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Ebola virus circulation in a non-epidemic Guinean rural area: A mixed-method approach to assessing endemicity

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ABSTRACT

Objectives: This study aimed to investigate the prevalence of *orthoebolavirus* antibodies in Madina Oula, a non-epidemic rural area in Guinea, in 2022.

Methods: A cross-sectional study was conducted from March 14 to April 3, 2022 involving recording household and socio-demographic characteristics, lifestyle data, and collecting dried blood spots from 878 individuals in 235 households. Dried blood spots were tested using multiplex serology to detect antibodies to different *orthoebolaviruses*: Ebola virus, Bundibugyo virus, Sudan virus, Reston virus, and Bombali virus. Seroprevalence was estimated with a 95% confidence interval and a Z-test was performed to compare the seropositivity between children aged under 15 years and those over 15 years. Household and participant characteristics were analyzed using descriptive statistic, and socio-historical conditions were discussed.

Results: The serological analysis conducted in 2022 on 878 participants revealed varying reactivity to *orthoebolavirus* antigens, notably, with glycoprotein antigens, particularly, glycoprotein Sudan virus (16%). A total of 21 samples exhibited reactivity with at least two antigens, with a median age of 27 years (interquartile range 10.00-35.00), ranging from 2 to 80 years. There is no significant difference between seropositivity in children aged under 15 (2.86%) years and those over 15 (2.14%) years. The antibody presence varied per village, with the highest prevalence observed in Ouassou and Dar-es-Salam.

Conclusions: Serological data in a region unaffected by recent Ebola outbreaks indicate possible orthoebolavirus endemicity, emphasizing the need for preparedness against known or novel orthoebolaviruses with potential cross-reactivity.

Introduction

The Republic of Guinea became the focus of renewed attention for hemorrhagic fever due to the large Ebola virus (EBOV) disease (EVD) outbreak in the Mano River countries (Guinea, Sierra Leone, Liberia) that affected this West African region between 2014 and 2016 [1]. The results of a seroprevalence survey carried out in this area in 1982, 6 months after an outbreak of unknown origin, were published in 1987. The authors hypothesized that it was a case of hemorrhagic fever linked to the Lassa or EBOV. In October 2014, at a time when Guinea was experiencing what was presented as the first epidemic, the article published in 1987 was rescued from oblivion.

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The large outbreak in 2014, resulting in more than 28,000 cases and over 11,000 recorded deaths, was the first outbreak of this scale in history. Since then, Guinea has been the site of multiple research programs aimed at identifying the possible animal reservoir of EBOV to prevent the next pandemic [2]. Some studies have indeed shown the possible circulation of EBOV in the region before the 2014 outbreak [3–6] and insisted on the need to pay a closer look to circulation of *orthoebolaviruses* within the population, which was, up to now, poorly studied [7]. During the 2014 epidemic, data from more than 1700 EVD survivor contacts showed that the EBOV seroprevalence ranged from 3.06% among asymptomatic contacts who did not participate in funeral rituals to 5.98% among those who did. Similarly, the seroprevalence ranged from 7.17% among pauci-symptomatic contacts who did not participate in funeral rituals to 17.16% among those who did in Guinea [8]. Other enzyme-linked immunosorbent assay–based serological surveys have shown different antibody prevalence rates among populations living in areas where no Ebola cases have ever been reported, suggesting that EVD can have asymptomatic forms in humans [9]. In addition, serology suggests EBOV circulation in samples taken 2 years before the 2014-2016 epidemic [10], showing that EBOV was potentially already present in some localities in Guinea. Similar data were found in Central African areas where apparently healthy individuals have been found to be seropositive for EBOVs [11].

For some years now, Madina Oula has been considered as a hotspot of epidemics, particularly, for Lassa [12] but Ebola remained a pending question articulated with a question regarding the possibility of herd immunity based on the old reports from 1987. The region, although being in the area of the Ebola outbreak in 2014, did not report cases during the 2014-2016 pandemic. We aimed to analyze the seroprevalence of EBOV disease in a non-epidemic period in a rural region, with suspicion of a similar outbreak in the 80s.

Material and methods

Study setting

Madina Oula is a district in Guinea. It borders the Republic of Sierra Leone and is located 75 km east of Kindia city (Figure 1). The district of Madina Oula covers an area of 1432 km² and has 27,874 inhabitants and 3947 households, with a mainly agropastoral population [9].

Study design, sampling, and data collection

A cross-sectional study for estimating seroprevalence to *orthoebolaviruses* was conducted between March 14 and April 3, 2022 in all the districts in Madina Oula. Participation in the study was voluntary. Based on this, 878 people were interviewed from the 235 households that agreed to participate in the study (Figure 1d). Blood samples were collected in the form of dried blood spots (DBS) on an adult's fingertip or on children's heel. Samples were sent to the Centre de Recherche et de Formation en Infectiologie de Guinée's laboratory in Conakry for serological testing. Heads of households provided household characteristics. All who were surveyed provided information on socio-demographic characteristics, lifestyle, and feeding habits. These data were collected using REDCap mobile data collection tool.

Historical and ethnographic data

Historical data were collected based on literature reviews, archive work in Guinean and French national archives, and ethnography. Fieldwork was carried out in Madina Oula district villages and comprised observation of practices associated with epidemic management, collection of data regarding human, hon-human, and environmental evolution, and epidemic experiences since the 19th century. Interviews in local languages (Soso, Maninka) or French were held with key informants, such as Guinean researchers, members of families exposed to former outbreaks, health care workers, and key informants (men and women of old age) who were able to recall the outbreak and relate the history of Madina Oula.

Analysis of blood samples and seropositivity criteria

DBSs were tested using the Luminex technology (Luminex Corp, Austin, TX, USA) [13] and more precisely on MagPix instrument, which allows the simultaneous detection of up to 50 analytes in a single well of a 96-well flat-bottom plate, thus limiting the volumes of rare biological samples. The assay included 11 commercially available recombinant *orthoebolavirus* proteins, glycoprotein (GP), nucleoprotein (NP), and viral protein 40 (VP40) from five different *orthoebolavirus* species: EBOV, previously called Zaire (NP, strain Kissidougou-Makona 2014; VP40, strain Kissidougou-Makona 2014; GP-k, strain Kissidougou-Makona 2014; GP-m, strain Mayinga 1976); Sudan virus (SUDV), Sudan (NP, strain Gulu; GP, strain Uganda 2000; and VP40, strain Gulu,); Bundibugyo virus (BDBV), Bundibugyo (GP, strain Uganda 2007; VP40 strain Uganda 2007); Reston virus, Reston (GP); and Bombali virus, Bombali (GP). Recombinant proteins were produced in insect cells and purchased from Sinobiologicals (Beijing, China), except for REST GP (IBT, Gaithersburg, MD). We first reconstituted blood from DBS in 200 µl of dilution buffer. A final sample dilution of 1/1000 was incubated with magnetic beads coated with recombinant protein (2 µg protein/1.25 × 106 beads) in 96-well flat-bottom chimney plates (Greiner bio one, Frickenhausen, Germany) on a plate shaker at 400 rpm for 16 hours at 4°C in the refrigerator. After washing, we added 50 µl of 4 µg/ml Biotin mouse anti-human immunoglobulin (Ig) G to each well and incubated for 30 minutes at 400 rpm at room temperature. After washing, we added 50 µl of 4 µg/ml streptavidin-R-phycoerythrin (Fisher Scientific/Life Technologies, Ill-kirch, France) per well and incubated for 10 minutes at 400 rpm at room temperature. Reactions were read with MagPix (Luminex, Austin, TX, US). At least 50 events were read for each bead set, and the results were expressed as median fluorescence intensity (MFI) per 50 beads. Samples were considered reactive when MFI values exceeded 400 for GP, 600 for NP, and 650 for VP40 for each virus species [14]. A subset of samples that showed positive signals were repeated to validate the results. Samples were considered positive for previous infections with *orthoebolaviruses* (EBOV, SUDV) when antibodies to two antigens from different genomic regions were positive. Positivity was established based on reactivity to two antigens of the same species. For EBOV: NP + GP-k or NP + VP40 or VP40 + GP-k, VP40 + GP-m; for SUDV: NP + GP or NP + VP40 or GP + VP40; and for BDBV: GP + VP40. Cross-reactivity was established based on positivity to two virus species simultaneously, considering the same MFI threshold values.

Analysis of data

Data were processed and analyzed with R 3.5.3 software [15]. Descriptive statistics were used to describe quantitative (median and interquartile range were calculated against the normality violation of the data with Shapiro–Wilk test) and categorical variables (absolute frequency and percentages) of households characteristics in Madina Oula, the socio-demographic characteristics, and the lifestyle of the members of these households. The seroprevalence of the respondents to the EBOV was examined using percentage distributions with their 95% confidence intervals (CIs). These CIs were calculated using the Wilson score method [16]. A Z-test

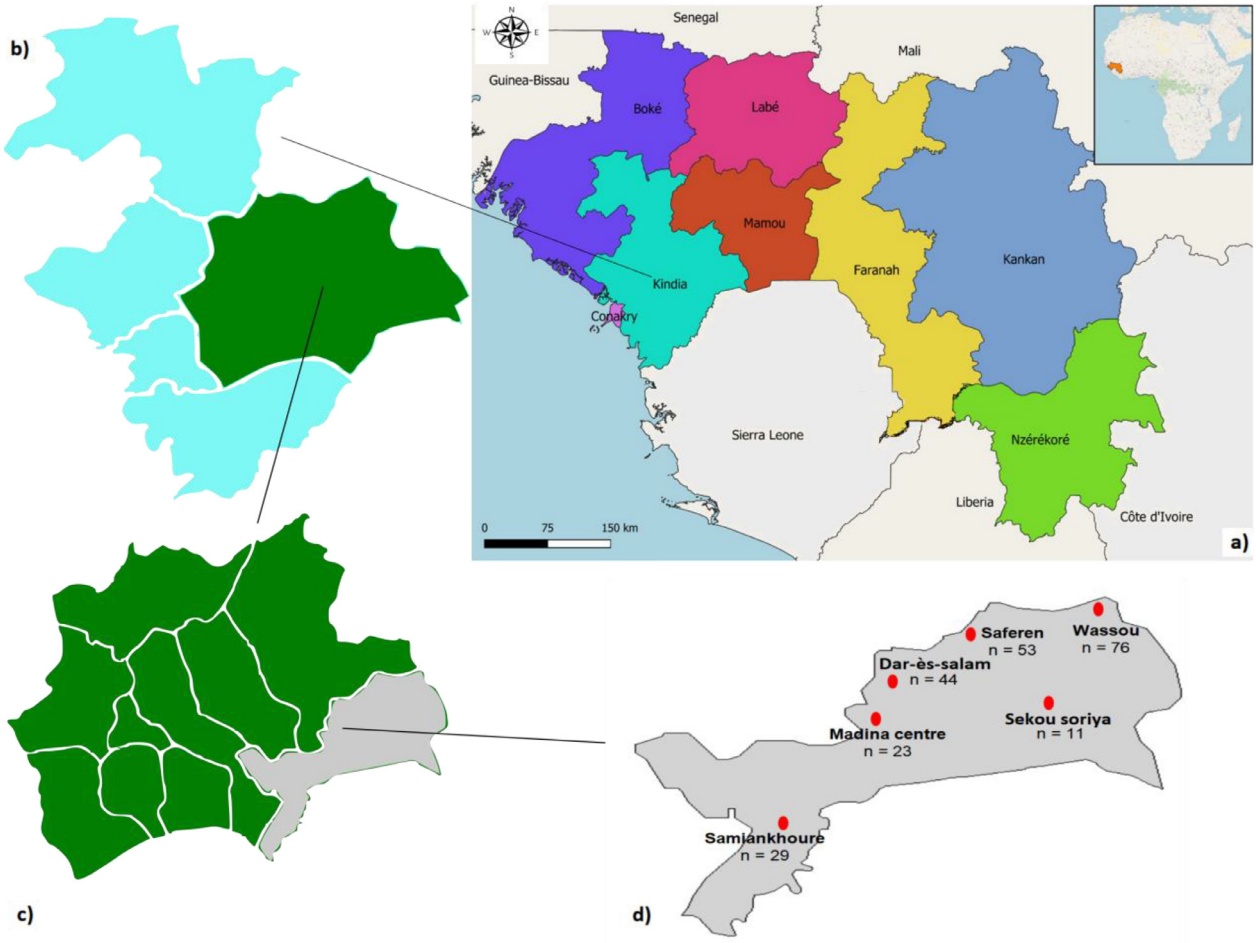

Figure 1. Map of Madina Oula showing survey and blood sample collection sites, and the size of households surveyed (red dots).

was performed to compare the seropositivity between in children under 15 years and those over 15 years. A significant difference is observed if P <5%. Cluster analyses were performed, considering each household as a cluster where seroprevalences were determined for reactivity to at least the antigens of the same orthoebolavirus species. The overall estimate of household seroprevalence was then calculated with the mean and 95% CI.

Interviews have been transcribed in vernacular languages and translated into French. Together with data collected through observation and archives, they have been subjected to thematic analysis.

Ethical aspects

Ethical approval for the study was obtained from the Guinea Ethics Committee (100/CNERS/19).

Results

Population

The population of Madina Oula is predominantly Soso and Maninka. The villages are spread over the plain as far as the Tamisso mountains and are linked by a road that is not very passable during the rainy season. The population maintains family ties with neighboring populations in Sierra Leone and lives mainly from agriculture (rice, peanuts, arboriculture, and market gardening) and livestock farming. Fishing is also practiced in the rivers. Hunting is regularly practiced for sale and provides an occasional protein resource.

History of epidemics in Madina Oula

Between March-August 1982 and January 1983, several illnesses had occurred in this region and the authors retrospectively described the cases as follows: cough, cold, asthma, chest pain, asthenia, and, sometimes, anorexia and emaciation [4]. In some patients, vomiting (sometimes with blood), diarrhea, and hematuria were also reported. The illness lasted 7-12 days and 17% of the villagers were affected and 60% of them were children. The illness began with a sudden high fever, pronounced toxicosis, and frequent hemorrhaging. A total of 137 deaths were recorded in the region, corresponding to a case fatality rate of 38%. However, this paper received little attention from the scientific community, which questioned the sensitivity of the tests used to characterize Ebola. During the Ebola outbreak in 2014-2016 in Guinea, although Madina Oula is located in the epidemic region between Kindia and Forecariah, no cases were recorded, raising the question of the possibility of herd immunity. In the 2000s, Madina Oula had been the site of the Lassa fever outbreak associated with the settling of Sierra Leonean refugees fleeing the rebellion in Sierra Leone [17]. Seen today as a remote place between floods and mountains at the Sierra Leonian border, today's Tamisso used to be an important nexus for traveling from east to west toward the port of Benty on the Guinean shores of the Atlantic Ocean. Until now, its population (except for the refugee camp) had remained relatively ho-

Table 1
Antibody detection rates in the rural population of Madina Oula to antigens of different *Orthoebolavirus* species according to districts.

Species	Antigen	Dar-es-Salam, n (%) N = 183	Madina center, n (%) N = 95	Saferen, n (%) N = 221	Samiankhoure, n (%) N = 106	Sekou Sorya, n (%) N = 46	Ouassou, n (%) N = 227	Total, n (%) N = 878
	NP	1 (0.1)	..	3 (0.3)	2 (0.2)	1 (0.1)	..	7 (0.8)
EBOV	GP Kiss	17 (1.9)	5 (0.6)	3 (0.3)	5 (0.6)	4 (0.5)	24 (2.7)	58 (6.6)
	GP May	16 (1.8)	7 (0.8)	5 (0.6)	3 (0.3)	3 (0.3)	29 (3.3)	63 (7.2)
	VP40	8 (0.9)	2 (0.2)	3 (0.3)	9 (1)	1 (0.1)	9 (1)	32 (3.6)
	NP	2 (0.2)	..	..	4 (0.5)	1 (0.1)	..	7 (0.8)
SUDV	GP	28 (3.2)	14 (1.6)	17 (1.9)	8 (0.9)	6 (0.7)	66 (7.5)	139 (16)
	VP40	5 (0.6)	1 (0.1)	4 (0.5)	5 (0.6)	1 (0.1)	10 (1.1)	26 (3.0)
BDBV	GP	12 (1.4)	5 (0.6)	2 (0.2)	4 (0.5)	1 (0.1)	20 (2.3)	44 (5.0)
	VP40	12 (1.4)	0 (0)	4 (0.5)	3 (0.3)	1 (0.1)	13 (1.5)	33 (3.8)
RESTV	GP	1 (0.1)	1 (0.1)	..	..	..	6 (0.7)	8 (0.9)
BOMV	GP	2 (0.2)	1 (0.1)	..	..	..	2 (0.2)	5 (0.6)

BDBV, Bundibugyo; EBOV, Ebola virus; GP1, glycoprotein; NP, nucleoprotein; RESTV, Reston; SUDV, Sudan; VP40, 40 kDa viral protein.

Table 2
Antibody detection rates in the rural population of Madina Oula to at least two antigens of the same *orthoebolavirus* species according to districts.

Species	Antigens	Dar-es-Salam N = 183 n (%)	Madina Oula center N = 95 n (%)	Saferen N = 221 n (%)	Samiankhoure N = 106 n (%)	Sekou Sorya N = 46 n (%)	Ouassou N = 227 n (%)	Overall N = 878 n (%)	n (%); IC95% per species
	NP + GP_k	..	..	1 (0.1)	..	..	..	1 (0.1)	
	NP + VP40	..	..	..	..	1 (0.1)	..	1 (0.1)	12 (1.4);
EBOV	VP40 + GP-k	1 (0.1)	..	..	1 (0.1)	..	3 (0.3)	5 (0.6)	IC 95% = (0.7-2.4)
	VP40 + GP-m	1 (0.1)	..	..	1 (0.1)	..	3 (0.3)	5 (0.6)	
	NP + GP	..	..	..	1 (0.1)	..	..	1 (0.1)	12 (1.4);
SUDV	NP + VP40	1 (0.1)	..	..	1 (0.1)	..	..	2 (0.2)	IC 95% = (0.7-2.4)
	GP + VP40	4 (0.5)	..	1 (0.1)	..	1 (0.1)	3 (0.3)	9 (1)	
BDBV	GP + VP40	4 (0.5)	..	..	..	..	3 (0.3)	7 (0.8)	7 (0.8); IC 95% = (0.4-1.7)

BDBV, Bundibugyo; EBOV, Ebola virus; GP, glycoprotein; GP-k, kissoudougo-makona EBOV strain; GP-m, mayinga EBOV strain; IC, confidence intervals; NP, nucleoprotein; SUDV, Sudan; VP40, 40 kDa viral protein.

mogeneous. Population movements concerned mainly the inhabitants of the mountain villages who got closer to the floods at the opening of the road and French colonial domination. Interestingly, the signing of the protectorate between France and the Kingdom of Tamisso in May 1889, guaranteeing France the establishment of a secure trade route between the interior of Guinea and the port of Benty, coincides with the period when, according to phylogenetic research [18], it is believed that the Lassa virus entered Guinea. The entry of certain viruses, therefore, coincides with the intensification of trade in the region. In 1917, outbreaks have been described along the road from Ouassou to Benty by indigenous health workers with symptoms close to what is known as hemorrhagic fever (rash, diarrhea, death within few days, and high contagiousness) [19].

Socio-demographical characteristics of the 2022 survey

A total of 879 participants from 236 households were included. The median number of people in each household was 7 (interval interquartile range [IQR] 3-11) and the mean number of rooms per household was 6.99 ± 8.03. The distance between health facilities and households was between 0 and 5 km in 196 (83.05%) households. between 6 and 10 km in 39 (16.53%), and more than 10 km in 1 (i.e. 0.42%). Meat consumption was 67% and consisted of bushmeat and domestic animals. A total of 57% of the meat consumed was either beef, sheep, or goat. The bushmeat was either purchased (64%) or obtained from hunting (9.3%).

The majority of participants were female (55%) and the median age of the study population was 25.00 (IQR 9.00-42.00) years. The majority of participants were women (20.62%), children (23.92%), or grandchildren (13.78%) of the head of the household. More than half were farmers (56.15%). Those who reported that they had been in contact with someone with signs of EBOV numbered 29, representing 3.30%.

Seroprevalence to orthoebolaviruses in the 2022 survey

Among the different antigens, the highest reactivity was observed with GP antigens, especially for GP SUDV (16%) (Table 1), followed by GP EBOV (7.2% for EBOV-m and 6.6% for EBOV-k) and GP BDBV (3.8%), and only few reactivity and at lower MFIs was observed with GP RESTV (0.8%) and GP BOMV (0.6%). The presence of antibodies to *orthoebolavirus* antigens varied per village and was the highest in Ouassou and Dar-es-Salam.

Table 2 shows the numbers and percentages for the different *orthoebolaviruses* and by locality. Significantly, there were less samples that had antibodies to two or more antigens from the same *orthoebolavirus* species. The total number of samples reactive with at least two antigens of the same virus species was 21. EBOV and SUDV each represented 12 cases of 878 (1.4%; 95% CI 0.7-2.4). BDBV accounted for seven cases of 878 (0.8%), with 95% CI 0.4-1.7.

Whatever the species, the highest seroprevalence was found in Dar-es-Salam and Ouassou. No cases were observed in Madina Oula center (Table 2). In Dar-es-Salam, four cases (0.5%) were found seropositive for SUDV (GP + VP40) and BDBV (GP+VP40). In Ouassou, three cases (0.4%) were seropositive: one for SUDV (GP+VP40), a second for BDBV (GP+VP40), and a third one for EBOV (VP40+GP Kissidougou).

The socio-demographic characteristics and lifestyle of 21 samples reactive with at least two antigens of the same virus species are summarized in Table 3, together with localities. More than half of the seropositive participants who have two or more antigens from the same *orthoebolavirus* species were farmers (11) and uneducated (10) and very few had primary and secondary education (9.52% and 4.76%, respectively). Their median age was 27 (IQR 10.00-35.00) years and varied from 2.0 to 80.00. The seroprevalence in children under 15 years was 2.86%. The analysis of the Z-test showed that there is no significant difference (P >5%) be-

Table 3
Socio-demographic characteristics of respondents seropositive for one or more Ebola virus species in the Madina Oula district.

District	Sex	Age	Relationship head of household	Current occupation	Education level	Bushmeat purchased	Bushmeat hunted	Antigens: combination per species
Dar-es-Salam	Male	2	Child	NA	NA	NA	NA	Sudan: (GP+VP40) Bundibugyo: (GP+VP40)
Dar-es-Salam	Female	4	Child	NA	NA	NA	NA	Bundibugyo: (GP+VP40)
Dar-es-Salam	Male	10	Child	NA	NA	NA	NA	Sudan: (GP+VP40)
Dar-es-Salam	Male	10	Child	NA	NA	NA	NA	Sudan: (GP+VP40)
Dar-es-Salam	Female	11	Child	NA	NA	NA	NA	Bundibugyo: (GP+VP40)
Dar-es-Salam	Female	20	Female	Farmer	Primary	Yes	No	Bundibugyo: (GP+VP40);
Dar-es-Salam	Male	24	Head of household	Farmer	Illiterate person	No	No	Sudan: (NP+VP40)
Dar-es-Salam	Female	35	Female	Farmer	Illiterate person	Yes	No	Zaire: (VP40+GPKiss, VP40+GPMay)
Dar-es-Salam	Female	80	Sister/Brother	Farmer	Illiterate person	Yes	Yes	Sudan: (GP+VP40)
Saferen	Female	35	Female	Farmer	Illiterate person	Yes	No	Zaire: (NP+GPKiss)
Saferen	Female	40	Female	Framer	Illiterate person	No	No	Sudan: (GP+VP40)
Samiankhoure	Male	9	Sister/Brother	NA	NA	NA	NA	..
Samiankhoure	Female	9	Child	NA	NA	NA	NA	Sudan: (GP+VP40) Zaire: (VP40+GPKiss)
Samiankhoure	Female	25	Sister/Brother	Farmer	Illiterate person	Yes	Yes	..
Sekou Sorya	Male	14	Child	Trainee Driver	Secondary	No	No	Sudan: (GP+VP40)
Sekou Sorya	Male	25	Child	Blacksmiths	Illiterate person	Yes	No	Zaire: (NP+VP40)
Ouassou	Male	10	Child	NA	NA	NA	NA	Bundibugyo: (GP+VP40); Sudan: (GP+VP40); Zaire: (VP40+GPKiss,VP40+GPMay)
Ouassou	Female	30	Female	Farmer	Primary	Yes	No	Bundibugyo: (GP+VP40)
Ouassou	Female	33	Female	Farmer	Illiterate person	No	No	Bundibugyo: (GP+VP40); Sudan: (GP+VP40); Zaire: (VP40+GPKiss,VP40+GPMay)
Ouassou	Female	35	Female	Farmer	Illiterate person	Yes	No	Sudan: (GP+VP40)
Ouassou	Male	40	Head of household	Farmer	Illiterate person	Yes	No	Sudan: (GP+VP40); Zaire: (VP40+GPKiss)

GP, glycoprotein; IC, confidence intervals; NA, not available; NP, nucleoprotein; VP40, 40 kDa viral protein.

Table 4
Antibody detection rates in the clusters (households) of the rural population of Madina Oula to at least two antigens of the same *orthoebolavirus* species.

Species	Antigens	Overall seroprevalence per household, N = 235 Mean (IC 95%)	Overall seroprevalence per household and per species Mean (IC 95%)
EBOV	NP + GP_k	0.14 (−0.14, 0.42)	0.93 (0.14, 1.72)
	NP + VP40	0.05 (−0.05, 0.15)	
	VP40 + GP-k	0.74 (0.01, 1.47)	
	VP40 + GP-m	0.74 (0.01, 1.47)	
SUDV	NP + GP	0.21 (−0.21, 0.63)	1.81 (0.54, 3.08)
	NP + VP40	0.09 (−0.03, 0.21)	
	GP + VP40	1.51 (0 .3, 2.71)	
BDBV	GP + VP40	1.02 (−0.03, 2.07)	1.02 (−0.03, 2.07)

BDBV, Bundibugyo; GP, glycoprotein; GP-k, kissoudougo-makona EBOV strain; GP-m, mayinga EBOV strain; IC, confidence intervals; NP, nucleoprotein; SUDV, Sudan; VP40, 40 kDa viral protein.

tween seropositivity in children under 15 (2.86%) and those over 15 (2.14%) years.

The overall seroprevalence of households (clusters) in the rural population of Madina Oula for at least two antigens of the same orthoebolavirus species (Table 4) was 1.81% for SUDV species, with a 95% CI 0. 54-3.08, for BDBV it was 1.02%, with a 95% CI −0.03 to 2.07, and for EBOV, 0.93% with a 95% CI 0.14-1.72; each represented in 12, six, and seven households with cases out of 235, respectively.

Socio-anthropo-historical analysis of seroprevalence

A seen from the vantage of anthropological research, the 2022 results echo the memories of the 1982 outbreak. Ouassou (60 clinical cases declared, 30 deaths) and Dar-es-Salam (40 cases and 15 deaths) have been the most affected villages and where we observe today the highest reactivities to *orthoebolavirus* antigens. At the time of the survey, these villages still hosted families able to name their parents lost during the 1982 outbreak. Only Sekou Sorya presented with less cases (six cases recorded and three deaths) [3]. In the interviews held in these villages, the clinical signs referred to by inhabitants to describe the epidemic show that local nosography cannot strictly be translated in biomedical nosologies, which is a fact very well known in medical anthropology [20]. Thus, referring to the 1982 outbreak, an old man in Dar-es-Salam showed what were obviously scars of smallpox, whereas others described what appeared to be a cholera outbreak. Nevertheless,

all remember collective and individual reactions to the epidemic (in Soso: *wuganyi*, epidemic, or *wugan fure*, big illness). In Ouassou, people recalled that it started with a young man coming back from Simbaraya (a neighboring village) into the village and being sick. Facing his illness, his sister advised him to stay in quarantine in a bush camp to avoid contamination of his family. He died there, together with those staying with him, and the illness started spreading along the road from Ouassou to Madina Oula. A Fulani settlement (cattle herders) totally disappeared next to Saferen in the Tamisso mountains. Witnessing the death of their parents, the surviving Fulani abandoned the site. At that time, no health center was present in the area (except in Madina Oula city). Nevertheless, it is worth noting that the population did not remain stagnant. Ancestors have been worshipped and readings happened at the mosque. At home, separation was organized among siblings. Children presenting with rash, fever, and diarrhea with blood were asked to keep separated from their siblings when eating (in Soso, *taxunyi* means to divide and by extension separation). They washed themselves in a separate place and slept alone on a bed made of banana leaves. In Ouassou and Saferen, a separate cemetery was dedicated in the bush to those who died because of this bad disease. The outbreak followed the road from Ouassou to Madina Oula City. This road structures the regional space. It is used by the population to attend rural markets (between Ouassou and Simbaraya in Madina Oula) or to link families together. For example, the founders of Ouassou have many family members in Madina Oula city and extensive marriages are occurring within families spread in different villages located in the district of Madina Oula.

Discussion

Our results suggest the circulation of *orthoebolaviruses* in the general population in a rural area that reported no EVD cases during the 2014-2016 and 2021 epidemics in the country but had reported a possible hemorrhagic fever outbreak in the 80s. In this area, we found antibodies to ebolaviruses (EBOV and SUDV), which are at the origin of the EVD epidemics in Africa.

The source of contamination of the seropositive individuals in this study is unclear. Three hypotheses could be formulated. First, these people were contaminated during the 2014-2016 epidemic. Our previous studies showed that some people that come into contact with Ebola can remain pauci- or asymptomatic [6,8]. The proportion of these asymptomatic forms is highest in people who have been in contact with patients with EVD. Second, contamination could have occurred during the probable outbreak in the 1980s. The memory of this probable Ebola outbreak is vivid among elders in the area. However, the age of some young people does not support this hypothesis. Only two seropositive cases were over 40 years, i.e. present in the district in 1980s. Recent studies have suggested that recovery from EBOV infection triggers the development of cell-mediated and humoral immune responses, which may diminish over time [21,22]. Substantial drops in immunoglobulin G levels have also been reported among healthy survivors [21–23]. The third hypothesis is that these people encountered the virus through wildlife and/or domestic animals, suggesting that asymptomatic cases of EVD sometimes occur in this area. Domestic animals are exposed to wildlife through divagation, and humans may be exposed to the viruses through activities performed in areas where wildlife is present. Previous studies carried out on samples taken before the first Ebola outbreak in Guinea, together with data on bats, showed that the virus could be present in Guinea before without being detected [24,25]. Seroprevalence studies carried out on thousands of bats sampled between 2015 and 2021 showed the presence of antibodies against EBOV and SUDV in insectivorous and frugivorous bats in Guinea, Cameroon, and the Democratic Republic of Congo. One of the sites where bats have been captured in Guinea were close to the Madina Oula area. Other studies in humans in Guinea showed also the presence of antibodies against EBOV in humans, probably due to exposure to other Ebolavirus species or related filoviruses [26], as is the case in other West and Central African countries [7,27].

Our study showed the presence of antibodies to SUDV, although this species has never been reported in Guinea; this could indicate a possible circulation of this species in this area or cross-reactivity with other known or not yet documented *orthoebolaviruses* that circulate in humans and animals in Guinea**.** We previously reported cross-reactivity with SUDV and BDBV antigens among EBOV survivors in Guinea. We also observed cross-reactivity in bat populations, especially among GP antigens [28]. Importantly, the assay used in the current study is the same as the one used to study antibody kinetics over time in EBOV survivors, evaluate pauci- and asymptomatic infections, and evaluate the presence of antibodies in 2012 in Guinean forests and bats; data are, thus, in contrast with other studies, where a wide variety of tests and antigens are used. Given the high cross-reactivity of antibodies among GP proteins, it cannot be excluded that the observed antibodies correspond to cross-reactivity with other not yet identified *orthoebolaviruses* that circulate among bats and humans. Finally, false-positive reactivity or cross-reactivity with other pathogens cannot be excluded. The latest SUDV epidemic was reported in Uganda in 2023; however, further studies are needed to assess the risk of its occurrence in Guinea. This work could help the health authorities to prepare for a new epidemic, especially because the vaccine that is available and was used during the 2014 and 2021 epidemics is only dedicated to the EBOV strain.

One of the interesting results of our study is that children from an area that has never officially reported a case of Ebola tested positive for serology. Over 40% of people with positive Ebola serology are children aged between 2 and 14 years. However, a detailed analysis showed that there is no statistically significant difference between seropositivity in children aged under 15 and those over 15 years. The presence of antibodies in children, some of whom were born a few years after the 2014-2016 epidemic in an area that has not reported any confirmed case of Ebola, raises important questions when analyzing the risks of emerging hemorrhagic fever epidemics. Two hypotheses could explain these results. They could be antibodies acquired from the mother or correspond to asymptomatic forms of the disease. With a minimum age of 2 years, any antibodies from the mother would have probably disappeared by this age because, for example, for HIV, maternal antibodies rarely persist for up to 24 months [29]. Therefore, the presence of antibodies in these children could be related to a probable contact with an *orthoebolavirus*, either EBOV or a not yet documented virus from the same genus cross reacting with EBOV. The hunting activities of children and their consumption of small game (e.g. rodents) are often overlooked by those studying possible links between hemorrhagic fever outbreaks and bushmeat consumption [28]. Our finding suggests a need for a more systematic exploration of this avenue.

Although asymptomatic cases have been described previously, it is difficult to hypothesize that there have been so many asymptomatic cases with no symptomatic cases detected by the health care system. Not all filoviruses are pathogenic to humans, for example, the Bombali and Reston *orthoebolaviruses* have not been documented or are not pathogenic in humans, respectively, and the observed seropositivity can also be due to a not yet documented non-pathogenic *orthoebolavirus*.

The seroprevalence results and their juxtaposition to historical and anthropological data raise several questions that deserve further explorations. First, it invites consideration of the long socio-economic dynamics in the spread of viruses and in the emergence of hemorrhagic fever outbreaks. Second, the hunter figure or bush-

meat consumer appears as an easy target when profound socio-economic dynamics shape the context in which the virus found its way within the population [30]. Third, this raises questions about our sources of knowledge. In contexts with a deficient health system, we must accept the possibility of having overlooked the low-level presence of the virus [29]. In this context, the recognition of the population's exposure to viruses invites us to consider community experience as one form of knowledge on hemorrhagic fever and one dimension of preparedness [2,5]. It invites us to shift from an understanding of the hemorrhagic fever outbreak as an emergency issue calling for humanitarian response to the acknowledgment of its endemicity and the need to develop a public health approach built on a collaboration with the population because it has been "living with viruses" [31]. It invites to provide the health system with tools to triage "good" and "bad" fevers on an ordinary basis, i.e. rapid tests.

This study may have some limitations. First, because the study was cross-sectional, we were unable to carry out an in-depth analysis of the source of the contamination. Second, the study focused on a well-defined area; however, this was a border area with the potential for regular travel to Sierra Leone, another country that regularly experienced cases of viral hemorrhagic fevers, including Lassa, and we did not test for antibodies to Lassa or Marburg viruses, which are also reported in Guinea. Finally, our data did not explore the frequency and type of meat consumed by the population for an in-depth analysis of this hypothesis of contamination. Despite these limitations, this study provides information that will be useful in preparing the country for future epidemics.

Conclusion

In short, the results of this study suggest EBOV circulation in an area that reported no cases during the last two epidemics in Guinea. Antibodies were found in people of all ages, suggesting the need to strengthen the surveillance and the development of an early detection system in Guinea. These results, along with those published previously, increasingly raise the question of the endemicity of Ebola in certain sub-Saharan African countries and show that these rural populations have been living with viruses and, in the past, adopted strategies to stop their spread, which should be considered in prevention strategies.

Declarations of competing interest

The authors have no competing interests to declare.

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Author contributions

FLM, AA, MP, AKK, ED, and AT designed the study and developed the protocol. CGH, FLM, DK, MD, AKS, HD, GT, SCC, AA, MP, AKK, ED, and AT acquired the data, had direct access to the data, and have verified the data reported in the manuscript. CGH, FLM, MP, AA, AKK, and AT analyzed the data. All authors had full access to the data, critically reviewed the paper, approved its submission, and accept responsibility for the contents.

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