most effective approaches and can balance both conservation and human health needs. The need to better understand the ecology of Filoviruses in their natural hosts and factors that facilitate transmission could not be timelier, as an unprecedentedly large human Ebola virus outbreak is currently ravaging Guinea (Olival *et al.*, 2013; Wood *et al.*, 2012).

### CONCLUSION AND RECOMMENDATIONS

Ebola virus outbreaks continue to emerge in rural settings where public health surveillance is lacking or non-existent. 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 bush meat. 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. 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.

Therefore, based on the above conclusion remarks the following recommendations are forwarded:

- ◆ In order to have a better protection the communities from outbreaks rule out other endemic infections, surveillance and rapid diagnostic testing needs to be implemented in order to ensure an early public health response.
- ◆ Educational campaigns about the dangers of contracting *Ebola virus* from infected wildlife should be undertaken to prevent possible outbreaks. Take in consideration the Fruit bats as have reservoir for Ebola virus.
- ◆ A rigorous effort between international governments, healthcare officials, and the communities affected by Ebola will have to be made in preventing strategies.

- ◆ Further studies should be carried out in designing of treatment and vaccination for the disease.
- ◆ Train the public health experts up on the diagnosis techniques, prevention and control strategies since the extent and severity of the virus highly depend on the ability of the public health officials to diagnose and respond it.

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