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MAJOR ARTICLE

High seroreactivities to orthoebolaviruses in rural Cameroon: a case-control study on non-human primate bites and a cross-sectional survey in rural population.

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Background : Ebola (EBOV) and Sudan (SUDV) orthoebolaviruses are responsible for lethal haemorrhagic 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 non-human 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 two 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 three 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.

Conclusion: 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 orthoebolavirus, or of other not yet documented filoviruses, in these forested regions.

KEY WORDS: Filoviridae, Ebolavirus, Sudan virus, Cameroon, Central Africa, Pygmy, Serologic Tests.

Article's main point: Our study found high seroreactivities to Ebola and Sudan orthoebolaviruses' 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.

INTRODUCTION

To date, six distinct species of orthoebolaviruses have been identified, with four of them involved in fatal haemorrhagic fever outbreaks in West and Central Africa. Since 1976, more than 30 Ebola virus disease (EVD) outbreaks occurred in Africa, characterized by high mortality rates ranging from 24% to 88% [1,2]. The predominant causative agent, Ebola virus (EBOV) previously known as Zaire Ebolavirus, caused the largest recorded EVD outbreak in West Africa from 2014 to 2016, resulting in over 28,600 cases and 11,300 fatalities [3,4]. Sudan virus (SUDV) has been reported in eight outbreaks in Uganda and South Sudan between 1976 and 2022, while Bundibugyo virus (BDBV) caused two distinct outbreaks in Uganda and the Democratic Republic of Congo (DRC) in 2007 and 2012, respectively [1]. Tai Forest virus

(TAFV) led to two outbreaks among chimpanzee populations in Ivory Coast and, notably, a single non-fatal human case after exposure during the autopsy of an infected chimpanzee [5]. 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 USA from 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 one study has detected EBOV RNA in three 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 sources 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 NHP 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 South Cameroon, adjacent to the regions of EVD outbreaks in Congo and Gabon. Lastly, we aim to determine the specificity of observed seroreactivities against the different *orthoebolavirus* antigens.

METHODS

a. 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.

b. 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 Leukaemia virus type 1 (HTLV-1) infections [23,24]. For each individual with a history of NHP bite, two individuals from the same cross-sectional survey were enrolled as controls, 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 South-East 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 .

c. Microsphere-based multiplex immunoassay (MIA)

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 MIA based on the Luminex technology (Luminex Corp, Austin, TX, USA) [25]. The MIA used eleven recombinant proteins from different genomic regions (nucleoprotein NP, glycoprotein GP, and viral protein VP-40) of five *Orthoebolavirus* species; EBOV, SUDV, BDBV, BOMV and RESTV, detailed in Appendix Table 1. Reactivity to each antigen was defined by median intensity fluorescence (MFI) values above previously defined thresholds (400 per 100 beads for GP, 600 for NP, and 650 for VP40) [25].

d. Quantification of total immunoglobulin G (IgG) concentrations

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

E. Analysis of antibody binding by Surface plasmon resonance (SPR) 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 glycoproteins using SPR. Additionally, the antibody avidity to each *Orthoebolavirus* antigens (NP, GP and VP40) was assessed using an avidity MIA. A

comprehensive description of the methods employed is provided in the supplemental (Figures S9-S10). Five sera from EVD survivors of the outbreak in 2014-16 in Guinea were used as positive controls [26]. In total, twenty-one samples with different antibody profiles: positive (i.e. antibodies to at least two antigens) for EBOV (n=2), SUDV (n=5), or both EBOV and SUDV (n=7), indeterminate (antibodies to one antigen, n=4) and negative (n=3) from the case-control on NHP bite study in Cameroon were tested in SPR and avidity MIA.

f. **Plaque-neutralizing assay**

A subset of 28 samples from the case-control study, were tested in EBOV and SUDV plaque-neutralizing assay, including twenty seropositive samples (thirteen reactive to two antigens and seven to three antigens), five indeterminate (reactive to one SUDV or EBOV antigen only) and three negatives. Plasma was diluted 1:20; 1:80; 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 were obtained were considered positive.

Statistical analyses

Seropositivity for each *Orthoebolavirus* species, was defined by the presence of reactivity toward at least two different antigens, as previously used [28]. Proportions were compared between groups using chi square 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 0.05. All analyses were performed using STATA 15.0 software (Stata Corporation, College Station, TX, USA) and R (version 4.2.2).

RESULTS

Ebolavirus seroprevalence in the case-control study on non-human primate (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 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 supplemental (Table S1). 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 orthoebolaviruses antigens (Supplemental Figure S1). Of the 795 tested samples, two (0.3%) exhibited reactivity to all three EBOV proteins (NP, GP, and VP40) and ten to three SUDV proteins (1.3%) (Table 1). Considering seropositivity as the

presence of antibodies to at least two antigens from the same viral species, the overall seroprevalences were 10.2% (95%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).

Ebolavirus seroprevalence in the rural forest population survey

To further study the above observed difference, we analysed 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), 31.6 and 38.6 years for the Pygmy and Bantu group respectively, and 40% of the participants were men (40% and 38% in the Pygmy and Bantu group respectively).

Two (0.3%) and ten samples (1.4%) had antibodies against three EBOV and SUDV antigens, respectively (Table 1). Considering presence of antibodies to at least two antigens as seropositivity, overall seroprevalence was 15.6% (n=97, 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 three 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 (supplemental Table S1, Figure S3).

Ebolavirus 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) for Bantus and higher for Pygmies with 60 mg/mL (range, 4.1-156, Mann-Whitney test $p<0.001$) (Supplemental Figure S4). There was a significant correlation between total IgG level and MFI values for all *Orthoebolavirus* antigens (Supplemental Table S2).

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 that were higher in the Bantu group, and for VP40 protein of EBOV and BDBV for which there was no difference between Pygmies and Bantus (Supplemental Figure S5).

Average affinity to *Orthoebolavirus* glycoproteins

We evaluated the anti-GP antibody average affinity by SPR assays on a subset of samples from our study, and from five EBOV survivors from the 2014-2016 outbreak in Guinea as controls (details of methods and results are in the supplemental Figures S6-S8). Twenty-one samples from Cameroon were tested twice by SPR. The antibody binding titers, measured in resonance units (RU), could be obtained for fourteen of the twenty-one, including ten with reactivity to at least two antigens for EBOV (n=1), SUDV (n=3), or both EBOV and SUDV (n=6), three indeterminate profiles (only one reactive antigen) and one negative samples (without reactivity to any antigen). The five EVD survivors had high antibody binding levels for GP EBOV, with a mean of 237 RU [101-372RU], and levels were significantly lower for GP SUDV (mean 110 RU, range 59-206 RU, $p < .0001$) (Figure 3). GP EBOV levels were significantly lower in reactive samples from our study population in Cameroon (mean 79 RU, range 3.5-172, $p = 0.0043$), as compared to EVD survivors. However, anti-GP SUDV binding levels were comparable between EVD survivors (mean 162 RU, range 78-206) and reactive samples from Cameroon (mean 160 RU, 124-225, NS) (Figure 2).

Avidity index (AI) to ebolavirus antigens

Avidity index (AI) was measured for the same samples tested in SPR analysis, five 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 glycoprotein conformation, impeding the avidity assay (Supplemental Figure S10). Therefore, only AI on NP and VP40 were analysed. Cameroonian samples had lower AI 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 higher than 50% for either SUDV NP or VP40. By analogy with EVD survivors with EBOV antigens, this suggest 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; three negative, five indeterminate (reactive to one SUDV or EBOV antigen only), thirteen with reactivity to two antigens (6 for EBOV, 5 for SUDV, 2 to both) and seven reactive to all three antigens (2 for EBOV, 5 for SUDV). Only four samples had a titer of 1:20, one for EBOV (with a negative profile in MIA) and three for SUDV (one negative, one indeterminate with reactivity toward glycoproteins only, and one reactive to three 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 neighbouring 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 Yaounde, the capital city, was minimal (0.3% for EBOV and 0% for SUDV) based on positivity criteria presence of antibodies to at least two reactive antigens (unpublished data). Even with the most stringent criteria, which require concomitant reactivity to three 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 Yaounde. 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 three, or even two antigens diminishes over time among survivors, suggesting that the criterion of three 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 1,517 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 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 (CAR) [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).

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 non-neutralizing 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 (Supplemental Figure S1) [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 glycoprotein 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 sero-reactivities in general could represent cross-reactivity resulting from exposure to a potentially non-pathogenic and uncharacterized filovirus that circulates in Cameroon and Central Africa. In our study, the probability of seroreactivity was significantly higher in Pygmies for all three viruses (EBOV, SUDV and BDBV). Higher seroprevalence to filoviruses have 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 CAR, 24% of Aka Pygmies were seropositive for EBOV, SUDV or Marburgvirus in 1987, and 13% to EBOV in 1995 using IFA, and ELISA respectively [37,44]. In Cameroon, seroprevalence was 15% in Pygmies from South and Eastern regions using IFA [33]. Though, 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 IgM, 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 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 Ebola virus (EBOV) and Sudan virus (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 spill-over events with orthoebolaviruses in Cameroon and other regions with similar ecological contexts.

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Conflict of Interest: The authors declare to have no conflict of interest.

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FIGURES AND TABLES

Table 1: Numbers and percentages of samples from the two surveys in rural forest Cameroon reactive to different antigen combination of orthoebolaviruses (EBOV, SUDV, BDBV)

	NHP case control study (N=795) n (%)	Rural survey (N=622) n (%)
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 two	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 two	76 (9.6%)	88 (14.1%)
BDBV		
GP+VP40	28 (3.5%)	38 (6.1%)

n (%): number (and proportion) of samples with combined seroreactivity to orthoebolaviruses antigens, in two studies in Cameroon. 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. NP: nucleoprotein; GP: glycoprotein; VP40: 40-kDa viral protein; EBOV: Ebola virus; SUDV: Sudan virus; BDBV: Bundibungyo virus.

Table 2: seroprevalence to Ebola viruses in a case-control study on NHP bite in rural Cameroon (N=795)

	N	EBOV seropositivity			SUDV seropositivity			BDBV seropositivity		
		n+	%	<i>p</i>	n+	%	<i>p</i>	n+	%	<i>p</i>
Sex										
Male	753	77	10.2%	1.00	73	9.7%	0.79	25	3.3%	0.18
Female	42	4	9.5%		3	7.1%		3	7.1%	
Age category										
<40	290	25	8.6%	0.52	29	10.0%	0.72	9	3.1%	0.68
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%	<0.001	39	7.4%	0.003	13	2.5%	0.02
Pygmy	267	42	15.7%		37	13.9%		15	5.6%	
NHP Bite										
Control group	530	61	11.5%	0.09	54	10.2%	0.39	23	4.3%	0.09
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 two different antigens. EBOV: Ebola Zaire virus; SUDV: Sudan virus; BDBV: Bundibugyo virus. n+ number of seropositive samples and % seroprevalences; p: p-value. NHP: Non-human primate.

Table 3: Seroprevalence to Orthoebolaviruses in a rural population survey in Cameroon (N=622)

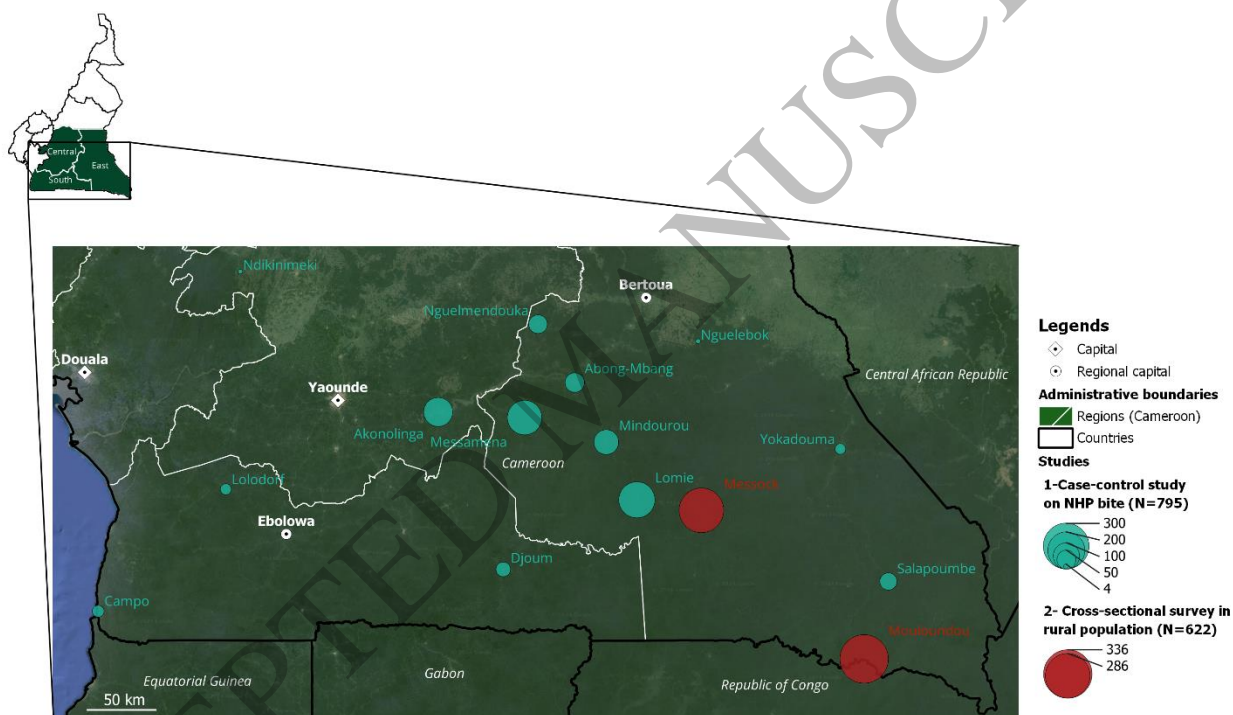
	N	EBOV seropositivity			SUDV seropositivity			BDBV seropositivity		
		n (%)	ORa	p	n (%)	ORa	p	n (%)	ORa	p
Sex										
Male	247	43 (17.4%)	1	0.41	41 (16.6%)	1	0.20	21 (8.5%)	1	0.06
Female	375	54 (14.4%)	0.8 (0.5-1.3)		47 (12.5%)	0.7 (0.5-1.2)		17 (4.5%)	0.5 (0.3-1)	
Age category										
<25	203	38 (18.7%)	1	0.36	35 (17.2%)	1	0.33	15 (7.4%)	1	0.77
25-40	218	33 (15.1%)	0.7 (0.4-1.2)		29 (13.3%)	0.7 (0.4-1.1)		15 (6.9%)	0.8 (0.4-1.8)	
>40	201	26 (12.9%)	0.9 (0.5-1.6)		24 (11.9%)	0.9 (0.5-1.6)		8 (4%)	0.7 (0.3-1.8)	
Ethnic group										
Bantu	286	11 (3.8%)	1	<0.001	13 (4.5%)	1	<0.001	3 (1%)	1	<0.001
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%)		

Seropositivity to each pathogenic *Ebolavirus* species, was defined by reactivity toward at least two different antigens. EBOV: Ebola Zaire virus; SUDV: Sudan virus; BDBV: Bundibugyo virus. n+ number of seropositive samples and % seroprevalences; p: p-value; ORa: odds ratio adjusted on ethnic group.

FIGURES LEGENDS

Figure 1. Geographic distribution of participants from the two serological studies on Ebola viruses in rural Cameroon

Geographical location and number of individuals included in the two serosurveys on Ebola viruses in Cameroon. (1) the case-control study on NHP bite (in blue) included 795 people from South, East and Center Cameroon: 265 people bitten by a NHP and 530 controls, adjusted on age, sex, ethnic group and location. (2) The second study (in red) was carried out in two rural villages in East Cameroon and included 691 people. Google Earth satellite imagery was used as the map background (Map data ©2024 Google).

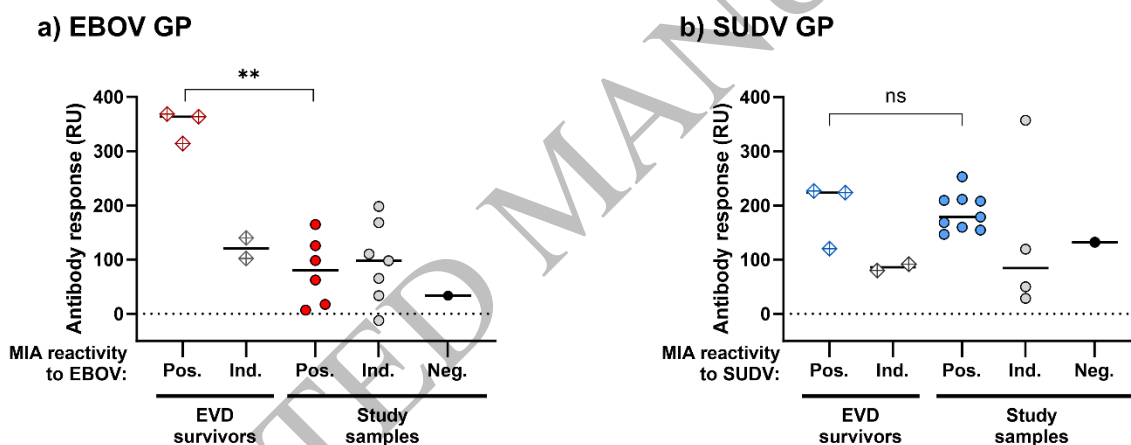


Maps realized with QGIS software.

Figure 2. Average IgG response against EBOV and SUDV glycoproteins measured by Surface Plasmon Resonance (SPR).

Comparison of antibody response on EBOV GP (a) and SUDV GP (b) between EVD survivors and participants in the serosurveys from Cameroon. Antibody binding titers, measured in resonance units (RU), were obtained for five EVD survivors from Guinea, and fourteen samples from the case-control study on NHP bite in Cameroon, including ten seroreactive samples to EBOV or SUDV (at least two antigens), three indeterminate (reactive to only one SUDV or EBOV antigen respectively) and one negative (without reactivity to any antigen). Among the ten reactive samples, 6 are reactive to both EBOV and SUDV, 2 to SUDV only (indeterminate for EBOV), and one to EBOV only (indeterminate for SUDV).

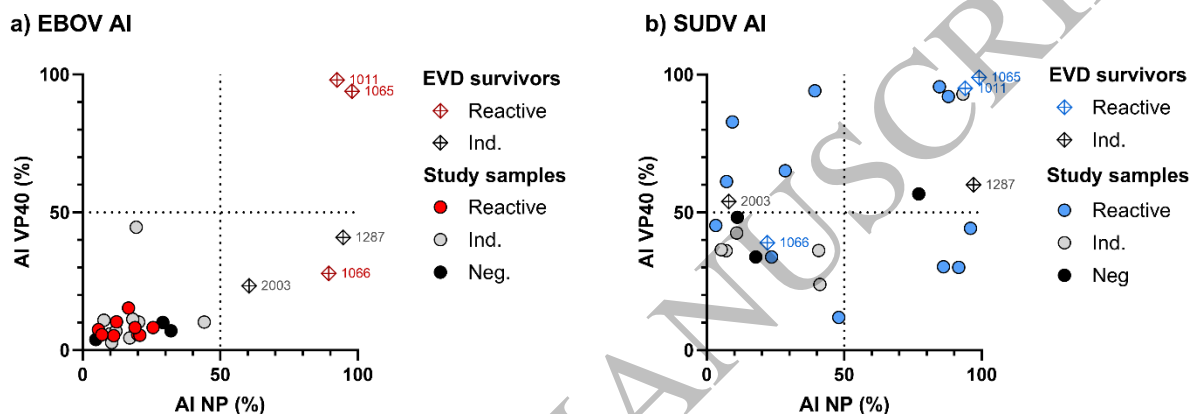
Detailed results are given in the supplemental figure S8. Each sample was tested twice. Differences between EVD survivors and Cameroonian samples were assessed using the Mann-Whitney test.



Pos.: positive MIA (at least two antigens); Ind.: Indeterminate (reactive to only one SUDV or EBOV antigen); Neg.: Negative; EVD: Ebola virus disease; GP: glycoprotein; RU: Resonance Units; SPR: Surface Plasmon Resonance. **<0.01; ns: not significant.

Figure 3. MIA avidity index (%) for IgG against ebolavirus antigens

MIA avidity index on a) EBOV and b) SUDV nucleoprotein and VP40 for 21 samples from the case-control study on NHP bite from Cameroon were tested, including 14 with reactivity to at least two antigens (2 to EBOV antigens, 5 to SUDV and 7 to both EBOV and SUDV), and seven negative samples (4 with indeterminate profiles i.e. only one reactive antigen, and three negatives to all antigens). Five sera from EBOV survivors of the outbreak in 2014-16 in Guinea were used as positive controls and are indicated with their labels in the figure.



AI: avidity index (%); NP: nucleoprotein; VP40: 40-kDa viral proteins; EBOV: Ebola virus; SUDV: Sudan virus; Ind. Indeterminate; Neg. Negative.