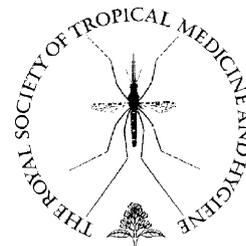




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# Morbidity and mortality of wild animals in relation to outbreaks of Ebola haemorrhagic fever in Gabon, 1994–2003

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**Summary** Antibody to Ebola virus was found in 14 (1.2%) of 1147 human sera collected in Gabon in 1981–1997. Six seropositive subjects were bled in the northeast in 1991, more than 3 years prior to recognition of the first known outbreak of Ebola haemorrhagic fever (EHF), whilst eight came from the southwest where the disease has not been recognised. It has been reported elsewhere that 98 carcasses of wild animals were found in systematic studies in northeastern Gabon and adjoining northwestern Republic of the Congo (RoC) during five EHF epidemics in August 2001 to June 2003, with Ebola virus infection being confirmed in 14 carcasses. During the present opportunistic observations, reports were investigated of a further 397 carcasses, mainly gorillas, chimpanzees, mandrills and bush pigs, found by rural residents in 35 incidents in Gabon and RoC during 1994–2003. Sixteen incidents had temporal and/or spatial coincidence with confirmed EHF outbreaks, and the remaining 19 appeared to represent extension of disease from such sites. There appeared to be sustained Ebola virus activity in the northeast in 1994–1999, with sequential spread from 1996 onwards, first westwards, then southerly, and then north-eastwards, reaching the Gabon–RoC border in 2001. This implies that there was transmission of infection between wild mammals, but the species involved are highly susceptible and unlikely to be natural hosts of the virus.

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## 1. Introduction

The filoviruses include Marburg virus and three serotypes of Ebola virus (Ebola-Zaire, Ebola-Sudan and Ebola-Ivory Coast) that cause outbreaks of haemorrhagic fever among humans in Africa, plus Ebola-Reston from the Philippines that affects monkeys but appears to be non-pathogenic for humans (Peters et al., 1996). Three species of bats were recently identified as potential reservoirs of Ebola-Zaire (Leroy et al., 2005), but the natural hosts of the other viruses remain unknown.

Marburg virus was discovered in 1967 when laboratory workers in Germany and Yugoslavia acquired fatal illness from contact with the tissues of imported monkeys, many of which were sick or dead on arrival from Uganda (Siebert et al., 1967). Attempts to find evidence of infection in non-human primates in Africa were inconclusive, partly because existing serological techniques lacked sensitivity and specificity. Since the animals appeared to be highly susceptible to infection, it was concluded that they were unlikely to be capable of serving as reservoir hosts responsible for perpetuation of the virus in nature (Malherbe and Strickland-Cholmley, 1971; Simpson et al., 1968; Slenczka et al., 1971). Small outbreaks of Marburg haemorrhagic fever were subsequently observed in South Africa in 1975, with infection apparently originating in Zimbabwe, and in Kenya in 1980 and 1987, whilst large epidemics were recorded in the northeastern Democratic Republic of the Congo (DRC) in 1998–2000 and in Angola in 2004–2005 (Gear et al., 1975; Johnson et al., 1996; Smith et al., 1982; WHO, 1999, 2005). Although various species, including bats, were investigated as potential reservoir hosts of the virus during the outbreaks in Africa, no disease or deaths were noted in domestic or wild animals subsequent to the original episodes in Europe (Conrad et al., 1978).

Ebola virus was discovered in 1976 when simultaneous outbreaks of haemorrhagic fever occurred in northern DRC and southern Sudan, which were found to be caused by two different serotypes of a new virus, designated Ebola-Zaire and Ebola-Sudan (Cox et al., 1983; McCormick et al., 1983; Richman et al., 1983). A single case of Ebola-Zaire infection was recognised in DRC in 1977, and an outbreak of Ebola-Sudan infection recurred in southern Sudan in 1979 (Baron et al., 1983; Heymann et al., 1980). No deaths of livestock or wild animals were observed during these four initial outbreaks of Ebola haemorrhagic fever (EHF), but the position changed when the disease re-appeared in Africa in November 1994 after an interval of 15 years, when a new serotype of the virus designated Ebola-Ivory Coast was isolated from a biologist who became infected during an autopsy on a chimpanzee in which the infection was confirmed by immunohistochemistry (Le Guenno et al., 1995). The virus is believed to have caused epidemics in the chimpanzee community that had been under observation for 14 years (Formenty et al., 1999).

During the same month (November 1994), there was an outbreak of EHF in gold mining camps in the Minkébé region of northeastern Gabon, where residents noted the simultaneous occurrence of deaths among gorillas, monkeys and bush pigs (red river hogs) in the surrounding forest. This proved to be the first of three epidemics to occur in Gabon from 1994–1997, each associated with deaths of wild

animals and each caused by a genetically distinct lineage of Ebola-Zaire virus (Georges et al., 1999; Georges-Courbot et al., 1997). After a 4-year interval during which no virus activity was detected, a further six epidemics occurred in Gabon and neighbouring northwestern Republic of the Congo (RoC) in 2001–2005. The first four of these six epidemics involved multiple incidents of humans acquiring infection from contact with wild animal carcasses and were associated with multiple genetic lineages of Ebola-Zaire virus (Leroy et al., 2004a; Rouquet et al., 2005). Meanwhile, there had been a large outbreak of EHF associated with Ebola-Zaire virus in the DRC in 1995, as well as epidemics caused by Ebola-Sudan virus in Uganda in 2000–2001 and in Sudan in 2004 (WHO, 2001, 2004).

The senior investigator (S.A.L.), who was conducting unrelated ecological studies in Gabon, was prompted by these outbreaks to make observations on morbidity and mortality in wild animals in Gabon and RoC from 1994 to 2003. The opportunity was also taken to perform tests for antibody to Ebola virus on human sera that had been collected earlier for an unrelated survey. The findings of that study are presented here and suggest that Ebola virus activity has been more widespread and sustained in Gabon than previously suspected, and provide an indication of the manner in which the infection may have spread.

## 2. Materials and methods

### 2.1. Study area

Gabon straddles the equator within the Guineo-Congolian phytogeographic region (White, 1983). The classic dense evergreen forest characteristic decreases with distance from the Atlantic coast towards the northeastern mixed evergreen semi-deciduous forest (Caballé, 1978). Approximately 15% of the habitat is savannah. Annual rainfall varies from a maximum of 3200 mm in the northwest to 1400–1600 mm in southern savannah regions. The three climatic regimes described are: (1) northeastern equatorial climate of two wet and two dry seasons; (2) central transitional tropical climate with a 3-month dry season and a 9-month variable wet season; and (3) southern tropical climate of 4–5 dry months and a 7–8-month wet season (Richard and Leonard, 1993).

### 2.2. Human serosurvey

From 1981–1997, 3531 serum samples were obtained by the Department of Tropical Medicine and Parasitology, University of Health Sciences, Libreville, Gabon, with informed consent from people in six rural communities in northeastern, southeastern and western Gabon for onchocerciasis research. The sera were stored at  $-70^{\circ}\text{C}$ . In 1998, 1147 (32.5%) of the samples were tested at the National Institute for Communicable Diseases (NICD), South Africa, for anti-Ebola IgG and IgM antibody using an ELISA with Ebola-Zaire virus antigen, as described previously (Ksiazek et al., 1999). In the absence of a panel of sera derived from known Ebola-infected individuals for proper validation and standardisation of the ELISA, the cut-off optical density (OD) value used for interpretation of results was based on

**Table 1** Location, date bled and number of subjects tested for Ebola IgG antibody in Gabon serosurvey, 1981–1997

Community	Region	Year	No. tested/no. bled	IgG positive		
				N (%)	M	F
Lastoursville	Central	1981	214/1301 (16.4%)	0	0	0
Makokou	Northeastern	1991	344/889 (38.7%)	6 (1.7%)	3	3
Matadi-Ngouassa	Western	1991	214/901 (23.8%)	2 (0.9%)	1	1
Moukoro	Western	1993	94/137 (68.6%)	2 (2.1%)	0	2
Doussiéoussou	Western	1996	139/139 (100%)	3 (2.2%)	3	0
Doussala	Western	1997	142/164 (86.6%)	1 (0.7%)	0	1
Total			1147/3531 (32.5%)	14 (1.2%)	7	7

M: no. of males; F: no. of females.

mean + 3 SD of OD values determined for negative control samples, an approach deliberately intended to be conservative (Heffernan et al., 2005; Ksiazek et al., 1999).

In 2003, repeat blood samples were obtained from six of the 14 persons initially found to be IgG antibody positive and from 14 control subjects comprising relatives and cohorts. A life history questionnaire was administered to each of the 20 participants. Information collected included the subject's age, sex, birthplace, occupation, personal and family medical history, location and duration of visits to and residency in other areas, relationship to the environment (hunting, farming, gathering), diet, food preparation techniques and observations on unusual events with regard to wildlife.

### 2.3. Animal disease outbreaks

Reported incidents of animal morbidity and mortality were investigated on an opportunistic basis during the course of Ebola-related investigations and other biological research projects conducted from November 1994 to November 2003. Information was provided by villagers, hunters, gold miners, fishing families, logging company employees, EHF outbreak survivors and families of victims, and wildlife survey teams at numerous sites in northeastern, central and western Gabon and northwestern RoC. People who live in remote areas often have clear recall of seeing dead animals because such encounters are rare in this sparsely populated and heavily forested country where carcasses decompose rapidly due to scavenger and insect activity and high ambient temperatures.

No faecal, blood or other soft tissue samples were obtained owing to delays in acquiring the information, which ranged from a week to 1 or more years, but in some instances it was possible to collect long bones and skulls that were tested at the NICD by RT-PCR for the presence of Ebola virus nucleic acid (Sanchez et al., 1999).

## 3. Results

### 3.1. Human serosurvey

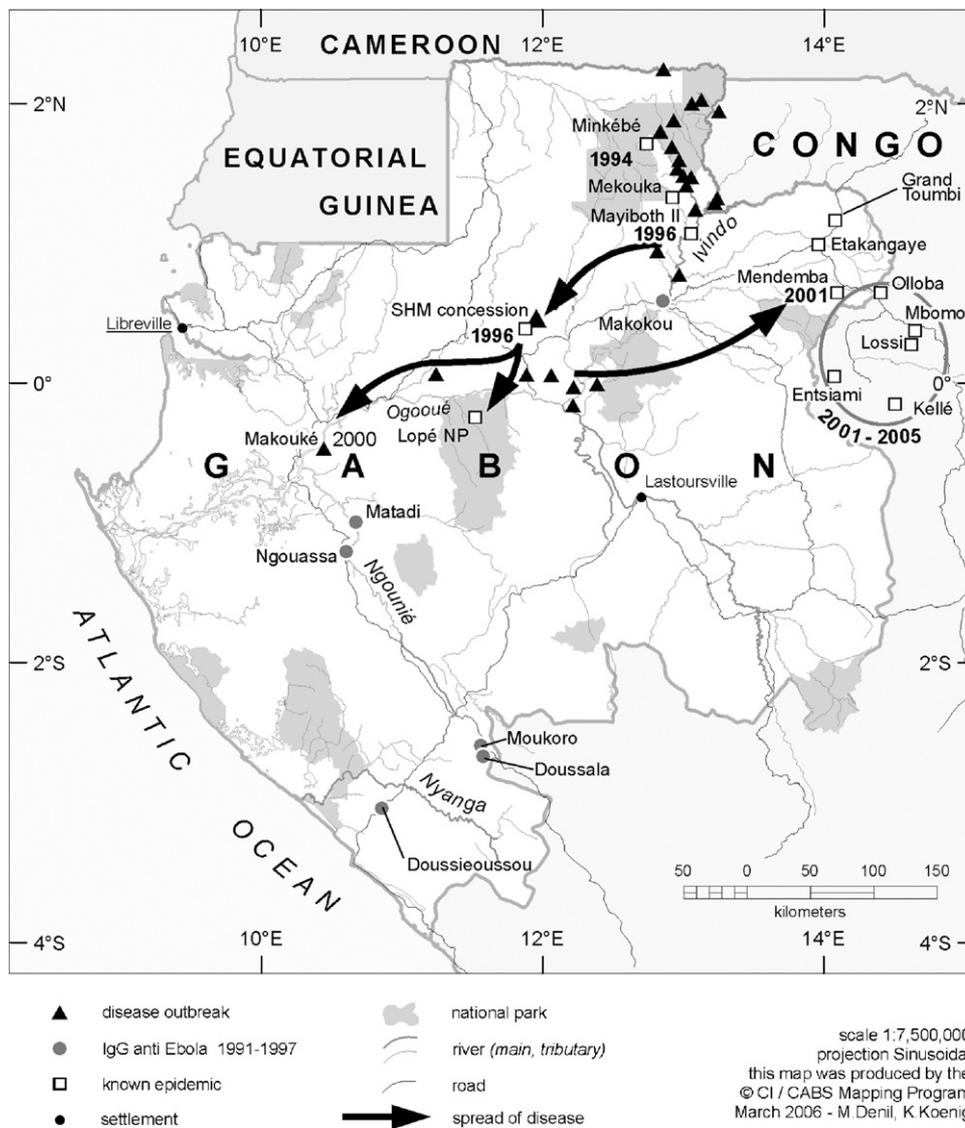
Of the 3531 sera originally obtained during 1981–1997, between 94 and 344 samples (comprising 16.4% to 100% of the samples) from each of the six communities were tested (Table 1). The habitat of Lastoursville, Makokou and

Matadi-Ngouassa is continuous forest, whereas the three other sites are in a forest–savannah mosaic. Fourteen sera from five of the communities were IgG antibody positive, with seroprevalence rates of 0.7–2.2%, comprising an equal number of males and females. No IgM antibodies were detected (Table 1; Figure 1). The ages of the 14 seropositive subjects ranged from 10 years to 79 years (mean 45 years, median 51.5 years), and five of the seven seropositive males were >40 years old. The mean seropositivity of the five communities was 1.5%, and seropositivity was 1.2% for the total sample population. The highest rates occurred in the mixed forest–savannah of southwestern Gabon, where eight individuals in four communities had antibodies in 1991–1997, although differences in seroprevalence between the northeastern and western communities were not significant (Fisher's exact test,  $P=0.786$ ; Zar, 1999).

In 2003, six of the 14 seropositive subjects were located and re-tested, along with 14 controls comprising two to three relatives or cohorts of each subject. The other eight seropositive persons could not be tested because they had died, moved away or were isolated by floods. In 2003, Ebola-specific IgG antibody was detected in only two (33%) of six previously seropositive subjects initially bled in 1991 and 1993 in northeastern and southwestern Gabon; one was a 56-year-old male from the Makokou area and the second was a 64-year-old female from Moukoro. No IgM antibodies were found in these subjects and all of the control subjects were negative for IgG and IgM antibodies.

Life history interviews were conducted with two of the subjects originally found to be seropositive in Makokou, northeastern Gabon, and with five persons in the west (one was investigated posthumously through questioning a relative) as well as all control subjects. Those at Makokou had spent most of their lives in the region engaged in hunting, trapping and agricultural activities. Two sons (controls) of one seropositive subject recalled suffering serious simultaneous illness with EHF-like symptoms in 1972. One remembered finding dead animals near his hunting camp during the two dry seasons in 1982.

None of the five seropositive subjects interviewed in western Gabon had ever been in the northeast. A man from Matadi village, who initially tested positive at age 10 years, had lived for several years at Makouké agroindustrial complex 100 km to the north. He recalled elders' concerns upon discovering dead animals in the forest when he was a child. This same farming complex was a site of reported animal



**Figure 1** Sites of Ebola haemorrhagic fever (EHF) outbreaks (□), reported animal mortality incidents (▲) and EHF seropositive humans (●) in Gabon and Republic of the Congo.

mortality in 2000. The two seropositive women at Moukoro village had lived there since at least 1967, whereas the woman resident in Doussala had lived in neighbouring RoC when she tested positive while visiting Gabon briefly in 1997, specifically to consult the visiting medical team.

### 3.2. Animal disease outbreaks

From November 1994 to November 2003, witnesses reported what were perceived as 44 separate outbreaks of morbidity and mortality in wild animals at 29 sites in Gabon and 10 sites in RoC, including all known EHF epidemics plus 35 undocumented incidents (Tables 2 and 3). It has been reported elsewhere that 98 carcasses, including 50 gorillas, 15 chimpanzees, 6 unspecified monkeys, 14 duiker antelopes and 13 less frequently encountered animals (1 brush-tailed porcupine, 3 genets, 1 elephant, 1 pangolin, 1 mongoose, 2 cane rats, 1 sitatunga antelope, 2 pythons and an unspecified bird of prey), were found in Mékambo district of northeastern

Gabon as well as in adjoining districts of northwestern RoC in the course of systematic investigations conducted during five EHF fever epidemics from August 2001 to June 2003 (Leroy et al., 2004a; Rouquet et al., 2005).

Excluding these 98 animal carcasses, reports were received during the present study of a further 397 carcasses found in the remaining 35 outbreaks of morbidity and mortality recorded within Gabon and RoC from November 1994 to December 2001 (Table 2), including 95 gorillas, 75 mandrills, 35 chimpanzees, 87 monkeys of various species, 17 duiker and 3 sitatunga antelopes, 64 bush pigs, 20 porcupines and 1 civet. Reports were verified by obtaining information from independent witnesses or by visiting sites to collect specimens whenever possible. The estimates are conservative since the lowest figures reported for each incident were recorded. The full range of species involved in all 35 outbreaks as reported to the present investigator (S.A.L.) included seven primates, five ungulates, one rodent and one carnivore, whilst in five instances carcasses were

**Table 2** Sites and mammal species involved in reported outbreaks of morbidity and mortality in Gabon and Republic of the Congo, November 1994 to April 2005

Date	Region <sup>a</sup>	Site	Type <sup>b</sup>	Species involved
<b>1994</b>				
Nov	NE Gabon	Mékouka	GC	Human <sup>c</sup> , gorilla, white-nosed guenon, mustached guenon, black colobus, bush pig
Nov	NE Gabon	Andok	GC	Human <sup>c</sup> , gorilla
Nov	NE Gabon	Minkebé	GC	Human <sup>c</sup> , white-nosed guenon, bush pig
Nov	NE Gabon	Mbokamabaka	GC	Chimpanzee
Nov	NE Gabon	Téka Téka	GC	Chimpanzee, gorilla
<b>1995</b>				
Dec	NE Gabon	Minkebé	GC	White-nosed guenon
Dec	NW Congo	Elir	GC	Mandrill, bush pig, duikers
<b>1996</b>				
Jan	NE Gabon	Mayiboth II	V	Human <sup>c</sup> , chimpanzee <sup>c</sup> , gorilla, sitatunga
Jan	NE Gabon	Citron	FC	Chimpanzee
Jan	NE Gabon	Asso	GC	Gorilla, white-nosed guenon, mandrill
Jan	NE Gabon	Ngnavôme	GC	Mandrill
Aug	C Gabon	SHM	TC	Human <sup>c</sup>
Sep	C Gabon	Lopé	NP	Chimpanzee <sup>c</sup>
<b>1997</b>				
Jan	NE Gabon	Mékouka	GC	Mandrill
Jan	NE Gabon	Masoko	GC	Chimpanzee
Jan	NW Congo	Été	V	Gorilla, white-nosed guenon, mandrill, bush pig, yellow-backed duiker
Feb	C Gabon	SHM	TC	Chimpanzee, gorilla, mandrill, bush pig, brush-tailed porcupine
May	NE Gabon	Mbokamabaka	GC	Mandrill
July	NE Gabon	Bibeh zone 1	HC	Chimpanzee, gorilla
Dec	NE Gabon	Mékouka	GC	Mustached guenon
Dec	NE Gabon	Upper Ayina	HC	Chimpanzee, gorilla, white-nosed guenon, guereza colobus, black colobus, sitatunga
<b>1998</b>				
Jan	NE Gabon	Bibeh zone 2	HC	Gorilla
Jan	NW Congo	Pounga	GC	Black colobus
Jan	NE Gabon	Oua River #1	FC	Mandrill
Jan	NE Gabon	Oua River #2	FC	Mandrill
May	NE Gabon	Andok	GC	Bush pig
June	NE Gabon	Mékouka	GC	Peter's duiker
<b>1999</b>				
Jan	NE Gabon	Minkebé	GC	White-nosed guenon
<b>2000</b>				
Feb	W Gabon	Makouké	AZ	Bush pig, brush-tailed porcupine
Aug	C Gabon	GEB 1	TC	Monkeys, bush pig, brush-tailed porcupine
Aug	C Gabon	Rougier #1	TC	Mandrill
Aug	C Gabon	Rougier #2	TC	Gorilla, mandrill, bush pig
<b>2001</b>				
Aug	C Gabon	GEB 2	TC	Mandrill
Aug	C Gabon	Rougier #3	TC	Mandrill
Aug	NE Gabon and NW Congo	Mendemba area to Lossi	V&C	Human <sup>c</sup> , chimpanzee <sup>c</sup> , gorilla <sup>c</sup> , bay duiker <sup>c</sup> , mustached guenon, monkeys, brush-tailed porcupine
Dec	C Gabon	Rougier #4	TC	Chimpanzee
<b>2002</b>				
Jan	NW Congo	Entsiami	V	Human <sup>c</sup> , gorilla, duiker
March	Gabon	Grand Toumbi	V	Human <sup>c</sup> , gorilla <sup>c</sup> , civet

Table 2 (Continued)

Date	Region <sup>a</sup>	Site	Type <sup>b</sup>	Species involved
April	Gabon	Zambakangaka	V	Sitatunga
May	NW Congo	Olloba	V	Human <sup>c</sup> , chimpanzee <sup>c</sup>
Oct	NW Congo	Kéllé-Mbomo	V&T	Human <sup>c</sup> , gorilla, duiker
2003				
Nov	NW Congo	Mbanza-Mbomo	V&T	Human <sup>c</sup> , monkeys, bush pig
2004				
Apr	NW Congo	Odzala	NP	Gorilla
2005				
April	NW Congo	Etoumbi	V	Human <sup>c</sup> , gorilla, monkeys

<sup>a</sup> NE: northeastern; NW: northwestern; C: central; W: western.

<sup>b</sup> GC: gold camp; V: village; FC: fishing camp; TC: timber concession; V&C: villages and camps; NP: national park; HC: hunting camp; AZ: agroindustrial zone; V&T: village and town.

<sup>c</sup> Diagnosis of Ebola haemorrhagic fever confirmed in a laboratory.

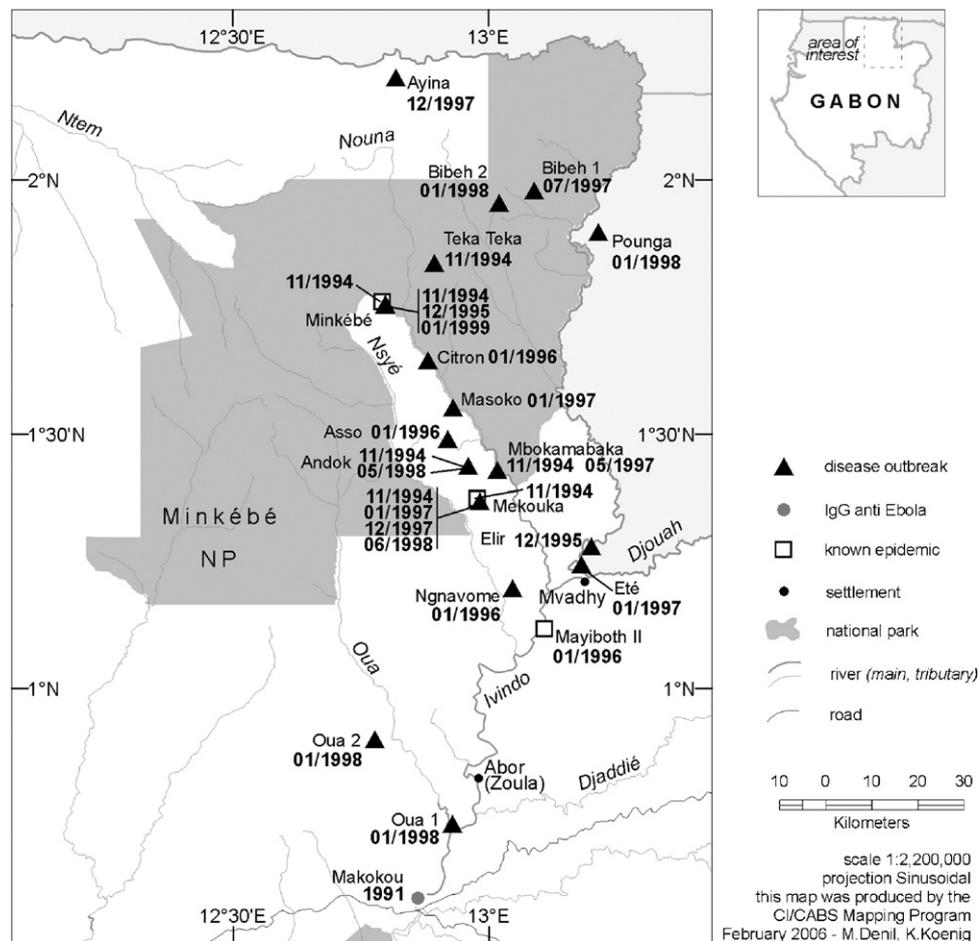
simply identified as monkeys or duikers, as summarised in Table 3 (this excludes the 13 less frequently encountered species detected in the systematic study cited above).

Moribund and dead animals were seen either singly or in groups of two or more individuals, as for example when 10 mandrills were observed dying in a timber concession. Whenever single animals were involved, other incidents usually

occurred in close proximity during the same period. In the majority (23/35) of incidents, only carcasses were reported. At 12 sites, witnesses described symptoms of moribund or dying animals, such as: (a) vomiting and/or diarrhoea, some loss of hair and emaciation (mandrill: Elir, GEB 2, Rougier 1 and 3; bush pigs: Elir, Andok); (b) severe diarrhoea and emaciation (mandrill: Mékouka, Ngnavôme; chimpanzee: Citron,

Table 3 Numbers of reported outbreaks of morbidity or mortality in wild mammals in Gabon and Republic of the Congo, November 1994 to April 2005, summarised in relation to species involved and month of occurrence

Common name	Scientific name	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total
Total outbreaks per month		13	2	1	3	3	1	1	7	1	1	6	5	44
Species frequency in outbreaks														
Chimpanzee	<i>Pan t. troglodytes</i>	3	1			1		1	1	1		2	2	12
Gorilla	<i>Gorilla g. gorilla</i>	5	1	1	1			1	3		1	3	1	17
White-nosed guenon	<i>Cercopithecus n. nictitans</i>	3										2	2	7
Mustached guenon	<i>Cercopithecus c. cephus</i>								1			1	1	3
Mandrill	<i>Mandrillus sphinx</i>	6	1			1			4				1	13
Guereza colobus	<i>Colobus guereza</i>												1	1
Black colobus	<i>Colobus satanas</i>	1										1	1	3
Unspecified monkeys					1				2			1		4
African brush-tailed porcupine	<i>Atherurus africanus</i>		2						2					4
African civet	<i>Civettictis civetta</i>								1					1
Red river hog	<i>Potamochoerus porcus</i>	1	2			1			2			3	1	10
Western sitatunga	<i>Tragelaphus spekei gratus</i>	1			1								1	3
Yellow-backed duiker	<i>Cephalophus sylvicultor</i>	1												1
Peters' duiker	<i>Cephalophus callipygus</i>						1							1
Bay duiker	<i>Cephalophus dorsalis</i>								1					1
Unspecified duikers		1									1		1	3



**Figure 2** Sites of Ebola haemorrhagic fever (EHF) outbreaks (□), reported animal mortality incidents (▲) and EHF seropositive humans (●) in northeastern Gabon.

Masoko); and (c) vomiting and bleeding from nostrils (white-nosed guenon: Minkebé, Asso; mandrill: Asso; black colobus: Pounga) (Table 1; Figures 2 and 3). Fourteen decomposed bone samples tested by RT-PCR were found to be negative for the presence of Ebola virus nucleic acid.

### 3.3. Seasonality of Ebola epidemics and wild animal mortality incidents

Although rainfall varies between years and subregions, there was a clear tendency for outbreaks of disease in wildlife in Gabon and RoC, including confirmed EHF, to occur mainly in transitional and dry months. There is no evidence to suggest that the confirmed EHF events and wildlife mortality reports are drawn from different populations: no significant differences were found between the numbers of unconfirmed incidents and combined confirmed EHF epidemics and animal carcasses either for the two wet and two dry seasons ( $G$ -test,  $G = 2.96$ , d.f. = 3, not significant (NS)) or for the wet and dry seasons combined ( $G = 2.50$ , d.f. = 1, NS) (Zar, 1999) (Figure 4).

January was the peak month for wild animal morbidity and mortality, followed by August, November and December (Tables 2 and 3). The transitional month of December plus the short dry season of January–February and the long

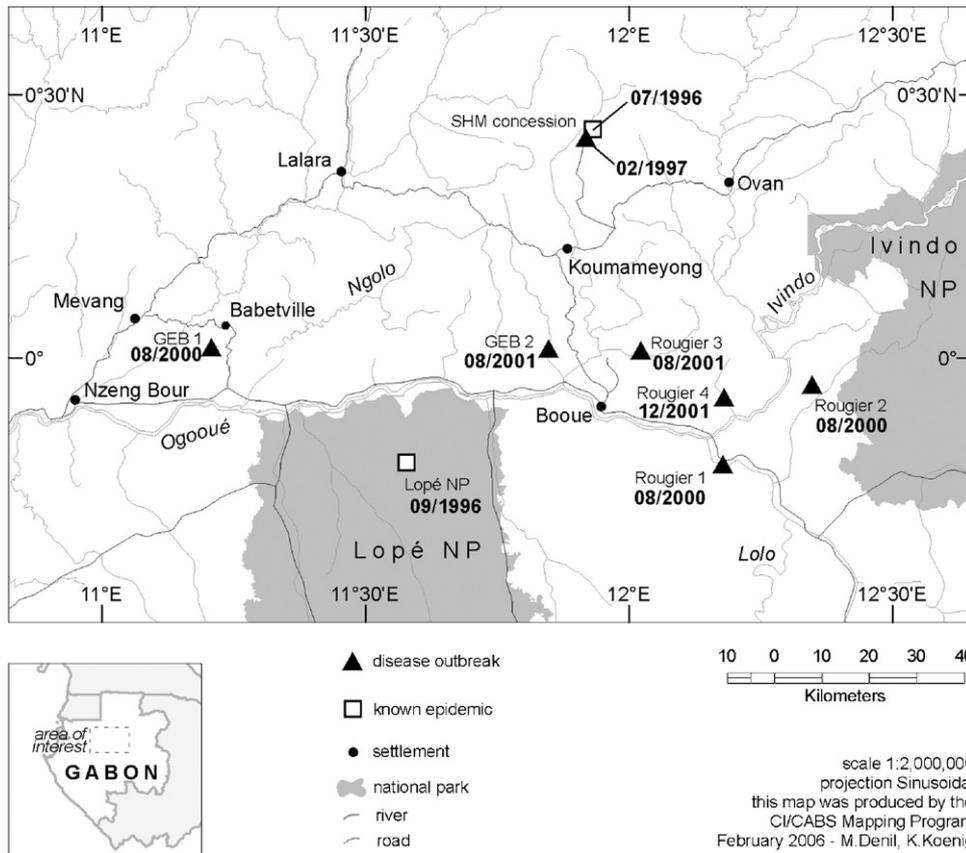
dry season of July–September together accounted for 29/44 (65.9%) of all outbreaks.

### 3.4. Distribution of EHF epidemics and wild animal mortality incidents

#### 3.4.1. Northeastern Gabon

There were 22 reports of deaths of single or multiple species from November 1994 to January 1999 in the upper Ivindo River Basin extending from the Oua River approximately 50 km north of Makokou to the RoC–Cameroon border, an area of approximately 8000 km<sup>2</sup> to which there is no direct access by road, with the Ivindo River and its tributaries serving as transport routes (Table 2; Figure 2). Animal deaths were initially observed during the first EHF epidemic recognised in Gabon in November 1994 near five gold camps in the Minkébé region, west of the Ivindo River. Residents found dead gorillas, monkeys and bush pigs, and recalled seeing solitary gorillas following people or frequenting the periphery of camps, but no animal samples were obtained for laboratory investigation (Table 2) (Georges et al., 1999).

More animal deaths were observed in the region in 1995–1996 (Table 2), and in January 1996 the second EHF epidemic in Gabon occurred in Mayiboth II village on the

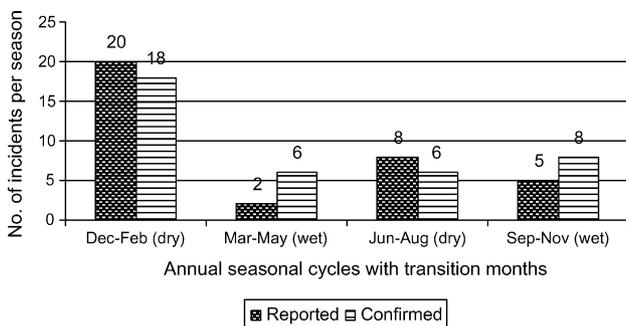


**Figure 3** Sites of Ebola haemorrhagic fever outbreaks (□) and reported animal mortality incidents (▲) in central Gabon.

east bank of the Ivindo River, when villagers butchered and consumed an infected chimpanzee carcass (Georges et al., 1999). Residents of Elir gold camp and Été village in RoC saw a variety of animal carcasses while hunting in RoC in 1995 and between the Ivindo and Nouna Rivers in Gabon in 1997, respectively, including mandrills, gorillas, duikers, porcupines and bush pigs. The 1995 Elir outbreak in RoC preceded the 1996 Mayiboth epidemic in Gabon by 1 month. Baka hunters in Gabon found various dead mammals near the Cameroon border in December 1997, whilst other hunters discovered dead mandrills 140–160 km to the south on the Oua River in January 1998. Finally, white-nosed guenon carcasses were seen near Minkébé gold camp in January 1999,

where the same species had been found dead in 1994 and 1995.

In August 2001, villagers began finding animal carcasses further east in the Mékambo region, and in October the first human victims at Mendemba were recognised in what proved to be an EHF epidemic that occurred on both sides of the Gabon–RoC border and lasted until May 2002. This was followed by a further five epidemics in northwestern RoC in 2002–2005. Altogether this series of six epidemics affected humans and animals within an area of approximately 20 000 km<sup>2</sup> straddling the border between the two countries. During the first five epidemics, 98 animal carcasses were found in systematic studies (Leroy et al., 2004a; Rouquet et al., 2005). Laboratory confirmation of Ebola virus infection was obtained for the carcasses of 10 gorillas, 3 chimpanzees and a duiker (Leroy et al., 2004a; Rouquet et al., 2005). Unlike previous outbreaks of EHF, the 2001–2003 epidemics were associated with multiple introductions of infection into the human population. Strong circumstantial evidence was obtained indicating that people had acquired infection from contact with the carcasses of four gorillas, three chimpanzees and four duikers in separate incidents (Leroy et al., 2004a; Rouquet et al., 2005). Unconfirmed wildlife mortality reports at 13 northeastern sites coincided spatially and temporally with the 1994, 1996 and 2001–2003 epidemics (Figure 2).



**Figure 4** Number of reported animal mortality incidents and number of combined observations of confirmed animal remains and Ebola haemorrhagic fever epidemics per season in Gabon and Republic of the Congo, 1994–2005.

**3.4.2. Central and western Gabon**

No animal deaths were reported at the start of the third recorded EHF epidemic in Gabon, which began at the Socié

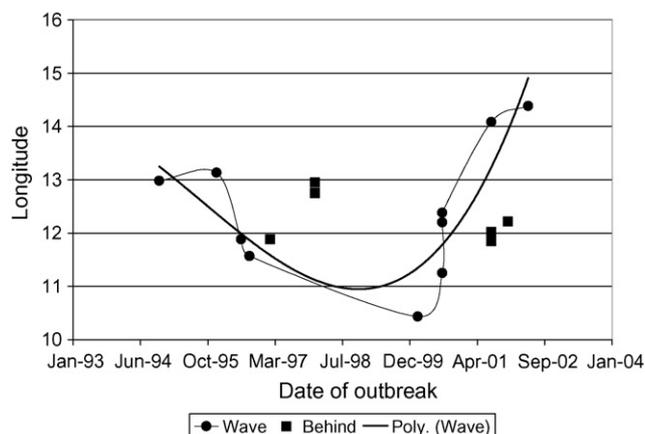
de la Haute Mondah (SHM) timber concession 60 km north of Booué in July 1996 (Georges et al., 1999; Georges-Courbot et al., 1997). However, family members and a hunting companion of the deceased putative index patient revealed that he had killed several mandrills prior to developing fatal illness. Shortly thereafter, in September 1996, an Ebola-infected chimpanzee carcass was found 85 km to the south across the Ogooué River in Lopé National Park (Georges-Courbot et al., 1997), and in February 1997 forestry workers reported the occurrence of a disease outbreak involving five wild animal species at the SHM timber concession (Table 2; Figure 1). During the short dry season in February 2000, dead bush pigs and brush-tailed porcupines were found at Makouké agroindustrial complex in western Gabon (Figure 1), where animal deaths had also been observed some years prior to 1991, according to a human Ebola antibody-positive subject as reported above.

In August 2000, carcasses of mandrills and four other species were found in three additional timber concessions north and east of Makouké in central Gabon on either side of the Ogooué–Ivindo waterway. Deaths of animals were observed 1 year later at three further logging sites in the same area in August 2001 (Table 2; Figure 3). This coincided with the onset of disease outbreaks 260 km to the northeast in the Mendemba vicinity, which marked the start of a series of EHF epidemics as outlined above. These wildlife mortality reports in 2001 thus concurred temporally with this outbreak.

### 3.5. Geographic spread of confirmed and reported incidents

The spatiotemporal pattern of outbreaks illustrated in Figure 1 presents a scenario of perceived spread of infection south and west, then east and north in Gabon towards RoC during 1994–2002. The virus appears to have been circulating in the northeastern forests during the years 1994–1999, of which the two 1998 Oua River incidents are the southernmost extension of this persistent infection (Figure 2). From 1996 the infection spread from Mayiboth II southwest through the forests north of the Ogooué River, except for one isolated case south of the river at Lopé (Figure 3). It reached Makouké, its westernmost extent, in February 2000 (Figure 1). Both Makouké and Lopé lie south of the Ogooué River. In August of that year, cases were reported in logging camps east of Makouké (GEB 1) and east of Booué (Rougier 1 and 2) (Figure 3). Reported disease outbreaks east of Makouké in 2000–2001 occurred north of or near the Ogooué River. The area between Makouké and Lopé may also have been affected, but we have no data for this zone. The virus then spread eastwards to Mendemba and reached Olloba, RoC, in December 2001.

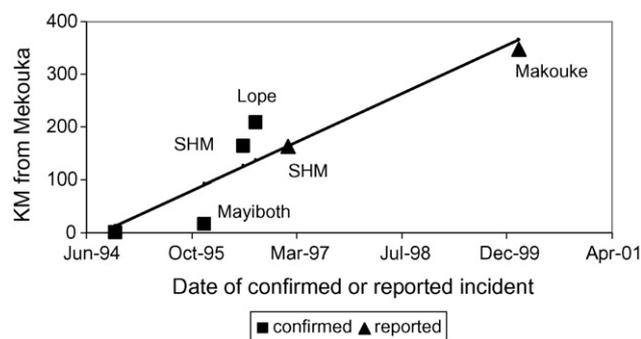
Three scenarios are possible. First, these represent random outbreaks in Gabon. Second, the virus spread southwest to Makouké, and from Makouké it returned east, and then passed through the large expanse of forest to Mendemba and beyond. Third, the virus spread from Mayiboth II to the SHM concession (Figure 1) and then took three separate paths: west to Makouké, south to Lopé, and finally southeast to the Rougier logging camps whence it went east to Mendemba. If the infection spread as a wave through the forest, rather



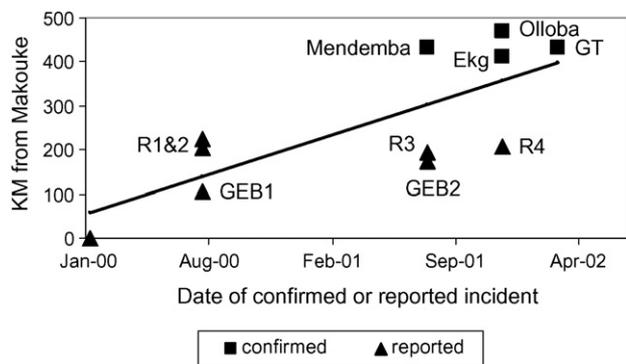
**Figure 5** Relationship between longitude and date of outbreaks hypothesised to move southwestwards and then eastwards: (●) the wave of outbreaks; (■) outbreaks that occurred behind the wave. The best-fit curve is a cubic polynomial.  $R^2 = 0.753$ ;  $P = 0.030$ ;  $Y = 13.83 - 0.04x - 0.001x^2 + 1.48 \times 10^{-5}x^3$ .

than outbreaks occurring at random across the landscape, a plot of longitude against time would show a straight line. If the infection spread to the southwest and then returned, a plot of longitude against time would be U-shaped. When the data were plotted (Figure 5), they showed a U-shape, thus the hypothesis of random outbreaks can be rejected. These data are consistent with, but do not confirm, the hypothesis that the virus moved towards the southwest and then towards the north and east to RoC.

There were distinct and statistically significant spatiotemporal patterns for EHF outbreaks and reported mortality incidents from 1994–2002. Spread rates were calculated for each proposed trajectory according to the number of months since 1 January 1994 corresponding to the date of each incident. The westward spread from Mékouka to Makouké, a distance of 347 km, was estimated at 67.3 km per annum from 1994 to 2000 (Figure 6). The subsequent eastward extension of the virus from Makouké towards Olloba, RoC, in 2000–2001 occurred at a rate of 162 km per annum (Figure 7).

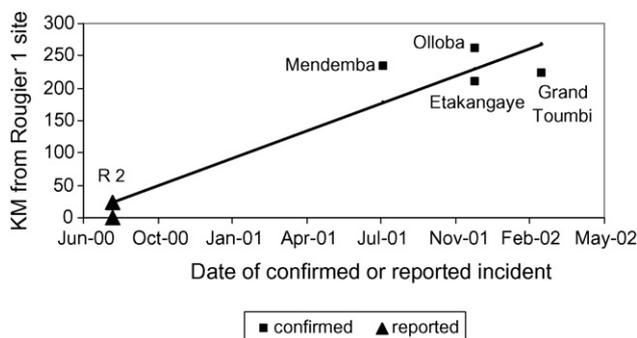


**Figure 6** Relationship between date and distance from origin of confirmed Ebola haemorrhagic fever outbreaks (■) and reported wildlife mortality incidents (▲) between Mékouka and Makouké, Gabon, 1994–2001.  $R^2 = 0.846$ ;  $P = 0.009$ ;  $Y = 5.61x - 48.74$ . SHM: Société de la Haute Mondah timber concession.



**Figure 7** Relationship between date and distance from origin of confirmed Ebola haemorrhagic fever outbreaks (■) and reported wildlife mortality incidents (▲) between Makouké, Gabon and Olloba, Republic of the Congo, 2000–2002. R 1–4: Rougier sites 1–4; Ekg: Etakangaye; GT: Grand Toumbi.  $R^2 = 0.573$ ;  $P = 0.007$ ;  $Y = 13.508x - 940.61$ .

The third hypothesis, shown in Figure 1, is the three-pronged scenario with infection extending from the SHM logging concession south to Lopé, west to Makouké, and northeast towards Mendemba from other timber concessions east of Booué. First, an EHF-confirmed chimpanzee carcass was discovered at Lopé in September 1996 during the SHM epidemic 85 km to the north. Second, infection also spread west to Makouké, with wildlife mortality reported at a site midway between the latter two sites several months later (GEB 1), perhaps resulting from continuing virus circulation in the area. Third, from the Rougier 1 and 2 sites, where animal mortality incidents were reported in August 2000, infection spread east and north across a large expanse of sparsely inhabited forest towards Mendemba (Figure 8). Meanwhile, the virus continued to circulate in the vicinity of the Rougier logging sites, where wildlife mortality was reported in three other logging concessions within 60 km west of Rougier 1 and 2 in August 2001. Thus, the virus had already spread to Mendemba by this time, resulting in simultaneous outbreaks in wildlife populations at these sites 260 km apart. The chimpanzee carcass reported at Rougier 4 site in December 2001 may have been part of this sustained epidemic in the south-eastern central region (Figure 3). The estimated spread rate



**Figure 8** Relationship between date and distance from origin of confirmed Ebola haemorrhagic fever outbreaks (■) and reported wildlife mortality incidents (▲) between Rougier 1 site in central Gabon and Olloba, Republic of the Congo, 2000–2002. R 2: Rougier 2 site.  $R^2 = 0.893$ ;  $P = 0.004$ ;  $Y = 12.918x - 1010.6$ .

from Rougier 1 to Olloba, RoC, is 155 km per annum, similar to that of Makouké–Olloba.

Alternatively, this latter scenario shown in Figure 8 may also describe the second half of the Makouké–Olloba epizootic path, in which virus spread east from Makouké through a series of logging concessions from GEB 1 towards the central zone in 2000 and then spread further east towards Mendemba and the RoC border.

#### 4. Discussion

The indirect immunofluorescence test, widely used in early filovirus studies, often detected a high prevalence of antibody in the absence of a corresponding history of disease. In surveys conducted on sera collected in 1980–1987 in Gabon and the neighbouring countries of Cameroon, Central African Republic, Chad and Equatorial Guinea, 6–37% of subjects were found to react with Ebola and 0–5% with Marburg virus antigens (Bouree and Bergmann, 1983; Gonzalez et al., 1989; Ivanoff et al., 1982; Johnson et al., 1993a, 1993b). Antibody tended to be more prevalent in hunter–gatherer populations than in agriculturalists, as well as in residents of equatorial forests than in town dwellers, but there were discrepancies. Moreover, results obtained with Ebola-Zaire and Ebola-Sudan antigens were inconsistent in successive studies. It was concluded either that sera reacted non-specifically in the indirect immunofluorescence test, particularly with Ebola virus antigen, or that there were non-pathogenic strains of the virus in circulation (Bouree and Bergmann, 1983; Gonzalez et al., 1989; Ivanoff et al., 1982; Johnson et al., 1993a, 1993b). Although the sensitivity and specificity of serological tests ostensibly improved with the introduction of ELISA in the 1990s, antibody activity to filoviruses was detected in 6.9% of people tested in Germany in 1992 and in 43.3% of monkeys originating in the Philippines, China and Uganda, from which it was concluded that filoviruses are not confined to Africa and cause non-fatal infections in humans and non-human primates (Becker et al., 1992).

Asymptomatic Ebola virus infections were described during the second and third EHF epidemics in Gabon (Leroy et al., 2000). Antibody to the virus was detected by ELISA in 9.7–16.6% of people in the affected communities in the upper Ivindo River Basin sampled in 1995–1996 during and shortly after the occurrence of the initial epidemics (Bertherat et al., 1999; Georges et al., 1999). In contrast, antibody to the virus was found in only 0.9% of people in Kwélé fishing communities along the Ivindo River in 1997, but also in 11.1% of hunter–gatherer Baka pygmies inhabiting the same area (Heffernan et al., 2005).

In the present study, IgG antibody to Ebola virus was detected in 1.2% (14/1147) of sera that had been collected in Gabon in 1981–1997. Most of the 14 seropositive subjects were >40 years old and none of the six questioned when re-bled could recall suffering from disease compatible with EHF, but non-fatal Ebola virus infection would be difficult to differentiate from malaria and other common tropical infections of the region. Antibody was found in only two of six seropositive subjects when re-bled in 2003, but there is no information available on persistence of antibody to Ebola virus, apart from the fact that one person in the Philippines

retained antibody activity to Ebola-Reston virus for at least 4 years (Miranda et al., 1999). The dates of infection of our 14 seropositive subjects are unknown. However, since the two persons who remained seropositive in 2003 had originally been found positive on serum collected in 1991 and 1993, the present findings indicate that antibody to Ebola virus may remain detectable for at least 10–12 years.

Six of the seropositive subjects detected in the present study were residents of Makokou who had been bled in 1991, more than 3 years prior to the first recognised EHF epidemic in Gabon. This finding is not surprising in view of the subsequent evidence of repeated Ebola virus activity in the northeastern region of the country. Although the prevalence of Ebola antibody detected in Makokou in this study is low (1.7%; 6/344), it is significantly higher than the 0.9% ( $P < 0.001$ ) subsequently found in Bakwélé fishing communities along the Ivindo River upstream from the town in 1997 (Heffernan et al., 2005). The difference may be related to the fact that the Kota, Fang and Bichibé participants in the Makokou study, like the Baka, hunt more often than the Kwélé (Lahm, 1993, 2000). Thus, there has been a clear tendency for higher prevalences of Ebola virus antibodies to occur in populations that have greatest contact with wild animals, and strong evidence was obtained during the EHF epidemics in Gabon to indicate that people acquired infection from scavenging gorilla, chimpanzee and duiker carcasses (Georges et al., 1999; Leroy et al., 2004a; Rouquet et al., 2005). Like hunting, scavenging is an ancient foraging strategy of humans and their ancestors (Barsh and Marlor, 2003; Lewis, 1997) and is still widely practiced in Central Africa (Bahuchet, 1985; Lahm, 1993).

In addition to the six seropositive people detected in northeastern Gabon, Ebola antibody was also found in eight people tested in 1991–1997 in five western communities more than 500 km from the northeast where most of the known EHF epidemics occurred (Table 1). Nevertheless, as indicated above, outbreaks of mortality in wild animals have been observed in this area on at least two occasions, and more recently the results from a serological survey of wild-born primates in Cameroon, Gabon and RoC confirmed that Ebola virus is widely distributed within the Central African forest region (Leroy et al., 2004b).

Although Ebola virus infection was not confirmed in any of the decomposed wild animal carcass material examined in the present study, 16 of 44 incidents investigated had temporal and spatial coincidence with confirmed EHF outbreaks, including those in which Ebola virus-infected animal carcasses were found (Table 2; Figure 1) (Georges et al., 1999; Georges-Courbot et al., 1997; Leroy et al., 2004a; Rouquet et al., 2005). The remaining incidents appear to represent extension or spread of virus activity from confirmed foci, similar to the perspectives of Pinzon et al. (2004) and Walsh et al. (2005). Thus, the 22 incidents of wild animal morbidity and mortality recorded in the upper Ivindo River Basin included two confirmed EHF epidemics, in one of which people were infected by a chimpanzee carcass. The multiple incidents in the northeast imply the occurrence of almost continuous Ebola virus activity in the region from November 1994 to January 1999, possibly facilitated by prolonged dry conditions in the environment (Pinzon et al., 2004). This probably involved sequential introductions of infection into discontinuously distributed mammal popu-

lations. Despite the depletion of apes in the vicinity of EHF epidemics, isolated groups of chimpanzees and gorillas continued to thrive, in one instance only 20 km from a location where an Ebola-infected gorilla carcass was found (Lahm, 2000, 2002).

In summary, the onset of ostensibly sustained Ebola virus activity in the upper Ivindo River Basin of northeastern Gabon in 1994 appeared to be followed by spread of infection in successive waves over the next decade, first in a southwesterly direction to central Gabon, then eastwards and finally northeastwards towards Mendemba and northern RoC. The strong genetic relationship between virus isolates from the Gabon–RoC border outbreaks and the Booué area, rather than with those from the spatially closer Mékouka–Mayiboth sites, is consistent with the hypothesis of eastward spread from the central zone (Figure 3) (Walsh et al., 2005). Unexpectedly low numbers of apes recorded during a series of biological surveys in the remote, sparsely populated region between the central Gabon southeastern sites and Mendemba in 2001–2002 (Figure 1) suggest that wildlife populations there may also have been affected by EHF (Lahm, 2001a, 2001b).

This pattern of spread represents a departure from previously observed outbreaks propagated solely by human-to-human transmission and suggests that there may have been some transmission of infection within wild animal populations, particularly in view of the evidence that there were multiple introductions of genetically distinct lineages of Ebola virus infection into human populations as a result of contact with animal carcasses (Leroy et al., 2004a; Rouquet et al., 2005).

Incidents of morbidity and mortality involving gorillas, chimpanzees, mandrills and bush pigs constituted 63% (53/84) of all reports for individual species (Tables 2 and 3), but the occurrence of Ebola virus infection was confirmed only in gorillas, chimpanzees and a duiker antelope (Leroy et al., 2004a; Rouquet et al., 2005). Chimpanzees are omnivorous and their diet includes driver ants (*Dorylus* spp.), which feed on carcasses. They share feeding sites with lowland gorillas, and manifestations of social behaviour that favour transmission of infection include fighting and predation (Kuroda et al., 1996; Yamagiwa et al., 1996). Gorillas are vegetarian, but populations were denser than those of chimpanzees in some of the locations worst affected by the Ebola outbreaks (Lahm, 2000, 2002). Intergroup encounters and emigration that facilitate transmission of disease are common in gorillas (Bermejo, 2004; Doran-Sheehy et al., 2004; Stokes et al., 2003; Tutin, 1996). Indeed, social structure appears to have strongly influenced the spread of EHF in a gorilla population in northwestern RoC (Caillaud et al., 2006).

Reported incidents of morbidity and mortality were comparatively frequent for mandrills (15.5%; 13/84) (Tables 2 and 3), a species not previously cited as being involved in Ebola outbreaks. Antibody to the virus was found in mandrills in a recent survey (Leroy et al., 2004b). Mandrills live in groups of 25 to >700 (Hoshino et al., 1984; Lahm, 1986; Tutin, 2000). They are largely frugivorous but eat insects and arachnids including predaceous doryline and ponerine ants as well as small animals ranging from amphibians to rodents, including brush-tailed porcupines (Lahm, 1986). Opportunities for transmission of infection could arise

during social and aggressive interactions within groups and between solitary males. Porcupines are frugivorous but gnaw bones and are exposed to bat and rodent excreta in dens within caves and hollow logs (Emmons, 1983). Bush pigs were involved in 12% (10/84) of incidents of morbidity and mortality reported for individual species. They are omnivorous but scavenge meat, and disease transmission could occur at crowded communal sleeping and wallowing sites. Group sizes observed in Gabon varied from  $\leq 10$  in hunted areas to  $>50$  in undisturbed locations (Lahm, 1993). Reported incidents of mortality were infrequent for individual duiker species and sitatunga antelopes collectively (10.7%; 9/84), and sparse for other species.

Although the implication is that there was intraspecies and interspecies transmission of infection in wild mammals, the species putatively involved appear to be highly susceptible to the virus because populations of gorillas, chimpanzees and duiker antelope were found to be drastically reduced following outbreaks of Ebola virus infection (Leroy et al., 2004a; Rouquet et al., 2005; Walsh et al., 2003). This suggests that these animals are unlikely to be natural hosts responsible for perpetuation of the virus in nature. Moreover, circulation of multiple genetic lineages of Ebola virus within the recent outbreaks in Gabon and RoC is in contrast to the serial transmission of a single lineage of virus in humans as observed in previous outbreaks and implies repeated exposure of humans and susceptible wild animals to unidentified natural hosts (Leroy et al., 2002, 2004a; Rodriguez et al., 1999; Rouquet et al., 2005). It has been hypothesised that the reservoir hosts of filoviruses are small mammals (Peterson et al., 2004) and there has been unsubstantiated evidence to suggest that bats or rodents could be involved (Morvan et al., 1999; Swanepoel et al., 1996). Three species of bats were identified recently as potential reservoir hosts for Ebola-Zaire (Leroy et al., 2005).

It is notable that, in general, neither the major Ogooué–Ivindo waterway nor numerous tributaries and adjacent wetlands that constitute barriers to the movement of many terrestrial vertebrates appeared to curb the spread of Ebola virus. One inference is that the virus may be associated with flying creatures, including insects, birds and bats, but patterns of spread of infection observed in human populations are more consistent with contagion than with transmission by haematophagous insect vectors.

Recent analyses of environmental and climatic factors associated with EHF epidemics in Africa indicate that outbreaks of the disease can be expected to occur in tropical forest areas approximately 2–4 months after the onset of markedly drier conditions at the end of wet seasons (Peterson et al., 2004; Pinzon et al., 2004; Tucker et al., 2002). The long dry season (July–September) of 1994 was not exceptionally long but was extremely dry in northeastern Gabon where the first EHF outbreak of the 1994–1997 series occurred in November 1994. This season was also exceptionally dry in 2001 throughout the northeast, where epidemic EHF re-appeared in August 2001 in the Mendemba region, to be followed by a series of outbreaks in neighbouring RoC. The short dry season (January–February) of 2002, which normally ends in mid March, also extended through April–May in the region (Lahm, 2002).

In conclusion, the sites of confirmed EHF epidemics together with locations where outbreaks of disease assumed

to be EHF were observed in wild animals during the present study constitute an area of approximately 135 000 km<sup>2</sup>, and the eight seropositive human subjects detected in western Gabon are clustered in an adjoining 23 000 km<sup>2</sup> (Figure 1). These areas, which cover 59% of Gabon extending from the northeastern borders to within 100 km of the Atlantic Ocean, should be considered as having the potential for continued EHF outbreaks. The same is true for adjoining parts of Cameroon and RoC, as confirmed by recent detection of antibody to Ebola virus in the sera of wild-caught primates from all three countries (Leroy et al., 2004b). Clearly, many more species and individuals within mammal communities in Gabon and RoC have been affected by disease outbreaks between 1994 and 2003 than previously reported.

The directions of the arrows in Figure 1 are concordant with those published by Walsh et al. (2005) on the basis of Ebola DNA analysis of sequential virus isolates across the geographic region, and are borne out by the wildlife mortality observation dates reported in Figures 1–3 and Table 2. Pinzon et al. (2004) also suggested this direction of disease spread in which the two 1996 outbreaks appear to have originated from ongoing transmission in the 1994 epidemic area further north and east of the 1996 sites (Mayiboth, SHM) in northeastern and central Gabon, respectively. There also appeared to be ongoing transmission in the southeastern part of the central zone in 2000–2001, with subsequent extension east towards Mendemba beginning in 2000.

Although we recognise that other contagious pathogens and parasitic diseases cause mortality in wildlife (Karesh et al., 1995; Njiokou et al., 2004; Stockenstrom et al., 1997) and we cannot confirm that all of the reported disease outbreaks were caused by EHF, the circumstances in which they occurred lead us to believe that the majority can be attributed to EHF: Ebola-Zaire, other Ebola strains or possibly new undiscovered related viruses. Less virulent strains of EHF most likely infected human populations in western Gabon. We conclude that our detection of (generic) antibody to Ebola virus in three regions of Gabon, one area subsequently subject to repeated outbreaks of the disease and the other two free of reported disease but with the presence of antibody in a non-human primate and in five human communities, agrees with this supposition.

Mammals found dead from unknown causes within these areas should be regarded as potentially infected with EHF virus, and public education should discourage scavenging and promote safe procurement of meat. Continued monitoring of wildlife mortality (Rouquet et al., 2005), utilising the knowledge and skills of rural residents, could help safeguard public health and provide key evidence for understanding the natural history of the disease.

#### Conflicts of interest statement

The authors have no conflicts of interest concerning the work reported in this paper.

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