SUPPLEMENT ARTICLE

Risk Factors for *Zaire ebolavirus*–Specific IgG in Rural Gabonese Populations

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Background. In Gabon, several Ebolavirus outbreaks have occurred exclusively in the northeastern region. We conducted a large serosurvey to identify areas and populations at risk and potential demographic, clinical, and behavioral risk factors.

Methods. Blood samples and clinical and sociodemographic data were collected from 4349 adults and 362 children in a random sample of 220 villages in the 9 provinces of Gabon. An enzyme-linked immunosorbent assay was used to detect *Zaire ebolavirus* (ZEBOV)–specific IgG, and thin blood smears were used to detect parasites. Logistic regression was implemented using Stata software (Stata), and a probability level of <.05 was considered to be statistically significant.

Results. The prevalence of ZEBOV-specific IgG was 15.3% overall, increasing to 32.4% (*P*< .001) in forest areas. No sociodemographic risk factors were found, but the antibody prevalence increased linearly up to 20 years of age. Chronic arthralgia and amicrofilaremia were the only factors associated with ZEBOV seropositivity.

Conclusions. These findings confirm the endemicity of ZEBOV in Gabon and its link to the ecosystem. Human antibody positivity would appear to be to the result of exposure to contaminated fruits.

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Ebolavirus (EBOV) and Marburgvirus (MARV) are among the most virulent pathogens for humans and nonhuman primates, causing outbreaks of fulminant hemorrhagic fever (HF). The EBOV genome consists of a single negative strand of RNA that encodes 7 linearly arranged gene products. There is only 1 known species of MARV (*Lake Victoria marburgvirus)*, whereas EBOV is subdivided into 5 species: *Zaire ebolavirus* (ZEBOV), *Côte d'Ivoire ebolavirus* (CIEBOV), *Sudan ebolavirus* (SEBOV), *Reston ebolavirus* (REBOV) [1], and *Bundibugyo ebolavirus* (BEBOV), recently discovered in Uganda [2]. EBOV pathogenicity depends on the species. The case-fatality rate can reach 90% with ZEBOV infection, 50%–55% with SEBOV infection, and 26% with BEBOV infection. CIEBOV has caused a single recorded human case of nonfatal HF, and REBOV does not seem to be pathogenic for humans. After an incubation period of 4–6 days, clinical manifestations occur in 3 successive phases, which often overlap. Disease onset is characterized by general symptoms, such as pyrexia, headache, arthralgias, and myalgias. The second phase begins 2–4 days later, with gastrointestinal symptoms and a morbilliform rash. In general, the third, terminal phase begins 5–7 days after onset, with hemorrhagic manifestations usually leading to death within 3–5 days [3, 4]. After lengthy and laborious convalescence, survivors developed a variety of sequelae, including persistent arthralgias [5].

To date, 16 outbreaks of EBOV infection and 9 outbreaks of MARV infection have been reported [6]. Since the discovery of EBOV in 1976 in Nzara (Sudan) and Yambuku (Democratic Republic of Congo [DRC]) [7, 8], epidemics have been reported in other central African countries. ZEBOV infection epidemics have only been recorded in Gabon, RC, and DRC, whereas SEBOV infection epidemics have occurred in Sudan and Uganda, and a BEBOV infection epidemic

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was recorded in Uganda. During 1994–2005, northeastern Gabon and northwestern RC experienced a total of 7 outbreaks. Four outbreaks occurred in Gabon during 1994–2002, affecting >20 villages and towns, with 208 cases and 151 deaths (overall case-fatality rate, 72.6%) [9–13]. During 2001–2002, outbreaks consisted of multiple independent introductions of the virus into human populations [14].

Since the first epidemic in 1976, many serosurveys have been conducted to understand EBOV circulation and to identify factors associated with human outbreaks. The results vary according to the type of test used. Immunofluorescent antibody test (IFAT)-based surveys showed higher prevalence rates (up to 33%) than did enzyme-linked immunosorbent assay (ELISA)–based studies [15–21], but ELISA is more specific and sensitive than are IFAT tests [22]. For instance, in Gabon, of 253 and 213 human serum samples collected in 1980 in Ambinda and in 1985 in Franceville, both situated in a nonepidemic area (Haut Ogooue Province, southeastern Gabon) 6.3% and 10.3% of samples, respectively, were IFAT positive for anti-EBOV IgG [19–23]. Another IFAT-based survey showed an antibody prevalence of up to 21% [16]. In contrast, ELISA-based serosurveys of epidemic areas have given seroprevalence rates of 1.2%–16.6% [11, 24–26]. Similarly, in an IFAT-based survey conducted during 1985–1987 in 6 central African countries, high prevalence rates were found in nonepidemic localities: 32.7% of 327 serum samples in Bangui, Central African Republic (CAR) [16] and 21.3% of 4295 serum samples collected in CAR [20]. The seroprevalence was highest in forest areas, especially among hunters, identifying this ecosystem as at risk and the main source of contamination in humans [18, 27]. Pygmy populations (mainly hunter-gatherers) living in the rainforest had a higher seroprevalence than did farmers [17, 18, 21]. The 2001–2002 epidemics first affected hunters who handled infected animal carcasses [14, 28].

Recently, with a specific ELISA method, we showed that ZEBOV IgG positivity in rural human populations of Gabon is associated with cellular and humoral immune responses. We suspected that this seropositivity could be attributable to asymptomatic infection, mild disease, or simple exposure to viral particles [29]. In our study, we attempted to identify potential geographic, demographic, behavioral, and clinical risk factors for ZEBOV seropositivity.

MATERIALS AND METHODS

Area of Study

The survey covered rural Gabonese populations and included all ecosystems. Gabon is located in central Africa, is crossed by the equator, is limited to the west by an 800-km Atlantic coast, and has a surface area of 267 667 km^2, nearly 80% of which is covered by rainforest [30]. Gabon is divided into 9 provinces, 47 departments, 50 municipalities, and 2048 villages. The population is ~1.5 million inhabitants, 73% of whom live in urban areas. Rural populations, located along roads and rivers, belong to 10 ethnic groups with different customs, faiths, and languages. Subsistence farming, hunting, gathering, and fishing are the main activities. Geographically, the country is divided into 3 ecosystems: forest, savannah, and lakeland. The forest extends from west to east and is divided into grassland and deep forest. Grassland lies at low elevations, forming a coastal strip 20–300 km wide, juxtaposing the deep forest, which is composed of mountains and plateaus, extending to the Congo border. The mountains form a band 60–100 km wide, parallel to the coast. The plateaus are divided into interior and northeastern forests. The deep forest is characterized by the presence of okoumé trees, except in the northeast. The south and southeast contain scattered areas of savannah and steppe. Lakeland, a coastal and continental marine ecosystem, is located in Estuaire, Ogooué Maritime, and Moyen Ogooué Provinces, around the mouth of the Ogooué River (Figure 1).

Ethical Considerations

The study protocol was approved by the Gabonese Ministry of Health (research authorization 00093/MSP/SG/SGAQM) (Supplementary Figure 1). The health director of each province and the chiefs of each village received written information. In each village, the inhabitants received information on the study, and individual written consent was required for obtainment of blood samples (parental consent for children) (Supplementary Figure 2).

Study Population

The survey was conducted by a multidisciplinary team, including a doctor from the Gabonese Ministry of Health, a nurse, an epidemiologist, a veterinarian, and laboratory technicians, during nine 1-month field missions (from June 2005 through September 2008). Stratified random sampling was used. The stratification was based on the 9 provinces, and the sample size was calculated as $n = \varepsilon^2 [p(1-p)]/e^2$; with $\varepsilon = 1.96$ (α risk, 5%), e (precision) = 2%, and p (expected prevalence) of 2%–10%. Then, in each province, 10–40 villages were randomly selected. All healthy volunteers aged >16 years who had been residing in the village for >1 year and who accepted obtainment of blood samples were included in the study. Another specific field mission was performed to enroll children who were randomly selected from 6 villages of Ogooué Ivindo Province. In addition to a free medical examination, blood smears for malaria diagnosis and field blood typing were proposed, and basic medicines were provided to all participants. All the villages were geolocated.

Epidemiological Records

Questionnaire. Adult participants were asked to complete an administered 2-page questionnaire, generally in French or in the local language, with the help of a local health care worker (Supplementary Figure 3). The following information was collected: demographic data (age, sex, marital status, and level of

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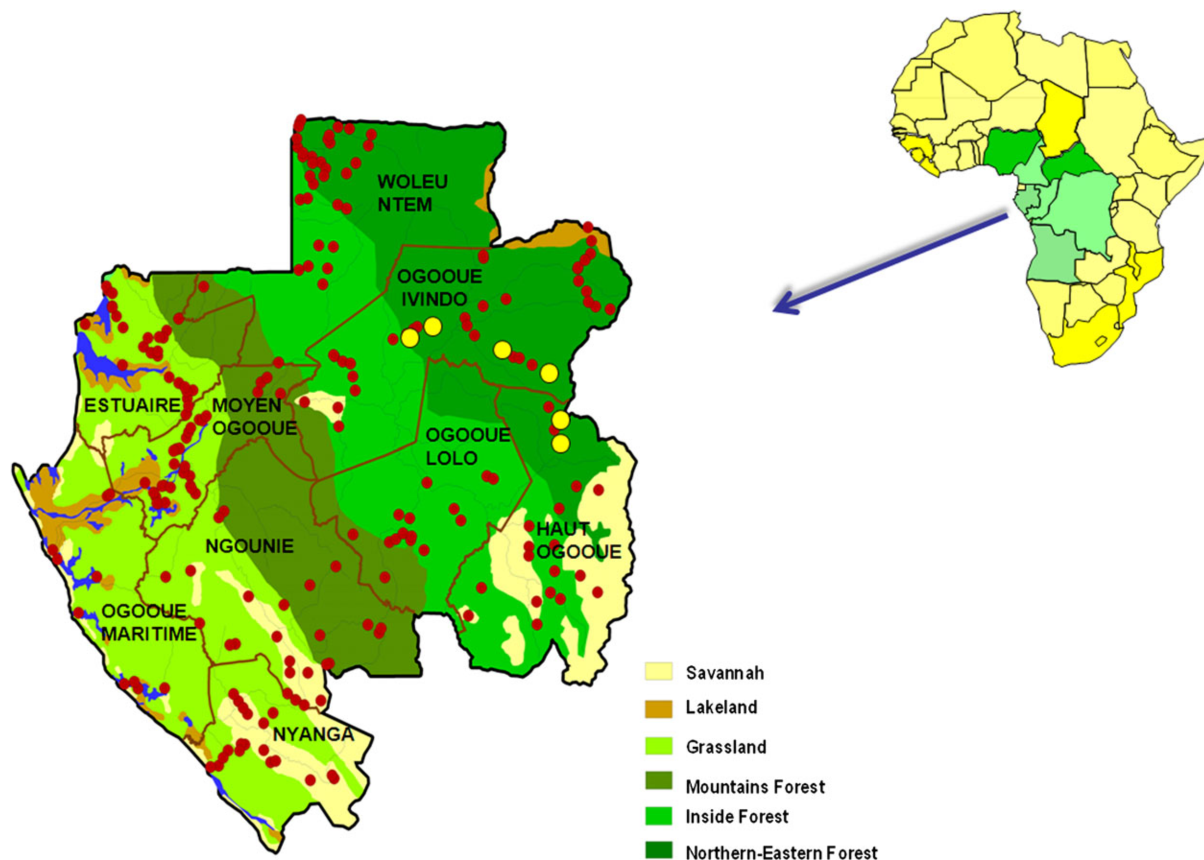

Figure 1. Map of Gabon with limits of administrative regions and location of villages surveyed (red circles), strictly georeferencied and generated by MAPINFO. All ecosystems are represented in thorough colour. Villages where children under 16 years were included are indicated by yellow circles.

education), geographic location (name of the village, length of residence, and province), tattooing and scarification, and contact with wild animals (monkeys, duikers, and bats) through hunting, eating, cooking, butchering, or pet-keeping. Only age and sex data were collected for children.

Medical Data. All the participants were interviewed to record medical history (hemorrhagic fever, prolonged fever, chronic arthralgias, skin diseases, reasons for hospitalization if any, adult *Loa loa* worm crossing the subconjunctiva, and swellings). The participants were also briefly examined for anemia, lymphadenopathies, cutaneous and neurological disorders, and swelling of joints or abdominal organs.

Laboratory Analyses

Blood Sample Collection. The team resided in the main town of each administrative region, and laboratory facilities were established inside the general hospital. Blood samples were collected, usually at the village health care center, in two 7-mL EDTA Vacutainer tubes (VWR International). The tubes were stored in the dark at 4°C until arrival at the laboratory. Plasma was obtained by centrifugation (10 minutes, 2000 *g*) each evening and stored in aliquots at −20°C. At the end of the field mission, the aliquots were transferred to the Centre International de Recherches Médicales de Franceville, Gabon, on dry ice, and were stored at −80°C until analysis.

ZEBOV-Specific IgG Detection and Cutoff Calculation. An IgG ELISA method was used, as described elsewhere [22], with antigens kindly provided by the Special Pathogens Branch, Centers for Disease Control and Prevention. In brief, Maxisorp plates (Nunc) were coated with ZEBOV antigens diluted 1:1000 in phosphate-buffered saline (PBS) overnight at 4°C. Control plates were coated with uninfected Vero cell culture antigens in the same conditions. Serum samples were diluted 1:1600 in 5% nonfat milk in PBS-Tween 20 (0.1%) and were incubated in the wells overnight at 4°C. Binding was visualized by using a peroxidase-labeled antibody to human IgG (Sigma) and the TMB detector system (Dynex Technologies). Optical density (OD) was measured at 450 nm with an ELISA plate reader. For each sample, we calculated the corrected OD as the OD of the antigen-coated well minus the OD of the corresponding control well. A panel of 104 serum samples from inhabitants of Marseille, France, who had never visited Africa, were used as negative controls for cutoff calculation (all tested negative), with Western blot confirmation, as described elsewhere [29].

Parasitological Analyses. *L. loa* microfilaremia was detected by direct reading of 10 μL of fresh uncoagulated blood

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between a microscope slide and coverslip, using an optical microscope with a x100 objective. When microfilaria were found, their total number on the slide was multiplied by 100 to express parasitemia as mf/mL of blood. When no microfilaria were found, Knott's concentration technique was used: 1 mL blood was diluted with 9 mL of PBS in a conical tube, and 200 µL of saponin (2%) was added to induce hemolysis. The tube was then centrifuged (10 minutes, 500 *g*), and the supernatant was discarded. The entire pellet was scanned for microfilaria under a microscope with a x100 objective. Filarial speciation was based on size and movement.

To detect *Plasmodium falciparum,* thick and thin blood films were stained with Giemsa and examined at x1000 magnification. A sample was considered to be negative if no parasites were detected after examining 1000 erythrocytes.

Statistical Analysis

To take into account the linear association between ZEBOV seroprevalence and the age group, we used a trend test across ordered groups. All variables were screened to describe our population, and the χ^2 test (Fisher's exact test when appropriate) was used to identify statistical associations with ZEBOV seroprevalence. Overall and subgroup-specific ZEBOV seroprevalence rates were estimated. Potential differences between risk factors and seroprevalence by ecosystem were found. Multivariate logistic regression models stratified by ecosystem were constructed from risk factors with a significance of ≤0.10 in univariate analysis, using a backwards stepwise elimination procedure. Odds ratios (ORs) and exact 95% confidence intervals (CIs) were used to assess the association between potential risk factors and ZEBOV seroprevalence. $P < .05$ was considered to be statistically significant. Stata software, version 10 (Stata) was used for all analyses.

RESULTS

Characteristics of the Study Population

Ten field missions lasting a total of 38 weeks were performed from June 2005 through September 2008. The survey covered 220 villages, representing 10.7% of all villages in Gabon. We enrolled a total of 4349 adults and 362 children, representing 1.2% of the 387 670 rural inhabitants in Gabon. From 0.6% through 1.9% of inhabitants in each province were enrolled (Table 1). A total of 3458 individuals (79.5%) were enrolled in the forest ecosystem, 448 (10.3%) in savannah, and 443 (10.2%) in lakeland. Fourteen outbreak villages, with a total of 265 inhabitants, were also selected. The male-to-female sex ratio of the adult population was 0.89, and their mean age (±standard deviation [SD]) was 46 ± 14 years (range,15–90 years). They included 1273 hunters (29.3%), and 2874 participants (69%) had resided in their village for >10 years (Table 2). Among the 362 children enrolled, 56.2% were male (sex ratio, 1.3), 51 (12.9%) were aged 1–5 years, 156 (39.5%) were aged 6–10 years, 168 (42.5%) were aged 11–15 years, and 20 (5.1%) were aged 16–20 years.

Table 1. Regional Distribution of the Study Population

	Villages			Population		
Provinces	Total	Surveyed	%	Total	Tested	%
Estuaire	100	30	30	53 459	314	0.6
Haut Ogooué	171	18	10.5	50 196	364	0.7
Moyen Ogooué	226	31	13.7	31 089	618	1.9
Ngounié	233	22	9.4	52 115	455	0.8
Nyanga	113	16	14	20 544	403	1.9
Ogooué Ivindo	197	41	20.8	34 540	618	1.8
Ogooué Lolo	190	18	9.5	36 877	421	1.1
Ogooué Maritime	188	10	5.3	12 940	205	1.6
Woleu Ntem	630	34	5.4	95 910	951	0.9
TOTAL	2048	220	10.7	387 670	4349	1.02

Geographic Distribution of ZEBOV Seroprevalence

The overall prevalence of ZEBOV-specific IgG in the study population was 15.3%.

The highest prevalence (21.5%) was found in Ngounié, and the lowest (1.5%) was in Ogooué Maritime. The prevalence was 20.1% in the epidemic Ogooué Ivindo Province and 18.9% and 19.2% in the nearby Woleu Ntem and Ogooué Lolo Provinces, respectively (Table 3).

The seroprevalence was significantly higher ($P < .0001$) in the forest region (17.6%) than in savannah (10.5%) and lakeland (2.7%). The seroprevalence was significantly lower ($P < .0001$) in lakeland than in savannah. In the forest ecosystem, the

Table 2. Sociodemographic Characteristics of the Study Population (n = 4349) and Distribution According to the Ecosystem

Characteristics		Number	%
Sex	Male	2049	47.1
	Female	2300	52.9
Age	<15	362	
	15–30	746	17.1
	31–45	1159	26.7
	46–60	1695	39
	>60	749	17.2
Occupational activity	Nonhunters	3076	70.7
	Hunters	1273	29.3
Length of residence	<10 years	1281	31
	≥10 years	2874	69
Geographic location	Lakeland	443	10.2
	Savannah	448	10.3
	Forest	3458	79.5
	Grassland	*918*	*21.1*
	Deep forest	*2540*	*58.4*

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Table 3. Prevalence of ZEBOV-Specific IgG in Gabon According to the Administrative Region

Provinces	+/Total	Prevalence (%)	95% CI	Extreme limits
Estuaire	48/314	15.3	11.5–19.8	0–27.5
Haut Ogooue	46/364	12.6	9.4–16.5	0–26.9
Moyen Ogooue	36/618	5.8	4.1–8	1–13.2
Ngounie	98/455	21.5	17.8–25.6	12.1–32.4
Nyanga	51/403	12.7	9.6–16.3	0–21.2
Ogooue Ivindo	124/618	20.1	17–23.4	7.7–25.6
Ogooue Lolo	81/421	19.2	15.6–23.3	6.1–28.3
Ogooue Maritime	3/205	1.5	.3–4.2	0–6.9
Woleu Ntem	180/951	18.9	16.5–21.6	11.9–26.1
TOTAL	667/4349	15.3	14.3–16.5	0–32.4

seroprevalence was significantly lower ($P < .0001$) in grassland than in mountains and interior and northeastern forest zones but higher ($P < .0001$) than in lakeland (Table 4). There was no difference between grassland and savannah ($P = .3$) or between mountains or interior and northeastern forest. No statistically significant difference in seroprevalence was observed between epidemic (20%) and nonepidemic (19.6%) villages of the northeastern forest in Ogooué Ivindo Province ($P = .9$) or between villages of the 1996–1997 outbreaks (18%) and villages of the 2001–2002 outbreaks (20.1%; $P = .6$).

Sociodemographic Characteristics

Univariate analysis of the adult population showed no correlation between seropositivity and sociodemographic factors in the deep forest or savannah. In grassland, the seroprevalence was significantly higher in male individuals (OR, 1.75; 95% CI, 1.16–2.63; $P = .007$) and in individuals who ate bats (OR, 1.74; 95% CI, 1.09–2.75; $P = .02$) (Supplementary Table 1). In lakeland, individuals who butchered game had a significantly higher seroprevalence, compared with other participants ($P = .02$) (Supplementary Table 2).

The overall seroprevalence in the child population was 12.9% (95%CI, 9.8%–16.7%), with no statistically significant difference between male (12.2%) and female individuals (13.9%). The youngest seropositive person was 2 years of age. Increasing age was an important risk factor, with a statistically significant difference ($P = .02$) across the age groups. A linear association was observed when we added individuals aged ≤20 years who lived in the same department and were had samples obtained during the initial field study. For persons >20 years of age, the trend was sinusoidal, both overall and in each ecosystem (Figure 2).

Medical Data

None of the participants declared a history of Ebola-like HF or had been in contact with a known EBOV-infected person. Associations between the seroprevalence rate and the participant's medical history were examined separately in each ecosystem. In the deep forest, eye worm, joint swelling, and abdominal pain were significantly more frequent in IgG-positive persons ($P = .03$) (Supplementary Table 3), whereas eye worm was more frequent in northeastern forest (OR, 1.42 95% CI, 1–2; $P = .05$) and joint swelling was more frequent in the mountain forest (OR, 2.33; 95% CI, 1.07–5.08; $P = .03$). Abdominal pain (OR, 0.42; 95% CI, .18–.99; $P = .04$) and chronic arthralgias (OR, 2.94; 95% CI, 1.40–6.17; $P = .003$) were significantly more frequent in savannah (Supplementary Table 4). No medical risk factors for EBOV seropositivity were noted in the other ecosystems (interior forest, grassland, and lakeland).

Parasitological Findings

Overall, 3.8% of individuals had both specific IgG and *L. loa* carriage. This association was significantly stronger in the forest ($P < .0001$). Among IgG-positive individuals, 25% were *L. loa* carriers, whereas 17% of *L. loa* carriers had specific IgG. One percent of individuals had both specific IgG and *P. falciparum* carriage. In lakeland, a positive correlation was noted between IgG positivity and *L. loa* carriage (OR, 5.4; 95% CI, 1.70–17.3; $P = .007$). Among the 700 amicrofilaremic individuals with eye worm but *L. loa* negativity, 20% ($n = 141$) were ZEBOV IgG positive. In the deep forest ecosystem, the risk of being ZEBOV IgG positive was 1.25-fold higher among amicrofilaremic individuals than among microfilaremic individuals (95% CI, 0.99–1.56-fold; $P = .054$) (Supplementary Table 5).

Logistic Regression Analysis

Multivariate analyses were performed separately for each ecosystem when possible. The only variables that remained significantly associated with ZEBOV IgG positivity after adjustment for other risk factors were amicrofilaremia in deep forest

Table 4. Prevalence of ZEBOV-Specific IgG in Gabon According to the Ecological Region

Ecosystems	Villages	+/Total	Prevalence (%)	95% CI	Min–Max	*P*-value
All population	*220*	*667/4349*	*15.3*			
Lakeland	24	12/443	2.7	1.4–4.7	0–13.2	< .0001
Savannah	22	47/448	10.5	7.8–13.7	0–16.1	
Forest	174	608/3458	17.6	16.3–18.9	3.8–32.4	
Grassland	62	114/918	12.4	10.3–14.7	3.8–30	< .0001
Deep forest	112	494/2540	19.4	18–21.2	5–32.4	

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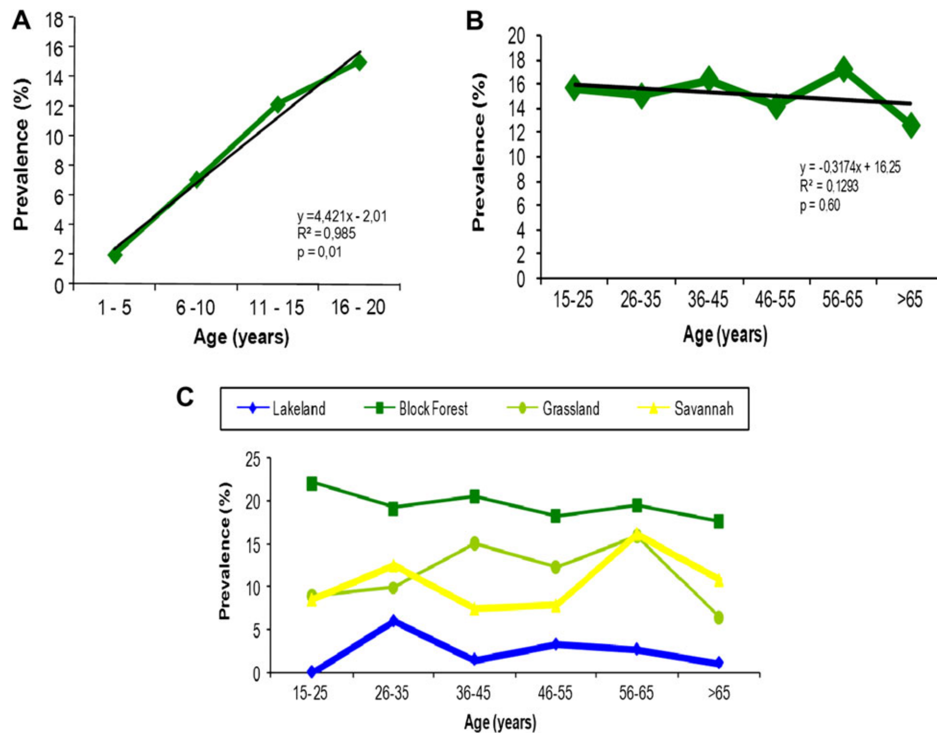

Figure 2. *A,* Prevalence of ZEBOV-specific IgG, by age, in children group. *B,* Overall prevalence of ZEBOV-specific IgG, by age, in adults group. *C,* Prevalence of ZEBOV-specific IgG, by age in adults group and by ecosystem.

(adjusted OR, 1.26; 95% CI, 1–1.59; *P* = .04) and chronic arthralgias in grassland (adjusted OR, 1.88; 95% CI, 1–3.55; *P* = .05) and savannah (adjusted OR, 3.40; 95% CI, 1.58–7.29; *P* = .002) (Table 5).

DISCUSSION

We conducted a serosurvey of *Zaire ebolavirus* in 220 rural villages in Gabon, to define and characterize the geographic distribution and circulation of ZEBOV and to identify risk factors for human exposure.

The prevalence of ZEBOV-specific IgG was surprisingly high, at 15.3% overall. It reached 32.4% in one village, the highest rate ever detected with an ELISA method. Seroprevalence rates in previous ELISA-based studies conducted in CAR and DRC never exceeded 20% [15, 17, 21]. In Gabon, during the second ZEBOV outbreak, 205 samples were collected in 3 villages, and ZEBOV IgG was found with this method in 14.9%–30% of individuals [10]. In contrast, a prevalence of only 1.4% was found in 8 villages in the Gabonese outbreak area [25]. Of 1147 serum samples collected during 1981–1997 in 6 rural communities in central, northeastern, and western Gabon, only 14 (1.2%) contained ZEBOV-specific IgG [26]. This discrepancy may be attributable to differences in the screening and population sampling methods (random vs nonrandom). Our survey included rural populations in all ecosystems and used the most specific available ELISA method.

The test used here lacks cross-reactivity with other known EBOV species and with MARV [22]. Recently, a Western blot

Table 5. Adjusted Seroprevalence and Odds Ratios (OR) for the Presence of *Zaire Ebolavirus* Antibodies According to Potential Risk Factors Stratified by Ecosystem, in Gabon

Ecosystem	Risk factor		Adjusted OR	95% CI	*P* value
Deep forest	Sex	Male	0.83	.68–1	.06
		Female	Referent		
	Nodes	Yes	2.85	.98–8.3	.054
		No	Referent		
	Amicrofilaremia	Yes	1.26	1–1.59	.04
		No	Referent		
Grassland	Sex	Male	0.55	.3–1.02	.06
		Female	Referent		
	Fever	Yes	0.51	.22–1.15	.1
		No	Referent		
	Arthralgias	Yes	1.88	1–3.55	.05
		No	Referent		
Savannah	Abdominal pain	Yes	0.51	.21–1.22	.13
		No	Referent		
	Arthralgias	Yes	3.40	1.58–7.29	.002
		No	Referent		

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study confirmed its specificity on highly diluted serum samples (1:1600), and memory T cells responses were found in IgG-positive individuals [29]. Furthermore, there was no difference in the ranges of antibody titers between persons with and without a history of HF [31].

Marked variability in seroprevalence rates was noted across the administrative regions in this survey, suggesting that specific surveillance programs should be focused on particular regions. Regions with a high prevalence (Ngounie, Woleu Ntem, Ogooue Ivindo, and Ogooue lolo) were mainly situated in the forest ecosystem. Previous serosurveys, together with the geographic pattern of outbreaks, have highlighted the potential role of the ecosystem [18, 27, 32], and an increased risk among forest populations has previously been described [15, 18]. Our study confirms that the forest, particularly the deep forest, is the environment most at risk. This is the area harboring animals susceptible to the virus, such as great apes and bats, the latter representing a viral reservoir.

Previous studies have shown a higher prevalence in adults, women, and hunter-gatherers [15, 17, 20]. In contrast, we found no role of sex or activity. There is no individual susceptibility, but susceptibility at the population scale.

No statistically significant difference was observed between children and adults. Antibodies appeared early, from 2 years of age, and their prevalence increased linearly until 20 years, then remained stable. Children appear to be exposed as soon as they become autonomous. The high seroprevalence among children indicates the same source(s) of exposure as in adults, either inside or near villages.

Both arthralgias and amicrofilaremia were associated with EBOV seropositivity. Arthralgias were described both among fatalities during the Kikwit (DRC) outbreak and among survivors, in whom they were sometimes long-lasting [3, 5]. In our study, arthralgias were the only clinical factor associated with IgG seropositivity. They may be because of the long-term reaction to the virus, as described with other viruses [33, 34], rather than being a symptom of mild infection. Athralgias are also encountered in many other chronic tropical infectious diseases, such as loiasis. Alternatively, arthralgias may have been related to loaisis, which was highly prevalent in our population. *L. loa* amicrofilaremia also influenced ZEBOV seropositivity. Amicrofilaremic status is generally associated with humoral and cellular responsiveness [35, 36]. Immune anergy associated with loaisis could lead to undetectable ZEBOV-specific IgG levels and, thus, to an underestimation of the prevalence.

No difference in seroprevalence was found between epidemic and nonepidemic areas, indicating an animal source prevalent throughout the country and to exposure to a less lethal or nonlethal strain [2, 37–39], although a cross-reactive antibody response cannot be out-ruled.

Great ape infection is often lethal, and direct contact with humans is rare. Fruits bats therefore represent the most likely common animal source of ZEBOV exposure. These animals, previously identified as a potential reservoir [40], are abundant in the forest ecosystem and consume fruits on trees located in or around villages. Infected fruits bats led to the 2007 Luebo outbreak in DRC [41] and have been implicated in many filovirus outbreaks in Africa. Seropositive participants in our survey could have been exposed to low viral inocula, inactivated virus, or isolated viral antigens while gathering or consuming fruits contaminated by bat saliva. Studies of epidemic areas are underway in an attempt to isolate the virus from both humans and bats.

In conclusion, the widespread ZEBOV seropositivity observed in rural Gabon suggests that outbreaks may potentially occur anywhere in the country. Clinical awareness and surveillance programs should be implemented in all apparent areas of endemicity. Such programs should provide training for all health care workers to facilitate early outbreak recognition and patient treatment and should be adapted to the ecosystem and region.

Supplementary Data

Supplementary Data are available at *The Journal of Infectious Diseases* online.

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