Review

The natural history of Ebola virus in Africa

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Abstract

Several countries spanning the equatorial forest regions of Africa have had outbreaks of Ebola hemorrhagic fever over the last three decades. This article is an overview of the many published investigations of how Ebola virus circulates in its natural environment, focusing on the viral reservoir, susceptible animal species, environmental conditions favoring inter-species transmission, and how the infection is transmitted to humans. Major breakthroughs have been made in recent years but many outstanding questions must be dealt with if we are to prevent human outbreaks by interfering with the viral life cycle.
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1. Introduction

Ebola hemorrhagic fever is caused by a negative-strand RNA virus. The viral genome encodes seven structural proteins and one non-structural protein (soluble glycoprotein) [1]. Morphologically, the virus consists of a linear genome entirely enclosed in an envelope, which is coated by the membrane glycoprotein, organized in homotrimers [2]. Genetic and antigenic characterization of Ebola virus (EBOV) isolates during human outbreaks has led to the identification of four subtypes [3]—*Ebola Sudan*, *E. Zaire*, *E. Ivory Coast* and *E. Reston*. In contrast to *Ebola Reston*, which originates in Asia and has never been reported to cause human disease [4,5], the other three subtypes circulate on the African subcontinent and are pathogenic for humans, causing a specific febrile hemorrhagic disease. After an incubation period of about a week, victims rapidly develop high fever, diarrhea,

vomiting, respiratory disorders and hemorrhaging. Death ensues within a few days. The case fatality rates are about 80% with *E. Zaire* [6,7] and 50% with *E. Sudan* [8,9].

Ebola virus hemorrhagic fever is a zoonotic disease transmitted accidentally by direct contact with infected live or dead animals. Since the first recorded human outbreak in 1976, many teams have attempted to unravel the natural history of EBOV in Africa. In this setting the natural history can be defined as the sum of the different ways in which the virus circulates in its natural environment, from its natural host(s) to susceptible intermediate animal species (i.e. those which develop fatal infection), and then to humans, either directly via the natural host or through an intermediate species.

This article reviews many previous studies designed to elucidate the natural history of EBOV. We describe current knowledge and outstanding questions in the following order: first, results from studies of human outbreaks, which help to throw light on the ecological context of EBOV emergence and to identify sources of infection; second, the way in which the virus infects humans; third, naturally infected intermediate animal species (how they are infected by the reservoir and how the infection spreads within their populations); and finally, attempts to identify the EBOV reservoir.

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2. Human outbreaks

2.1. 1976–1979: The first recorded Ebola outbreaks

EBOV first emerged in the form of two nearly simultaneous outbreaks, one due to *E. Sudan* and the other to *E. Zaire*. The first outbreak was due to *Ebola Sudan* (Fig. 1). As its name implies, it occurred in Sudan, near the border with the Democratic Republic of the Congo (DRC) (formerly Zaire), mainly affecting the towns of Nzara and Maridi [8], between June and November 1976. The mortality rate was 53% (150 of 284 victims), which is characteristic of the *Sudan* subtype.

The second outbreak was due to *E. Zaire* (Fig. 1). It occurred in DRC, near the borders with Sudan and the Central African Republic (CAR), between August and November 1976 [10]. The epicenter was in Yambuku, about 800 km from Nzara. This previously unknown disease was named for the river Ebola, which flows past Yambuku. The mortality rate was 89% (284 deaths among 318 declared victims).

EBOV made its third appearance in June 1977, infecting a 9-year-old girl living in Tandala, a DRC town close to the border with CAR [11], 325 km from Yambuku (Fig. 1). She

died with typical clinical manifestations of hemorrhagic fever due to *E. Zaire*. No secondary cases were identified.

The third outbreak (the second due to *E. Sudan*) occurred between July and October 1979 in the same region as the first, i.e. in Nzara, Sudan [9]. As in 1976, the first persons to be infected worked in the Nzara cotton factory. This outbreak was less extensive than the first, affecting 34 people and killing 22 (mortality 65%).

2.2. 1994–1997: Ebola resurgence

After a 15-year period in which no further cases were recorded, Ebola re-emerged in 1994 for a 3-year period. This new phase was marked by the identification of a new subtype, *E. Ivory Coast*, and by an escalation of outbreaks due to *E. Zaire*.

The first human case to be diagnosed in Africa during this period occurred in June 1994, when a female Swiss ethnologist became ill a few days after autopsying a chimpanzee found dead in Tai National Park (Fig. 1) located in Ivory Coast, not far from the border with Liberia [12,13,14]. EBOV infection was confirmed in both the woman and the chimpanzee.
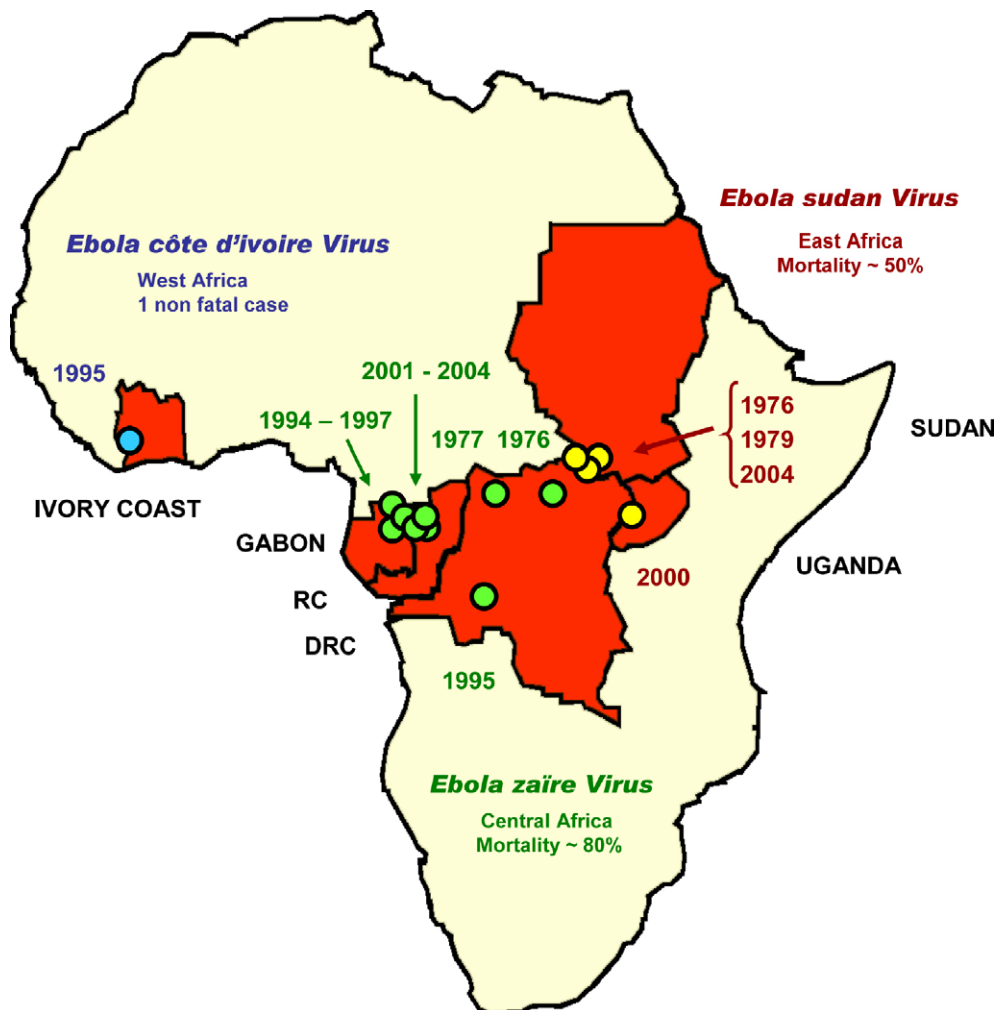

Fig. 1. Human EBOV outbreaks in Africa.
RC: Republic of Congo; DRC: Democratic Republic of Congo.

Although this case occurred in a biotope similar to that of the first outbreaks, and at the same distance from the equator (about 5° north), it was the first and only human case observed in West Africa, and the only case clearly attributed to *E. Ivory Coast*.

The first outbreak during this period (the fourth in total) occurred in the town of Kikwit, situated about 500 km southeast of the DRC capital, Kinshasa [15] (Fig. 1). Between January and July 1995, the virus killed 256 of its 315 victims, a mortality rate (81%) characteristic of the Zaire subtype. Despite the use of more sophisticated scientific and medical resources than those available in 1976, this outbreak was as large as the 1976 outbreak, probably because it affected a town of several hundred thousand inhabitants where person-to-person transmission was occurring primarily in two of the hospitals in the town.

Finally, three further outbreaks, all due to *E. Zaire*, occurred in northeast Gabon [16] (Fig. 1), the first in Mekouka between 1994 and 1995, the second in Mayibout in early 1996, and the third in Booué between 1996 and 1997.

The first of these three outbreaks occurred in northeastern Gabon, close to the border with Cameroon, between December 1994 and February 1995 [17]. It took place in two successive waves: the first affected three gold-miners' camps located in the heart of the forest, in December 1994, with 32 recorded clinical cases. Some of the victims left the camps for the nearest hospital, located in Makokou, where they infected other patients and caused a second wave. Sixteen cases were retrospectively identified in neighboring villages. In total, 49 clinical cases and 29 deaths were recorded (mortality rate 59%). Epidemiological data on this outbreak are somewhat imprecise, and not all clinical cases were recorded: EBOV infection was diagnosed retrospectively, and only in patients whose stored clinical samples were available.

In February 1996 the second outbreak hit the villages of Mayibout I and II, located about forty kilometers south of Mekouka along the river Ivindo [16]. The first victims were 18 children in Mayibout II village who had helped to carry and butcher a chimpanzee carcass found in the forest. These 18 children infected their families and friends, who in turn transmitted the disease to neighboring villages—Mayibout *I* to the south and Mvadi to the north. In total, this outbreak affected 31 people and caused 21 deaths (mortality rate 67.7%).

The third outbreak, the Booué outbreak, occurred between October 1996 and March 1997. Officially, it began on 5 October 1996, when the first patients tested positive by antigen capture [16]. However, epidemiological investigations indicated that it in fact began 2 months earlier; the index case was a hunter who died with hemorrhagic fever. Several carcasses of chimpanzees were found in the surrounding forest at the same time, and the CDC in Atlanta detected EBOV antigens in tissue samples taken from one of them. At the end of the same month, a second hunter died with similar symptoms, while one of his friends fell ill 12 days later and was taken to Booué hospital. This patient left the hospital for a village close to Booué, where he received traditional treatment, including scarification, from a *nganga*. The traditional healer, his assistant and some of his subsequent patients also became infected and in turn transmitted the disease to inhabitants of several towns and villages in Gabon. Fifteen cases and 11 deaths were recorded in Libreville, together with three cases in Lastourville, 130 km southeast of Booué. This outbreak also caused a case in South Africa: a Gabonese physician working in Libreville, unaware that he was infected by EBOV, traveled to Johannesburg for treatment, where he infected a nurse, who died a few days later. This outbreak, with 60 cases and 45 deaths occurring over a 6-month period, was noteworthy for its wide geographic range (which also probably explains its size).

2.3. 2000–2004: An emerging geographic pattern of E. Zaire and resurgence of E. Sudan

This period saw multiple outbreaks of *E. Zaire* in a relatively limited area (the border region of Gabon and the Republic of Congo), and by the resurgence of *E. Sudan* in Sudan and Uganda (Fig. 1). The other outstanding feature of this period is the occurrence of Ebola outbreaks in large animal species (mainly gorillas and chimpanzees).

2.3.1. E. Zaire outbreaks

The first outbreak during this period was the Mekambo outbreak, which occurred between October 2001 and May 2002 [18] (Figs. 2 and 3). The Mekambo outbreak was in fact a series of independent outbreaks [19]. The first occurred in the village of Mendemba on 25 October 2001 (Fig. 3). This was the most severe, and generated several secondary foci (in Mekambo, and also the RC villages of Ntolo, Ilahounene, Ekata and Olloba). The secondary focus in Mekambo itself generated a tertiary focus in Makokou, following the evacuation of a patient from Mekambo to the Makokou hospital. This outbreak appears to have started when hunters manipulated an antelope carcass they had found. Two other outbreaks occurred more or less simultaneously (on 28 November 2001 and 1 December 2001) in the villages of Ekata and Olloba on the other side of the border, in the Congo. These outbreaks apparently started when local hunters found and manipulated the carcasses of an antelope (Ekata) and a gorilla (Olloba). Two other outbreaks occurred 3 weeks later, some distance apart: the first on 22 December 2001 in Ekata village located on the road leading south from Mekambo towards RC, and the second on 29 December 2001 in Etakangaye village located on the northern axis. The source of the first outbreak has not been identified, while the second resulted from the handling of a chimpanzee carcass. The last clearly identified outbreak occurred on 27 March 2002 when hunters from Grand-Etoumbi village (on the northern axis) butchered and ate a gorilla carcass they had found in the forest. The outbreaks that occurred on the Mekambo-Olloba axis then spread to the other side of the border, as far as the RC town of Mbomo, located about 80 km from Olloba.
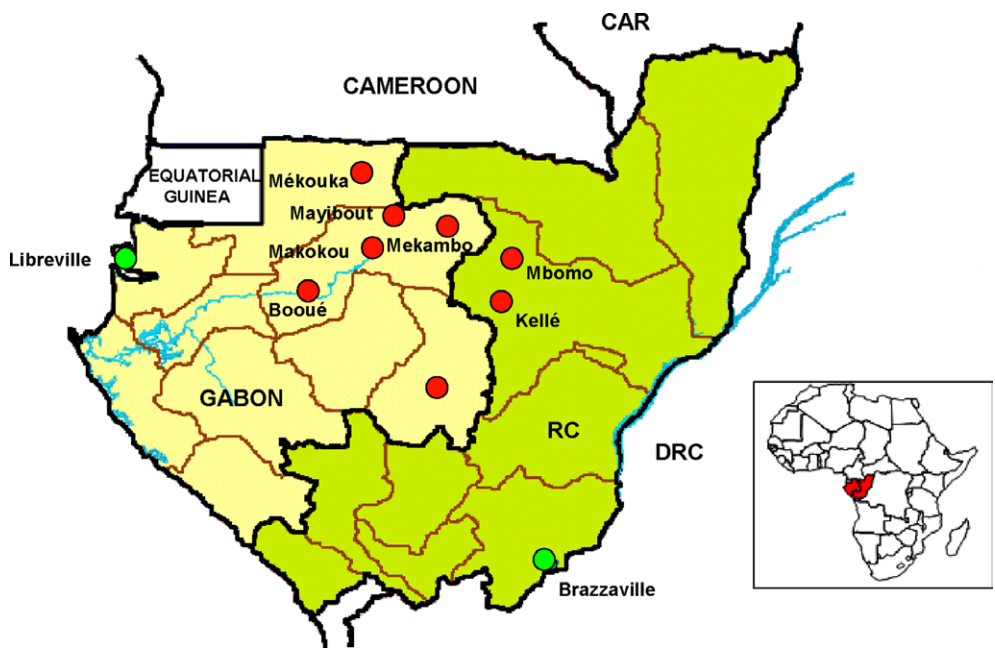
Fig. 2. EBOV outbreaks in the border region of Gabon and the Republic of Congo.
RC: Republic of Congo; DRC: Democratic Republic of Congo; CAR: Central African Republic.

Simultaneously, a second outbreak occurred in mid-January in the RC villages of Entsiami, Abolo and Ambomi, located close to the Gabonese border, and nearly 200 km south of Olloba (Fig. 3). The disease then spread to the town of Kelle about 65 km away (Fig. 2). The origin of this outbreak is unknown. Sequencing of viral isolates confirmed the existence of several concurrent outbreaks.

The third outbreak, confirmed by laboratory tests, again affected the region of Mbomo in RC, between December 2002 and May 2003 [20] (Fig. 3). This outbreak had two independent sources, one in Yembelengoye (near Entsiami) and one in Mvoula, a gold-mining camp located further east, following the handling of animal carcasses. The Mvoula focus mainly affected Mbomo, while the Yembelengoye focus
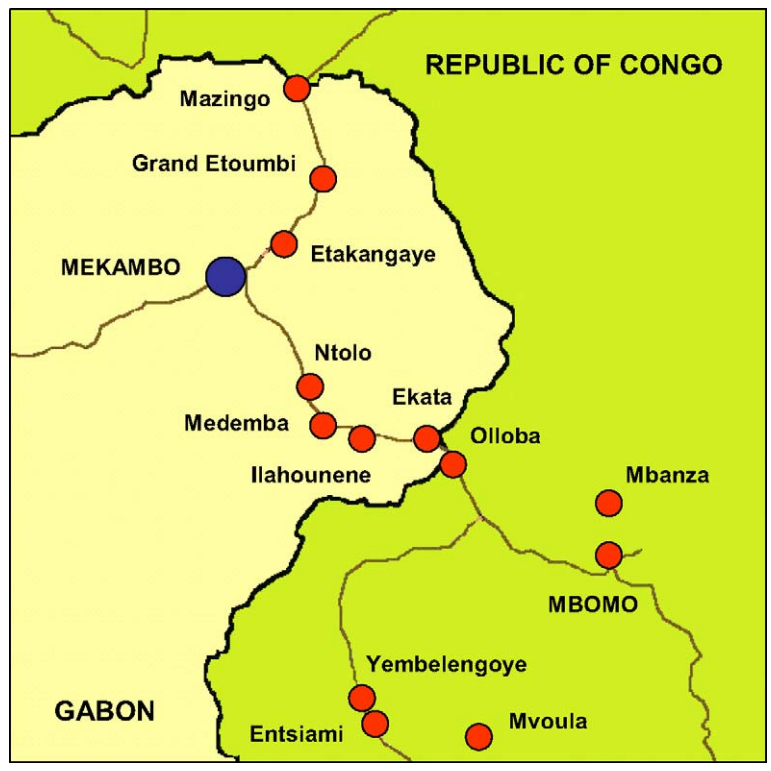

Fig. 3. EBOV outbreaks in the border region of Gabon and the Republic of Congo.

mainly affected Kelle. This outbreak involved 143 cases and 128 deaths.

The last outbreak to occur during this period again affected the region of Mbomo, between October and December 2003 [21]. The first cases occurred in Mbanza, a village located about 30 km north of Mbomo (Fig. 3). No animal source was clearly identified. There were 35 cases and 29 deaths.

Simultaneously with these human outbreaks, major Ebola outbreaks also affected animal species, including gorillas, chimpanzees and duikers, apparently accounting for the sharp population declines observed in these regions [22,19,23].

2.3.2. E. Sudan outbreaks

Two outbreaks of *E. Sudan* occurred during this period (Fig. 1). The site of the first was in Uganda between October 2000 and January 2001, causing 173 deaths among its 425 victims (mortality rate 40.7%) [24]. This was numerically the largest of all recorded outbreaks, and comprised three foci, one in the immediate area of Gulu, one in the town of Masindi, and another smaller outbreak in Mbarara, where a military patient carried the outbreak.

In contrast, the second outbreak was the smallest ever recorded, with 17 cases and seven deaths during May and June 2004; this mortality rate (41%) is characteristic of *E. Sudan* [25]. The outbreak occurred in Sudan, in a town called Yambio, located only a few dozen kilometers from Nzara and Maridi, which had been the sites of outbreaks in 1976 and 1979.

Thus, since the discovery of EBOV in 1976, there have been 13 outbreaks in Africa (nine due to *E. Zaire* and four to due to *E. Sudan*) and two isolated cases (due to *E. Zaire* and *E. Ivory Coast*). These outbreaks took place during three distinct periods (three between 1976 and 1979, four between 1994 and 1997, and six between 2000 and 2004). In total, EBOV-infected about 1850 people and caused nearly 1300 deaths. The different EBOV subtypes showed a certain geographic pattern, *E. Ivory Coast* affecting West Africa, *E. Sudan* east Africa, and *E. Zaire* central Africa. In contrast, the ecological circumstances of human outbreaks due to the three African EBOV subtypes were very similar, all involving forest regions located near the equator. These regions share a number of climatic and ecological characteristics, such as dense vegetation and a tropical climate, with two peaks of rainfall separated by dry seasons.

3. EBOV transmission to humans

3.1. Transmission from infected animals

In most outbreaks the source of the human index case was not identified. This includes all the outbreaks occurring from 1976 to 1979, the outbreaks of Mekouka (Gabon) in 1995, Booué (Gabon) in 1996, Kikwit (DRC) in 1995, and all the outbreaks of *E. Sudan* in Sudan (1976, 1979 and 2004) and Uganda (2000).

In contrast, several other outbreaks were clearly linked to animal carcasses. As mentioned above, a Swiss ethnologist became infected by *E. Ivory Coast* in 1994 while autopsying a chimpanzee (this remains the only recorded case of human infection by this subtype). The animal was found to be infected by the same EBOV strain as the ethnologist [12]. Similarly, the 1996 Mayibout outbreak in Gabon started among children who had found and butchered a chimpanzee carcass in the forest [16]. Similar sources have been reported for Marburg virus, the other member of the *Filoviridae* family: the 1967 outbreak in Marburg and Belgrade was linked to the handling of organs and tissues from vervet monkeys imported from Uganda [26,27].

The sources of the outbreaks that occurred in the border region of Gabon and RC between 2001 and 2003 are well documented. All these outbreaks (some of which had multiple sources) occurred after people had handled animal carcasses found in the forest (mainly gorillas, chimpanzees and duikers) [19]. We use the term "epidemic chain" to designate all human cases of infection arising from a given index case and thus from a given animal carcass. Gorilla carcasses were the source of the epidemic chains in Olloba 2001, Grand-Etoumbi 2002, Entsiami 2002 and Yembelengoye 2002. Chimpanzee carcasses appear to have been the source of the epidemic chains in Etakangaye 2001 and Olloba 2002. Finally, duiker carcasses apparently caused the epidemic chains in Mendemba 2001, Ekata 2001 and Mvoula 2003.

3.2. Transmission from the reservoir

There is currently no firm proof that humans can be directly infected by the (unknown) EBOV reservoir species, but several lines of evidence support this possibility.

3.2.1. Bats: epidemiological evidence

Bats were noted on several occasions in the warehouses of the cotton factory, which employed the first people to be infected during the 1976 and 1979 outbreaks in Nzara, Sudan. 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 [28]. However, when baboons and vervet monkeys were placed in cages inside the same caves, none became infected [29].

Two families of viruses (the *Rhabdoviridae* and *Paramyxoviridae*) are genetically related to the *Filoviridae* [30]. The order *Mononegavirales* encompasses all viruses with genomes consisting of a single negative-strand of non-segmented RNA.

The other two principal families in this order are the *Paramyxoviridae* (Hendra virus, Nipah encephalitis virus, measles virus, etc.) and the *Rhabdoviridae* (including rabies virus). Sequencing studies of the EBOV genes encoding the RNA polymerase (protein L) and nucleoprotein (NP) revealed that the *Paramyxoviridae* and *Rhabdoviridae* share many nucleotide stretches [30,31], supporting the genetic relatedness of these two viral families with the *Filoviridae*. Several studies have raised the possibility of direct human infection by bats carrying some of these viruses. For example, Hendra virus and Nipah virus can apparently be transmitted directly to humans by the consumption of fruit contaminated with fruit bat saliva [32]. Similarly, some rabies virus variants can be transmitted directly to humans by the bites of insect-eating bats [33,34].

3.2.2. Arthropods: several epidemiological clues

Although arthropods have never been successfully infected following inoculation [35,36], several observations suggest they can transmit Ebola virus to humans. A study done in 1968 showed that Marburg virus could persist for more than 3 weeks in *Aedes* mosquitoes after experimental inoculation [37]. It is also noteworthy that the Australian patient infected by Marburg virus in 1975 in Zimbabwe had been bitten, 6 days before symptom onset, by an arthropod (a spider, scorpion, ant, or wasp).

4. EBOV-susceptible animals: species, transmission and spread

4.1. Species naturally infected by EBOV

Numerous dead animals were found in the surrounding forest during the outbreaks in Gabon and RC between 2001 and 2004 [38]. To date, we have analyzed a total of 34 carcasses at the Centre International de Recherches Médicales de Franceville (CIRMF), Gabon, between 2001 and 2003. The carcasses were in a state of decomposition such that we could only sample muscle and skin and, in some cases, only bone tissue. We used a panel of highly sensitive techniques, including detection of EBOV-specific IgG and viral antigens in homogenized soft tissues (skin and muscle); virus isolation on soft-tissue cell lines; detection of DNA fragments by gene amplification from soft tissues and bone; and viral protein detection by immunohistochemistry on soft tissues fixed in 10% formalin.

Fourteen of the 34 animals (10 gorillas, three chimpanzees and one duiker) tested positive for Ebola infection, indicating that these three animal species can be naturally infected by EBOV. Most infected animals probably die rapidly, as suggested by the rapidly fatal nature of experimental EBOV infection in a variety of non-human primate species. However, disease severity and progression after experimental inoculation both depend on the dose, the portal of entry, and the viral subtype. In general, symptoms are more severe and long last-

ing when the inoculum is large [39,40]. Moreover, African EBOV subtypes are more pathogenic than *E. Reston* (the only Asian subtype) in macaque monkeys [41]: the mortality rate is higher, symptoms are more severe, hemorrhaging is more frequent and more generalized, and death is more rapid. *E. Sudan* shows intermediate pathogenicity between *E. Reston* and the other two African subtypes (*Zaire* and *Ivory Coast*): macaques inoculated with *E. Sudan* often survive, while almost all those inoculated with *E. Zaire* succumb [39,41].

Analyses of animal carcasses show that the great apes of the central African forests are particularly at risk from Ebola. This was confirmed by a serologic survey based on 790 samples taken from about 20 primate species in Cameroon, Gabon and RC [42]: for example, 12.9% of wild chimpanzees in Cameroon, Gabon and RC were found to have anti-EBOV IgG. Interestingly, some positive samples largely preceded the first human outbreaks in these regions. Together, 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 (five drills, one baboon, one mandrill and one *Cercopithecus* monkey), suggesting that EBOV circulation may be very complex, involving far more than simple direct passage from the reservoir to gorillas and chimpanzees. 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.

4.2. Spread of Ebola in wild animal populations

As stated above, the different outbreaks that occurred between 2001 and 2003 in Gabon and RC were the first to be linked to concurrent animal mortality, mainly affecting gorillas, chimpanzees and duikers. The discovery of EBOV-infected corpses of these three species strongly suggests a major role of the virus in the observed deaths. Between 2001 and 2003 the carcasses of 50 gorillas, 15 chimpanzees and 14 duikers were found in the outbreak areas of Gabon and RC. Given that a carcass disappears completely in less than 3 weeks in the rain forest, and that most of these carcasses were discovered within 3–4 h walking distance of villages, it is largely possible that several hundreds or even thousands of animals perished during this 2-year period in the border region of northeast Gabon and RC. Furthermore, calculated numbers of animal populations in certain areas, based on signs such as excreta, paw imprints, broken vegetation and nests, revealed a large increase in the mortality of some animal species, just before and also during human outbreaks. Gorilla and duiker populations are estimated to have fallen by 50% between 2002 and 2003 in the Lossi sanctuary (320 km^2) situated in RC, while chimpanzee populations fell by 88% [19]. Even if these are only approximations—for example, the demise of a dominant male gorilla provokes the dispersal of the group, making the remaining members diffi-

cult to count—they strongly suggest that EBOV provokes massive outbreaks in these animals, with hundreds or even thousands of deaths during a very short period (note that the possibility of such massive population declines due to EBOV may have major implications for animal conservation).

These recent studies showing an increase in animal mortality coinciding with human outbreaks, and providing biological confirmation of EBOV infection of animal carcasses, confirm the results of other studies conducted some years previously in other regions. First, a team of primatologists showed that gorilla and chimpanzee population densities fell strongly between 1994 and 1998 in the forest block of Minkebe to the northeast of Gabon, near the border with Cameroon [23]. The occurrence of human Ebola outbreaks in 1995 and 1996 in the same areas (Mekouka and Mayibout outbreaks) suggests that this marked decline in gorilla and chimpanzee populations may have been due to EBOV. Similarly, another study conducted in the Tai Forest of Ivory Coast showed the disappearance of 11 members (26%) of a group of 43 chimpanzees during the month of November 1994 [43]. The occurrence of the only human case of infection by *E. Ivory Coast*, together with the immunohistochemical positivity of tissues taken from a chimpanzee carcass, both events taking place in the same area and during the same period, suggests that the deaths in this group of chimpanzees may also have been due to EBOV.

4.3. EBOV emergence in animals

The chronological and geographic characteristics of the different outbreaks in Gabon and DRC between 1995 and 2003 point to a drift from northeast Gabon towards DRC. This raises the possibility that gorillas and chimpanzees are succumbing to a single outbreak that has been devastating animal populations for about 10 years, spreading along a northwest/southeast axis [22].

The EBOV genome is particularly stable. For example, the rate of mutations between strains *Booué* 96 (Gabon) and *Zaire* 76 was only 1.7% in the membrane glycoprotein gene (GP), 1.3% in the nucleoprotein (NP), 1.2% in the 40-kDa structural protein VP40, and 0.9% in the 24-kDa structural protein VP24, even though these strains were isolated more than 1000 km, and 20 years apart [44]. Similar genetic differences were found between the 2001 and 2003 outbreak strains and the strains *Mekouka* 94 (Gabon) and *Zaire* 76 [19]. Moreover, sequencing of a 249-nucleotide stretch in the most variable part of the GP gene showed no genetic differences between isolates recovered from nine patients (five of whom survived) who were sampled during the 1995 Kikwit outbreak in Zaire (DRC) [45].

In light of these results, and in order to understand how great apes are infected, we amplified and systematically sequenced the coding part of the GP gene (the most variable gene of the EBOV genome) in samples from all gorilla and chimpanzee carcasses. We found a distinct viral sequence in each carcass [19], even in carcasses belonging to the same

species (gorilla or chimpanzee) that had been discovered at the same time and only a few hundred meters apart (Leroy and Wittmann, personal data). As we had previously shown that a given outbreak strain undergoes no genetic variations, even after several human passages, these results argued against intraspecies transmission. On the contrary, the observed genetic diversity suggests that great ape infection results from simultaneous but independent transmission events from the reservoir species [19]. In this scenario the sequence variations probably result from viral adaptation to the natural host, which likely consists of several subspecies or genetically distinct groups. Thus, Ebola outbreaks in great apes appear to result not from the propagation of a single outbreak from one individual to another, but rather from massive simultaneous infection by the animal reservoir in particular environmental conditions (outbreaks always occur at the same times of the year, during the transitional periods between the dry and rainy seasons). Human infection occurs secondarily, and is generally linked to handling of infected animal corpses. Unfortunately, the precise environmental conditions required for EBOV to emerge from its reservoir are unknown, and the reservoir species remain(s) to be identified.

5. The reservoir species: 30 years of fruitless research?

The reservoirs (natural hosts) of Ebola virus and Marburg virus remain a mystery. Since the first recorded human outbreak in 1976, several laboratory and field studies have been conducted to identify the animal(s)—vertebrate or invertebrate—that can harbor the virus asymptomatically.

5.1. Wild animal capture studies

The first field study dates back to the Yambuku outbreak in 1976 in DRC (Table 1). An attempt was made to infect EBOV-permissive cell lines with samples of arthropods captured in and near patients' homes. The arthropods were pooled and crushed, and the resulting suspension was used to inoculate monkey kidney epithelial cells (Vero cells). Vero cells were also inoculated with centrifuged serum prepared from whole-blood samples of vertebrates captured near the homes and villages [10]. No viruses were isolated, despite the capture of 818 bedbugs, 1500 mosquitoes, 10 domestic pigs, one cow, seven bats, 123 rodents, eight squirrels, six *Cercopithecus* monkeys, and three small antelopes. A similar study of samples from 499 vertebrates obtained at the time of the 1976 outbreak in Sudan also gave negative results [46].

After the isolated case of *E. Zaire* infection in Tandala, DRC, in 1977 [11], intensive investigations were undertaken in 1979 in a very large northern region of DRC, including Tandala and Yambuku, which are about 300 km apart (Table 1). This study, conducted between June and August 1979, during the dry season, focused on two regions: the first centered on Yambuku and covered an area of 2800 km^2 with about 50,000 inhabitants, while the second, of roughly the

Table 1
Animals captured in field studies designed to identify the EBOV reservoir

		Yambuku 1976 (OMS 1978)	Nzara 1976 (OMS 1978)	Cam–Zaïre 1979–80 (Breman 1999)	Kikwitt 1995 (Leirs 99, Reiter 99)	Taï 1996–1997 (Formenty 99)	RCA 1999 (Morvan 99)	Total
Mammals	Chiroptera	7				652		
	Megachiroptera		4	41	125		19	189
	Microchiroptera		174	422	414		4	1014
	Rodents	131	309	661	1759	283	163	3306
	Insectivores		7	53	114	398	56	628
	Carnivores			27	28			55
	Primates			267	12	27		312
	Pholidotes			66	29			95
	Artiodactyles	13		27	22			62
Birds				67	184			533
Reptiles and amphibians			5	33	127	282		165
Total vertebrates		157	499	1664	2814	1642	242	7018
Arthropods		2318			27,843			30,161

Arthropods: bedbugs, mosquitoes, ticks, lice, flies.

same size and with 80,000 inhabitants, centered on Tandala [47]. The animals captured in this study comprised 267 primates, 463 bats, 661 rodents, 173 other mammals, 67 birds, and 33 reptiles and amphibians. Once again, no viruses were isolated.

Two other field studies were performed in 1995 near Kikwit, immediately after the outbreak (Table 1). A total of 2814 vertebrates [48] and 27,843 arthropods [49] were captured during respective periods of 3 and 6 months after the end of the outbreak. Viral isolation was attempted from both categories of animals, and vertebrates were also tested for *E. Zaire*-specific IgG. No positive IgG reactions were obtained, and no viruses were isolated on Vero cells. The vertebrates included 1759 rodents, 539 bats, 114 insectivores, 184 birds, and 127 reptiles and amphibians. The arthropods included 15,118 mosquitoes, 124 blood-sucking flies, 6538 bedbugs, 144 fleas, 103 lice, and 5816 ticks.

In Tai Forest, Ivory Coast, 1642 vertebrates (652 bats, 283 rodents, 398 insectivores, 27 monkeys and 282 birds) were captured and autopsied between 1996 and 1997 [50]. Organs were analyzed with the same methods as those described above. Except for a colobe bai monkey (red colobus, *Colobus badius*) that was seropositive for EBOV, no specific IgG reactions were detected and no viruses were isolated on cell lines (Table 1).

Finally, 242 vertebrates (24 bats, 163 rodents and 56 insectivores) were captured in CAR in 1998 [51] (Table 1). Small EBOV-specific genetic sequences were amplified from organs of six mice (*Mus setulosus* and *Praomys* sp) and a shrew (*Sylvisorex ollula*). However, although these results constitute the only biological evidence that EBOV may be present in healthy animals, no firm conclusions as to the EBOV reservoir status of these animals can be drawn, given the lack of specific serologic responses, the lack of nucleotide specificities in the amplified viral sequences, the failure of virus isolation, and the non-reproducible nature of the results.

5.2. Experimental infection

Attempts have also been made to infect various plant and animal species [35,36], including rodents (mice, shrews, rats, guinea pigs, etc.). Bats, birds (mainly pigeons), reptiles (tortoises, snakes, geckos, frogs), mollusks (mainly snails), arthropods (spiders, cockroaches, ants, centipedes, mosquitoes and butterflies) and more than 33 plant varieties belonging to 24 species (tomatoes, cucumbers, wheat, cotton, lupin, corn, tobacco, etc.).

All attempts to inoculate rodents, birds, reptiles, mollusks and plants failed to yield evidence of virus replication. Likewise, intrathoracic inoculation of arthropods with *Ebola Zaire* and *Ebola Reston* led to no detectable viral replication.

In contrast, some bat species belonging to the genera *Epomophorus* and *Tadarida* developed transient viremia lasting nearly 4 weeks after intravenous inoculation. However, given the non-reproducibility of these experiments, and the very particular conditions of inoculation (dose and route), these results fail to prove that bats are indeed the EBOV reservoir. They nonetheless open up new research perspectives, notably for field studies.

5.3. Conclusion

The natural reservoirs of Ebola and Marburg viruses remain elusive, as the only positive results are fragmentary and non-reproducible, and were obtained in questionable conditions. However, a trail of epidemiological clues (see above) makes bats the most likely candidates. Furthermore, several viruses belonging to virus families that are genetically related to the *Filoviridae,* such as *Rhabdoviridae* and *Paramyxoviridae*—rabies virus [52], Hendra virus [53] and Nipah virus [54]—are known to have bat reservoirs.

6. Conclusion

This article reviews all available data on the natural history of Ebola virus in Africa. Although several questions

remain, our knowledge of how the virus circulates in its natural environment has improved markedly in recent years. Despite the apparent geographic disparity of human outbreaks and the marked genetic diversity of the three "African" EBOV subtypes, all three subtypes circulate within a homogeneous ecological entity composed of the African equatorial forest block. The virus reservoir has not yet been identified, but many of the steps leading to human outbreaks are now fairly well understood. Several animal species, including great apes, are naturally infected by the virus, probably directly from the reservoir. These intermediate animal species develop a disease, which is usually fatal. Their carcasses remain infectious for humans, who may in turn transmit the disease through the community. These infected animals thus spread the virus to other ecological niches linked to their environment and behaviors. The large number of EBOV-permissive animal species, their variable sensitivity to the disease, and their very different patterns of behavior suggest that EBOV transmission cycles within its natural environment are highly complex. Identification of the reservoir and of the natural life cycle of the virus should help in the development of strategies for preventing human outbreaks and for reducing the impact of EBOV on animal species such as great apes, whose wild populations are very seriously threatened in endemic regions.

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