

Review

Chiropteran and Filoviruses in Africa: Unveiling an ancient history

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***Ebolavirus* and *Marburgvirus* belong to the Filovirus family and are responsible for hemorrhagic fevers in Africa. The first documented Filovirus outbreak in Africa occurred in Central Africa and was attributed to *Ebolavirus* species. In the last four decades, Filoviral hemorrhagic fevers (FHF) outbreaks caused by Ebola and Marburg viruses have been on the increase in Africa. The 2013-2015 outbreak has been the largest outbreak in human and has had the most devastating human and economic impact. Epidemics usually originate from a primary single introduction of the virus into simian or human population followed by an interspecies spill over. Multiple, short and isolated transmissions to humans have been also observed. Since the 1976 Yambuko (Democratic Republic of Congo) and Nzara (Sudan) epidemics, several investigations of different animal species have been undertaken but failed to identify the natural reservoirs of *Ebolavirus*. Further studies identified bats as probable reservoirs of *Ebolavirus* in Gabon, and major natural reservoirs of *Marburgvirus* in Uganda, supposed central forested areas of Africa as the epicenter where these viruses originated from, before dissemination. Chimpanzees, gorillas and duikers have been identified as highly sensitive hosts of *Ebolavirus* within wildlife. However, the relative importance of potential vertebrate hosts in the FHF emergence into human population remains unclear. Different transmission routes involving bats have been proposed. Filoviruses have a zoonotic origin; amplified and maintained in nature between potential reservoirs in a jungle cycle. *Ebolavirus* mostly escapes these natural foci, when other sensitive secondary simian are infected and transmit the virus to human population via hunting, bat's saliva infected wild fruit collection or land monitoring, while *Marburgvirus* emergence was linked to monkey's tissues handling or human entry into bat sheltering habitats. This review discusses the dissemination of filoviruses circulating within their possible chiropteran reservoir species. Vertebrate hosts suspected in the maintenance/transmission cycles are reviewed and their bioecological features discussed. Despite the importance of the findings about reservoirs' discovery, several other questions such as plurispecific associations, migration routes, breeding cycles need to be addressed and are pointed out in this review, in order to generate risk maps for filoviruses' (re)emergence in West Africa.**

Key words: *Ebolavirus*, *Marburgvirus*, Chiropteran, emergence, bioecology, West Africa.

INTRODUCTION

Filoviral hemorrhagic fevers (FHF) are endemic to Africa. Certainly confined in a jungle cycle for a long time, their etiological agents, namely Ebola and Marburg viruses circulated silently without any manifestation in human population until 1976, when *Ebolavirus* hemorrhagic fever was first simultaneously diagnosed from human communities in Yambuko (Democratic Republic of Congo, DRC) (Johnson, 1978) and Nzara and Maridi (Sudan) (Smith, 1978). Its closest relative, *Marburgvirus* was first recognized in Marburg, Germany and Belgrade, Serbia (formerly Yugoslavia) in 1967 causing an outbreak of severe viral hemorrhagic fever among laboratory workers. African green monkeys (*Chlorocebus aethiops*) imported from Uganda for research purpose were the source of the infection (Smith et al., 1967; Siegert et al., 1968). In Africa, it appeared first in Johannesburg, South Africa (Gear et al., 1975). Since those first recorded emergences, filoviruses increasingly manifest their pathogenic potential, sporadically emerging or re-emerging, enlarging their areas of incidence into Africa and threatening public health and animal biodiversity. There has been a mystery overlapping their natural emergence for decades. Nowadays, bats are much more known involved in their transmission cycle. The emergence of *Ebolavirus* in West Africa inspired several interrogations and request detailed research-action studies in order to understand the extent that the viral amplification, within the reservoir species, has reached. It is likely that the 2013 Guekedou emergence in Guinea was induced by a fruit bat, *Eidolon helvum* (Funk and Piot, 2014). If the virus circulates within the local West African fauna, it will then have the opportunity to set in new ecological niches, in a West African sylvatic cycle, and sporadic epidemics are predictable in West Africa. Surveillance study programs across West African countries, along a west-east prospection transect bordering the northern limit of the forested areas of Central Africa needs to be entirely undertaken. This will aim to detect virus circulation or specific antibodies in reservoir and incidental hosts using serology and RT-PCR for viral nucleic acid sequences detection from wild samples in order to infer the natural history of *Ebolavirus* circulation, and map the geographic range of the virus' amplification. This review discusses the filoviruses associated with bats, and proposes future directions for epidemiological and ecological studies that need to be undertaken, in order to better understand the involvement of chiropteran populations and the patterns of FHF emergence.

We reviewed the literature on chiropteran found naturally infected with filoviruses in Africa. Other bat species or wild animals from which filovirus nucleic acid

sequences or serological evidence of filovirus circulation has been detected are also listed. Considering the ecological and ethological features so far known about chiropteran (Rosevear, 1965; Walker, 1999), we speculate on the potential filoviruses' extension due to their migration, roosting and reproduction.

A literature analysis allowed us to discuss each potential reservoir species' implication in the epidemiology of Ebola and Marburg viruses. Future orientation studies are proposed to pinpoint the areas at risk for eventual filovirus' emergence in West Africa. Systematic terminology of chiropteran used in this paper follows Rosevear (1965) and Walker (1999), while classification of filoviruses follows the revised filovirus taxonomy of the 9th report of the International Committee on Taxonomy of Viruses (ICTV) (Kuhn et al., 2010; 2013). The distribution maps of bats are documented from the available bibliographic data and unpublished collection data from the IRD laboratory of medical zoology, in Dakar, Senegal. We hypothesize the potential amplifying mechanisms, and the ways from which human populations might become infected from sylvatic cycles. We also specify the eventual role of various potential bat reservoir species.

BACKGROUND OF FILOVIRAL HEMORRHAGIC FEVER OUTBREAKS

Filoviruses, the causative agents

The causative agents of FHF are non-segmented, enveloped negative-sense, single-stranded RNA viruses, that morphologically resemble rhabdoviruses and functionality paramyxoviruses, similar also in their genome organization, expression and replication (Feldmann et al., 1993; Beer and Kurth, 1999). RNA viruses have a high ability to rapidly evolve in response to changing host and environmental circumstances via multiple genetic mechanisms, what classify them among the most dangerous emerging and re-emerging pathogens (Morens and Fauci, 2013). The family *Filoviridae* (filo derived from: *filum*, Latin) comprises three genera: *Ebolavirus*, *Marburgvirus* and *Cuevavirus*. The two first ones are the most known because they were described during deadly filoviral hemorrhagic fever epidemics. A third genus, *Cuevavirus*, (species *Lloviu cuevavirus*) less known than the precedents, was only described after a filoviral outbreak [viral pneumonia due to Lloviu virus (LLOV)] which affected a population of the Schreiber's bats, *Miniopterus schreibersii* Kuhl, 1817 in Spain, Europe (Negredo et al., 2011). The genus *Ebolavirus* includes

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five genetic and antigenic subtypes: *Bundibugyo ebolavirus* (BEBOV), *Zaire ebolavirus* (ZEBOV), *Reston ebolavirus* (REBOV), *Sudan ebolavirus* (SEBOV) and *Tai Forest ebolavirus* (TAFEBOV) or *Ivory Coast ebolavirus* (ICEBOV). The genus *Marburgvirus* accounts for a single species, *Marburgvirus marburgvirus* (formerly *Lake Victoria marburgvirus*), which consists of two very divergent “viruses”: Marburg virus and Ravn virus, approximately 20% divergent at a genetic level (Carroll et al., 2013; Kuhn et al., 2010, 2013; Towner et al., 2006, 2009). This is in contrast to the known diversity for Ebolavirus species, with *Zaire ebolavirus* having only a 2.7% nucleotide difference between sequences, *Sudan ebolavirus* 5.2%, and *Reston ebolavirus* 4.5% (Lauber and Gorbalenya, 2012; Carroll et al., 2013).

Despite increasing numbers of viruses being detected, some species are represented by single viral lineage (for example, *Tai Forest ebolavirus* by Forest virus and *Lloviu cuevavirus* by Lloviu virus). During the 1998 Marburg Viral Disease outbreak that occurred in northeastern DRC, nine genetic lineages of the virus were involved (Bausch et al., 2006). In 1976, when *Ebolavirus* described 9 years after *Marburgvirus* presented the same filament-like structure as *Marburgvirus*, both were included in the same family of *Filoviridae*, newly described (Kiley et al., 1982). With the growing awareness of the rising threats to humans and wildlife caused by filoviruses, the importance of bats as potential reservoirs of viruses are much more investigated and will probably provide more divergent lineages within *Filoviridae*, that will enrich these taxonomic classifications.

Discovery of filoviruses

Ebolavirus

The first emergences of *Ebolavirus* were documented from Yambuko (DRC), Nzara and Maridi (Sudan) in 1976 with very high case fatality rates of 88 and 53%, respectively, caused by two distinct species of *Ebolavirus*: *Z. ebolavirus* (ZEBOV) (Johnson, 1978), and *Sudan ebolavirus* (SEBOV) (Smith, 1978). The source of transmission remains unknown. The causative agent was then named *Ebolavirus* after the Ebola River running along the Yambuko village, in the North Equator province of the Democratic Republic of Congo (formerly Zaire), where it was first diagnosed in the human population in 1976, simultaneously as in Nzara, Sudan (Smith, 1978). The number of cases has risen steeply and *Ebolavirus* outbreaks re-emerged after a long silent period (1980-1993), with increased frequency and new species discovery: *Côte d'Ivoire ebolavirus* (CIEBOV) in 1994 in the Ivory Coast and, *Bundibugyo ebolavirus* (BEBOV) in 2007 in Uganda (Towner et al., 2008). While re-emerging in Gabon and Republic of the Congo, *Ebolavirus* incidence in human was concomitant with a marked mortality amongst

gorillas and chimpanzees infected with the ZEBOV strain. *Ebolavirus* epidemics occurred between latitudes 10°N and 10°S, on both sides of the equator (Peterson et al., 2004; Groseth et al., 2007), approximately corresponding to the Afrotropics, with exception of *S. ebolavirus* which emerged at the extreme Eastern. The disease spread from Central to West Africa. Four of the known *Ebolavirus* species have emerged in sub-Saharan Africa, causing deadly outbreaks: *S. ebolavirus* (SEBOV), *Ivory Coast ebolavirus* (CIEBOV), *Bundibugyo ebolavirus* (BEBOV), and *Zaire ebolavirus* (ZEBOV) recently incriminated in the biggest Ebola epidemic ever recorded touching Guinea, Sierra Leone, Liberia (Baize et al., 2014) and lastly Nigeria, Senegal and Mali. From the past, epidemics have occurred in the Democratic Republic of Congo, Sudan, Gabon, Republic of Congo and Uganda (Smith, 1978; Le Guenno et al., 1995, 1999).

Marburgvirus

The other member of the *Filoviridae* family is *Marburgvirus*, the silent cousin of Ebola. The virus Marburg was named after Marburg in Germany, but originated from Uganda, in Central Africa. Vervet monkeys [*Chlorocebus aethiops* (Gray, 1821)] importation for research purpose in Marburg and Belgrade (formerly Yugoslavia) brought the virus to these countries in 1967 (Smith et al., 1967). The first manifestation of *Marburgvirus* in Africa was a sporadic and fatal case, documented in Johannesburg, South Africa, in February 1975 from an Australian who came from Zimbabwe. Marburg hemorrhagic fever epidemiology will be discussed below. Ebola and Marburg viruses occurred in Africa, and at a much lesser extent in a primatology research center, in Manila, Philippines where *Reston Ebolavirus* (REBOV) has been described from cynomolgus monkeys (*Macaca fascicularis* Raffles, 1821) imported into America (Philadelphia, 1989; Alice, Pennsylvania, 1990, 1996) and Italy (1996) (Rollin et al., 1999; WHO, 1992).

Epidemiology of Filoviral hemorrhagic fevers

Ebola hemorrhagic fever (EHF) or ebola virus disease (EVD)

EHF (EVD, International Classification of Diseases, ICD-10) is of major public health concern in the rural areas of sub-Saharan Africa, where *Ebolavirus* reached human population, after escaping its sylvatic foci first, then spread into rural/urban areas where it caused deadly hemorrhagic manifestations in human population. Multiple *Ebolavirus* species are co-circulating in endemic areas and the emerging zoonosis remains one of the most important zoonotic viral diseases of human in sub-Saharan Africa, because there is no approved treatment

and no licensed vaccine. EVD outbreaks occurred sporadically in Africa, scattered, within 10° latitude of the equator (Peterson et al., 2004; Groseth et al., 2007). This area is of dense and humid rainforest, characterized by succession of two rainy seasons and two dry seasons, providing the ecological niches favorable for *Ebolavirus* spp. amplification, maintenance and circulation.

It is likely that the vertebrate animals involved in *Ebolavirus* circulation find the optimal conditions necessary for sheltering, feeding and breeding and that the factors modulating *Ebolavirus* emergence are associated with those ecosystems. Spatio-temporal distributions of human *Ebolavirus* spp. outbreaks in Africa have already been well documented and mapped (Peterson et al., 2004; Pourrut et al., 2005; Groseth et al., 2007; Changula et al., 2014; Rougeron et al., 2015). *Ebolavirus* epidemics arose generally at the same time of the year (end of the dry season-beginning of the rainy season), when reservoir species of the virus gather with other sensitive hosts because of scarcity of food source, modification of ecological habitats which imply encroachment of different vertebrate animals. Also, population dynamic over time (physiological status such as reproduction time, demographic explosion of sensitive naive species) and space (migration) might conduct to amplification and emergence of *Ebolavirus*.

***Ebolavirus* dissemination**

When the optimal conditions for *Ebolavirus* spp. circulation into those ecosystems are met, their probability to escape from these foci is enhanced. Peterson et al. (2004) used an ecologic niche modeling of outbreaks and sporadic cases of filovirus-associated hemorrhagic fever (HF) to provide a large-scale perspective on the geographic and ecologic distributions of Ebola and predicted that EVD would occur in the humid rain forests of central and western Africa. They observed that filovirus' transmission to humans is not common, and most occurrences can be traced to a single index case (WHO, 1978), followed by a spillover reaching the population. The following hypotheses can be considered for the introduction of the virus to nonhuman primate populations: 1) Non-human primates might have shared and eaten fruit rests containing virus in residual bat saliva and directly infected themselves. Gonzalez et al. (2007) theorized this pathway, stating that chronically *Ebolavirus* spp. infected bats might drop down partially eaten and masticated fruit spats or pulp picked from the canopy to the ground, promoting indirect transmission of the virus to some terrestrial dwelling mammals. Viral particles shed in bat saliva infected by the way, infect the rests of fruits secondly eaten by ground mammals. It has been shown that females chimpanzees mostly gave some collected fruit to their depending offspring and that adult male share meat with females and juveniles (de Wall, 1989); 2)

Infected individuals can contaminate their group during care and social behavior, 3) Great apes also hunt and share other primates preys such as vervets, galagos and colobes and can be infected with contaminated meat. Assessing that infection of primates colonies begin with a single index case is then more difficult to support. Several individuals can contract the virus at the same time and contribute to disseminating it, because of their social behavior, 4) Natural secretions such as feces, urine, body fluid, placental rest and secretion might be shed in nature and represent a potential source of contamination to other small terrestrial mammals. Great apes and forest duikers fed on fruit rests become infected and might later represent the first link of a human transmission chain if rural communities enter into contact with those wild animals, via hunting. It is an epidemiological schema that might transpose the virus in a human population.

Olival and Hayman (2014) summarized, in their proposed transmission dynamic, that chiropteran are the potential reservoirs maintaining an intra-interspecies *Ebolavirus* circulation, and transmitting it to non-human primates and forest duikers; while direct transmission to human as well as rodents and pigs remain to be elucidated. Also, there is no yet evidence that wild animals, excepted non-human primates, can transmit directly the virus to human populations. The role of mosquitoes in their transmission model is questionable, interhuman transmission via natural secretions favors the virus spreading. Bausch et al. (2007) tested several body fluids as saliva, stool, semen, breast milk, tears, and nasal blood and concluded that EBOV is shed in a wide variety of bodily fluids during the acute period of illness but that the risk of transmission from vomits in an isolation ward and from convalescent patients is low. Humans can transmit the virus as soon as symptoms appear and continue to be infectious during the later stages of the disease as well as after death. Burial ceremonies in which mourners have direct contact with the body of the deceased person can also play a role in the transmission. *Ebolavirus* has been detected in semen for up to 82 days, and *Marburgvirus* for up to 13 weeks (Martini and Smith, 1968; Bausch et al., 2007), after the onset of illness, suggesting that these viruses could be eventually transmitted by sexual route (Bausch et al., 2007).

Analyzing the origin of contaminations

After the first Ebola outbreaks that occurred between 1976 -1979 (DRC and Sudan), the second waves of *Ebolavirus* spp. epidemics occurred between 1994-1997, after a silent period of 15 years; a first case was linked to a chimpanzee autopsied by a Swiss ethnologist in Ivory Coast, West Africa, and was attributed to a new strain, CIEBOV. The Kikwit epidemic (DRC), Mekouka, Mayibout and Booue (Gabon) were due to ZEBOV reemergence (Amblard et al., 1997; Georges et al., 1999). The source was a deep forest gold-mining camp, suggesting that

workers of the mine entered the reservoir/vectors biota. Mayibout outbreak was related to Mekouka's epidemic. Booue epidemic also began by an infected hunter who accidentally entered the sylvatic cycle at this time, while a high viral sylvatic amplification was going on as suggested by died chimpanzees that tested positive for *Ebolavirus* infection. From 2000 to 2004, multiple epidemics were recorded and attributed to ZEBOV at the border of Gabon and the Republic of Congo and to SEBOV in Sudan and Uganda, affecting simultaneously large populations of gorillas and chimpanzees (Leroy et al., 2002, 2004b; Bermejo et al., 2006). The first findings that the Swiss ethnologist was infected by a chimpanzee and the fact that the Mayibout outbreak originated in deep forest and was related to a gold-mine, drew the schema of an implication of forest mammals, more specifically cave dwelling mammals. ZEBOV reemerged in 2005 in the Republic of Congo, in 2007-2009 in Democratic Republic of Congo, twelve years after the 1995 Kikwit outbreak. Two successive epidemics arose in the Luebo region (Kasai Occidental Province, DRC) in 2007 and 2008 and were caused by *Zaire ebolavirus* (Grard et al., 2011). Phylogenetic analyses performed on the full-length genomes of the two Luebo strains revealed that they were nearly identical, but not related to the lineage including ZEBOV strains from the 1976-1996 outbreaks (DRC and Gabon), nor to the descendants of the lineage including animal-derived sequences since 2001 and the human strains from the Mbandza-Mbomo 2003 and Etoumbi 2005 outbreaks (Gabon-RDC), with which they do, however, share a common ancestor (Grard et al., 2011). The Luebo 2007 outbreak represented an independent viral emergence, favored by a viral spillover caused by a dispersed reservoir species. Like the 1994-1997 Gabonese epidemics, these cross-border outbreaks were concomitant to marked wildlife epizootics (Leroy et al., 2004b; Rouquet et al., 2005; Lahm et al., 2007).

Chimpanzees, gorillas and duikers were susceptible hosts responsible for viral introduction into human populations. SEBOV emergence was also recorded in Uganda from 2011-2012, as in the DRC in 2012 (<http://www.cdc.gov/vhf/ebola/resources/outbreak-table.html>). In their modeling of geographic distribution of filovirus disease across Africa, Peterson et al. (2004) predicted the eastern extreme as the predilection area of *S. ebolavirus*, but this species emerged in DRC, the viral spillover being probably favored by widely dispersed reservoirs. In the past decades, in particular, FHF incidences have increased and have been seen in areas they were not reported previously. Before, FHF have never been recorded in Guinea until December 2013 when the first cases arose in the Southeast (Baize et al., 2014). Ebola virus disease was spreading unrecognized, while typical hemorrhagic fever cases such as Lassa fever or yellow fever, endemic in the area, were suspected but not proven. The hemorrhagic disease has

been spreading quietly until late March 2014 when the diagnosis was finally confirmed Ebola virus disease. Human to human transmission via contact of fluids favored a spillover and the disease reached the neighboring countries of Sierra Leone and Liberia bordering the original epicenter of the outbreak. Lastly, the outbreak reached unexpected proportion in two months (Baize et al., 2014; Gire et al., 2014; Pigott et al., 2014; Wauquier et al., 2015), overwhelming the fragile health system in those developing West African countries. The epidemic touched the cities of Conakry (Guinea), Freetown (Sierra Leone), Monrovia (Liberia), Lagos (Nigeria), Dakar (Senegal) and Kayes (Mali), reaching the specter of a regional, even international dissemination. In fact, imported cases have been noticed in the USA (Dallas, Texas; Chevalier et al., 2014), Spain (Madrid; Parra et al., 2014) and the United Kingdom (London; Kuhn et al., 2014). Also, contaminated healthcare workers have been transferred to Hamburg (Germany) and Lyon (France) for care. The disease spread from Central Africa to West Africa. Among the known *Ebolavirus* species, four have emerged in sub-Saharan Africa, causing deadly outbreaks: *S. ebolavirus* (SEBOV), *Ivory Coast ebolavirus* (ICEBOV), *Bundibugyo ebolavirus* and *Z. ebolavirus* (ZEBOV) recently incriminated in the biggest Ebola epidemic ever recorded. The forested area of Guinea has been the epicenter and the source of contamination is discussed subsequently. While the Guinean EVD outbreak was spreading in the neighboring countries of West Africa, *Ebolavirus* reemerged in July 26, 2014, for the seventh time, in Democratic Republic of Congo, in Inkanamongo village, in the vicinity of Boende town (Equateur province). A total of 69 cases were reported, including 8 cases among health care workers, with 49 deaths (Maganga et al., 2014). A coding-complete genome sequence of EBOV that was isolated during this outbreak showed 99.2% identity with the most closely related variant from the 1995 outbreak in Kikwit (DRC) and 96.8% identity to EBOV variants that are currently circulating in West Africa (Maganga et al., 2014). The two outbreaks were in fact caused by two novel EBOV variants, consensually named Makona (West Africa) and Lomela (Middle Africa), after the Makona River close to the border between Liberia, Guinea and Sierra Leone and the Lomela River that runs through DRC's Boende District, respectively (Kuhn et al., 2014). The genetic characterization of the virus, combined with the geographic location of the outbreak, demonstrate that the DRC outbreak is an independent event, without any epidemiologic or virologic connection with the continuing epidemic in West Africa (Kuhn et al., 2014; Maganga et al., 2014).

Marburg hemorrhagic fever (MHF) or Marburg viral disease (MVD)

Marburgvirus was described from the Behring laboratory,

in Marburg, Germany from Vervet monkeys (*Chlorocebus aethiops*) imported from Uganda (Smith et al., 1967). Infected monkeys presented typical hemorrhagic fever clinical tables (Jahrling et al., 1990; Peters et al., 1992). That first Marburg outbreak reported with severe viral hemorrhagic fever was related to the handling of organs and tissues from those green monkeys (Smith et al., 1967; Martini, 1969). Eight years later, the first manifestation of *Marburgvirus* in Africa happened, in Johannesburg, South Africa, in February 1975, sporadic and fatal. It concerned an Australian just returning from a trip to Zimbabwe where he slept frequently in the open and once in an abandoned house which loft was inhabited by numerous bats (Gear et al., 1975). The third recognized Marburg manifestation affected a French engineer in Kenya in 1980 that subsequently infected his doctor before dying. He visited the Kitum cave (Mont Elgon National Park) where large populations of bats were sheltering. Next, another Marburg case has been reported and concerned a Danish who died after visiting the Kitum cave in August 1987 (Kenyon et al., 1994). After a silent period of more than 30 years, Marburg virus, the long neglected Ebola virus relative, called for attention in its cradle of Central Africa, hitting twice recently, and in large proportion: 1) 1998-2000, a gold-mining community in Durba, in the northeastern region of the Democratic Republic of the Congo, was affected with a high mortality rate reaching 83% (Rec, 1999; Baush et al., 2006); 2) 2004 and 2005, a second and large Marburg outbreak followed in northern Angola (West Africa), in the province of Uige (Rep, 2005; Towner et al., 2006) with a mortality rate higher than that during the 1998-2000 outbreak of Durba above cited (Towner et al., 2006).

Surprisingly, an Ebola outbreak was expected because of the large area affected reaching a big community since a first single infected case working in a gold-mining company. In July and September 2007, miners working in Kitaka Cave, Uganda, were diagnosed with MHF (Towner et al., 2009). At the same time (June-September 2007), 4 miners from Ibanda District contracted MHF through exposure to bats secretions in a mine in Kamwenge District, Uganda (Adjemian et al., 2011). Genetically diverse viruses isolated from tissues of the Egyptian Fruit Bat as well as detection of RNA MARV from these bats supported that *Rousettus aegyptiacus* was responsible for the epidemic. In late 2007, an American tourist contracted MVD in the python cave and in July 2008, another tourist from Netherlands was also infected with MARV in the same cave, from which diverse genetically MARVs were also isolated from *R. aegyptiacus* (Amman et al., 2014). Confined in a jungle cycle as *Ebolavirus*, *Marburgvirus* emerged and expressed its pathogenic potential, such as that one for *Ebolavirus*, without any doubt. As for *Ebolavirus* epidemics, *Marburgvirus* outbreaks in Africa were also well mapped and documented (Bausch et al., 2006; Feldmann, 2006; Brauburger et al.,

2012; Rougeron et al., 2015). Imported human cases of Marburg virus infection from Uganda have been also reported in the USA (Timen et al., 2009) and in Netherlands (Fujita et al., 2010). Practically, all MARV emergences have been related to bat shelters (caves, gold-mining areas) and contact with infected monkeys (Cercopithecidae). These events clearly traced back the source of contamination to chiropters and primates Cercopithecidae. Both filoviruses are afrotropical, originally infectious of fruit bats (Chiroptera, Pteropidae) that seem playing the major role in their epidemiology, namely their maintenance and circulation in nature that will be discussed in a comparative manner in this review. *Ebolavirus* emerged mostly than *Marburgvirus*, but in terms of epidemiology both filoviruses are very similar. They share bats as the same vertebrate hosts.

Clinical manifestations and pathology of Ebola and Marburg viral diseases

At several times that a FHF arose in Africa, other endemics diseases such as Lassa fever, Yellow fever, malaria, cholera or typhoid fever were suspected. That has been the case for this ongoing Ebola epidemic in West Africa, where local Guinean healthcare workers attributed the first reported hemorrhagic cases to Lassa fever (Vogel, 2014). In 2007, the RDC ZEBOV emergence was also concomitant to an epidemic of typhoid and shigellosis. Then, the clinical table of filovirus-infected patients is non-specific and difficult to separate from other endemic diseases. The asymptomatic incubation period of filoviruses is 2-21 days. Symptoms usually manifest abruptly by a fever (greater than 38.6°C), severe headache, muscle pain and malaise. Secondly, severe diarrhea, nausea, vomiting, respiratory disorders, abdominal pain and weakness appear, accompanied with a lack of appetite. Hemorrhagic manifestations are observed in 30-50% of patients and vary in severity. Spontaneous abortion has been recorded within pregnant woman (Baize et al., 2014; Vogel, 2014). The pathogenesis of these hemorrhagic fevers includes necrosis of many organs, particularly liver (Martines et al., 2014). It has been suggested that the hemorrhages and shock manifestations may be a consequence of endothelial cell infection, with consequent loss of endothelial integrity leading to rapid hypovolaemic shock, multiple effusions and bleeding (Fisher-Hock et al., 1985). Death ensues within few days but some infected people recover.

However, patients who die usually have not developed a significant immune response to Ebola infection. *Z. ebolavirus*, *S. ebolavirus*, *Bundibugyo ebolavirus* and *Forest ebolavirus* cause severe illness in humans, although Forest virus infections have rarely been documented. *Reston ebolavirus* does not seem to be pathogenic for humans, but people may seroconvert after exposure to infected nonhuman primates or pigs.

Infection with *Marburgvirus* develops an acute illness for up to three weeks at least, accompanied by the following signs and symptoms: fever, generalized body pain, nausea and vomiting, headache, anorexia, malaise, abdominal pain, diarrhea, dyspnea, dysphagia, hiccups, conjunctivitis, rash or petechiae and abnormal bleeding from the nose, mouth, gastrointestinal tract, or genitourinary tract (Bausch et al., 2006). Death arises within few days, but as for EVD, some MVD infected people recovered.

The reservoir search

Several investigations targeting different vertebrate animals have been undertaken to identify the natural vertebrates that host and lurk Ebola virus in nature, after the first emergences. Arata and Johnson (1977) tested 100 specimens from 501 vertebrates collected in 1977 from Sudan; Germain (1978) screened more than 800 bedbugs and 147 mammals in DRC; Breman et al. (1999) collected 1664 animals of 117 species around the areas where the 1976 Ebola hemorrhagic fever occurred in the DRC and in Cameroon; Leirs et al. (1999) screened 3000 animals primarily from forest areas near the home of the index case after the Kikwit Ebola epidemic (DRC). Samples were representative of the different class of mammalia, reptilia and birds; even plants were suspected and tested. Globally, no evidence of *Ebolavirus* infection was found. Swanepoel et al. (1996) conducted experimental inoculation of thirty-three varieties of 24 species of plants with *Z. ebolavirus*, no evidence of infection was observed. Vertebrate animals inoculated included pigeons, young snakes, rodents, laboratory mice colonies, tortoises, lizards, frogs, toads and bats. Two microchiroptera of the family Molossidae, the Angola free-tailed bat, *Tadarida condylura* and the little free tailed bat, *Tadarida pumila* and one megachiroptera of the family Pteropidae, the Wahlberg's epauletted fruit bat, *Epomophorus wahlbergi* were able to asymptotically replicate the ZEBOV with high viral titers, 4 weeks after inoculation, demonstrating for the first time that bats might be reservoirs hosts of *Ebolavirus* (Swanepoel et al., 1996). Invertebrates as cockroaches, leafhoppers, spiders, social ants, myrmicine ants, millipede and land snails were also inoculated but did not yield any proof of virus replication (Swanepoel et al., 1996). Turell et al. (1996) negatively tested the ability of three mosquitoes *Aedes albopictus*, *Aedes taeniorhynchus* and *Culex pipiens* (Diptera, Culicidae), and one soft tick, *Ornithodoros sonrai* (Ixodida, Argasidae) for *Ebolavirus*. Arthropods have never been successfully infected following inoculation (Swanepoel et al., 1996, Turell et al., 1996), although several observations suggest they can transmit Ebola virus to humans, as demonstrated by Kunz et al. (1968) who showed that Marburg virus persist for more than 3 weeks in *Aedes* mosquitoes after experimental inoculation. Since their first emergences in

1976 (*Ebolavirus* in Yambuko, RDC and Nzara, Sudan), and in 1975 (*Marburgvirus* in Johannesburg, South Africa), natural reservoirs of filoviruses remained elusive for 3 decades and any investigation was not able to reveal where these viruses persist in nature, during inter-epidemic periods until 2005 when Leroy et al. (2005) provided the first evidence of bats as possible natural reservoirs.

The first documented primary infections of natural MVD outbreaks in Africa have been linked to human visiting caves inhabited by bats: gold mining in Kitaka Cave in the Kamwenge District, Uganda (Adjemian et al., 2011); visit of python Cave in Maramagambo Forest Uganda (Fujita et al., 2010; Timen et al., 2009). These findings provided the first clues that bats might play an important role in the transmission cycle of MVD (Monath, 1999; Peterson et al., 2004; Bausch et al., 2003), and evidence of MARV circulation in bats was only been documented when Towner et al. (2007) first detected MARV nucleic acids and antibodies from the common Egyptian fruit bat, *Rousettus aegyptiacus* in 2002 and 2005 in Gabon, without any virus isolation. Swanepoel et al. (2007) also found MARV nucleic acid and antibody to the virus in the serum of insectivorous and fruit bats trapped in the Goroubwa Mine, in northeastern DRC, but their attempts to isolate the virus were unsuccessful. Later, Towner et al. (2009) isolated MARV nine months apart from Egyptian fruit bats of the Kitaka cave in Uganda, demonstrating long-term virus circulation among the bat reservoir species. Genome sequences of MARV isolated from bats closely matched those isolated from miners during this epidemic, indicating that common Egyptian fruit bats represent major natural reservoir and source of Marburg virus with potential for spillover into humans. Despite the isolation of MARV from naturally infected Egyptian fruit bats captured in the Kitaka cave near Ibanda, in Western Uganda (Towner et al., 2009) and the python cave in the Queen Elisabeth National Park, Uganda (Amman et al., 2014), experimental inoculation of *R. aegyptiacus* with MARV were conducted and showed that the species is a natural reservoir host for MARV and demonstrated routes of viral shedding via rectal and oral routes capable of infecting humans and other animals (Amman et al., 2015). While the *Marburgviruses* exhibit high overall genetic diversity (up to 22%), only 6.8% nucleotide difference was found between the West African Angolan viruses and the majority of East African viruses, suggesting that the virus reservoir species in these regions are not substantially distinct. Remarkably, few nucleotide differences were found among the Angolan clinical specimens (0 to 0.07%), consistent with an outbreak scenario in which a single (or rare) introduction of virus from the reservoir species into the human population was followed by person-to-person transmission with little accumulation of mutations. This is in contrast to the 1998 to 2000 *Marburgvirus* outbreak, where evidence of several virus

genetic lineages (with up to 21% divergence) and multiple virus introductions into the human population was found (Towner et al., 2006).

Wild vertebrate hosts sensitive to Filoviruses

With the exception of *Reston ebolavirus*, all African filoviruses cause severe illness in nonhuman primates and some other animals. While there is no formal evidence for a causative role in some species, *Ebolavirus* outbreaks have been linked to reports of massive die-off of gorilla (*Gorilla gorilla*) and chimpanzee (*Pan troglodytes*) populations. An outbreak of Ebola decimated in November 1994, 25% of a wild chimpanzee community of 43 members in the Taï National Park, in Ivory Coast (Formenty et al., 1999), as did another in great apes of Minkebe Forest, north-eastern Gabon and in western equatorial Africa (Huijbregts et al., 2003; Walsh et al., 2003). Between 2001 and 2003, the epidemics that occurred in Gabon and Republic of Congo were also, for the first time, linked to concurrent animal mortality, mainly gorillas, chimpanzees and duikers (Leroy et al., 2004b; Bermejo et al., 2006). Detection of EBOV infected corpses in these three species strongly incriminated *Ebolavirus* as the causative agent.

Their population decreased and duikers were estimated to have fallen by 50% between 2002 and 2003 in the Lossi sanctuary, Republic of Congo, while chimpanzees lost 88% of their populations (Leroy et al., 2004b). *Ebolavirus* was also incriminated in a marked decline in gorilla and chimpanzee populations in the same areas, at the same point in time in Mekouka and Mayibout outbreaks. Small EBOV-specific genetic sequences were amplified from organs of six mice (*Mus setulosus* and *Praomys* sp., Rodentia, Muridae) and a shrew (*Sylvisorex ollula*, Insectivora, Erinaceidae), in Central African Republic and provided the first documented biological evidence of EBOV presence in healthy animals (Morvan et al., 1999), however this data was not sufficient enough, to attribute a reservoir status to these animals, being given lack of specific serologic responses, nucleotide specificities in the amplified viral sequences, failure of virus isolation, and the non-reproducible nature of the results. *Ebolavirus* infects a large variety of animal species, as attested by exploration of dead wild animal carcasses analyses. During the Gabon and RC epidemics (2001- 2004), the remains of animals were found in the surrounding forest (Rouquet et al., 2005). Thirty four samples taken from those carcasses (bones, muscles and skin) were analyzed using a panel of highly sensitive techniques, such as reverse-transcription polymerase chain reaction (RT-PCR), serology, histopathology and immunohisto-chemistry (IHC). Fourteen of them (10 gorillas, 3 chimpanzees and 1 duiker) tested positive for Ebola infection, indicating that these three animal species can be naturally infected by EBOV.

Most infected animals probably died rapidly, as suggested

by the rapidly fatal nature of experimental EBOV infection in a variety of non-human primate species (Pourrut et al., 2005). Analyses of animal carcasses show that the great apes of the central African forests are particularly at risk for Ebola. This was confirmed by a serologic survey based on 790 samples taken from about 20 primate species in Cameroon, Gabon and Republic of Congo (Leroy et al., 2004a). Interestingly, some positive samples largely preceded the first human outbreaks in these regions, suggesting a viral sylvatic amplification chronologically happening before human contact with the virus. The results suggest that these animals are in regular contact with the EBOV reservoir, that some of them survive the infection, and that EBOV has probably been present for a very long time in the central African forest region. EBOV-specific antibodies were also found in other monkey species such as mandrills (*Mandrillus* sp.), vervets (*Cercopithecus* sp.), baboon, and drills suggesting that EBOV circulation between Cercopithecidae may be very complex, and some of their representative might be amplifying hosts because some great apes developed an Ebola viremia after eating their congeners Cercopithecidae. *Ebolavirus* epidemiology might involve other reservoir/amplifying hosts' species different to bats, and the passage of the virus to gorillas and chimpanzees might be more complex than a simple direct contact from the main reservoir. It is also possible that there are several reservoir species, and that many other animal species are susceptible to the virus and thereby participate in the natural EBOV life cycle (Figure 1). These include duikers (forest antelope, *Cephalophus dorsalis*, Unguligrades, Artiodactyla, Bovidae) and bush pigs (red river hog, *Potamochoerus porcus*, Unguligrades, Artiodactyla, Suidae). Overall, non-human primates of the family Cercopithecidae (colobus, baboons, mandrills, vervets and guenons) seem less sensitive to *Ebolavirus* infection as compared to non-human primates of the family Hominidae (chimpanzees and gorillas).

The Egyptian fruit bat is the potential reservoir of MARV. Marburg virus has been circulating in this species between the python cave and the Kitaka cave in Uganda as suggested by virus' isolation obtained by Towner et al. (2009) and Amman et al. (2014). The fact that Marburg and Belgrade epidemics were caused by *Chlorocebus aethiops* imported from Uganda support a typical reservoir role of this green monkey for the virus Marburg. In fact, the monkeys that carried the virus to Europe in 1967 were kept on Lake Victoria island, in a holding facility where large numbers of fruit bats were sheltering (Swanepoel et al., 2007). Uganda represents a "hotspot" for MARV circulation. It's actually known that transmission cycle can be schematized as presented in Figure 2.

Chiropteran as probable natural reservoirs of filoviruses

Enquiries were carried out in Central Africa, aiming to

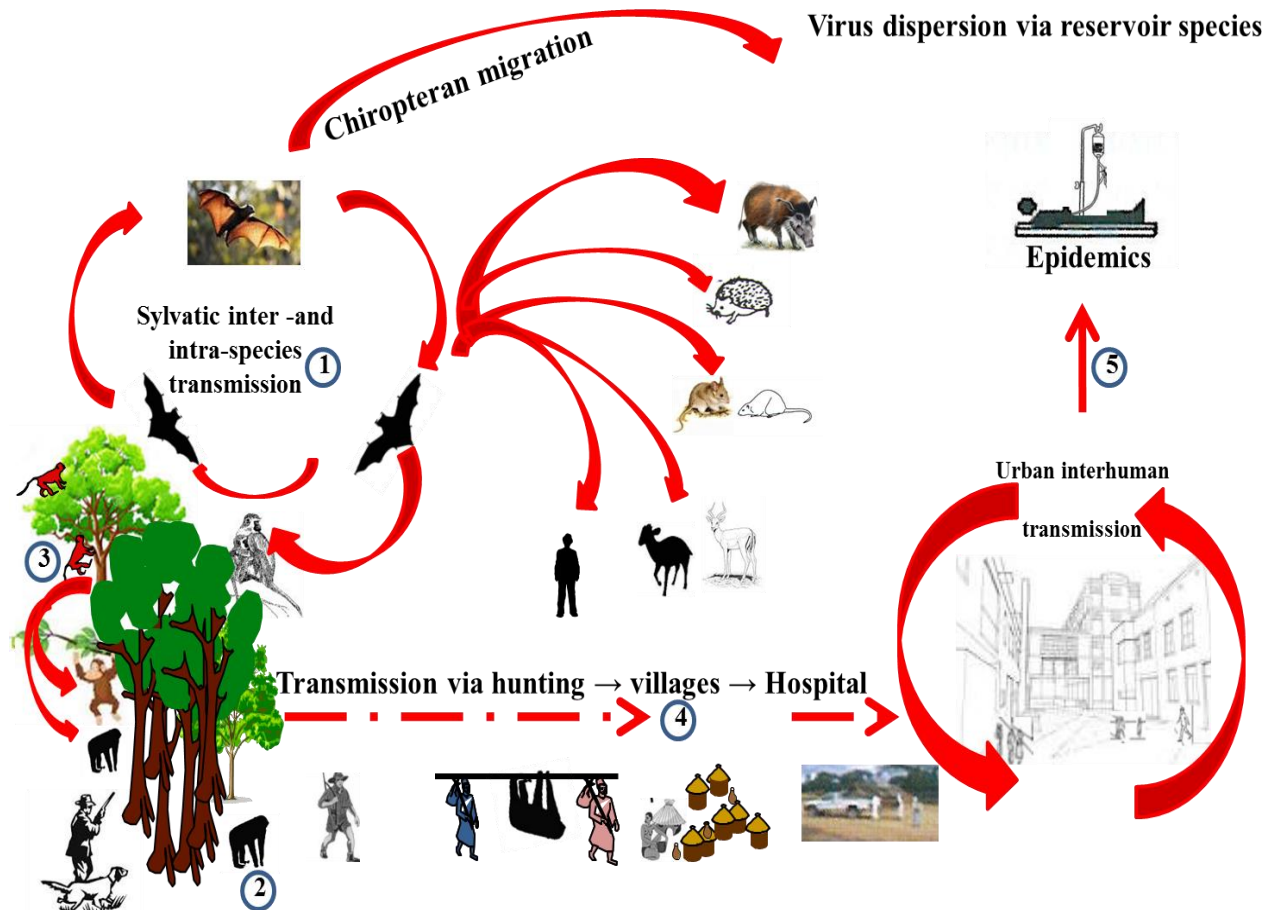


Figure 1. Ecoiagram of *Ebolavirus* transmission in nature. Fruit bats infected with *Ebolavirus* partially eat wild fruits in the forests (1). Partially chewed fruit contain virus particles enrobed in bat's saliva and dropped down from trees, contaminate other ground animals such as rodents, Insectivora, Onguligrades and non-human primates (2). Infected bats and Cercopithecidae are also eaten by great apes that are subsequently infected (3). Man can also be infected after intrusion in the canopy (caves and bat shelters) receiving directly bat's secretion infected with *Ebolavirus*. Mostly, hunting and handling of bushmeat (4) transposed ebolavirus from a sylvatic to an rural/urban transmission cycle causing deadly epidemics (5).

identify the natural reservoirs species of filoviruses (Leroy et al., 2005; Gonzalez et al., 2007; Pourrut et al., 2009). They found that bats belonging to the family Pteropidae were the major susceptible population, asymptotically infected by the virus as attested by antibodies and viral nucleic acid detection. Serological studies conducted allowed to detect specific anti Ebola IgG from 16 bats: 4 Hammer-headed Fruit Bat, *Hypsignathus monstrosus* H. Allen, 1861, 8 Franquet's Epauletted bat, *Epomops franqueti* Tomes, 1860 and 4 Little Collared Fruit bat, *Myonycteris torquata* Dobson, 1878 (Chiroptera, Pteropidae) (Leroy et al., 2005; Gonzalez et al., 2007; Pourrut et al., 2009). Their studies also detected viral nucleic acid sequences in the tissues of 13 bats (3 *H. monstrosus*, 5 *E. franqueti* and 5 *M. torquata*) and provided the first evidence of bats' role as probable potential reservoirs of *Ebolavirus* in nature (Table 1). Swanepoel et al. (2007) investigated the reservoir hosts

for Marburg virus (MARV) after the epidemic that hit the gold mining-community in Durba and detected MARV viral nucleic acid sequences from two insectivorous bats, the Greater Long-fingered Bat, *Miniopterus inflatus* Thomas, 1903 and the Eloquent horseshoe bat, *Rhinolophus eloquens* K. Anderson, 1905 (Microchiroptera, Rhinolophidae), and the Egyptian fruit bat, *Rousettus aegyptiacus* E. Geoffroy, 1810 (Megachiroptera, Pteropidae). Serological evidence of MARV circulation was detected by ELISA in *R. eloquens* and *R. aegyptiacus*. They concluded that these bats were implicated in *Marburgvirus* circulation around the Goroubwa mine and its immediate surroundings. Towner et al. (2007) detected MARV-specific RNA, IgG antibody from *R. aegyptiacus* and isolated MARV for the first time from this species in Gabon, acting now as a typical reservoir of *Marburgvirus* (Towner et al., 2007). Pourrut et al. (2009) documented that both Ebola and

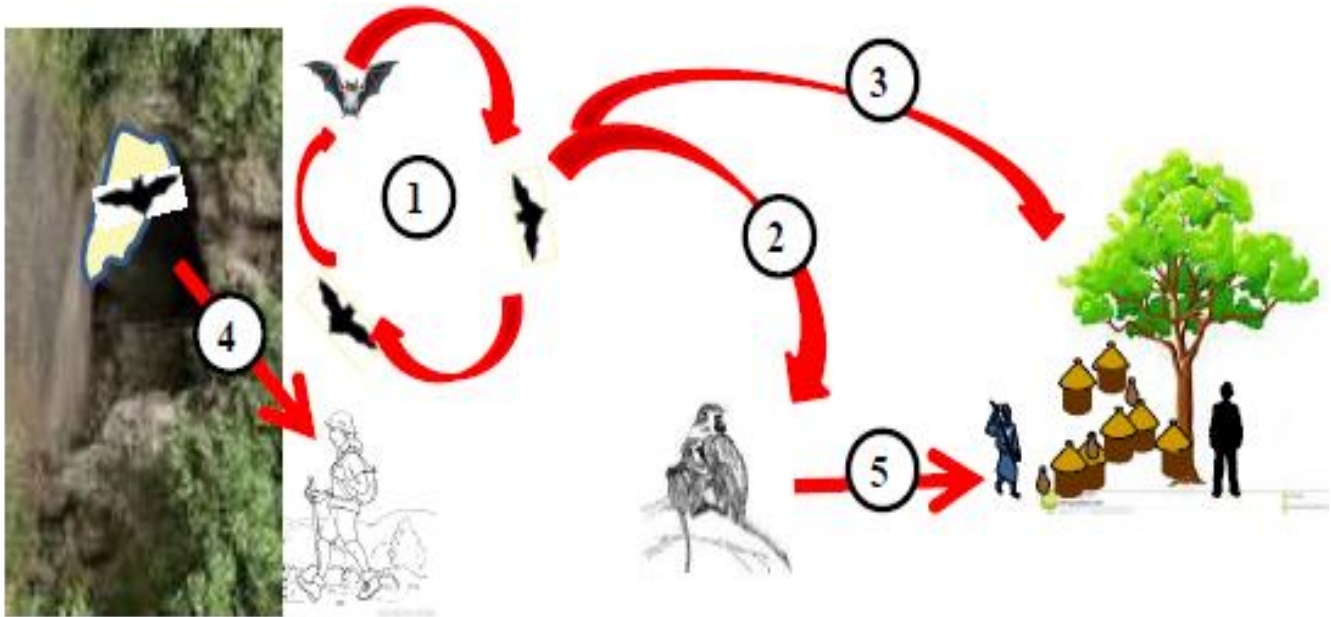


Figure 2. Ecodiagram of *Marburgvirus* transmission in nature. High intra-interspecific contact in roost facilitates rapid transmission of MARV between bats (1). Partially chewed fruit containing virus particles shed in bat's saliva and dropped down from trees, contaminate Cercopithecidae (2) and Hominidae (3). Man can also be infected after intrusion into the sylvatic (caves and bat shelters) receiving directly bat's secretion infected with MARV (4). Handling of monkeys tissues also directly infect human beings (5).

Marburg viruses co-circulated within the Egyptian Fruit Bat. Hayman et al. (2010) detected Zaire EBOV (ZEBOV) antibodies in a single Straw-colored Fruit Bat, *Eidolon helvum* Kerr, 1792 (Megachiroptera, Pteropidae) from a roost in Accra, Ghana; another fruit bat *Epomophorus gambianus* Ogilby, 1835 (Megachiroptera, Pteropidae) has been found infected with *Ebolavirus* by Hayman et al. (2012), as well as *E. franqueti* and *H. monstrosus*. Serological evidence of EBOV antibodies has been also detected in a serum sample of the Little flying Cow, *Nanonycteris veldkampii* Matschie, 1899 (Megachiroptera, Pteropidae) (Hayman et al., 2012). ZEBOV-IgG were detected again in *E. franqueti*, *H. monstrosus*, *R. aegyptiacus* and *M. torquata*; while the Lesser Epaulet bat, *Micropteropus pusillus* Peters, 1867 (Megachiroptera, Pteropidae) and *Mops condylurus* Lesson (Microchiroptera, Molossidae) tested for the first time ZEBOV-IgG positive in nature (Pourrut et al., 2009). MARV-IgG were also found in *R. aegyptiacus* and *H. monstrosus* (Pourrut et al., 2009). Amman et al. (2012) investigated the Python Cave inhabited by the Egyptian Fruit Bat in Uganda and detected viral nucleic sequences of MARV; also seven of the bats yielded Marburg virus isolates (Table 1). Using an enzyme-linked immunosorbent assay based on the viral glycoprotein antigens, Ogawa et al. (2015) detected IgG ZEBOV, and MARV in serum samples collected from the fruit bats (*Eidolon helvum*) in Zambia during 2006-2013. Distinct specificity for *Reston ebolavirus*, so far known only from Philippines and China, in Asia (Barrette et al., 2009; Pan et al., 2014), has been shown also from

E. helvum for the first time in Zambia (Ogawa et al., 2015). Serological evidence of antibodies directed against flaviviruses and detection of viral nucleic acid incriminate those chiroptera as potential reservoirs of filoviruses in nature. The isolation of MARV in nature supports a typical status of *Marburgvirus* reservoir species for *R. aegyptiacus*. Overall, these findings suggest a closer follow-up of the other bats, particularly of the family Pteropidae that can play the major role. Researches on the role of bats as reservoirs of filoviruses, particularly *Ebolavirus* are still ongoing, several vertebrate animals as Great apes and duikers are naturally infected by this virus, probably directly from the reservoir, but the pathways of its emergence in human environment is not yet fully understood. However, the epidemiological scenario so far advanced, make bats the most probable reservoir candidates for filoviruses.

Domestic vertebrate animals sensitive to filoviruses

Dogs and pigs are the only domestic animals so far identified as species that can be infected with EBOV. A survey conducted in Gabon on dogs eating dead animals showed over 30% seroprevalence for EBOV during the Ebola outbreak in 2001-2002 (Allela et al., 2005). Dogs asymptotically incubate the virus; while pigs experimentally infected with EBOV can develop clinical disease, depending on the virus species. Pigs were experimentally able to transmit Zaire-Ebola virus to naive pigs and macaques; however, their role during Ebola

Table 1. *Marburgvirus* (MARV) and *Zaire ebolavirus* (ZEBOV), antibodies (IgG), and viral RNA sequences detected from bats in Africa.

Date	Bat species	Vernacular name (Order, Family)	Filovirus isolated				Filoviral event	
			Ebolavirus		Marburgvirus		Locality	Reference
			Antibodies detected	RNA sequences	Antibodies detected	RNA sequences		
January 2008	<i>Eidolon helvum</i>	Straw-colored Fruit Bat	IgG	PCR (-)	-	PCR (-)	Ghana Zambia*	Hayman et al., 2010 Ogawa et al., 2015*
May-June 2007	<i>Epomophorus gambianus</i>	Gambian Epauleted Bat (Megachiroptera, Pteropidae)	IgG	PCR (-)	-	PCR (-)	Ghana	Hayman et al., 2012
June 2003- March 2008	<i>Epomops franqueti</i>	Franquet's Epauleted Bat (Megachiroptera, Pteropidae)	IgG	PCR (+)	IgG	PCR (-)	Gabon, Ghana*	Pourrut et al. (2005, 2007; 2009) Hayman et al., 2012*
May-June 2007	<i>Hypsignathus monstrosus</i>	Hammer-headed Fruit Bat (Megachiroptera, Pteropidae)	IgG	PCR (+)	IgG*	PCR (-)	Gabon*, RC*, Ghana	Pourrut et al., 2009* Hayman et al., 2012
June 2003- March 2008	<i>Micropteropus pusillus</i>	Lesser Epauleted Bat (Megachiroptera, Pteropidae)	IgG	PCR (-)	IgG	PCR (-)	Gabon	Pourrut et al., 2009
June 2003- March 2008	<i>Myonycteris torquata</i>	Little Collared Fruit Bat (Megachiroptera, Pteropidae)	IgG	PCR (+)	-	PCR (-)	Gabon, RC	Pourrut et al., 2009
June 2003- March 2008	<i>Hyposideros gigas</i>	Giant Leaf-nosed Bat (Microchiroptera, Hyposideridae)	-	PCR (-)	IgG	PCR (-)	Gabon, RC	Pourrut et al., 2009
June 2003- March 2008	<i>Mops condylurus</i>	Greater Mastiff Bat (Microchiroptera, Molossidae)	IgG	PCR (-)	IgG	PCR (-)	Gabon	Pourrut et al., 2005; 2007; 2009
May-October 1999	<i>Miniopterus inflatus</i>	Greater Long-fingered Bat (Microchiroptera, Vespertilionidae)	-	PCR (-)	-	PCR (+)	DRC	Swanepoel et al., 2007
May-October 1999	<i>Rhinolophus eloquens</i>	Eloquent Horseshoe Bat (Microchiroptera, Rhinolophidae)	-	PCR (-)	IgG	PCR (+)	DRC, Gabon*	Swanepoel et al., 2007 Pourrut et al., 2009*
June 2003- March 2008	<i>Nanonycteris veldkampii</i>	Little flying Cow (Megachiroptera, Pteropidae)	-	PCR (-)	IgG	PCR (-)	Ghana	Hayman et al., 2012
May-October 1999	<i>Rousettus occidentalis</i>	Egyptian Fruit Bat (Megachiroptera, Pteropidae)	-	PCR (-)	IgG	PCR (+)	RDC	Swanepoel et al., 2007
May-June 2007	<i>Rousettus occidentalis</i>	Egyptian Fruit Bat	IgG	PCR (-)	IgG*	PCR (-)	Ghana, Gabon*, DRC*	Hayman et al., 2010 Pourrut et al., 2009*
June 2003- March 2008	<i>Rousettus occidentalis</i>	Egyptian Fruit Bat	IgG	PCR (+)	IgG	PCR (-)	Gabon, Republic of Congo	Pourrut et al., 2009

Table 1. Contd

2005-January 2006	<i>Rousettus occidentalis</i>	Egyptian Fruit Bat	-	PCR (+)	Virus isolation	PCR (+)	Gabon*	Towner et al. (2007 ; 2009)*
Aug 2008-Nov 2009	<i>Rousettus occidentalis</i>	Egyptian Fruit Bat	-	PCR (-)	Virus isolation	PCR (+)	Uganda	Amman et al., 2012
June-July 2007	<i>Rousettus occidentalis</i>	Egyptian Fruit Bat	-	PCR (-)	-	PCR (+)	Kenya	Kuzmin et al., 2010

When several documented filoviral events happened in different localities, the mark on the locality's name refer to the author with the same mark. Republic of Congo (RC), Democratic Republic of Congo (DRC).

outbreaks in Africa needs to be clarified (Weingartl et al., 2013). In 2009 Reston-EBOV was the first EBOV reported to infect swine with possible transmission to humans (Weingartl et al., 2013).

ECOLOGY OF BATS AS POTENTIAL RESERVOIRS OF FILOVIRUSES

Hypsignathus montrosus, *Epomops franqueti* and *Myonycteris torquata* approximately share the same vital domains, the two last species being sympatric (Pourrut, 2007). They are confined to the tropical Central Africa and extent their distribution range to the wetter part of West Africa (Figure 3). They are found natively along and on either side of the equator, between latitudes 10°N and 10°S. They have been also recorded eastwards to Uganda and southwards to Angola and Congo (Rosevear, 1965). *H. montrosus* is the less gregarious species among these; living in companies of a maximum of 20 individuals hanging close together daily up in trees or low down in shrubs. The Hammer-headed Fruit Bat has a preference for the closed forest what affiliate it to the Guinean woodlands where it finds dense patched of forest, with a variety of fruits maturing successively over seasons. Rosevear (1965) postulated that a little is known about its

mode of life. Dispatched records of *H. montrosus* occurrence have been noted, but nobody gave information about its migration range north and south the equator according to the season. Other bioecological features related to mating, breeding, feeding and roosting are not well known. Sanderson (1940) recorded a little colony of the Hammer-headed Fruit Bat resting into rocks, what seems unusual in current scientific literature, the species might have switched to a tree sheltering bat, because of scarcity of cave-dwelling structures. The Franquet's Epauletted bat, *E. franqueti*, occurs in West Africa, from Ghana to Loanda in Angola, and across the continent to the great Lakes as far south as Tanganyika. As the Hammer-headed Fruit Bat, it is a closed forest species and does not appear to be gregarious too; only few specimens have been found roosting together, hanging freely from trees or low bushes (Rosevear, 1965). Its bioecological features are not also well known. The Little Collared Fruit bat, *M. torquata*, shares the same predilection areas as the previous two other Ebola probable reservoirs, but a little is known about its habits (Rosevear, 1965). *R. occidentalis*, a potential filovirus reservoir species, is common and widely distributed in Africa (Figure 4). Its migration range can lead to a large variety of epidemiological situations. Over the ten species of the genus *Rousettus* known worldwide, *Rousettus*

occidentalis is the mostly represented in Africa, numbering several subspecies, *R. a. arabicus* of the Arabic Peninsula (Saudi Arabia, Yemen, Oman, Pakistan, Iran), *R. a. aegyptiacus* in Egypt, Turquia, Syria, *R. a. unicolor* in West Africa, *R. a. leachi* in East, *R. a. angolensis* (or *Lissonycteris angolensis*) from Guinea to Kenya and from South Angola to Zimbabwe and *R. a. princeps*, *R. a. tomasi*, *R. a. unicolor* on the islands of Guinea gulf. The genus *Rousettus* is widely distributed and colonizes a large range of areas including dry and humid ecosystems, within altitudes reaching 4000 m. It is the only megachiroptera actually found roistering into caves and treeholes, thousands of individuals can also shelter into roofs of non-occupied human habitations, bridges. Bats of the genus *Rousettus* leave their shelter at sun down and fly around 30 km for feeding. A little is known about their migratory behavior (1 individual has been caught 500 km far away from its previous shelter in South Africa few days after). Widely common in sub-Saharan Africa (Figure 5), *Eidolon helvum* live in large colonies reaching 1, 000, 000 individuals of both sex (Walker, 1999), hanging on trees, often in cities. This fruit bat is of interest in *Ebolavirus* epidemiology because of its wide range migration, reaching more than 2,500 km (Richter and Cumming, 2008). The typical predilection area of the Straw-colored Fruit Bat is the forested areas of Central Africa where it is

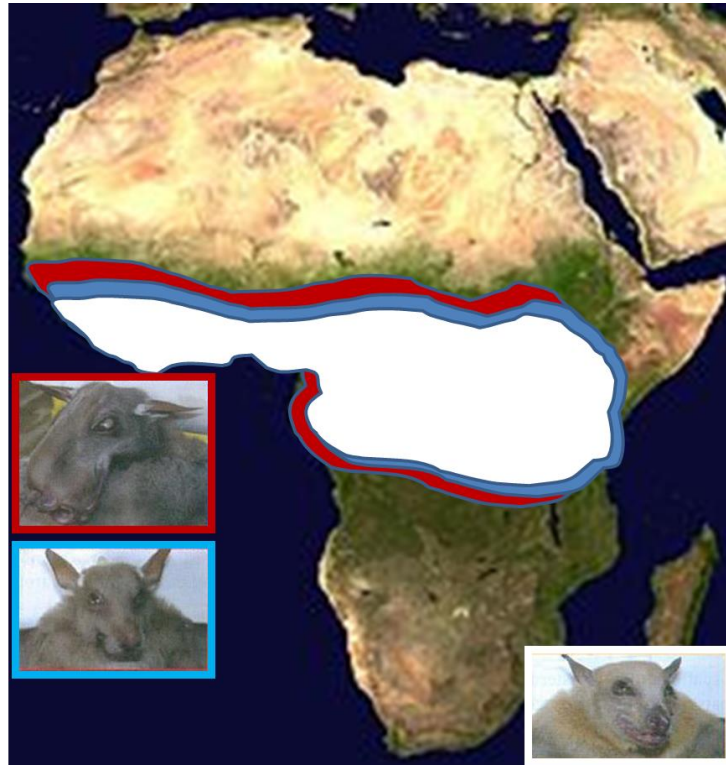


Figure 3. Distribution of *Hypsignathus monstrosus* (red), *Epomops franqueti* (white) and *Myonycteris torquata* (blue) in Africa. The vital domains of the three species are overlapping.



Figure 4. Distribution of *Rousettus aegyptiacus occidentalis* in Africa.

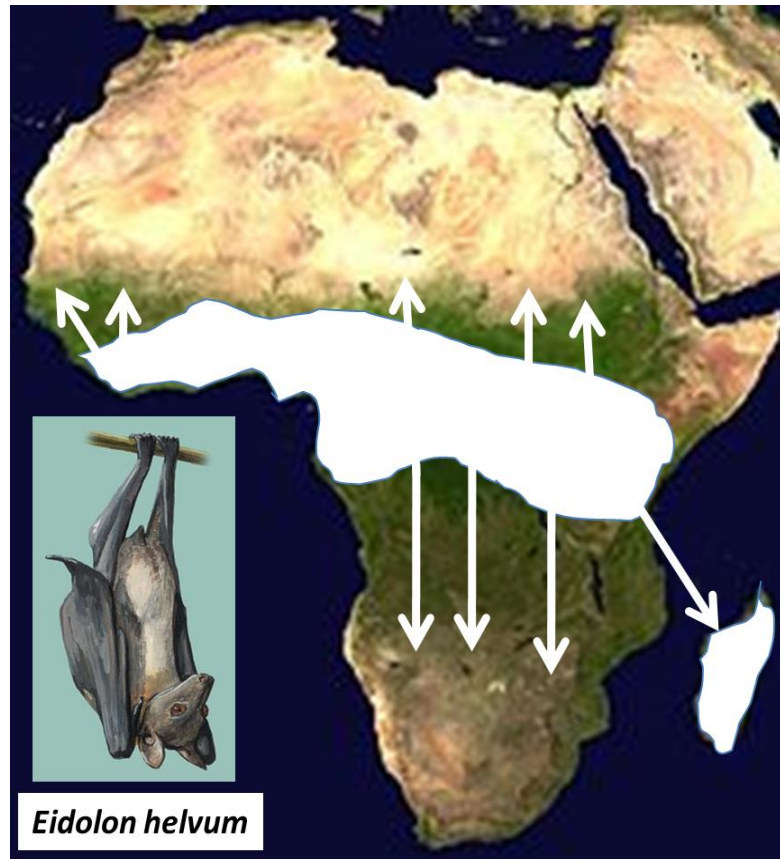


Figure 5. Distribution of *Eidolon helvum* in Africa. Arrows indicate their migration routes.

present year-round but its migration routes conduct numerous colonies of the fruit bat to North and South of Africa. Anderson (1907) reported its distribution from Somalia, Djibouti, southeastern Ethiopia and Sudan in the northeast; Senegal, Gambia and Mali in the northwest, to Malawi, South Africa and Zimbabwe in the south. The transition of filovirus species causing outbreaks in Central and West Africa during 2005-2014 seemed to be synchronized with the change of the serologically dominant virus species in the species *E. helvum* (Ogawa et al., 2015), but surveillance programs seem too limited over time and space to state that the serological status of these bats has changed. *Epomophorus gambianus*, contrarily to the other Pteropodids suspected to be reservoirs of *Ebolavirus*, is not associated with the forested areas of Central Africa. Indeed, the Gambian Epauleted bat prefers open grasslands, woodlands and savannah of Western Africa (Figure 6). It has been recorded in the forest edges, and occurs from Senegal to Southern Sudan and Ethiopia (Rosevear, 1965). The Sahel Acacia-wooded grassland and deciduous bush land form its northern limit of predilection. Its particular ecological features might involve it in a less manner in *Ebolavirus* ecology; in fact the species roosts singly or in

groups of a maximum of 50 individuals (Rosevear, 1965), and does not compete with the other known Ebola potential reservoirs. *N. veldkampii* migrates northward from the forest of Ivory Coast and into the savannah during rainy season. They can fly 500 km and roost in small groups of well-spaced individuals (Reeder, 1999). Plurispecific associations have been noted between bats of the genus *Rousettus* and other microchiroptera such as the Giant Leaf-nosed Bat, *Hipposideros gigas* (Wagner), the Benito Leaf-nosed Bat, *Hipposideros beatus* K. Anderson, 1906 and the High-crowned Bat, *Miniopterus inflatus* (Thomas, 1903) in Gabon (Pourrut, 2007). Considering that ecological feature, an eventual role of microchiroptera as reservoir or amplificatory hosts of filoviruses needs to be investigated. In fact, Saez et al. (2015) recently suspected that *M. condylurus* might be involved in the zoonotic origin of the ongoing 2013-2015 West African EVD epidemic. The Eloquent horseshoe bat, *Rhinolophus eloquens* is found in Eastern Africa (Ethiopia, Kenya, Rwanda, Somalia, South Sudan, Tanzania and Uganda). This cave dwelling microchiroptera is associated with natural habitats of the subtropical or tropical moist lowland forests, dry savanna and moist savanna. The Greater Long-fingered Bat, *Miniopterus inflatus* is a



Figure 6. Distribution of *Epomophorus gambianus* in Africa.

species inhabiting high forested areas where they roost in colonies reaching 1000 of individuals in caves, crevices and rocks sometimes in association with other insectivorous bats as *Hyposideros caffer* or fruit bats as *Lyssonycotis angolensis*. It is common in Central Africa (Cameroon, Gabon, Central African Republic, Democratic Republic of the Congo, Equatorial Guinea, Uganda) and East Africa (Ethiopia, Rwanda, Tanzania and Kenya). It has been recorded in West Africa (Guinea, Liberia) and south to Africa (Mozambique, Namibia and Zimbabwe). Epidemiological scenarios can be amplified by a response to environmental modifications, often resulting from human activities. *Ebolavirus* amplification in nature has been documented by Pourrut (2007) who found that it was correlated with reproduction time, changing from a country to another because of climatic specificities. *Hyposideros gigas*, *Mops condylurus*, *Miniopterus inflatus*, and *Rhinolophus eloquens* are the microchiroptera so far suspected as potential reservoirs of *Ebolavirus* spp. They proliferate in most of the African biota south to Sahara and in the island of Madagascar, of the Indian Ocean. Generally, microchiroptera are not migratory bats. Their seasonal movements are not well

studied but seem to be local. The four microchiroptera so far found associated with *Ebolavirus* in nature are present between the latitudes 10°N and 10°S, on both sides of the equator. Occurrence areas of *H. gigas* and *R.s. eloquens* almost overlap (Figures 7 and 8), covering the western central part of Africa; while some dispatched records are noted for *M. inflatus* which share the same ecosystems with the two precedents (Figure 9). This species has been recorded in Ethiopia, Uganda, Kenya and Tanzania in East Africa; and from Namibia, Zimbabwe and Mozambique in southern Africa. The predilection areas of *Mops condylurus* are much larger; this species is widely distributed over much of sub-Saharan Africa, ranging from Senegal, Gambia and Mali in the west, to the Sudan, Ethiopia and Somalia in the east (Figure 10). It has been also recorded southwards through much of eastern and southern Africa, and Swaziland. The species appears to be largely absent from the Congo Basin (Figure 10). As most of the microchiroptera, they eat insects that abound in greater or less profusion all year long under the tropics (Rosevear, 1965). Involved in the filoviruses' epidemiological cycle, microchiroptera will then maintain local enzootic cycles of



Figure 7. Distribution of *Hyposideros gigas* in Africa.



Figure 8. Distribution of *Rhinolopus eloquens* in Africa.



Figure 9. Distribution of *Miniopterus inflatus* in Africa.



Figure 10. Distribution of *Mops condylurus* in Africa.

infection and play an important role in the perpetuation of filoviruses within ecosystems.

The microchiropters, at the opposite of megachiropters which include the single family of Pteropidae, account for fifteen different families known worldwide among which eight have an Afrotropical biogeographical distribution: Emballonuridae, Megadermatidae, Molossidae, Myzopodidae (Malagasian Subregion), Nycteridae, Rhinolophidae/Hipposideridae, Vespertilionidae (<http://planet-mammiferes.org>). Rosecvear (1965) noticed that they breed at most times of the year, though there are indications of preferences for the dry season.

INVESTIGATION OF THE ZONOTIC ORIGIN OF FILOVIRAL HEMORRHAGIC FEVERS

The natural source of the first Ebola outbreaks occurring from 1976 to 1979 has never been elucidated despite several research tentative targeting different vertebrate animals (Breman et al., 1999; Germain, 1978; Arata and Johnson, 1977; Leirs et al., 1999). Later, the Swiss ethnologist's infection with *Ebolavirus* was related to a chimpanzee she was autopsying (Le Guenno et al., 1995). Similarly, the 1996 Mayibout outbreak in Gabon originated from children who found and butchered a chimpanzee in the forest (Georges et al., 1999). Similar sources have been reported for Marburg virus which caused the 1967 outbreak in Marburg and Belgrade linked to the handling of organs and tissues of *C. aethiops* monkeys imported from Uganda (Smith et al., 1967; Martini, 1969). Practically all the sources of *Ebolavirus* outbreaks in Democratic Republic of Congo and Gabon were related to animal carcasses of gorillas, chimpanzees and duikers, hunted and handled since the forest (Olloba, 2001; Grand-Etoumbi, 2002; Entsiami 2002; Yembelengoye, 2002; Leroy et al., 2004b) as well as for the epidemics of Etakangaye 2001, Olloba 2002, Mendemba 2001, Ekata 2001 and Mvoula 2003. The presence of bats were recorded several times in the warehouses of the cotton factory, where the first people infected during the 1976 and 1979 outbreaks in Nzara, Sudan were working. No other likely source of infection was identified in either outbreak. It is also noteworthy that the Australian who was infected by Marburg virus (and subsequently infected two other people in Johannesburg in 1975) had just returned from a trip to Zimbabwe, during which he had slept frequently in the open and once in an abandoned house, the loft of which was inhabited by numerous bats. A few days before becoming ill, the French engineer who was infected by Marburg virus in Kenya in 1980 (and who subsequently infected his doctor) had visited caves containing large bat populations (Smith et al., 1982). However, when baboons and Vervet monkeys were placed in cages inside the same caves, none became infected (Johnson, 1996 personal communication), the experience might be set up into the

caves out of the virus' amplification period in bats, or monkeys were resistant to infection and had developed an immunity following a previous contact with the virus. The fact that bats have already been implicated as source of infection in some previous filovirus outbreaks such as the Marburg hemorrhagic fever outbreak of Durba (Democratic Republic of Congo) inspired the IRD Research Unit 178 (Fundamentals and Domains of Disease Emergence) and opened the way to investigation of an eventual role of bats as reservoirs of those mysterious filoviruses. Swanepoel et al. (1996) experimentally proved that the Angola free-tailed bat, *Tadarida condylura* and the little free tailed bat, *Tadarida pumila* (Microchiroptera, Molossidae) and the Wahlberg's epauletted fruit bat, *Epomophorus wahlbergi* (Megachiroptera, Pteropidae), were able to asymptotically replicate ZEBOV with high viral titers, 4 weeks after inoculation, but the first attempts to isolate the virus from bats in nature were not successful (Germain, 1978; Arata and Johnson, 1977; Breman et al., 1999; Leirs et al., 1999). The mystery was dissipated when an IRD (UR 178) team based at the CIRMF first discovered that bats of the family Pteropidae might be involved in replication, incubation and filoviruses (Ebola and Marburg) maintenance and transmission in nature (Pourrut et al., 2005; Leroy et al., 2005; Towner et al., 2007) and enhanced future directions for the research on reservoir species. Hypothetical transmission routes that seem plausible are proposed (Gonzalez et al., 2007; Olival and Heyman, 2014); however more investigations are needed to elucidate the ways that filoviruses borrow from the reservoir to nonhuman primates and to humans. While the struggle for containing the deadly EVD outbreak in West Africa was going on, few studies searched to figure out where it came from, and what was its zoonotic carrier. It is hypothesized that the ongoing EVD epidemic originated from a little 2 years old girl who might have been infected by *Eidolon helvum* in Guekedou (Funk and Piot, 2014). There has been no handling or consummation of bush meat in the village, the toddler might have collected a partially chewed fruit dropped from a tree by the straw-colored fruit bat and subsequently became infected with virus particles in residual bat saliva (1st hypothesis). Saez et al. (2015) investigated the zoonotic origin of the West African *Ebolavirus* outbreak around Meliandou where the toddler first contracted the ZEBOV strain, but did not find any evidence of virus circulation in wildlife. Particularly, bats belonging to the incriminated species (*E. helvum*) that were captured and tested did not allow any virus isolation or ZEBOV sequences detection. Also, their enquiries conducted on wildlife did not reveal any decline of sensitive wild animals, but observed that there was a tree with large hollow in the index home, inhabiting microchiroptera among which *M. condylurus* has been identified. This insectivorous bat already tested ZEBOV-IgG positive (Pourrut et al., 2009) and might be the source

of the infection, because kids usually caught and played with bats in this tree (2nd hypothesis). Free-tailed bats have been already incriminated in such infection as for the first Sudan Ebola virus outbreaks (World Health Organization/International Study Team, 1978). Cases of Marburg virus infection via exposure to bat colonies have been already documented with the Kitum cave in Mont Elgon National Park, Kenya, and in Zimbabwe. A total of 12 bats have been suspected to be potential hosts of Ebola and Marburg viruses in the Afrotropical biogeographic region (Table 1). They include 8 megachiropters of the family Pteropidae: *H. monstrosus*, *M. torquata* and *E. franqueti*, mostly associated with the forested areas as previously discussed. *E. gambianus*, *E. helvum* and *R. occidentalis* found positive for filoviruses have tested negative in June 2006, in Senegal supposed Ebola free and used as a control site (Pourrut, 2007), *M. pusillus* and *N. weldkampii*. 4 microchiropters are identified as probable reservoirs: *M. condylurus*, *M. inflatus*, *H. gigas* [Pourrut et al. (2009) list it as IgG ZEBOV positif], and *R. eloquens*.

PLACE OF CHIROPTERS IN THE EPIDEMIOLOGY OF EMERGING ZONOTIC DISEASES

Bats harbor a potential role as reservoirs for zoonotic diseases. About 66 different viruses have been isolated from bats (Calisher et al., 2006) and serological evidence for infection of bats with many viruses has been found (Kuno, 2001; Messenger et al., 2003; Gonzalez et al., 2008). Studies of their bioecology, dynamic and natural behavior have been enhanced from the 1970s since they have been incriminated in zoonoses' emergence due to coronaviruses, filoviruses and paramyxoviruses. They considerably participate on diseases dispersal across a vast range of regions where they are involved in the increasing threat of emerging infectious diseases to human societies: the severe acute Middle East respiratory syndrome-like coronavirus (MERS-CoV) (Ithete et al., 2013; Memish et al., 2013), paramyxoviruses Nipah virus (NiV) in Malaysia and Bangladesh (Luby, 2013), Hendra (HeV) in Australia (Clayton et al., 2013), and lyssavirus disease in America, Europe and Australia (Warrell and Warrell, 2004; Van der Poel et al., 2006) plus the emerging filoviruses, Ebola and Marburg in Africa (Leroy et al., 2005; Calisher et al., 2006). It has been already established that rabies virus infections in France have been associated with the migratory routes of the Nathusius' pipistrelle, *Pipistrellus nathusii* Keyserling and Blasius, 1839 (Brosset, 1990). In Africa, the widely separated geographic locations of Ebola outbreaks have supported that the reservoir and the transmission cycle are probably closely associated with the rainforest ecosystem, assertion supported by antibodies distribution. The fact that outbreaks seldom occur suggests the presence of a rare or ecologically isolated reservoir species having few contact with human and non-human primate

species (Gonzalez et al., 2005). In the Class Mammalia of the vertebrate animals, the order Chiroptera represents the second in terms of species diversity, behind the order of Rodentia, but is the most important because of its potential for harboring zoonotic pathogens. It includes the suborders of Microchiroptera and Megachiroptera; the last accounting for the unique family of Pteropidae which include the Old World fruit bats or flying foxes found in tropical and subtropical Africa and east to the Western Pacific. Most of the actually suspected filoviruses' reservoirs belong to that family. The Microchiroptera are found throughout most of the world and include small insectivorous bats, few bat species fruit and flower feeders, few carnivorous bats, and lastly vampire bats which have a Neotropical geographic distribution, found in tropical areas of the American continent, principally in Mexico, Chile, Argentina and Brazil. Rodents are terrestrial and commensally mammals, closely associated with human environment and carry significant diseases with a real public health concern (Mills, 2006). As examples, Hantavirus pulmonary syndrome and hemorrhagic fever with renal syndrome are due to hantaviruses pathogens hosted by rodents of the family Muridae (Schmaljohn and Hjelle, 1997). Lymphocytic choriomeningitis, Lassa fever, Argentina, Bolivian, Venezuelan and Brazilian hemorrhagic fevers are caused by rodent's arenaviruses. These small mammals are also incriminated in Congo Crimean Hemorrhagic Fever and Rift Valley Fever epidemiology (Camicas et al., 1990; Pretorius et al., 1997). They become less studied than bats which do not directly interact with human environment, because they are phytophilous (associated with forest vegetation) or lithophilous (associated with caves, rocks and similar sheltering structures) (Rosevear, 1965). Compared with rodents, bats are unique in their propensity to host zoonotic viruses, they are natural reservoirs of a number of high-impact viral zoonoses. In their quantitative analysis, Luis et al. (2013) demonstrated that bats indeed host more zoonotic viruses per species than rodents, because their sympatry with other species of the same taxonomic order promote interspecific transmission and zoonotic viral richness.

THE PROBABLE ROLE OF ANIMALS INVOLVED IN FILOVIRAL HEMORRHAGIC FEVERS

In the light of reservoir species theory of Rodhain (1998), the following criteria can be considered: 1-Efficient vertebrate reservoirs (or good reservoirs) of filoviruses need to be receptive to these viruses, not just slightly sensitive. They must be able to asymptotically replicate the virus, develop an efficient and sufficient viremia, and once infected, the animal must survive; ensuring maintenance and circulation of the virus in nature, and therefore the foci's continuity. 2- the reservoir species must be of an abundant and prolific population, able to replicate and disseminate the pathogen. Neonate

or naive individuals are non-immune, which allow their receptivity to the virus and infection, ensuring continuation. 3- The viremia must be of a high viral titer, last longer enough, the time to allow it to infect other receptive hosts of the same population for virus perpetuation.

In its natural foci, a filovirus circulates between several vertebrate hosts, playing different roles in its epidemiology. For Ebola and Marburg viruses, bats are the potential candidates for the reservoir status: 1) Filovirus RNA characterization associated with virus specific antibodies and virus isolation within some bats species provided clues that chiropters might be incriminated; 2) It is also likely that the reservoir species are ecologically isolated, associated with the rainforest ecosystem with an important potential of migration which might justify the scattered geographic occurrences of Ebola outbreaks. Bats satisfy this statement. Other vertebrates are just activating the foci for a while, acting as amplifying hosts: in this category, belong some monkeys of the family *Cercopithecidae* such as vervet, *Chlorocebus aethiops*, found infected with a filovirus in Marburg (Smith et al., 1967) and the red colobus, *Procolobus badius*, hunted and eaten by chimpanzees, who subsequently became infected by *Ebolavirus* (Boesch, 1994). The virus can also reach some other non-susceptible animals unable to replicate it or who just present a temporary short viremia with a low viral titer: the dead-end hosts. Birds that tested refractory to *Ebolavirus* (Swanepoel et al., 1996) must be listed in this category. Widely divergent orders or families of the avian fauna were unable to experimentally replicate *Ebolavirus*. Then, efforts on field reservoir search should focus more on other animals able to replicate the virus than birds. Migratory vertebrates will disseminate the virus: bats again fit in this case, spreading pathogens through migration; and other sensitive hosts will serve as sentinel hosts or biological markers, allowing the epidemiologists to detect the virus' activity. That's the case for great apes (chimpanzees and gorillas) which have a wide range of vital domain but do not move as far as migratory bats. Once in contact with the virus, they die, promoting about a probable emergence. Animals involved in a filovirus' activity are not necessarily all reservoirs. Incidental hosts are just accidentally involved in the cycle, like mosquitoes that might be infected after a viremic blood meal taken on a wild animal. In addition, filoviruses generally do not replicate in arthropods or arthropod cell lines (Peterson et al., 2004). Due to their dispersal, several index cases should be reported if anthrophilic mosquitoes were able to disseminate filoviruses. A filovirus can adopt several different reservoirs, in different environmental conditions; an animal species might be a good reservoir in a certain environment and a bad one in another. In the case of bats for example, food is found in some restricted areas, depending to the phenology of wild fruit trees, which varies from season to season (even month to month). If the availability of food is good, they stay around for

several nights or even weeks, and chronically infected bats would increase the length of time during which they can infect other receptive species and are qualified as good reservoirs in such environmental conditions. If their survival conditions are not met, they must necessarily travel further afield and will not stay longer enough to perpetuate their carried pathogen in this specific ecosystem and are circumstantially qualified as bad reservoirs. Mostly wild vertebrates (birds and mammals) act as usual reservoirs for most of the pathogens. Domestic or commensal mammals, as well as human beings, are rarely involved as reservoirs. In the case of many arboviruses, arthropods are involved in their maintenance because of their longevity and their vectorial competence allowing them to replicate and transmit the virus through vertical transmission to the offspring. The bats might do the same for filoviruses, but will transmit the virus to the offspring through placental exchanges. In fact, Leroy et al. (2006) postulated that great apes might be contaminated while touching bat placental tissues and biological fluids, during parturition. Bat's ability for long distance flying provides an intensive selective force for coexistence with viral parasites through a daily cycle that elevates metabolism and body temperature analogous to the febrile response in other mammals (O'Shea et al., 2014). These factors imply a large diversity of epidemiological situations according to the virus, the bat reservoir species and the region. Understanding epidemiological situations need a comprehension of the evolution of these linked systems in correlation with the modification of ecosystems, often resulting from human induced activities on the environment. Repeated passages of filoviruses from a vertebrate host to another will, sooner or later, develop modifications of their viral genome in response to new environmental adaptation, by emergence of reassortants during coinfections. In such conditions two situations are predictable: 1- the virus might lose some virulence and this can lead to extinction of its foci, 2- after genome modification, the foci are activated after a short silent interval, increasing the ability of the virus to last longer. This last scenario happened in Sierra Leone and contributed to maintaining the virus' adaptation. In Fact, Gire et al. (2014) tracked *Ebolavirus*' evolution during this West African epidemic and found that it was changing as it spread. Their genetic analysis revealed that the outbreak in Sierra Leone was sparked by at least two distinct viruses, introduced from Guinea at about the same time. One of this disappearing from patients sampled later in the outbreak, while a third lineage appeared. Then, for several different reasons, it appears puzzling, to predict the ending of the outbreak because of those mutations, and to set efficient preventive measures axed at level of natural reservoirs.

FACTS, THEORY AND HYPOTHESIS

Zoonoses are diseases that originate from wildlife and

strike living animals, threatening animal biodiversity and public health (Daszak et al., 2000; Leroy et al., 2004a; Woolhouse et al., 2005; Lahm et al., 2007; Jones et al., 2008). Filoviral hemorrhagic fever asymptotically develops in the wild vertebrate host and cause fatal manifestations when it reaches human beings (anthropozoonose). Filoviruses are circulating in a sylvan cycle among reservoir species and other sensitive hosts. EVD is an anthropozoonose, benign within the reservoir species, fatal within sensitive human population where it is associated with a mortality rate ranging from 50 (SIEBOV) (Smith, 1978; Baron et al., 1983) to 80% (ZEBOV) (Bwaka et al., 1999; Nkoghé et al., 2004), depending on the virus species (Johnson, 1978). The duikers and great apes (gorilla and chimpanzee) are also sensitive to *Ebolavirus* infection and represent intermediate hosts that can bridge the virus to human population. Humans entering the forest can be infected while hunting bats or other apes, antelopes and sensitive hosts. It is in that occasion that the virus reaches rural population, spreading from human to human, causing outbreaks and even epidemics affecting several villages and towns. These outbreaks can provide a source for potentially devastating urban epidemics, which are the most dangerous, because of concentration of susceptible people; typically higher mortality rates associated with urban situation are recorded after prolonged human-to-human transmission. However, the role of bats with their spectrum of behavioral variation, in the forested areas of central Africa where the virus originated from is unclear.

ECONOMIC AND SOCIAL IMPACT OF FILOVIRUSES EMERGENCE

The public health and economic burden imposed by FHF on the developing world with limited medical coverage are enormous. The West African EVD outbreak caused global societal and economic impact due to the unexpected magnitude of the epidemic killing thousands of people; the socioeconomic impacts in Guinea, Sierra Leone and Liberia include job losses, smaller harvests and food insecurity. Travel, global business and other life activities were affected, taking a significant human toll as well as cause public fear, economic loss and other adverse outcomes. While the primary cost of this tragic outbreak is in human lives and suffering, the crisis will secondly worsen already entrenched poverty. The Bank Group estimates that Guinea, Sierra Leone and Liberia will lose at least US\$1.6 billion in economic growth in 2015 (<http://www.worldbank.org>). As of April 2015, the World Bank Group's response to the Ebola crisis has mobilized US\$1.62 billion to support the affected countries containing and preventing the spread of infections, providing treatment and care, and improving public health systems. They also mobilized funds for providing 10,500 tons of maize and rice to seed more than 200,000 farmers in Guinea, Liberia and Sierra Leone, averting hunger in Ebola-affected countries and reviving

agriculture. In terms of morbidity and mortality, EVD accounts largely among the global disease burden of humankind. As of April 19, 2015, 23816 cases of EHF (14893 laboratory confirmed were reported, accounting for 10736 deaths in Guinea, Sierra Leone, Liberia and to a less degree, in Nigeria and Mali (<http://www.cdc.gov/vhf/ebola/outbreaks/2014-west-africa/index.html>) (Figure 11). The bulk of FHF mortality occurs in sub-Saharan Africa where it is seeded by the lethal emergence of the most deadly *Ebolavirus* species, *Z. ebolavirus* (ZEBOV) and the existence of a wide range of potential bat reservoirs. Despite the rarity and ecologically isolation of the reservoir species, the force of FHF transmission in some areas of sub-Saharan Africa is extremely high (25,907 cases suspected, probable and confirmed), intensively driven by interhuman transmission. FHF are socially devastating diseases of the developing world and the risk of epidemics remains. Since the last emergence of ZEBOV in Gueckedou and Macenta, Southeastern Guinea (Baize et al., 2014), on December 2013, the disease continues to sicken and kill thousands of people in the affected countries of sub-Saharan Africa. It is difficult to control because of repetitive health care workers, medical doctors and laboratory diagnosis personnel direct contamination. Nosocomial infections occurred in the hospital, during the Yambuko epidemic (1976), a Belgian nuns inadvertently started the epidemic by giving vitamin injections to pregnant women, through reuse of unsterilized syringes, needles or other medical equipment contaminated with body fluids (Piot, personal communication). Inadequate dispositions for contact with Ebola infected patients throughout herbalist care, burial preparation, including body washing and long intimate funeral ritual greatly increased the risk of the virus spillover, by fluid transmission. By September 14, 2014, a total of 318 cases, including 151 deaths, had been reported among health care workers (WHO Ebola Response Team, 2014).

It is the first West Africa Ebola outbreak and the largest ever recorded in history; morbidity and mortality recorded are higher than in all previously Ebola outbreaks combined in Africa. This EVD epidemic is very similar to the 1976 outbreak. Both were caused by *Z. ebolavirus*, hitting rural forest communities first, before spreading into urban areas, without any link to bush meat handling. Hemorrhagic cases were suspected due to malaria, typhoid, Lassa fever, yellow fever or influenza. From the past, epidemics have occurred in the Democratic Republic of Congo, Sudan, Gabon, Republic of Congo and Uganda (Smith, 1978; Le Guenno et al., 1995, 1999). Filoviruses and mammals co-evolved since the Paleocene. The existence of orthologous filoviruslike elements shared among mammalian genera whose divergence dates have been estimated suggesting that filoviruses are at least tens of millions of years old (Taylor et al., 2010). Phylogenetic and sequencing evidence from gene boundaries was consistent with integration of filoviruses in mammalian genomes.

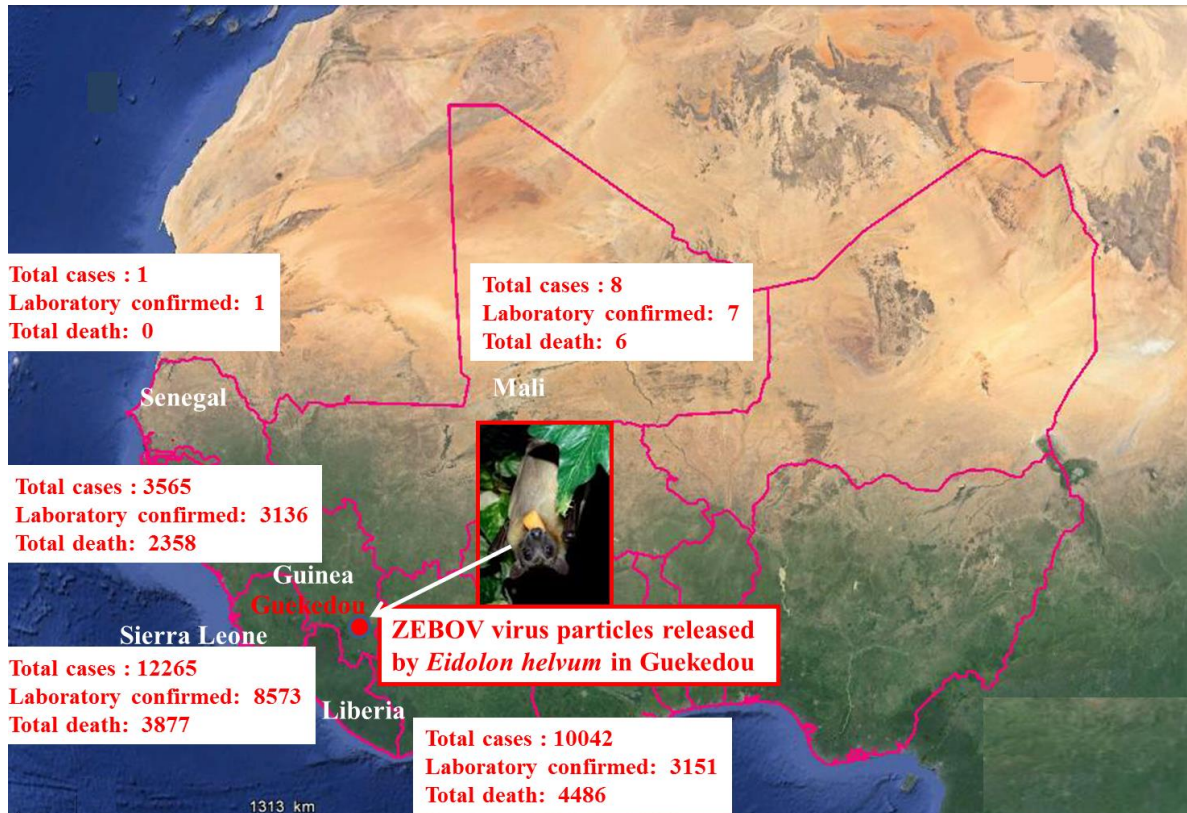


Figure 11. Incidence of ZEBOV activity in West Africa as at April 19, 2015.

FUTURE STUDIES

Despite the importance of the studies achieved on the epidemiology of filoviruses, a number of deficiencies have been pointed out and need to be addressed. A fundamental aim needs to assess the ecology of reservoirs in the rural/sylvan interface, where EVD transmission spills over into human populations. Filoviruses might silently breed in some West African forested ecosystems, introduced since the emerging areas of central Africa by some potential reservoirs as *E. helvum*. They can extend their amplification areas and reach other sensitive secondary hosts. Peterson et al. (2004) suggested that a large-scale ecologic and geographic comparison is an unexplored approach to identifying the natural reservoir of filoviruses in order to detect patterns of co-occurrence and co-distribution of viruses with potential hosts.

Studies extended to other Pteropidae sub families to see if any other potential reservoirs exist

Understanding the ecological features of the major suspected reservoirs of *Ebolavirus*, that is, *H. monstrosus*, *E. franqueti* and *M. torquata* is a major goal. Their principal known domains of occurrence is concern

with the central forested areas of Africa, but some studies recorded *H. monstrosus* in Southern Senegal (Feiler, 1986; Koopman, 1975; Koopman et al., 1978), as well as *E. franqueti* and *M. torquata* (Pourrut, 2007). The roosting behavior of *R. aegyptiacus* needs to be investigated. Plurispecific associations have been observed among Pteropidae (Kunz, 1982; Kuzmin et al., 2010). Many bat species are gregarious, living in dense colonies: for example, *Eidolon helvum* aggregations can reach a population of 50,000 to 100,000 individuals per roost (Jones, personal communication; Rosevear, 1965). Roosting sites can also account for assemblages of multiple species where high intra and interspecific contact rates of bats from different origins and unknown pathologic and immune status directly promote rapid transmission of pathogens and their spread. The Egyptian Fruit Bat roosts daily in trees or caves, often with large groups of other bats. High-densities bat colonies have been observed, sometimes numbering in the thousands. They emerge from the roost to forage for food in the late evening, and return just before dawn. They hang upside down, with their wings folded closely around their bodies (http://en.wikipedia.org/wiki/Rousettus_aegyptiacus). We hypothesize that following those pluri-specific associations, competition for territory conquest or simply daily association into shelters might lead to infection of potential reservoirs

such as *R. aegyptiacus* which is known widespread in all the Afrotropical biogeographic region excepted the Saharan domain (Figure 4). A scenario such as this one might extend the known occurrence area of *Ebolavirus* since its natural foci of central forested African areas, *R. aegyptiacus* acting as the bridge vector. 1) Occurrence areas of the three known potential reservoirs (*H. monstrosus*, *E. franqueti* and *M. torquata*) need to be updated and mapped as well as for the other potential filoviruses reservoirs. In fact, several vector-borne, parasitic or zoonotic diseases have (re)-emerged and spread within Africa these recent years, because of global and local changes caused by either climate change, human-induced landscape changes like constant reduction in size of natural forests tending to make the original epidemiologic sylvatic cycle somewhat a relic one, switching to a rural cycle. This implies encroachment of people and livestock into wildlife habitats and in another direction increases wildlife migration from degraded areas into rural and peri-urban regions. Impacted landscape variation induced by environmental factors and human behaviors (hunting, irrigation; deforestation; cattle breeding...), added to climatic changes, directly impact human health. 2) Their dynamic over time (reproduction period) and space (migration) need to be completely understood for modeling the risk of *Ebolavirus* emergence. It has been already proven that most reservoirs are efficient *filovirus* vectors during sexual activity (reproduction time). In fact, Amman et al. (2012) observed that birthing seasons represent times of increased infection among juveniles and that most human MVD cases coincided with those periods. 3) Serologic studies undertaken along a West-East transect study across West Africa will assess to what extent the *Ebolavirus* amplification has been observed. Other Pteropidae close to the known reservoirs such as *Rousettus angolensis smithi*, *Eidolon* spp., *Micropteropus* spp., *Nanonycteris*, etc., existing in Africa, need to be studied in order to discover other eventual filoviruses and bat reservoirs.

Migration routes and distribution areas of the potential bat species reservoirs

To fully understand their migration circuits and areas of predilection, the above cited transect study needs to be entirely prospected. The actually known EBOV serotypes might have circulating in a primeval cycle, among certain bat species (*Hypsignathus*, *Myonycteris*, *Epomops*...) without any symptomatic infection in the forest of Central Africa in a silent cycle. Man entering the forest gallery for the purpose of hunting might be occasionally involved in this cycle. Such a zoonotic reservoir of infection could exist in all forested areas (primary forest galleries, isolated patches of forest, forest-savanna mosaics) of West Africa. Ecosystems modification and environmental conditions linked to global change can influence spatial

and temporal distribution and dynamics of human pathogenic agents. A high viral amplification of *Ebolavirus* in the forest ecosystem probably favoured its escape from its naturally sylvatic cot increasing the probability for the virus to reach directly human population or via other sensitive hosts. As shown by the phylogenetic study from Baize et al. (2014), the bottom clade contains *Ebolavirus* (ZEBOV) described from Gabon, suggesting that the other top clades derived from it. In fact, the derived clades show that ZEBOV emerged in DRC in 1976, simultaneously as SEBOV in Sudan, in 1976 before the Ivory Coast emergence of CIEBOV. Their ancestor, the Gabon strain (ZEBOV) emerged later in 1994, probably confined in a jungle cycle, before its emergence. All available data about the implication of bats in the epidemiology of EVD are limited to Central Africa, because the disease first emerged in this area. Little information is obtained from West Africa. Senegal is the extreme limit of the geographical range of the known Ebola reservoir species, that is, *H. monstrosus*, *E. franqueti*, and *M. torquata*. Ninety eight (98) bats belonging to the genus *Eidolon* *helvum*, *Epomopus gambianus* and *Rousettus aegyptiacus occidentalis* were captured near Mbour (14°25' N, 16°57' W; MBour Dpt. [Thiès Reg.]), 80 km far away from Dakar in June 2006, and tested negative for EBOV (Pourrut et al., 2007). However, a serologic study of human and simian populations undertaken by Gonzalez et al. (2005) detected IgG from human population in Africa. The demonstration of neutralizing antibody to EBOV in the human sera suggested that there might be a sylvatic cycle of EBOV in West Africa. Marburg and Ebola viruses are endemic in Central African countries where outbreaks are unpredictable and just sporadically emerge.

Bioecology of the microchiropters, potential species reservoirs

Four species belonging to three different microchiropters' families (*Molossidae*, *Vespertilionidae*, and *Rhinolophidae*) are suspected for now in filoviruses' epidemiology. Some detailed studies need to be undertaken in order to clarify the following points: 1) are members of different families breeding at the same time of the year? 2) Do they successively breed over time? Responding to those questions will assess if seasonal amplification of a filovirus is short over time because of reproduction at the same period, with a sexual pause during which neonate bat species do not exist, corresponding to the inter-epizootic period. In the other case, the amplification period can last long and promoted by the opportunity of continuous contact of naive offspring with infectious bats in the colonies during a certain time of the year. This will conduct logically to a seasonal pulse of filoviruses in the ecosystem characterized by amplification periods separated by silent intervals. This scheme of amplification/silencing makes

sense if microchiropters were only proliferating in the ecosystem. Plurispecific associations include microchiropters and megachiropters, the last accounting individuals with large migration range (*Hyposideros* species and *R. aegypticus occidentalis* have been recorded together in the Kitaka cave, Uganda). Do both incubate filoviruses at the same time in nature? Are there reproduction/amplification periods synchronic? One might be a relay while another is entering a silent period. A comprehensive approach will investigate the natural reservoir of filoviruses which is large-scale ecologic and geographic comparisons in order to elucidate the patterns of (co) occurrence of viruses within potential hosts. Dynamic of the bat reservoir species of these filoviruses as well as interactions between sensitive hosts and bats in the rural/sylvan interface are not fully understood. Breman et al. (1999) conducted several researches aiming to identify the wild animal species hosting the virus in nature but failed to find the reservoirs. Extensive field and laboratory studies of the wide range of filoviruses activity in Central and West Africa need to be undertaken. The main emphasis will be the bioecology of the chiropteran with regard to the specific filovirus they carry. Sensitive serological assays need to be processed on a wide range of bats captured from diverse ecological forested areas as well as from other sensitive apes and Cercopithecidae in order to figure out the extent of the filoviruses amplification and dissemination. The 2013-2015 outbreak of EVD shows a higher fatality rate attributed to the strain ZEBOV, Quantitative Trait Loci maps of genetic factors that condition virulence of the Ebola strains isolated during these concomitant epidemics might be elucidated from a locality to another, and the already known Ebola virus strains so far isolated and incriminated during previous epidemics. Understanding the immune responses to filoviruses that ensure apathogenic, persistent infections in the reservoirs, without any sign of disease is a major goal.

Conflict of interests

The author(s) did not declare any conflict of interest.

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