The Journal of Infectious Diseases

MAJOR ARTICLE

High Seroreactivities to Orthoebolaviruses in Rural Cameroon: A Case-Control Study on Nonhuman Primate Bites and a Cross-sectional Survey in Rural Populations

Jill-Léa Ramassamy,[1,2] Ahidjo Ayouba,[2] Guillaume Thaurignac,[2] Chanceline Bilounga Ndongo,[3,4] Patrick Nnuka,[3] Edouard Betsem,[5] Richard Njouom,[6] Eitel Mpoudi Ngole,[7] Jessica Vanhomwegen,[8] Damien Hoinard,[8] Patrick England,[9] Alexandra Journeaux,[10,11] Caroline Picard,[10,11] Damien Thomas,[11,12] Delphine Pannetier,[11,12] Sylvain Baize,[10,11] Eric Delaporte,[2] Martine Peeters,[2] and Antoine Gessain[1]

[1]Unité d'Epidémiologie et Physiopathologie des Virus Oncogènes, Institut Pasteur, Université de Paris Cité, Centre National de la Recherche Scientifique, Unité Mixte de Recherche 3569, Paris; [2]Unité TransVIHMI, Université de Montpellier, Inserm, Institut de Recherche pour le Développement, Montpellier, France; [3]Direction de la lutte contre les Maladies, les Epidémies et les Pandémies, Ministère de la Santé Publique, Yaoundé; [4]Faculté de Médecine et des Sciences Pharmaceutiques, Université de Douala, Douala; [5]Faculty of Medicine and Biomedical Sciences, University of Yaounde I, Yaounde, Cameroon; [6]Unité de Virologie, Centre Pasteur du Cameroun; [7]Laboratoire de Virologie, Centre de Recherche sur les Maladies Emergentes et Re-Emergentes, Institut de Recherches Médicales et d'Etudes des Plantes Médicinales, Yaoundé, Cameroon; [8]Unité Environnement et Risques Infectieux, Institut Pasteur; [9]Plateforme de Biophysique Moléculaire, C2RT, Institut Pasteur, Centre National de la Recherche Scientifique, Unité Mixte de Recherche 3528, Paris; [10]Unit of Biology of Emerging Viral Infections, Institut Pasteur; [11]National Reference Center for Viral Hemorrhagic Fevers; and [12]Laboratoire P4 Jean Mérieux, US003, Inserm, Lyon, France

Background. Ebola (EBOV) and Sudan (SUDV) orthoebolaviruses are responsible for lethal hemorrhagic fever outbreaks in humans in Central and West Africa, and in apes that can be at the source of human outbreaks for EBOV.

Methods. To assess the risk of exposure to orthoebolaviruses through contact with nonhuman primates (NHP), we tested the presence of antibodies against different viral proteins with a microsphere-based multiplex immunoassay in a case-control study on bites from NHPs in forest areas from Cameroon (n = 795) and in cross-sectional surveys from other rural populations (n = 622) of the same country.

Results. Seroreactivities against at least 2 viral proteins were detected in 13% and 12% of the samples for EBOV and SUDV, respectively. Probability of seroreactivity was not associated with history of NHP bites, but was 3 times higher in Pygmies compared to Bantus. Although no neutralizing antibodies to EBOV and SUDV were detected in a selected series of highly reactive samples, avidity results indicate strong affinity to SUDV antigens.

Conclusions. The detection of high level of seroreactivities against orthoebolaviruses in rural Cameroon, where no outbreaks have been reported, raises the possibilities of silent circulation of orthoebolaviruses, or of other not yet documented filoviruses, in these forested regions.

Article's main point. Our study found high seroreactivities to Ebola and Sudan orthoebolavirus antigens in rural Cameroonian populations, especially among Pygmies, despite no reported outbreaks. This suggests potential silent circulation of orthoebolaviruses or unknown filoviruses, highlighting the need for further surveillance and research.

Keywords. Filoviridae; Ebola virus; Sudan virus; Cameroon; Central Africa.

To date, 6 distinct species of orthoebolaviruses have been identified, with 4 of them involved in fatal hemorrhagic fever outbreaks in West and Central Africa. Since 1976, >30 Ebola virus disease (EVD) outbreaks have occurred in Africa, characterized by high mortality rates ranging from 24% to 88% [1, 2]. The predominant causative agent, *Orthoebolavirus zairense* (Ebola virus [EBOV]), previously known as *Zaire ebolavirus*, caused the largest recorded EVD outbreak in West Africa from 2014 to 2016, resulting in more than 28 600 cases and 11 300 fatalities [3, 4]. Sudan virus (SUDV) was reported in 8 outbreaks in Uganda and South Sudan between 1976 and 2022, while Bundibugyo virus (BDBV) caused 2 distinct outbreaks in Uganda and the Democratic Republic of Congo (DRC) in 2007 and 2012, respectively [1]. Tai Forest virus (TAFV) led to 2 outbreaks among chimpanzee populations in Ivory Coast and, notably, a single nonfatal human case after exposure during the autopsy of an infected chimpanzee [5].

Received 07 June 2024; editorial decision 07 August 2024; accepted 08 August 2024; published online 10 August 2024

Presented in part: Conférence Internationale Francophone VIH/Hépatites/Santé sexuelle/Infections émergentes AFRAVIH 2024, Yaoundé, Cameroon, 16–19 April 2024; and EBOSURSY Symposium, Building capacity and surveillance for viral haemorrhagic fevers, Dakar, Senegal, 9–11 October 2023.

Correspondence: Jill-Léa Ramassamy, DVM, MPH, PhD, Institut de Recherche pour le Développement, 911 Avenue Agropolis, Montpellier 34090, France (jill-lea.ramassamy@ird.fr); Antoine Gessain, MD, PhD, Unité d'Epidémiologie et Physiopathologie des Virus Oncogènes, Institut Pasteur, 28 rue du Dr Roux, Paris 75015, France (agessain@pasteur.fr).

The Journal of Infectious Diseases®

https://doi.org/10.1093/infdis/jiae399

Reston virus (RESTV), the sole orthoebolavirus detected outside the African continent, was detected in monkeys and pigs in the Philippines and in *Macaca fascicularis* imported to the United States from the Philippines [6, 7]. Recently, Bombali virus (BOMV) was documented in bats captured in Sierra Leone, Guinea, and Kenya [8, 9]. Although no human infections have been reported with RESTV and BOMV, antibodies against RESTV have been identified in individuals in contact with infected animals, suggesting asymptomatic RESTV infections [7, 8].

The animal reservoir for most EVD outbreaks remains elusive [10]. Up to now, bats represent the most probable reservoir of orthoebolaviruses, as supported by the detection of antibodies against EBOV and SUDV in different bat species [11, 12]. Molecular evidence, however, remains scarce, as only 1 study has detected EBOV RNA in 3 frugivorous bat species in Gabon and the Republic of Congo [13]. Additionally, there have been limited investigations suggesting links between bats and human outbreaks [14, 15]. Molecular evidence also exists for other viruses within the Filoviridae family in bats, such as Marburg, Lloviu, and Mengla viruses [16–19]. EBOV and TAFV are highly pathogenic for apes, resulting in high mortality rates [5, 20]. Contact with infected carcasses poses a clear source of contamination and has been reported as a source of human outbreaks [5, 21, 22]. Moreover, up to now, none of the outbreaks caused by SUDV and BDBV have been linked to an animal source, emphasizing the ongoing challenge in uncovering the zoonotic origins of these viruses. This underscores the critical necessity to enhance our understanding of orthoebolavirus cycles, investigating both the species that contribute to maintaining these viruses between outbreaks and those acting as sources for animal-to-human transmissions.

The primary objective of this study is to investigate the seroreactivity to different orthoebolaviruses in human populations with documented exposure to nonhuman primates (NHPs) or identified as being at high risk for NHP exposure in Cameroon, a country considered at risk for outbreaks. We assessed the presence of antibodies to orthoebolaviruses, in a previously reported case-control study on NHP bites, and in a cross-sectional survey conducted in forest areas in southern Cameroon, adjacent to the regions of EVD outbreaks in Congo and Gabon. Last, we aim to determine the specificity of observed seroreactivities against the different orthoebolavirus antigens.

MATERIALS AND METHODS

Ethics

Studies received administrative and ethical clearance from the National Comity of Ethics (respectively, N°034/CNA/MP/06, N°2019/02/1136/CE/CNERSH/SP, and N°201/CNE/SE/2011; 2018/09/1090/CE/CNERSH/SE). Prior to field sampling, individual written informed consent was obtained by participants after detailed information and explanations of the study were provided. Consent for underage children was obtained from their parents.

Surveys

From 2004 to 2019, a cross-sectional survey was conducted in villages and settlements located in the rainforest of southern Cameroon. A standardized questionnaire was used to collect demographic data and information on injuries from NHPs. This study population has been previously described and demonstrated an association between NHP bite and presence of simian foamy virus (SFV) and human T-cell leukemia virus type 1 (HTLV-1) infections [23, 24]. For each individual with a history of NHP bite, 2 individuals from the same cross-sectional survey were enrolled as controls and matched on age (within a maximum margin of 3 years), sex, ethnicity, and geographic origin.

The second cross-sectional survey was performed in remote rural villages located close to Messok and Moloundou, cities in southeast Cameroon, in 2011 and 2012 (Figure 1). A standardized questionnaire was used to collect demographic data (age, sex, and ethnicity). Whole blood was collected from each consenting individual and serum/plasma was processed 48–72 hours after sampling and stored frozen at −80°C.

Microsphere-Based Multiplex Immunoassay

Plasma samples, diluted at 1:1000, were tested for the presence of immunoglobulin G (IgG) against different orthoebolavirus antigens, with a previously published in-house multiplex immunoassay (MIA) based on Luminex technology (Luminex Corporation, Austin, Texas) [25]. The MIA used 11 recombinant proteins from different genomic regions (nucleoprotein [NP], glycoprotein [GP], and 40-kDa viral protein [VP40]) of 5 orthoebolavirus species: EBOV, SUDV, BDBV, BOMV, and RESTV (detailed in Supplementary Table 1). Reactivity to each antigen was defined by median fluorescence intensity (MFI) values above previously defined thresholds (400 per 100 beads for GP, 600 for NP, and 650 for VP40) [25].

Quantification of Total IgG Concentrations

Total IgG levels were measured in a subset of 429 samples (272 Bantus and 157 Pygmies) of the total 622 from the second rural survey, using the commercial kit Bio-Plex Pro human isotyping assay (Bio-Rad, Hercules, California).

Analysis of Antibody Binding by Surface Plasmon Resonance and MIA Avidity Assay

To assess the specificity of the observed seroreactivity against orthoebolaviruses, we measured first the average antibody affinity on a subset of samples from the case-control study on NHP bite to EBOV and SUDV GPs using surface plasmon

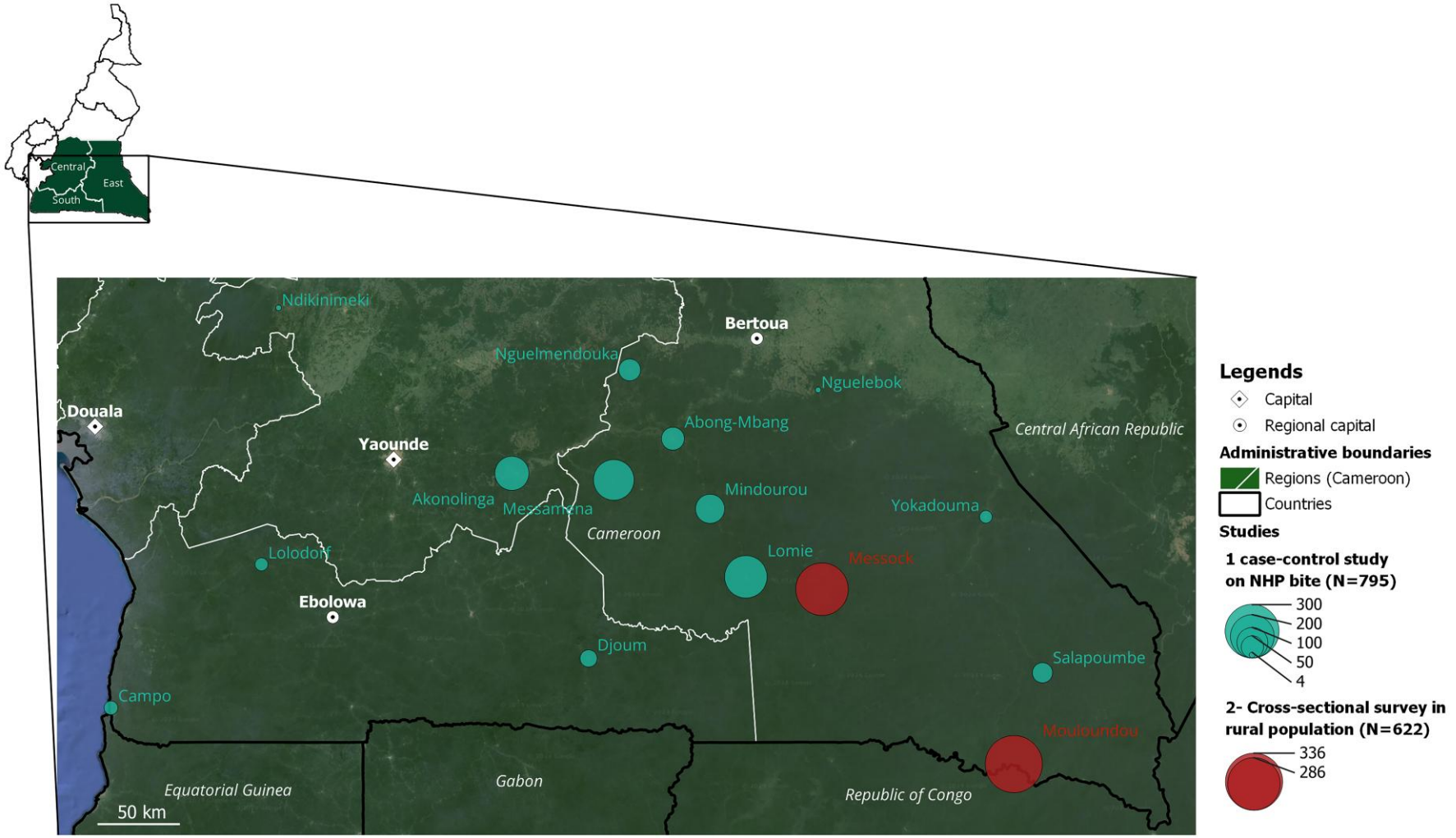

Figure 1. Geographic distribution of participants from the 2 serological studies on orthoebolaviruses in rural Cameroon. Geographical location and number of individuals included in the 2 serosurveys on orthoebolaviruses in Cameroon. The case-control study on nonhuman primate (NHP) bites (in blue) included 795 people from South, East, and Centre regions: 265 people bitten by a NHP and 530 controls, adjusted on age, sex, ethnic group, and location. The second study (in red) was carried out in 2 rural villages in East Cameroon and included 691 people. Google Earth satellite imagery was used as the map background (Map data ©2024 Google). Maps realized with QGIS software.

resonance (SPR). Additionally, the antibody avidity to each orthoebolavirus antigen (NP, GP, and VP40) was assessed using an avidity MIA. A comprehensive description of the methods employed is provided in Supplementary Figures 9 and 10. Five sera from EVD survivors of the outbreak in 2014–2016 in Guinea were used as positive controls [26]. In total, 21 samples with different antibody profiles—positive (ie, antibodies to at least 2 antigens) for EBOV (n = 2), SUDV (n = 5), or both EBOV and SUDV (n = 7); indeterminate (antibodies to 1 antigen, n = 4); and negative (n = 3)—from the case-control NHP bite study in Cameroon were tested by SPR and avidity MIA.

Plaque-Neutralizing Assay

A subset of 28 samples from the case-control study were tested in EBOV and SUDV plaque-neutralizing assay, including 20 seropositive samples (13 reactive to 2 antigens and 7 reactive to 3 antigens), 5 indeterminate (reactive to 1 SUDV or EBOV antigen only), and 3 negatives. Plasma was diluted 1:20, 1:80, and 1:320 and tested in plaque-neutralizing assay as previously described [27]. Neutralizing monoclonal and polyclonal antibodies directed against EBOV GP and SUDV were used as positive control, respectively. The foci were counted in each well and the plasma dilutions for which a neutralization above 50% of the viral inoculum was obtained were considered positive.

Statistical Analyses

Seropositivity for each orthoebolavirus species was defined by the presence of reactivity toward at least 2 different antigens, as previously used [28]. Proportions were compared between groups using χ^2 and Fisher exact test, as appropriate. For matched groups (bitten individuals and controls), *P* values were assessed with conditional logistic regression model. The significance threshold was set at .05. All analyses were performed using Stata 15.0 (StataCorp, College Station, Texas) and R (version 4.2.2) software.

RESULTS

Orthoebolavirus Seroprevalence in the Case-Control Study on NHP Bites

The case-control study on NHP bites included 795 participants from forest areas in South and East Cameroon: 265 individuals with a history of NHP bites and 530 matched controls. The study population consisted predominantly of males (95%), with a mean age of 45 years (range, 8–90 years) and 66% and 34% belonged to the Bantu or Pygmy ethnic group (Baka), respectively. Among the 265 cases, 160 were bitten by a monkey (60%), 78 by a gorilla (29%), and 30 by a chimpanzee (11%), mostly during hunting activities. Three individuals were bitten twice. MFI values and proportion of seroreactivity to each orthoebolavirus antigen are detailed in Supplementary Table 1. Highest proportions of reactivity were observed to the glycoproteins (62% to EBOV, 71% to SUDV, 46% to BDBV GP), even to RESTV GP (28%), which could suggest cross-reactivities between orthoebolavirus antigens (Supplementary Figure 1). Of the 795 tested samples, 2 (0.3%) exhibited reactivity to all 3 EBOV proteins (NP, GP, and VP40) and 10 to 3 SUDV proteins (1.3%) (Table 1). Considering seropositivity as the presence of antibodies to at least 2 antigens from the same viral species, the overall seroprevalences were 10.2% (95% confidence interval [CI], 8.2%–12.5%) for EBOV, 9.6% (95% CI, 7.6%–11.8%) for SUDV, and 3.5% (95% CI, 2.4%–5.1%) for BDBV. No statistically significant differences were observed between the case and control groups, or based on sex or age categories. However, orthoebolavirus seroprevalence was significantly higher in Pygmies compared to Bantus (Table 2).

Table 1. Numbers and Percentages of Samples From the 2 Surveys in Rural Forest Cameroon Reactive to Different Antigen Combination of Orthoebolaviruses

Orthoebolavirus	NHP Case-Control Study (n = 795) No. (%)	Rural Survey (n = 622) No. (%)
EBOV		
NP + GP + VP40	2 (0.3%)	2 (0.3%)
NP + GP	16 (1.8%)	16 (2.6%)
NP + VP40	5 (0.4%)	2 (0.3%)
GP + VP40	64 (7.8%)	83 (13.3%)
At least 2	81 (10.2%)	97 (15.6%)
SUDV		
NP + GP + VP40	10 (1.3%)	10 (1.6%)
NP + GP	53 (6.7%)	64 (10.3%)
NP + VP40	10 (1.3%)	11 (1.8%)
GP + VP40	33 (4.2%)	33 (5.3%)
At least 2	76 (9.6%)	88 (14.1%)
BDBV		
GP + VP40	28 (3.5%)	38 (6.1%)

The case-control study on bites from NHPs included 265 individuals bitten and 530 controls from rural forest areas of Cameroon. The rural survey included 622 individuals from rural areas of East Cameroon.

Abbreviations: BDBV, Bundibungyo virus; EBOV, Ebola virus; GP, glycoprotein; NP, nucleoprotein; SUDV, Sudan virus; VP40, 40-kDa viral protein.

Orthoebolavirus Seroprevalence in the Rural Forest Population Survey

To further study the above observed difference, we analyzed a second survey population from a previously described rural forest areas in East Cameroon [29]. Samples from 622 participants were tested: 286 Bantus (46%) and 336 Pygmies (54%). The mean age of the overall study population was 35 years (range, 4–86 years; 31.6 and 38.6 years for the Pygmy and Bantu groups, respectively), and 40% of the participants were men (40% and 38% in the Pygmy and Bantu groups, respectively).

Two (0.3%) and 10 samples (1.4%) had antibodies against 3 EBOV and SUDV antigens, respectively (Table 1). Considering presence of antibodies to at least 2 antigens as seropositivity, overall seroprevalence was 15.6% (n = 97;

Table 2. Seroprevalence to Ebola Viruses in a Case-Control Study on Nonhuman Primate Bites in Rural Cameroon (n = 795)

Characteristic	No.	EBOV Seropositivity			SUDV Seropositivity			BDBV Seropositivity		
		No. of Seropositive Samples	% Seroprevalence	*P* Value	No. of Seropositive Samples	% Seroprevalence	*P* Value	No. of Seropositive Samples	% Seroprevalence	*P* Value
Sex										
Male	753	77	10.2%	1.00	73	9.7%	.79	25	3.3%	.18
Female	42	4	9.5%		3	7.1%		3	7.1%	
Age category, y										
<40	290	25	8.6%	.52	29	10.0%	.72	9	3.1%	.68
40–50	252	27	10.7%		21	8.3%		8	3.2%	
>50	253	29	11.5%		26	10.3%		11	4.3%	
Ethnic group										
Bantu	528	39	7.4%	**<.001**	39	7.4%	**.003**	13	2.5%	**.02**
Pygmy	267	42	15.7%		37	13.9%		15	5.6%	
NHP bite										
Control group	530	61	11.5%	.09	54	10.2%	.39	23	4.3%	.09
Bitten	265	20	7.5%		22	8.3%		5	1.9%	
Total	795	81	10.2%		76	9.6%		28	3.5%	

Seropositivity to each pathogenic orthoebolavirus species was defined by reactivity toward at least 2 different antigens. Significant *P* values (<.05) are indicated in bold.

Abbreviations: BDBV, Bundibugyo virus; EBOV, Ebola virus; NHP, nonhuman primate; SUDV, Sudan virus.

95% CI, 12.8%–18.7%) for EBOV, 14.2% (n = 88; 95% CI, 11.5%–17.1%) for SUDV, and 6.1% (n = 38; 95% CI 4.4%–8.3%) for BDBV. Seroprevalence was significantly higher in Pygmies for all 3 orthoebolaviruses. Odds of seroreactivities for Pygmies compared to Bantus were 8.6 (95% CI, 4.3–17), 6.0 (95% CI, 3.2–11.4), and 11 (95% CI, 3.3–37) for EBOV, SUDV, and BDBV, respectively. After adjustment on ethnic group, there was no difference in seroprevalence according to age or sex (Table 3). In both studies, we found higher MFI values in Pygmies, suggesting higher concentration of anti-orthoebolavirus antibodies (Supplementary Table 1, Supplementary Figure 3).

Orthoebolavirus Seropositivity and Total IgG Titers Between Bantu and Pygmy Populations

To further study the observed differences of MFI values between Pygmies and Bantus, total IgG concentrations were measured for 422 individuals (269 Bantus and 153 Pygmies) from the above study. Mean total IgG titers were 28 mg/mL (range, 3.5–164 mg/mL) for Bantus and higher for Pygmies with 60 mg/mL (range, 4.1–156 mg/mL; Mann-Whitney test $P < .001$) (Supplementary Figure 4). There was a significant correlation between total IgG level and MFI values for all orthoebolavirus antigens (Supplementary Table 2).

MFI values were adjusted on IgG level for each individual and were then compared between Pygmy and Bantu population groups. Adjusted MFI remained significantly higher in Pygmies for most antigens, except for NP EBOV and GP BOMV, which were higher in the Bantu group, and for VP40 of EBOV and BDBV, for which there was no difference between Pygmies and Bantus (Supplementary Figure 5).

Average Affinity to Orthoebolavirus GPs

We evaluated the anti-GP antibody average affinity by SPR assays on a subset of samples from our study and from 5 EBOV survivors from the 2014–2016 outbreak in Guinea as controls (details of methods and results are in Supplementary Figures 6–8). Twenty-one samples from Cameroon were tested twice by SPR. The antibody binding titers, measured in resonance units (RU), could be obtained for 14 of the 21, including 10 with reactivity to at least 2 antigens for EBOV (n = 1), SUDV (n = 3), or both EBOV and SUDV (n = 6); 3 indeterminate profiles (only 1 reactive antigen); and 1 negative sample (without reactivity to any antigen). The 5 EVD survivors had high antibody binding levels for GP EBOV, with a mean of 237 RU (range, 101–372 RU), and levels were significantly lower for GP SUDV (mean, 110 RU [range, 59–206 RU]; $P < .0001$) (Figure 2). GP EBOV levels were significantly lower in reactive samples from our study population in Cameroon (mean, 79 RU [range, 3.5–172 RU]; $P = .0043$), as compared to EVD survivors. However, anti-GP SUDV binding levels were comparable between EVD survivors (mean, 162 RU [range, 78–206 RU]) and reactive samples from Cameroon (mean, 160 RU [range, 124–225 RU]; not significant) (Figure 2).

Avidity Index to Orthoebolavirus Antigens

Avidity index (AI) was measured for the same samples tested in SPR analysis, 5 EBOV survivors and 21 samples from Cameroon. There was a clear difference of AI between Guinean EVD survivors and Cameroonian samples for EBOV NP and VP40 antigens, but not for GP, which could be due to GP conformation, impeding the avidity assay

Table 3. Seroprevalence to Orthoebolaviruses in a Rural Population Survey in Cameroon (n = 622)

		EBOV Seropositivity			SUDV Seropositivity			BDBV Seropositivity		
Characteristic	No.	No. (%)	aOR (95% CI)	*P* Value	No. (%)	aOR (95% CI)	*P* Value	No. (%)	aOR (95% CI)	*P* Value
Sex										
Male	247	43 (17.4%)	1	.41	41 (16.6%)	1	.20	21 (8.5%)	1	.06
Female	375	54 (14.4%)	0.8 (.5–1.3)		47 (12.5%)	0.7 (.5–1.2)		17 (4.5%)	0.5 (.3–1)	
Age category, y										
<25	203	38 (18.7%)	1	.36	35 (17.2%)	1	.33	15 (7.4%)	1	.77
25–40	218	33 (15.1%)	0.7 (.4–1.2)		29 (13.3%)	0.7 (.4–1.1)		15 (6.9%)	0.8 (.4–1.8)	
>40	201	26 (12.9%)	0.9 (.5–1.6)		24 (11.9%)	0.9 (.5–1.6)		8 (4%)	0.7 (.3–1.8)	
Ethnic group										
Bantu	286	11 (3.8%)	1	**<.001**	13 (4.5%)	1	**<.001**	3 (1%)	1	**<.001**
Pygmy	336	86 (25.6%)	8.6 (4.3–17)		75 (22.3%)	6 (3.2–11.4)		35 (10%)	11 (3.3–36.9)	
Total	622	97 (15.6%)	…		88 (14.1%)	…		38 (6.1%)	…	

No. (%) indicates number of seropositive samples and percentage seroprevalence. Seropositivity to each pathogenic orthoebolavirus species was defined by reactivity toward at least 2 different antigens. Significant *P* values (<.05) are indicated in bold.

Abbreviations: aOR (95% CI), odds ratio adjusted on ethnic group (95% confidence interval); BDBV, Bundibugyo virus; EBOV, Ebola virus; SUDV, Sudan virus.

(Supplementary Figure 10). Therefore, only AI on NP and VP40 was analyzed. Cameroonian samples had lower AIs for EBOV antigens, ranging from 5% to 44% and 3% to 45%, respectively, for NP and VP40, as compared to EVD survivors. However, there was a wider variation of AI on SUDV antigens (NP AI range, 3%–96%; VP40 AI range, 12%–96%). Nine samples had AI >50% for either SUDV NP or VP40. By analogy with EVD survivors with EBOV antigens, this suggests stronger avidity to SUDV antigens (Figure 3).

Plaque-Neutralizing Assay for EBOV and SUDV

A subset of 28 samples were tested in the 50% plaque-neutralizing activity assay: 3 negative, 5 indeterminate (reactive to 1 SUDV or EBOV antigen only), 13 with reactivity to 2 antigens (6 for EBOV, 5 for SUDV, 2 to both), and 7 reactive to all 3 antigens (2 for EBOV, 5 for SUDV). Only 4 samples had a titer of 1:20, 1 for EBOV (with a negative profile in MIA) and 3 for SUDV (1 negative, 1 indeterminate with reactivity toward glycoproteins only, and 1 reactive to 3 SUDV antigens). All were negative at higher dilution.

DISCUSSION

Although no EVD cases have ever been officially reported, the tropical forest areas of southern Cameroon are deemed at elevated risk of outbreaks with orthoebolaviruses. This assessment is based on the occurrence of EVD outbreaks in neighboring countries, coupled with the ecological contexts of EVD index cases and the distributions of bat species suspected to be an animal reservoir [30]. In our study, we observed high percentages of reactivities to orthoebolavirus antigens among rural populations residing in these forested regions, with seroprevalence rates of 13% and 12% for EBOV and SUDV, respectively. Conversely, seroreactivity among 368 residents from Yaoundé, the capital city, was minimal (0.3% for EBOV and 0% for SUDV) based on positivity criteria presence of antibodies to at least 2 reactive antigens (unpublished data). Even with the most stringent criteria, which require concomitant reactivity to 3 antigens, the observed proportions were not negligible (0.3% for EBOV and 1.3% for SUDV) in rural forest areas in Cameroon, while they were nil in the urban study in Yaoundé. A decline of EBOV-specific IgG has been observed over time in survivors of both infections with EBOV and SUDV [31, 32]. Consequently, the probability of detecting antibodies to all 3 or even 2 antigens diminishes over time among survivors, suggesting that the criterion of 3 antigen reactivities may be overly stringent for detecting past orthoebolavirus infections.

Previous studies have reported EBOV seroreactivities in Cameroon. In 1980, a study found a 9.7% seropositivity rate among 1517 individuals using indirect immunofluorescence assay (IFA), with higher rates observed among Pygmies and individuals residing in forest areas [33]. Similarly, in 2011–2012, 1.3% of 160 hospitalized individuals with illness of unknown etiology were found to be seropositive for EBOV using enzyme-linked immunosorbent assay (ELISA) and a microneutralizing assay [34]. However, the absence of reported human outbreaks in Cameroon raises questions about the significance of these serological findings, which could suggest undetected circulation or asymptomatic infections. Pauci- and asymptomatic infections to EBOV have indeed been documented in individuals with known exposure to patients with confirmed infection [28, 35, 36]. In addition to Cameroon, antibodies against EBOV have also been detected in areas or countries without a history of outbreaks, such as the DRC or Central African Republic [33, 34, 37]. However, caution must be taken in interpreting these results, considering the diversity of serological assays employed and their specificity (lack of confirmatory tests).

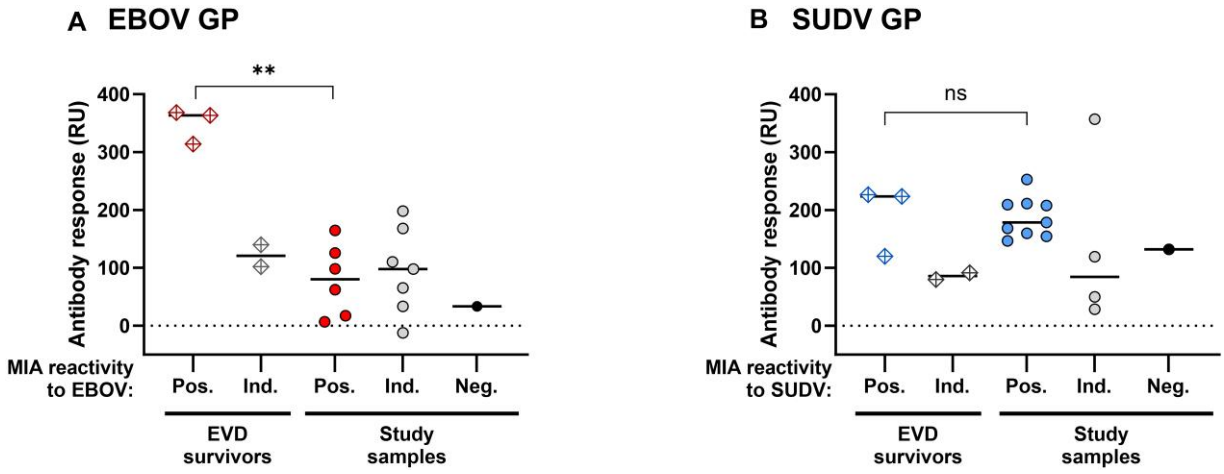

Figure 2. Average immunoglobulin G response against Ebola virus (EBOV) and Sudan virus (SUDV) glycoproteins (GPs) measured by surface plasmon resistance. Comparison of antibody response on EBOV GP (*A*) and SUDV GP (*B*) between Ebola virus disease (EVD) survivors and participants in the serosurveys from Cameroon. Antibody binding titers, measured in resonance units, were obtained for 5 EVD survivors from Guinea, and 14 samples from the case-control study on nonhuman primate bites in Cameroon, including 10 seroreactive (positive) samples to EBOV or SUDV (at least 2 antigens), 3 indeterminate (reactive to only 1 SUDV or EBOV antigen, respectively), and 1 negative (without reactivity to any antigen). Among the 10 reactive samples, 6 are reactive to both EBOV and SUDV, 2 to SUDV only (indeterminate for EBOV), and 1 to EBOV only (indeterminate for SUDV). Detailed results are given in Supplementary Figure 8. Each sample was tested twice. Differences between EVD survivors and Cameroonian samples were assessed using the Mann-Whitney test. $^{**}P < .01$. Abbreviations: EBOV, Ebola virus; EVD, Ebola virus disease; GP, glycoprotein; Ind., indeterminate; MIA, multiplex immunoassay; Neg., negative; ns, not significant; Pos., positive; RU, resonance units; SUDV, Sudan virus.

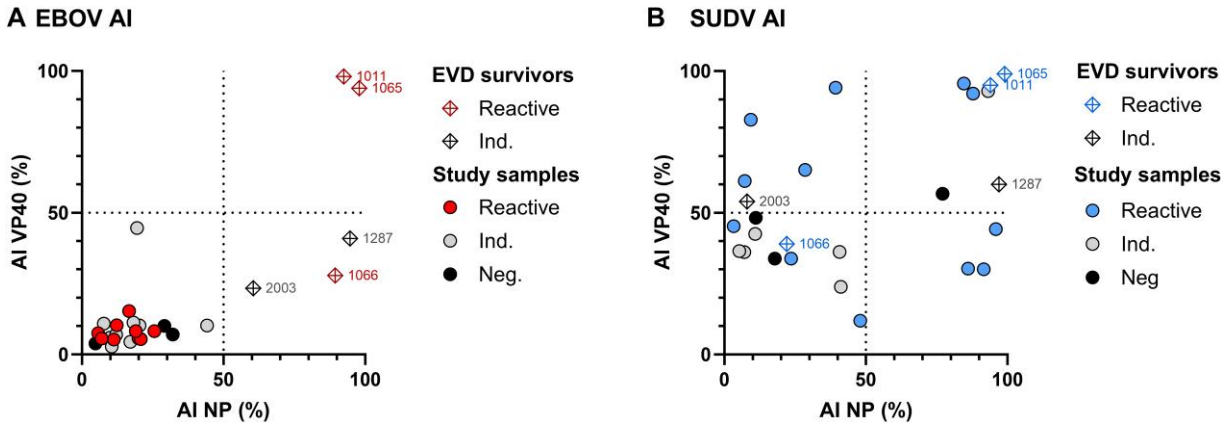

Figure 3. Multiplex immunoassay (MIA) avidity index (%) for immunoglobulin G against orthoebolavirus antigens. MIA avidity index on Ebola virus (EBOV; *A*) and Sudan virus (SUDV; *B*) nucleoprotein and VP40 for 21 samples from the case-control study on nonhuman primate bites from Cameroon were tested, including 14 with reactivity to at least 2 antigens (2 to EBOV antigens, 5 to SUDV, and 7 to both EBOV and SUDV), and 7 negative samples (4 with indeterminate profiles [ie, only 1 reactive antigen] and 3 negative to all antigens). Five sera from EBOV survivors of the outbreak in 2014–2016 in Guinea were used as positive controls and are indicated with their labels in the figure. Abbreviations: AI, avidity index; EBOV, Ebola virus; EVD, Ebola virus disease; Ind., indeterminate; Neg., negative; NP, nucleoprotein; SUDV, Sudan virus; VP40, 40-kDa viral protein.

In our study, no neutralizing antibodies to SUDV or EBOV were detected, suggesting no recent infection. However, this does not rule out the possibility of past infections, as declines in both neutralizing and nonneutralizing antibodies have been described among EVD survivors [38]. Moreover, our findings on affinity and avidity suggest a more specific reactivity against SUDV antigens, analogous to findings in EBOV survivors with EBOV antigens. Affinity assay has been used as a proxy for EBOV neutralizing antibody concentration [39], while avidity assays are used to enhance test specificity for other pathogens. In particular, urea treatment is currently used to increase test specificity for *Toxoplasma gondii* diagnosis and syphilis. It has also been used to differentiate primary to secondary infections and decrease cross-reactivities between arboviruses, especially for dengue viruses and Zika virus [40]. The similar antibody titers measured in SPR for SUDV GP between Cameroonian participants and EVD survivors suggest the presence of cross-reacting antibodies. However, a main limitation

of our study is the absence of SUDV-positive controls, which hinders the interpretation of these assay results.

Moreover, our study revealed significant cross-reactivity between antigens from different orthoebolaviruses, consistent with findings in EBOV survivors (Supplementary Figure 1) [31]. We also noted a high percentage of seroreactivity to the glycoprotein of RESTV, which is not typically found in the African continent [41]. Similarly, the seroreactivity against Bombali GP detected in our study, which has not previously been observed in human populations, cannot be conclusively distinguished from cross-seroreactivity at this stage, thus precluding any definitive conclusions regarding human infection. Overall, the observed seroreactivities in general could represent cross-reactivity resulting from exposure to a potentially nonpathogenic and uncharacterized filovirus that circulates in Cameroon and Central Africa. In our study, the probability of seroreactivity was significantly higher in Pygmies for all 3 viruses (EBOV, SUDV, and BDBV). Higher seroprevalence to filoviruses has been previously reported in Pygmy populations, both in countries with or without past outbreaks [42]. In DRC, 18% of Efe Pygmies tested positive for EBOV using ELISA [43]. In the Central African Republic, 24% of Aka Pygmies were seropositive for EBOV, SUDV, or Marburg virus in 1987, and 13% to EBOV in 1995 using IFA and ELISA, respectively [37, 44]. In Cameroon, seroprevalence was 15% in Pygmies from southern and eastern regions using IFA [33]. However, as previously mentioned, caution is warranted in interpreting seroprevalence due to the wide diversity of antibody assays used and low specificity of some ELISA and IFA assays. Importantly, we observed markedly higher total IgG levels among Pygmies compared to the local Bantu population. This hyperimmunoglobulinemia has been documented in older studies, revealing elevated concentrations of both total IgG and immunoglobulin M, exceeding the European range, in Pygmies from Cameroon, a phenomenon observed since infancy [45]. This difference could be attributed to genetic factors or higher exposure to various pathogens, although it remains poorly understood. However, the significant difference in total IgG concentrations could affect serological assays, increasing the risk of false-positive results for tests with low specificity. Nonetheless, in our study, MFI values normalized for total IgG concentration remained significantly higher in Pygmies compared to Bantus for most orthoebolavirus antigens. These findings suggest potential higher exposure to orthoebolaviruses or other filoviruses, likely associated to the hunter-gatherer lifestyle of Pygmies and their extensive interactions with the environment and potential reservoir species [46, 47].

Antibodies to EBOV and SUDV have been detected in several bat species captured in Cameroon [11, 12] and to a lesser extent in NHP populations [48, 49], suggesting the possible circulation of these filoviruses in wildlife in Cameroon and the subsequent risk of zoonotic transmission. In our study, we focused on direct contact with NHP, with exposure to body fluids through bites. Bites can serve as a proxy for the risk of exposure to viral pathogens, such as the retroviruses HTLV and SFV [23, 24, 50]. However, we found no association between seroreactivity to orthoebolavirus antigens and NHP bites. Although viral transmission can occur through contact with infected carcasses during EBOV outbreaks among ape populations [21, 22], our finding shows no evidence of transmission risk with NHP in a non-epizootic context. This observation aligns with the low prevalence reported in NHP populations [48] and the high mortality rates associated with EBOV outbreaks among apes. Exposure to orthoebolaviruses may result from interactions with other species or environmental factors that have yet to be identified.

In conclusion, our study highlights significant seroreactivity to orthoebolaviruses, notably EBOV and SUDV, among rural populations residing in forested regions of Cameroon, particularly among Pygmies. This underscores the potential circulation of orthoebolaviruses or not yet identified cross-reacting viruses that circulate in these areas, despite the absence of reported human outbreaks. Our findings emphasize the need for continued surveillance and research at the human–animal interface to screen for both known and novel filoviruses. These insights are crucial for guiding public health efforts to understand and mitigate the risk of zoonotic spillover events with orthoebolaviruses in Cameroon and other regions with similar ecological contexts.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online (http://jid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

Acknowledgments. We thank Pr Abdoulaye Touré and Pr Alpha Kabinet Keita, from Centre de Recherche et de Formation en Infectiologie de Guinée, Université Gamal Abdel Nasser de Conakry, Guinea, for the plasma samples from the PostEbogui cohort.

Disclaimer. The funders of this study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Financial support. This study was supported by the European Union (grant number FOOD/2016/379-660 to J. L. R.) as part of the EBO-SURSY project; and the Institut Pasteur, France and the Centre national de la recherche scientifique (UMR 3569) through the Investissement d'Avenir as part of a Laboratoire d'Excellence (LabEx) French research

program (Integrative Biology of Emerging Infectious Diseases, ANR10-LBX-62 IBEID to A. G.).

Potential conflicts of interest. The authors: No reported conflicts of interest.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. Centers for Disease Control and Prevention. Ebola disease outbreak locations. **2024**. https://www.cdc.gov/ebola/outbreak-map/?CDC_AAref_Val=https://www.cdc.gov/vhf/ebola/history/distribution-map.html. Accessed 10 August 2024.
2. Yamaoka S, Ebihara H. Pathogenicity and virulence of ebolaviruses with species- and variant-specificity. Virulence **2021**; 12:885–901.
3. Baize S, Pannetier D, Oestereich L, et al. Emergence of Zaire Ebola virus disease in Guinea. N Engl J Med **2014**; 371:1418–25.
4. World Health Organization. WHO: Ebola situation report 30 March 2016. https://iris.who.int/handle/10665/204714. Accessed 28 February 2024.
5. Formenty P, Hatz C, Le Guenno B, Stoll A, Rogenmoser P, Widmer A. Human infection due to Ebola virus, subtype Côte d'Ivoire: clinical and biologic presentation. J Infect Dis **1999**; 179(Suppl 1):S48–53.
6. Jahrling PB, Geisbert TW, Johnson ED, Peters CJ, Dalgard DW, Hall WC. Preliminary report: isolation of Ebola virus from monkeys imported to USA. Lancet **1990**; 335:502–5.
7. Barrette RW, Metwally SA, Rowland JM, et al. Discovery of swine as a host for the Reston ebolavirus. Science **2009**; 325:204–6.
8. Forbes KM, Webala PW, Jääskeläinen AJ, et al. Bombali virus in *Mops condylurus* bat, Kenya. Emerg Infect Dis **2019**; 25:955–7.
9. Goldstein T, Anthony SJ, Gbakima A, et al. Discovery of a new ebolavirus (Bombali virus) in molossid bats in Sierra Leone. Nat Microbiol **2018**; 3:1084–9.
10. Judson SD, Fischer R, Judson A, Munster VJ. Ecological contexts of index cases and spillover events of different ebolaviruses. PLoS Pathog **2016**; 12:e1005780.
11. De Nys HM, Kingebeni PM, Keita AK, et al. Survey of Ebola viruses in frugivorous and insectivorous bats in Guinea, Cameroon, and the Democratic Republic of the Congo, 2015–2017. Emerg Infect Dis **2018**; 24:2228–40.
12. Peeters M, Champagne M, Ndong Bass I, et al. Extensive survey and analysis of factors associated with presence of antibodies to orthoebolaviruses in bats from West and Central Africa. Viruses **2023**; 15:1927.
13. Leroy EM, Kumulungui B, Pourrut X, et al. Fruit bats as reservoirs of Ebola virus. Nature **2005**; 438:575–6.
14. Leroy EM, Epelboin A, Mondonge V, et al. Human Ebola outbreak resulting from direct exposure to fruit bats in Luebo, Democratic Republic of Congo, 2007. Vector Borne Zoonotic Dis **2009**; 9:723–8.
15. Marí Saéz A, Weiss S, Nowak K, et al. Investigating the zoonotic origin of the West African Ebola epidemic. EMBO Mol Med **2015**; 7:17–23.
16. Makenov MT, Boumbaly S, Tolno FR, et al. Marburg virus in Egyptian *Rousettus* bats in Guinea: investigation of Marburg virus outbreak origin in 2021. PLoS Negl Trop Dis **2023**; 17:e0011279.
17. Negredo A, Palacios G, Vázquez-Morón S, et al. Discovery of an *Ebolavirus*-like filovirus in Europe. PLoS Pathog **2011**; 7:e1002304.
18. Pawęska JT, Storm N, Markotter W, et al. Shedding of Marburg virus in naturally infected Egyptian Rousette bats, South Africa, 2017. Emerg Infect Dis **2020**; 26:3051–5.
19. Yang X-L, Tan CW, Anderson DE, et al. Characterization of a filovirus (Měnglà virus) from *Rousettus* bats in China. Nat Microbiol **2019**; 4:390–5.
20. Vogel G. Tracking Ebola's deadly march among wild apes. Science **2006**; 314:1522–3.
21. Leroy EM, Rouquet P, Formenty P, et al. Multiple Ebola virus transmission events and rapid decline of Central African wildlife. Science **2004**; 303:387–90.
22. Rouquet P, Froment J-M, Bermejo M, et al. Wild animal mortality monitoring and human Ebola outbreaks, Gabon and Republic of Congo, 2001–2003. Emerg Infect Dis **2005**; 11:283–90.
23. Betsem E, Rua R, Tortevoye P, Froment A, Gessain A. Frequent and recent human acquisition of simian foamy viruses through apes' bites in Central Africa. PLoS Pathog **2011**; 7:e1002306.
24. Filippone C, Betsem E, Tortevoye P, et al. A severe bite from a nonhuman primate is a major risk factor for HTLV-1 infection in hunters from Central Africa. Clin Infect Dis **2015**; 60:1667–76.
25. Ayouba A, Touré A, Butel C, et al. Development of a sensitive and specific serological assay based on Luminex technology for detection of antibodies to Zaire Ebola virus. J Clin Microbiol **2017**; 55:165–76.
26. Keita AK, Vidal N, Toure A, et al. A 40-month follow-up of Ebola virus disease survivors in Guinea (PostEbogui) reveals long-term detection of Ebola viral ribonucleic acid in semen and breast milk. Open Forum Infect Dis **2019**; 6:ofz482.
27. van Griensven J, Edwards T, Baize S. Efficacy of convalescent plasma in relation to dose of Ebola virus antibodies. N Engl J Med **2016**; 375:2307–9.

28. Diallo MSK, Rabilloud M, Ayouba A, et al. Prevalence of infection among asymptomatic and paucisymptomatic contact persons exposed to Ebola virus in Guinea: a retrospective, cross-sectional observational study. Lancet Infect Dis **2019**; 19:308–16.
29. Edoul G, Chia JE, Vidal N, et al. High HIV burden and recent transmission chains in rural forest areas in southern Cameroon, where ancestors of HIV-1 have been identified in ape populations. Infect Genet Evol **2020**; 84:104358.
30. Pigott DM, Millear AI, Earl L, et al. Updates to the zoonotic niche map of Ebola virus disease in Africa. eLife **2016**; 5: e16412.
31. Diallo MSK, Ayouba A, Keita AK, et al. Temporal evolution of the humoral antibody response after Ebola virus disease in Guinea: a 60-month observational prospective cohort study. Lancet Microbe **2021**; 2:e676–84.
32. Sobarzo A, Groseth A, Dolnik O, et al. Profile and persistence of the virus-specific neutralizing humoral immune response in human survivors of Sudan ebolavirus (Gulu). J Infect Dis **2013**; 208:299–309.
33. Bouree P, Bergmann J-F. Ebola virus infection in man: a serological and epidemiological survey in the Cameroons. Am J Trop Med Hyg **1983**; 32:1465–6.
34. Steffen I, Lu K, Yamamoto LK, et al. Serologic prevalence of Ebola virus in equatorial Africa. Emerg Infect Dis **2019**; 25: 911–8.
35. Glynn JR, Bower H, Johnson S, et al. Asymptomatic infection and unrecognised Ebola virus disease in Ebola-affected households in Sierra Leone: a cross-sectional study using a new non-invasive assay for antibodies to Ebola virus. Lancet Infect Dis **2017**; 17:645–53.
36. Leroy EM, Baize S, Volchkov VE, et al. Human asymptomatic Ebola infection and strong inflammatory response. Lancet **2000**; 355:2210–5.
37. Gonzalez JP, Nakoune E, Slenczka W, Vidal P, Morvan JM. Ebola and Marburg virus antibody prevalence in selected populations of the Central African Republic. Microbes Infect **2000**; 2:39–44.
38. Woolsey C, Geisbert TW. Antibodies periodically wax and wane in survivors of Ebola. Nature **2021**; 590:397–8.
39. Fuentes S, Ravichandran S, Coyle EM, Klenow L, Khurana S. Human antibody repertoire following Ebola virus infection and vaccination. iScience **2020**; 23:100920.
40. Teimouri A, Mohtasebi S, Kazemirad E, Keshavarz H. Role of *Toxoplasma gondii* IgG avidity testing in discriminating between acute and chronic toxoplasmosis in pregnancy. J Clin Microbiol **2020**; 58:e00505-20.
41. Natesan M, Jensen SM, Keasey SL, et al. Human survivors of disease outbreaks caused by Ebola or Marburg virus exhibit cross-reactive and long-lived antibody responses. Clin Vaccine Immunol **2016**; 23:717–24.
42. Becquart P, Wauquier N, Mahlakõiv T, et al. High prevalence of both humoral and cellular immunity to Zaire ebolavirus among rural populations in Gabon. PLoS One **2010**; 5:e9126.
43. Mulangu S, Borchert M, Paweska J, et al. High prevalence of IgG antibodies to Ebola virus in the Efé Pygmy population in the Watsa region, Democratic Republic of the Congo. BMC Infect Dis **2016**; 16:263.
44. Johnson ED, Gonzalez JP, Georges A. Filovirus activity among selected ethnic groups inhabiting the tropical forest of equatorial Africa. Trans R Soc Trop Med Hyg **1993**; 87:536–8.
45. Meazza C, Travaglino P, Pagani S, Laarej K, Duse M, Bozzola M. Hyperimmunoglobulinaemia in Babinga Pygmies is present from infancy. Int J Immunopathol Pharmacol **2009**; 22:1117–20.
46. Baudel H, Nys HD, Ngole EM, Peeters M, Desclaux A. Understanding Ebola virus and other zoonotic transmission risks through human–bat contacts: exploratory study on knowledge, attitudes and practices in southern Cameroon. Zoonoses Public Health **2019**; 66: 288–95.
47. Akoi Boré J, Timothy JWS, Tipton T, et al. Serological evidence of zoonotic filovirus exposure among bushmeat hunters in Guinea. Nat Commun **2024**; 15:4171.
48. Ayouba A, Ahuka-Mundeke S, Butel C, et al. Extensive serological survey of multiple African nonhuman primate species reveals low prevalence of immunoglobulin G antibodies to 4 Ebola virus species. J Infect Dis **2019**; 220:1599–608.
49. Leroy EM, Telfer P, Kumulungui B, et al. A serological survey of Ebola virus infection in Central African nonhuman primates. J Infect Dis **2004**; 190:1895–9.
50. Ramassamy JL, Ndongo CB, Nnuka P, et al. Epidemiological evidence of nosocomial and zoonotic transmission of HTLV-1 in a large survey in rural population of Central Africa. J Infect Dis **2023**; 227:752–60.