Spatial and Temporal Patterns of Zaire ebolavirus Antibody Prevalence in the Possible Reservoir Bat Species

X. Pourrut,1,2 A. Délatic,2 P. E. Rollin,3 T. G. Ksiazek,3 J.-P. Gonzalez,4 and E. M. Leroy1,2
1Institut de Recherche pour le Développement, UR178, 2Centre International de Recherches Médicales de Franceville, Franceville, Gabon; 3Special Pathogens Branch, Centers for Disease Control and Prevention, Atlanta, Georgia; 4Institut de Recherche pour le Développement, UR178, Mahidol University at Salaya, Nakhonpathon, Thailand

To characterize the distribution of Zaire ebolavirus (ZEBOV) infection within the 3 bat species (Epomops franqueti, Hypsignathus monstrosus, and Myonycteris torquata) that are possible reservoirs, we collected 1390 bats during 2003–2006 in Gabon and the Republic of the Congo. Detection of ZEBOV immunoglobulin G (IgG) in 40 specimens supports the role of these bat species as the ZEBOV reservoirs. ZEBOV IgG prevalence rates (5%) were homogeneous across epidemic and nonepidemic regions during outbreaks, indicating that infected bats may well be present in nonepidemic regions of central Africa. ZEBOV IgG prevalence decreased, significantly, to 1% after the outbreaks, suggesting that the percentage of IgG-positive bats is associated with virus transmission to other animal species and outbreak appearance. The large number of ZEBOV IgG–positive adult bats and pregnant H. monstrosus females suggests virus transmission within bat populations through fighting and sexual contact. Our study, thus, helps to describe Ebola virus circulation in bats and offers some insight into the appearance of outbreaks.

Ebola virus is a negative-stranded RNA virus belonging, like Marburg virus, to the Filoviridae family [1]. Four species of Ebolavirus have been described, 3 of which—Sudan ebolavirus, Zaire ebolavirus (ZEBOV), and Cote d’Ivoire ebolavirus—circulate in the tropical forests of Africa [2, 3]. The species Reston ebolavirus has been detected only in Asia and has never been reported to cause human disease [4–6]. ZEBOV is, by contrast, the most pathogenic species and causes a severe hemorrhagic fever syndrome in humans, associated with a case fatality rate of 80%–90% [7, 8]. After an incubation period of ~1 week, victims rapidly develop high fever, diarrhea, vomiting, respiratory disorders, hemorrhages, and multiorgan failure, leading to death in a few days in the severe forms.

ZEBOV first emerged in 1976 in Yambuku, Democratic Republic of the Congo (DRC) [9]. After a long epidemiological silence, the virus reappeared, causing 4 outbreaks: 1 in 1995 in Kikwit (DRC) [10] and 3 between 1994 and 1997 in northeastern Gabon, in the villages of Mékouka, Mayibout, and Bouqué [11]. There were then 5 outbreaks during 2001–2005 in the region around northeastern Gabon and northwestern Republic of the Congo (RC). The first outbreak occurred from October 2001 through May 2002 in Gabon and RC and consisted of multiple independent epidemic chains of transmission associated with several different viral strains [8]. Each time, the virus emerged in hunters infected from an animal source (chimpanzee, gorilla, or duiker carcasses) found in the forest; it then spread within their village and to the neighboring villages of the region through human-to-human transmission. The virus appeared in Mendemba, Ekata, Etakangaye,
and Grand Etoumbi (Gabon) and in Olloba (RC) during the 2001–2002 outbreak; it then spread to neighboring villages and to Mekambo and Makokou (Gabon) and Mbomo (RC). One human case was reported in January 2002 in Franceville in the south of Gabon, a substantial distance from this epidemic [12]. The following 4 outbreaks were in RC, in Entsimi (January 2002), Mvoula and Yembelengoye (December 2002), Mbandza (October 2003), and Etoumbi (May 2005), spreading to the towns of Kelle and Mbomo [7, 13]. All ZEBOV outbreaks since 1995 have been in the Gabon/RC region, suggesting that this region has been highly epidemic during this period. Interestingly, no human case or infected animal carcass has been reported since June 2005.

There have been major Ebola virus outbreaks in chimpanzees and gorillas in the same region, concomitant with the human epidemics since 2001, devastating local wild populations of these animal species [8, 14, 15]. Viral strains isolated from humans and great apes during outbreaks were not genetically identical, leading some authors to suggest that great apes are infected through independent transmissions of ZEBOV from the reservoir in some ecological conditions [8]. In an attempt to identify this reservoir species, we captured ~1000 small vertebrates, including many specimens of bats, birds, and small terrestrial vertebrates, from 2001 to 2003 in the epidemic Gabon/RC region, and we tested the collections for ZEBOV infection by use of a variety of ELISA and polymerase chain reaction techniques. Ebola virus–specific antibodies were detected in the serum of 7% (16/217) of fruit bats in 3 species—namely, Hypsiprymnodon monticolus, Epomops franqueti, and Myonycteris torquata—and Ebola virus nucleotide sequences were found in the liver and spleen of 3% (13/419) of these bats. It is, therefore, likely that these species are reservoirs of ZEBOV [16]. To characterize the geographical and temporal distribution of ZEBOV within these bat species and to elucidate the mechanisms of transmission of the virus to great apes, we conducted a large-scale serological survey of the 3 possible reservoir species. We tested for ZEBOV-specific IgG in 1390 blood specimens collected during 2003–2006 in both epidemic and nonepidemic regions.

**MATERIALS AND METHODS**

**Collection sites.** Samples were collected in RC, Gabon, and Senegal (figure 1). In RC, bats were captured in Mbomo (latitude [lat] 0°25′ N; longitude [long] 14°41′ E) and near the Odzala National Park, 15 km from Mbomo (figure 1). The climate is equatorial, with 2 dry seasons (December–February and June–August) and 2 wet seasons (March–May and September–November). The mean annual rainfall is 1500 mm. The vegetation is composed of dense semideciduous and evergreen forest with open and closed canopies.

In Gabon, bats were collected in 3 different regions (figure 1). One region was situated near Franceville (lat 1°37′ S; long 13°36′ E), the largest town of the Haut-Ogooué province in southeastern Gabon, 300 km from Mbomo. The climate is tropical transitional, with 1 clear dry season (June–August) and 1 long wet season (September–May), with lower rainfall in December and January. The mean annual rainfall is ~1800 mm. The vegetation is a savanna-forest mosaic. Three sites around Franceville, including both forested areas and savanna, were sampled.

The second region in Gabon was located around Lambarané (lat 0°41′ S; long 11°01′ E), the largest town of the Moyen-Ogooué province in western Gabon and ~500 km from Mbomo. The climate is tropical transitional and humid. The mean annual rainfall is ~2000 mm. The forested vegetation is semideciduous with a particular pattern in the littoral zone, including many lakes, rivers, and swamps. Bats were collected from 3 sites, each in a different biotope.

The third region in Gabon was around Tchibanga (lat 2°25′ S; long 11°01′ E), the largest town of the Nyanga province in southwestern Gabon, ~800 km from Mbomo. The climate is tropical with a long dry season (May–September) and a long wet season (October–April). The mean annual rainfall is ~1500 mm. The vegetation is composed of an evergreen forest and some savannas and caves. Samples were collected at 3 sites in different biotopes.

Senegal was chosen as a sampling site because this country is at the extreme limit of the geographical distribution of *H. monstrosus*, *E. franqueti*, and *M. torquata*. Bats were captured near Mbour (lat 14°25′ N; long 16°57′ E), 80 km from Dakar. The climate is sahelo-soudanian, with an annual rainfall of ~600 mm. The vegetation is degraded forest and savanna. This was the site most remote from the ZEBOV epidemic zone, ~4000 km from RC.

**Collection periods.** Bats were collected in RC both during the outbreak period (in June 2003, referred to as “Mbomo2003,” and in May 2005, referred to as “Mbomo2005”) and during the postoutbreak period (in May 2006, referred to as “Mbomo2006”). Bats were also collected in Gabon during these 2 periods. During the outbreak period, bats were collected in Franceville (February 2005; “Franceville2005”), and in Lambarané (April 2005; “Lambarané2005”). During the postoutbreak period, bats were collected in Franceville (October 2006; “Franceville2006”) and in Tchibanga (February 2006; “Tchibanga2006”). Bats were collected in Senegal in June 2006.

**Bat trapping and blood sampling.** Bats were trapped with mist nets. The nets were opened in the early evening and checked each morning. The captured animals were transported to a field laboratory set up specifically for this study a few kilometers from the capture sites. The animals were killed by ether inhalation. Weight, sex, body measurements, and direct species determination were recorded as described elsewhere.
Figure 1. Sampling countries and sites (green circles) for screening for Ebola virus antibodies in bats. The locations of outbreaks of Ebola virus infection in humans are indicated by red stars for primary cases (infection by direct contact with an infected animal), blue stars for secondary cases (infection by interhuman transmission), or by stars of both colors for both types of cases.

[17]. Blood was collected into EDTA tubes by cardiac puncture. Plasma was obtained after centrifugation at 800 g for 10 min, placed in Cryovials (VWR Prolabo), and then frozen in dry nitrogen. At the end of each capture period, the samples were transported to Franceville for laboratory analysis.

Serological testing. Laboratory testing was performed by the Centre International de Recherches Médicales de Franceville (CIRMF), Franceville, Gabon. Serum samples were tested with a standard IgG ELISA. Briefly, Zaire EBOV antigens diluted 1:1000 in PBS (BioMérieux) were passively adsorbed onto ELISA Maxisorp plates (VWR Prolabo) overnight at 4°C. Control wells were coated with uninfected cultured Vero cell antigens (mock antigens) in the same conditions. The plates were washed 3 times with PBS–0.1% Tween 20 solution. Two aliquots of 100 μL of each serum sample diluted 1:100 in PBS–0.1% Tween 20 containing 5% fat-free milk powder (Difco) were added to wells and incubated overnight at 4°C. After 3 washes, 100 μL of a 1:1 mixture of peroxidase-labeled protein A (1 mg/mL) (Sigma) and peroxidase-labeled protein G (1 mg/mL) (Sigma) diluted 1:1000 in PBS–0.1% Tween 20 containing 5% fat-free milk was added to each well. The plates were incubated for 1 h at room temperature and washed. TMB substrate (Thermo Electron Corporation) was used for detection. The optical density was measured at 450 nm with a PR 5100 ELISA plate reader (Biorad). The specific activity of each serum sample (corrected optical density) was calculated by subtracting the nonspecific background optical density (wells with mock antigen) from the optical density of wells with viral antigen. The cutoff value was calculated as \( M + (3 \times \sigma) \), where \( M \) is the mean corrected optical density for 98 tested bats from Senegal and \( \sigma \) is the SD. A sample was considered to be positive if its corrected optical density was higher than the cutoff value. The positive serum
samples were then tested using the same ELISA method to assign a titer to each sample. Serum samples were titrated at dilutions of 1:100, 1:400, and 1:1600.

Statistical analysis. Access software (version 2002; Microsoft) was used for data analysis. $\chi^2$ and Fisher’s exact tests were used to compare seroprevalence rates with Epi Info software (version 6.04; Epiconcept).

RESULTS

Bat collections. In all, 1390 (67%) of the 2070 bats captured belonged to the 3 possible ZEBOV reservoir species. The percentages of these 3 bat species differed between collections. These 3 species combined constituted 89% of all bats in Mbomo2003, 77% in Mbomo2005, 82% in Mbomo2006, 92% in Franceville2005, 94% in Franceville2006, 11% in Lambarene2005, and 49% in Tchibanga2006. Of these 1390 bats, 725 (52%) were E. franqueti, 111 (8%) were H. monstrosus, and 554 (40%) were M. torquata. The relative abundance of these 3 species in the various collections varied: 30%–87% for E. franqueti, 1%–34% for H. monstrosus, and 0%–68% for M. torquata (figure 2). None of the 115 bats captured in Senegal belonged to the species E. franqueti, H. monstrosus, or M. torquata.

In the captured population of the 3 selected bat species, 1127 (84%) were adults, and 209 (16%) were juveniles. The age ratio (numbers of adults per juvenile) was 5.0 in Mbomo2003, 5.5 in Mbomo2005, 8.6 in Mbomo2006, 1.5 in Franceville2005, 18.8 in Franceville2006, 4.8 in Lambarene2005, and 4.1 in Tchibanga2006.

The overall sex ratio (number of females per male) was 1.1:698 were females, and 638 were males. The sex ratio was 1.1 in Mbomo2003, 1.3 in Mbomo2005, 0.9 in Mbomo2006, 1.1 in Franceville2005, 0.8 in Franceville2006, 2.2 in Lambarene2005, and 1.8 in Tchibanga2006 (table 1).

The proportion of pregnant females varied greatly among collections and among bat species. The number of pregnant females among all adult females was 10 (41.7%) of 24 during the outbreak period and 6 (66.7%) of 9 after the outbreak period for H. monstrosus. The corresponding numbers were 74 (43.5%) of 170 and 57 (34.1%) of 167 for E. franqueti and 19 (20.9%) of 91 and 24 (15.4%) of 156 for M. torquata.

Serological test results. None of the 98 serum samples tested from Senegal contained ZEBOV IgG. In Gabon and RC, ZEBOV IgG was detected in 40 specimens belonging to the 3 bat species. These 40 serum samples again tested positive when titrated at 1:400 dilutions; the optical density decreased linearly with the dilution of the serum samples. The ZEBOV IgG prev-
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The ZEBOV IgG prevalence was 3.4% among female bats (outbreak period, 5.8%; post-outbreak period, 1.1%) and 2.5% among male bats (outbreak period, 4.7%; post-outbreak period, 0.8%), and the difference between sexes was not significant (overall, P = .2; outbreak period, P = .5; post-outbreak period, P = .3). Only 4 (1.9%) of 209 juvenile bats (2 females and 2 males) were ZEBOV IgG positive, whereas 36 (3.2%) of 1127 adult bats were ZEBOV IgG positive; the difference was not significant (P = .3). In Mbomo, although statistical comparison according to age was not possible (no infected juvenile bats were captured in Mbomo2003 or Mbomo2005), ZEBOV IgG was detected only in 23 (6%) of 375 adult bats and not in juvenile bats (0/70) (table 1). Similarly, although there was no significant difference during the outbreak period between pregnant and nonpregnant females of the 3 bat species, the ZEBOV prevalence among pregnant H. monstrosus females was high (3 [33.3%] of 10), relative to that among nonpregnant females (1 [7.1%] of 14) (table 2). We found no significant difference in ZEBOV IgG prevalence between sex and age groups for the 3 bat species.

**DISCUSSION**

In this study, we investigated the prevalence of ZEBOV IgG in 1390 bats collected during 2003–2006 in both epidemic and nonepidemic regions. To our knowledge, this is the first report of such a large geographical and temporal survey of Ebola virus antibodies in wild bats.

Since the first human Ebola outbreak in 1976, 6 large field and laboratory studies have been conducted to discover the reservoir of Ebola virus [3]. The first 2 field studies were conducted in 1976, during the first recorded outbreaks, in the DRC [9] and Sudan [18]. Two other studies were conducted in the DRC, in 1979 [19] and 1995 in the Kikwit region, just after the outbreak [20, 21]. The last 2 studies were conducted in 1995 in the Tai Forest, Ivory Coast [22], and in 1999 in the Central African Republic [23]. In total, ~7000 vertebrates and 30,000 invertebrates have been captured and tested for the presence of Ebola virus. Evidence of Ebola virus infection (RNA sequences) was shown in 6 mice (Mus setulosus and Praomys species) and 1 shrew (Sylvisorex ollula) during the Central African Republic study. However, no firm conclusion as to the Ebola virus reservoir status of these animals could be drawn. Many animal species, including bats, birds, reptiles, molluscs, arthropods, and >33 plant varieties have been experimentally infected with ZEBOV. Two fruit bat species (an Epomophorus

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**Table 1. Ebola virus antibody prevalence in bat reservoir species during outbreak and postoutbreak periods in Gabon and the Republic of the Congo.**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Female Adults</th>
<th>Male Adults</th>
<th>Female Juveniles</th>
<th>Male Juveniles</th>
<th>Undetermined</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mbomo2003</td>
<td>5/71 (7)</td>
<td>3/55 (5.4)</td>
<td>0/8 (0)</td>
<td>0/17 (0)</td>
<td>0/9 (0)</td>
<td>8/160 (5)</td>
</tr>
<tr>
<td>Mbomo2005</td>
<td>9/142 (6.3)</td>
<td>6/107 (5.6)</td>
<td>0/25 (0)</td>
<td>0/20 (0)</td>
<td>0/19 (0)</td>
<td>15/313 (4.8)</td>
</tr>
<tr>
<td>Franceville2005</td>
<td>3/54 (5.5)</td>
<td>2/34 (5.9)</td>
<td>1/36 (2.7)</td>
<td>0/3 (0)</td>
<td>8/151 (5.3)</td>
<td></td>
</tr>
<tr>
<td>Lambaréné2005</td>
<td>1/18 (5.4)</td>
<td>1/6 (16)</td>
<td>0/3 (0)</td>
<td>0/0 (0)</td>
<td>2/29 (6.8)</td>
<td></td>
</tr>
<tr>
<td>Mbomo2006</td>
<td>3/223 (1.3)</td>
<td>2/232 (0.8)</td>
<td>1/34 (2.9)</td>
<td>0/5 (0)</td>
<td>6/513 (1.1)</td>
<td></td>
</tr>
<tr>
<td>Franceville2006</td>
<td>1/56 (1.8)</td>
<td>0/57 (0)</td>
<td>0/6 (0)</td>
<td>0/16 (0)</td>
<td>1/135 (0.7)</td>
<td></td>
</tr>
<tr>
<td>Tchibanga2006</td>
<td>0/53 (0)</td>
<td>0/19 (0)</td>
<td>0/12 (0)</td>
<td>0/2 (0)</td>
<td>0/89 (0)</td>
<td></td>
</tr>
<tr>
<td>Total outbreak (2003–2005)</td>
<td>18/285 (6.3)</td>
<td>12/202 (5.9)</td>
<td>1/76 (1.3)</td>
<td>0/31 (0)</td>
<td>33/653 (5)</td>
<td></td>
</tr>
<tr>
<td>Total postoutbreak (2006)</td>
<td>4/332 (1.2)</td>
<td>2/308 (0.6)</td>
<td>1/52 (1.9)</td>
<td>0/23 (0)</td>
<td>7/737 (0.9)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>22/617 (3.6)</td>
<td>2/81 (2.5)</td>
<td>14/510 (2.7)</td>
<td>2/128 (1.6)</td>
<td>0/54 (0)</td>
<td>40/1390 (2.8)</td>
</tr>
</tbody>
</table>

**NOTE.** Data are no. of bats found to be positive/total no. of bats tested (% positive).
species and a *Tadarida* species) developed transient viremia lasting nearly 4 weeks after intravenous inoculation, without displaying any clinical sign [24, 25]. These results have not been confirmed, but they opened new avenues for research, particularly with regard to the study of bats in the field.

We then captured and tested numerous small vertebrates during outbreaks between 2001 and 2003 in Gabon and RC. For the first time, Ebola virus–specific antibodies were detected in serum, and nucleotide sequences were found in the liver and spleen of 3 fruit bat species (*H. monstrosus*, *E. franqueti*, and *M. torquata*), suggesting that fruit bats may be one the reservoirs of Ebola virus [16]. Indeed, we showed that 7% of the population (16/217) of these 3 bat species had specific ZEBOV IgG, and viral RNA was successfully amplified from 3% (13/419) of specimens [16]. These findings are consistent with the preliminary results obtained in a study of 2 other fruit bat species after experimental infection [25].

Here, we report additional biological evidence consistent with fruit bats being a possible reservoir of Ebola virus. We collected 1390 bats in Gabon and RC, both in epidemic regions and in 3 regions where neither human outbreaks nor animal mortality due to Ebola virus has been reported. Specific ZEBOV IgG was detected in 40 bats, distributed across all of the sites included in this study during the outbreak period; this confirms the status of these species as probable natural hosts and potential reservoirs. Furthermore, the ZEBOV IgG prevalences in Franceville2005 (5.3%) and Lambaréné2005 (6.8%) were not significantly different from those in Mbombo2003 (5.0%) and Mbomo2005 (4.8%), the 2 towns that have been the most affected by Ebola outbreaks during recent years. Indeed, ZEBOV-infected bats appear to be present throughout Gabon and RC and therefore, presumably, in all of the forested tropical regions of central Africa. Our findings confirm previous reports that Ebola virus is present in the forested regions of central Africa and suggest that there may well be unrecognized outbreaks or isolated cases in unmonitored areas [26–28]. Consequently, human outbreaks and animal mortality do not appear to be reliable indicators of the presence of the virus in a region. ZEBOV IgG prevalence may serve as an epidemiological indicator of Ebola virus activity in regions where no other means of virus detection are available. An increase in ZEBOV IgG prevalence in these bat species may reflect favorable conditions for spillover events from bats to other animal species and humans and, if this is the case, may be used to predict Ebola outbreaks.

However, the conditions of ZEBOV transmission from bats to great apes or even to humans are unknown. Outbreaks probably result from a combination of favorable conditions associated with animal demography, ecological phenomena, and viral factors. Increased great ape mortality has frequently been reported during the dry seasons (December 1995 in Mayibout, July 1996 in Bououé, July 2001 in Mekambo, December 2001 in Kelle, December 2002 in Kelle, and December 2003 in Mbandza). It may be significant that the 2 birthing periods of the 3 bat species identified as potential EBOV reservoirs also occur during the dry seasons and, moreover, at a time when fruit is scarce in the forest [29]. Field investigations indicate that great ape and bat populations compete for fruit during these periods, creating the conditions for closer and more frequent contact. Changes in immune function toward the end of pregnancy might lead to a transient resurgence of viral replication in bats, and this could favor transmission to other species via fetal tissues and associated fluids [30]. Interestingly, Hendra virus has been isolated from fetal tissues of experimentally infected bats, and bat placental tissue and associated fluids have been suggested as a possible source of outbreaks in horses [31, 32]. Thus, contact between bats and great apes, as well as higher viral loads in bats, may contribute to interspecies

| Table 2. Ebola virus antibody prevalence in the 3 bat reservoir species (*Hypsipetes monstrosus*, *Epomops franqueti*, and *Myonycteris torquata*) during outbreak and postoutbreak periods in Gabon and the Republic of the Congo. |

<table>
<thead>
<tr>
<th>Sampling period, species</th>
<th>Female</th>
<th>Male</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Adult</td>
<td>Adult</td>
</tr>
<tr>
<td></td>
<td>nonpregnant</td>
<td>pregnant</td>
</tr>
<tr>
<td></td>
<td>Juvenile</td>
<td>Total</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>Undetermined</td>
</tr>
<tr>
<td><em>H. monstrosus</em></td>
<td>0/14 (7.1)</td>
<td>0/3 (13.3)</td>
</tr>
<tr>
<td><em>E. franqueti</em></td>
<td>5/96 (5.2)</td>
<td>5/74 (6.7)</td>
</tr>
<tr>
<td><em>M. torquata</em></td>
<td>3/72 (4.2)</td>
<td>1/19 (5.3)</td>
</tr>
<tr>
<td>Total</td>
<td>9/182 (4.9)</td>
<td>9/103 (8.7)</td>
</tr>
<tr>
<td>Postoutbreak (2006)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>H. monstrosus</em></td>
<td>0/3 (0)</td>
<td>0/6 (0)</td>
</tr>
<tr>
<td><em>E. franqueti</em></td>
<td>3/110 (2.7)</td>
<td>1/57 (1.7)</td>
</tr>
<tr>
<td><em>M. torquata</em></td>
<td>0/132 (0)</td>
<td>0/24 (0)</td>
</tr>
<tr>
<td>Total</td>
<td>3/245 (1.2)</td>
<td>1/87 (1.1)</td>
</tr>
</tbody>
</table>

**NOTE.** Data are no. of bats found to be positive/total no. of bats tested (% positive).
ZEOBV transmission. The significant decrease in ZEOBV IgG prevalence among bats in 2006, both in Mbomo and in Franceville (from 5.0% to 1.1% and from 5.3% to 0.7%, respectively), suggests that outbreak periods and ZEOBV transmission from bats to other species coincide with larger proportions of the bat populations being infected. However, the factors that contribute to fluctuations in the number of infected bats still have to be identified; knowledge of these factors would improve our understanding of the mechanisms of the possible spillover from bats. They may include temporal fluctuations in wild bat populations as a result of environmental conditions, fluctuations in overall virus infection within bat populations as a result of virus evolution [33], and the pathogenicity of ZEOBV in bats. The presence and density of intermediary hosts undoubtedly affect the spread of the epidemic.

There was no significant difference in ZEOBV IgG prevalence among the 3 bat species or between males and females. In contrast, ZEOBV IgG was detected in 6% (23/375) of adult bats but in none (0/70) of the juvenile bats in Mbomo2003 and Mbomo2005. The sample sizes are too small for meaningful statistical analysis, but this nevertheless suggests horizontal rather than vertical virus transmission. Thus, ZEOBV transmission within bat populations may be mainly between adults and not from females to offspring. Virus transmission between males may result from fighting and biting, and transmission between males and females may result from sexual contact. Indeed, the ZEOBV IgG prevalence among pregnant Hypsig Nathus montrosus females during the outbreak period (Mbomo2003, Mbomo2005, and Franceville2005) was as high as 33.3% (3/10), compared with 7.1% (1/14) among nonpregnant adult females. This is consistent with possible sexual transmission of ZEOBV.

Our findings offer some insight into the emergence of Ebola outbreaks in great apes and in humans. They clearly show homogeneous ZEOBV infection in the wild populations of the bat species H. montrosus, E. franqueti, and M. torquata in Gabon and RC. This, presumably, reflects long-term persistence of the virus in the tropical forested regions of central Africa. Our study also indicates that the possible virus transmission from bats to great apes coincides with higher numbers of infected bats in the wild, suggesting that a high density of infected bats, together with high density of great ape populations, favors the appearance of outbreaks in humans. Finally, our findings suggest that virus transmission within bat populations occurs mainly between adults through fighting or mating.

It would be valuable to conduct ecological studies to document contacts between bats (possibly via biological fluids and placental tissues) and great apes. Similarly, virological analyses are required to isolate the virus from bat placenta or fetal fluids.

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