We used an ELISA to determine the prevalence of IgG antibodies specific for the Zaire subtype of Ebola virus in 790 nonhuman primates, belonging to 20 species, studied between 1985 and 2000 in Cameroon, Gabon, and the Republic of Congo. The seroprevalence rate of Ebola antibody in wild-born chimpanzees was 12.9%, indicating that (1) Ebola virus circulates in the forests of a large region of central Africa, including countries such as Cameroon, where no human cases of Ebola infections have been reported; (2) Ebola virus was present in the area before recent outbreaks in humans; (3) chimpanzees are continuously in contact with the virus; and (4) nonlethal Ebola infection can occur in chimpanzees. These results, together with the unexpected detection of Ebola-specific IgG in other species (5 drills, 1 baboon, 1 mandrill, and 1 *Cercopithecus*), may help to narrow the search for the reservoir of Ebola virus. They also suggest that future Ebola outbreaks may occur anywhere in the central African forest region.

Ebola virus (EBOV), like Marburg virus, belongs to the Filoviridae, a family of nonsegmented, negative-stranded RNA viruses [1]. EBOV is composed of 4 known species—namely, Ebola virus Zaire (EBOV-Z), Ebola virus Sudan, Ebola virus Côte d’Ivoire, and Ebola virus Reston. The Zaire, Sudan, and Côte d’Ivoire subtypes were isolated from patients in Africa and are highly lethal for both humans and nonhuman primates [2–4]; in contrast, the Reston subtype, isolated from Asian cynomolgus monkeys, is pathogenic in nonhuman primates (albeit much less so than are the African strains of EBOV) [5] but is apparently nonpathogenic in humans [6, 7]. EBOV-Z occurs in Gabon, the Republic
local outbreaks of Ebola threaten these species’ survival. In an attempt to explain Ebola outbreaks in nonhuman primates, it has been postulated that a single wave of Ebola infection is slowly advancing across central Africa, from southern Cameroon through northeastern Gabon and into the RC [9]; however, genetic studies suggest that EBOV has existed in its unidentified natural reservoir and that recent outbreaks have resulted from multiple infectious events occurring in specific environmental conditions [2]. Our serologic survey of wild nonhuman primates in central Africa was intended to determine which species the virus can infect, whether nonlethal natural infection occurs in nonhuman primates, and whether EBOV was present in these species before the recent Ebola outbreaks in this region.

MATERIALS AND METHODS

Between 1985 and 2000, samples were obtained from 790 nonhuman primates belonging to 20 species (table 1). The samples for epidemiologic and virologic studies were collected in Gabon and the RC by the Centre International de Recherches Médicales de Franceville (CIRMF) and the Centre Pasteur du Cameroun (CPC; Yaoundé); in Gabon, the blood samples were collected from wild-born animals housed in rescue centers (n = 51), wild-born animals housed at the CIRMF (n = 195), wild-born animals kept as pets by hunters and their families (n = 39), captive-born animals housed at the CIRMF (n = 145; 115 mandrills and 30 chimpanzees), and wild-born animals housed at either the CIRMF or in rescue centers (n = 7); in the RC, the blood samples were collected from wild-born orphan chimpanzees housed at the Habitat Ecologique et Liberté des Primates (HELP) Sanctuary in Conkouati-Douli National Park (n = 35); in Cameroon, the blood samples were collected from wild-born animals housed in either zoos or rescue centers (n = 311). In addition, muscle-tissue samples were collected from nonhuman primate carcasses (n = 7) found in Gabon during studies of EBOV reservoirs in Gabon and the RC. The 790 samples, therefore, can be subdivided into 145 from captive-born nonhuman primates, 638 from wild-born nonhuman primates, and 7 from carcasses.

Serum and plasma were obtained by centrifugation and Ficoll-diatrizoate density–centrifugation, respectively, and then aliquoted and stored at −80°C. Muscle tissue obtained from 5 gorilla and 2 chimpanzee carcasses was fragmented and homogenized in PBS, and the final supernatant was filtered for serological tests, antigen detection, and RNA amplification.

IgG ELISA was performed, as previously described [11], by coating Maxisorp plates (Nunc) with EBOV-Z antigens diluted to 1:1000 in PBS and incubating the solution in the wells, at 4°C overnight. Control plates were coated with uninfected Vero cell–culture antigens diluted to 1:1000 in PBS, and the solution was incubated in the wells, at 4°C overnight. Serum samples were diluted to 1:100 in 5% nonfat milk and 0.1% Tween 20 in PBS, and the solution was incubated in the wells, at 4°C overnight. Binding was visualized by use of a peroxidase-labeled antibody to human IgG (Sigma) and the TMB detector system (Dynex Technologies). Optical density was measured at 450 nm, with an ELISA plate reader. Sera from 145 captive-born nonhuman primates housed at the CIRMF were used as negative controls; for each sample, we calculated the optical density (OD) of the antigen-coated well and subtracted that of the corresponding control well, to obtain the corrected OD. The cutoff value (CO) was calculated as follows: CO = M + (3 × σ), where M is the average of the corrected OD of the 145 negative controls, and σ is the SD. A sample was considered seropositive when its corrected OD was greater than the CO. Plasma and serum samples, obtained from animals living in Gabon and the RC, that were seropositive in these serological assays were used for antigen detection [12] and viral PCR amplification, as described elsewhere [13].

RESULTS

In total, blood samples from 783 nonhuman primates and muscle-tissue samples from 7 nonhuman primates were screened for EBOV-specific IgG. The samples were collected, between 1985 and 2000, from 20 species of nonhuman primates. These animals were from Gabon (444 animals, 11 species), Cameroon...
In the 638 wild-born nonhuman primates, the seroprevalence of EBOV-specific IgG was 12.9% (29/225) in chimpanzees, 2.8% (6/215) in the genus *Mandrillus*, 4.0% (1/25) in baboons, and 0.9% (1/107) in the genus *Cercopithecus* (table 1; table 2). The seroprevalence rate was 2.7% (7/256) in the Papionini-genera tribe (in this study, composed of *Cercocebus torquatus*, *Mandrillus leucophaeus*, *Mandrillus sphinx*, and *Papio anubis*).

Seroprevalence rates of EBOV-specific IgG in nonhuman primates of the same species differed markedly between the different geographic areas; of the 292 wild-born animals sampled in Gabon, only 3 animals (all chimpanzees) had detectable EBOV-specific IgG in their blood. The seroprevalence rate of EBOV-specific IgG was 4.2% (3/71) in the wild-born chimpanzees tested in Gabon and 14.3% (5/35) in the chimpanzees sampled in the RC (table 2). In Cameroon, 21 of the 119 blood samples from chimpanzees (seroprevalence rate, 17.6%) and 5 of the 34 blood samples from drills were seropositive for EBOV-specific IgG; in contrast, only 3 of the other 141 animals (excluding gorillas) from Cameroon had detectable levels of EBOV-specific IgG in their blood (1 *Cercopithecus neglectus*, 1 mandrill, and 1 baboon; table 1). No EBOV antigens or genetic sequences were detected in the blood samples from animals in Gabon and the RC that were seropositive for EBOV-specific IgG.

**DISCUSSION**

Because 5 Ebola outbreaks in humans have occurred during the past 3 years in the border region between Gabon and the RC (figure 1), we conducted an ELISA-based survey of EBOV-specific IgG antibodies in blood and muscle-tissue samples from a wide range of nonhuman primate species in Cameroon, Gabon, and the RC. The ELISA method used EBOV-Z antigens, and it detected EBOV-specific IgG. Cross-reactions with antibodies to the Sudan and the Côte d’Ivoire EBOV subtypes can occur in such an assay, but the presence of these antibodies in our samples was highly unlikely, for the following reasons. First, each EBOV subtype circulates in a specific ecological region. The Sudan subtype is found in eastern Africa: 3 outbreaks occurred in Sudan, 1 in 1976, 1 in 1977, and 1 in 2004, all in the region bordering the DRC, and 1 occurred in Uganda in 2000. The Côte d’Ivoire subtype is found in western Africa: 1 nonhuman fatality occurred in Côte d’Ivoire (near Liberia) in 1995. The Zaire subtype is found in west-central Africa: 2 outbreaks occurred in the DRC, 1 in 1976 (around Yambuku near the Central African Republic), and 1 in 1995 (around Kikwit), and repeated outbreaks have occurred in Gabon and the RC since 1995. No EBOV subtype has ever been found outside its prescribed region. Second, Cameroon, Gabon, and the RC, which share a forested area in west-central Africa, are ecologically very different and several thousand kilometers distant from the regions where the other African strains of EBOV are found. Third, the region that we studied has been hit by 8 Ebola outbreaks, totaling ~500 reported human cases; all the patients tested (~300) were infected with the Zaire subtype only.

Fourth, we have identified EBOV in samples from several nonhuman primate carcasses in the region, and all the virus material that was detected belonged to the Zaire subtype [2]. We therefore assume that the antibodies detected in the present study were indeed specific for EBOV-Z.

None of the blood samples from the 145 captive-born nonhuman primates was seropositive for EBOV-specific IgG. In

**Figure 1.** Recent Ebola outbreaks in humans and habitats of seropositive animals in central Africa.

**Table 2.** Seroprevalence rates of Ebola-specific IgG in wild-born nonhuman primates in central Africa.

<table>
<thead>
<tr>
<th>Family, tribe, genus</th>
<th>Cameroon</th>
<th>Gabon</th>
<th>RC</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cercopithecidae</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cercopithecinae</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Cercopithecus</em></td>
<td>1/74</td>
<td>0/33</td>
<td>...</td>
<td>1/107 (0.9)</td>
</tr>
<tr>
<td>Papionini</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Papio</em></td>
<td>1/25</td>
<td>...</td>
<td>...</td>
<td>1/25 (4.0)</td>
</tr>
<tr>
<td><em>Mandrillus</em></td>
<td>6/52</td>
<td>0/163</td>
<td>...</td>
<td>6/215 (2.8)</td>
</tr>
<tr>
<td><em>Cercocebus</em></td>
<td>0/13</td>
<td>0/3</td>
<td>...</td>
<td>0/16 (0.0)</td>
</tr>
<tr>
<td><strong>Subtotal</strong></td>
<td>7/90</td>
<td>0/166</td>
<td>...</td>
<td>7/256 (2.7)</td>
</tr>
<tr>
<td>Pongidae</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Gorilla</em></td>
<td>2/17</td>
<td>0/13</td>
<td>...</td>
<td>2/30 (6.7)</td>
</tr>
<tr>
<td><em>Pan</em></td>
<td>21/119</td>
<td>3/71</td>
<td>5/35</td>
<td>29/225 (12.9)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>31/300</td>
<td>3/283</td>
<td>5/35</td>
<td>39/618 (6.3)</td>
</tr>
</tbody>
</table>
The 12.9% seroprevalence rate in these chimpanzees confirms that wild-born chimpanzees are naturally exposed to EBOV. Several Ebola outbreaks have occurred in chimpanzees in central Africa during the past 10 years, a period during which chimpanzee population levels have fallen by an estimated 80% [2, 9]. Many chimpanzee carcasses have been found in the forests of Gabon and the RC, and all those tested have been seropositive for EBOV. However, the case-fatality rate of natural (or even experimental) Ebola infection in chimpanzees is unknown. Experimental infection [16], in conjunction with the massive chimpanzee-population decline attributed to EBOV, initially suggested that lethality approached 100%; however, the 12.9% seroprevalence rate observed in the present study indicates that, like humans, chimpanzees in the wild can recover from EBOV infection or can be infected but remain asymptomatic [17, 18]. In a study conducted during the 1995 Ebola outbreak in humans in Kikwit City, DRC [19], that employed the same ELISA technique that was used in the present study, the seroprevalence rate in persons living in neighboring villages was 9.3%, compared with 2.2% in those living in Kikwit City. The authors of that report concluded that villagers were exposed to EBOV more often than were town dwellers. Thus, both chimpanzees and villagers from the forested areas of central Africa may live in close contact with the virus, or at least with its natural reservoir [20].

We found marked differences in the seroprevalence rates of EBOV-specific IgG in chimpanzee populations in different countries (notably, 17.6% in Cameroon and 14.3% in the RC). The high seroprevalence rate found in the chimpanzees in Cameroon is surprising, because no human or animal cases of Ebola infection had previously been reported there. This further confirms that Ebola is present in the forests of central Africa and suggests that isolated cases or unrecognized outbreaks have occurred periodically in Cameroon and, probably, in unmonitored areas of nearby countries. These findings suggest that Ebola outbreaks may occur in the future in Cameroon and in other central African countries that have tropical forest regions, and this possibility should stimulate awareness, among local health authorities, of the possibility of Ebola infection in humans.

In Gabon, the area, with the RC, hit hardest by Ebola outbreaks in humans (4 outbreaks since 1995), blood samples from only 3 (4.2%) of 71 wild-born chimpanzees were seropositive for EBOV-specific IgG. The difference in the seroprevalence rates of EBOV-specific IgG in chimpanzees in the 3 countries in the present study could result from the circulation of either different subtypes or several genotypes with differing virulence. Given both the geographic distribution of the Ebola subtypes and the lack of a relationship between virulence and genotype in outbreaks in humans, the most likely reason for this unexpected result is a sampling bias.

Blood samples from only 2 (6.7%) of the 30 wild-born gorillas were seropositive for EBOV-specific IgG, even though Ebola infections regularly devastate gorilla populations in both Gabon and the RC. EBOV genetic sequences and antigens were detected in muscle-tissue samples from 5 gorilla carcasses, but these samples were seronegative for EBOV-specific IgG, indicating that the animals may have died from Ebola infection but did not develop specific IgG responses. This situation is similar to that in humans, in whom fatal infection is characterized by an impaired humoral response and a failure to generate specific IgG [17]. Thus, the small number of gorillas seropositive for EBOV-specific IgG might be due to either a very high lethality in this species or, again, a sampling bias.

Surprisingly, blood samples from 5 drills, 1 mandrill, 1 baboon, and 1 Cercopithecus were seropositive for EBOV-specific IgG. Indeed, we have never found EBOV-infected carcasses of these nonhuman primate species in the wild, nor have we noted any increase in mortality or change in population density concomitant with Ebola outbreaks in humans; in addition, the natural habitats of drills and baboons are located in, respectively, the extreme west and the extreme north of Cameroon, where no human cases of Ebola have been described. If these are true seropositive reactions, they might, together with the seroprevalence rates of EBOV-specific IgG in great-ape populations, shed light on the EBOV natural reservoir of EBOV. Despite numerous studies designed to identify the reservoir of EBOV, no specific
animal has emerged as a major candidate. Epidemiological observations during the 1976 Ebola outbreaks in the DRC and Sudan implicated bats [21, 22], although EBOV nucleotide sequences and EBOV-like nucleocapsids were detected in organs of rodents such as mice and shrews in the Central African Republic [23]. Whatever the reservoir, its habitat probably coincides with that of certain nonhuman primates. Perhaps they feed on the same vegetation or fruits at the same time, with contamination occurring through direct physical contact.

In conclusion, the present study is the first serological survey of EBOV infection in nonhuman primates in central Africa, where several outbreaks in humans have occurred during recent years. The results show a high seroprevalence rate of EBOV-specific IgG in chimpanzees, indicating that Ebola has circulated for a long time in the forests of central Africa, that chimpanzees are continuously in contact with the virus, and that nonlethal Ebola infection can occur in chimpanzees, just as it does in humans.

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