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Short communication

Seroprevalence of Crimean-Congo hemorrhagic fever virus among people living with HIV in Brazzaville, Congo and among blood donors in Bamako, Mali

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ABSTRACT

Crimean-Congo hemorrhagic fever virus (CCHFV) is the causative agent of Crimean-Congo hemorrhagic fever (CCHF), a highly contagious and potentially fatal emerging disease. We assessed CCHFV seroprevalence by conducting a serological survey of two cohorts from Brazzaville, Congo and Bamako, Mali. We retrospectively screened 581 sera samples, including 352 from monitoring centers for people living with HIV (PLWH) in Brazzaville and 229 provided by the Blood Transfusion Center at Gabriel Touré Hospital in Bamako. An ELISA kit (ID Screen® CCHF Double Antigen Multi-species, Innovative Diagnostics) was used to detect total anti-CCHFV antibodies in serum. CCHFV seroprevalence was 0.6% in the PLWH cohort in Brazzaville, all in a peri-urban area near livestock/agriculture, and 1.75% in a cohort of blood donors in Bamako, half living in a peri-urban area near livestock/agriculture and the others performing risk-exposure activities, such as working as a butcher or with frequent rural travels. PLWH from Brazzaville were mostly female, older, and more highly educated, with a tertiary sector activity and living in an urban biotope without livestock/agricultural activities in the surroundings, in contrast to the blood donors of Bamako, who were younger and more likely to live in peri-urban/rural areas with livestock/agricultural activities in the surroundings. Despite a low CCHFV seroprevalence, our study indicates human contact with CCHFV in sub-urban areas of the capital cities of Congo and Mali associated with previously described CCHFV risk factors.

1. Introduction

Crimean-Congo hemorrhagic fever (CCHF) is an emerging highly contagious, tick-borne, potentially fatal disease with a case fatality rate of up to 30% (Ma and Hamza, 2021; Monsalve-Arteaga et al., 2020). The causative agent of this viral infection is the Crimean-Congo hemorrhagic fever virus (CCHFV), an enveloped RNA virus of the genus *Orthonairovirus* in the family *Nairoviridae* (Balinandi et al., 2022; Magyar et al., 2021; Nasirian, 2020). Although CCHFV infection in animals is generally asymptomatic, animal-to-human transmission occurs through infected blood or body fluids, which has raised public concern at the human-animal interface, especially for farmers, herders, veterinarians, hunters, butchers, and merchants involved in the above-mentioned sectors of activity. People living near livestock farms

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are also at an elevated risk of CCHFV transmission (Nasirian, 2019). CCHFV is endemic in more than 50 countries across Africa, Asia, Europe, and the Middle East. Its seroprevalence differs geographically between and within regions (Balinandi et al., 2022; Vawda et al., 2018). In sub-Saharan Africa, human CCHFV seroprevalence is disparate, with seroprevalence of 10.6% in Nigeria, 5.7% in Ghana, 4.4% in Cameroon, 1.3 to 1.5% in South Africa, and 0.5% in Madagascar (Akuffo et al., 2016; Andriamandimby et al., 2011; Bukbuk et al., 2016; Sadeuh-Mba et al., 2018; Vawda et al., 2018). CCHFV studies on human samples in the Republics of Congo and Mali are relatively rare. However, the circulation of CCHFV in southern Congo populations was reported in 1989 in agriculture and livestock farming areas (Gonzalez et al., 1989). In Mali, evidence of human contact with CCHFV and recent detection of two acute retrospective cases has been reported (Baumann et al., 2019). A recent CCHFV epidemiological study using the One-Health approach to define disease status in each African country highlighted an underestimation of CCHF cases or status due to a low number of seroprevalence studies on humans and animals, the absence of an active zoonosis surveillance system, especially for CCHF, and a lack of CCHFV-specific diagnostic capabilities (Temur et al., 2021). Based on this study, the Republic of Congo is classified as Level 3 (no CCHF cases reported and no robust surveillance established, but available data indicate the possibility of undetected/unreported CCHF cases) according to the One Health country-level classification scheme focusing on the epidemiology of CCHF (Blair et al., 2019; Temur et al., 2021), whereas Mali is classified as Level 2 (CCHF cases reported intermittently in the absence of robust surveillance). However, there have been no seroprevalence studies in the capitals of these countries, even though Brazzaville and Bamako represent millions of inhabitants and are well connected regionally and internationally. These two cities have a humid tropical climate but with different rainfall regimens and agricultural use of their surroundings. The population of Brazzaville and its suburbs experiences a bimodal rainfall regime and the climate is of type Aw according to the Köppen-Geiger classification (Geiger, 1961; Köppen, 2020; Köppen and Geiger, 1928). Alternating wet and dry seasons (from October to May and from June to September, respectively) and an extensive hydrographic network favors subsistence farming and livestock breeding. Bamako and its surroundings also offer fertile ground for agriculture and stockbreeding, although stockbreeding is the predominant activity of the population. Its climate is characterized by one rainy season from April to October, which peaks in August. The climate of Bamako corresponds to the BSh type according to the Köppen-Geiger classification (Geiger, 1961; Köppen, 2020; Köppen and Geiger, 1928). This would influence the ecological system of CCHFV and its transmission. Thus, we aimed to set up the first retrospective pilot serosurvey to estimate the seroprevalence of and associated risk factors for CCHFV among a Congolese population living with HIV (PLWH) and a Malian population of blood donors in urban and semi-urban areas of both capital cities.

2. Materials and methods

2.1. Study population and sample and data collection

We conducted a retrospective serological survey on sera provided from monitoring centers for PLWH in Brazzaville, Republic of Congo, and the Blood Transfusion Center at Gabriel Touré Hospital in Bamako, Mali and its surroundings. In total, 581 samples were collected, including 352 sera in Brazzaville from July to October 2019 and 229 in Bamako from November 2019 to January 2020. Although the use of these serum samples was circumstantial and the motivation was to study CCHFV seroprevalence in these two cities, the sample size for both capitals was appropriate for estimating the prevalence of exposure to CCHFV. The sera were stored at −80 °C before being transferred to the Virology Department of the Pitié-Salpêtrière Hospital, Paris, France for CCHFV serology. The characteristics of the study population, such as age, gender, area of residence, occupation, education level, and marital status were recorded using questionnaires. The eligibility criteria for the questionnaire and blood samples included participants being ≥18 years of age and consent to participate in the study.

2.2. ELISA serological screening

Serum samples were tested for the presence of total anti-CCHFV immunoglobulins (Ig) using a double-antigen ELISA kit (ID Screen® CCHF Double Antigen Multi-species, Innovative Diagnostics) according to the manufacturer's instructions. Previous studies used this double-antigen ELISA kit which has proven to be a useful tool for diagnosis and research (100% specificity, 99% sensitivity) (Frías et al., 2022; Negredo et al., 2021; Sas et al., 2018).

2.3. Data analysis

Statistical comparisons were performed using the Chi-squared test for categorical variables and the Mann-Whitney *U test* for continuous variables. Due to the low number of positive events, we were unable to perform regression analysis.

3. Results

The socio-demographic characteristics of 352 PLWH (97 men, 255 women) in Brazzaville and its outskirts and 229 blood donors (133 men, 96 women) from Bamako and its outskirts are presented in Table 1. The median age of the study participants was 39 [IQR: 29 – 49] years. Participants from Brazzaville were older, mostly female (72.4%), and more highly educated, with most working in the tertiary sector of economic activity and living in an urban biotope without livestock or agricultural

Table 1
Socio-demographic characteristics of the participants from Congo and Mali.

	Total N (%)	PLWHIV Brazzaville, N (%)	Blood donors Bamako, N (%)	P value
All	581 (100)	352 (61)	229 (39)	
Gender				<0.001
Male	230 (40)	97 (28)	133 (58)	
Female	351 (60)	255 (72)	96 (42)	
Age, median [IQR], years	39 [29–49]	45 [39–54	29 [23–35]	<0.001
Educational level				<0.001
Uneducated*	107 (18)	8 (2)	99 (43)	
Primary	158 (27)	98 (28)	60 (26)	
Secondary	219 (38)	177 (50)	42 (19)	
Superior	97 (17)	69 (20)	28 (12)	
Prof. economic sectors				<0.001
Primary	33 (6)	5 (1)	28 (12)	
Secondary	64 (11)	30 (9)	34 (15)	
Tertiary	280 (48)	193 (55)	87 (38)	
Other**	204 (35)	124 (35)	80 (35)	
Healthcare workers				0.87
Yes	16 (3)	10 (3)	6 (3)	
No	565 (97)	342 (97)	223 (97)	
Biotope				<0.001
Urban	421 (73)	287 (82)	134 (59)	
Peri-Urban	123 (21)	56 (16)	67 (29)	
Rural	37 (6)	9 (3)	28 (12)	
Livestock/ Agricultural areas residency				<0.001
Yes	116 (20)	9 (3)	107 (47)	
No	465 (80)	343 (97)	122 (53)	

Prof.: professional, *Uneducated: people who did not attend primary school, **Other: unemployed or students. Statistical comparisons were performed using the Chi-squared test for categorical variables and the Mann-Whitney *U test* for continuous variables.

activities in the surroundings. By contrast, the blood donors of Bamako were younger, mostly male (58.1%), with a lower level of education, and only one-third worked in the tertiary sector of economic activity. They were more likely to live in peri-urban or rural areas with livestock or agricultural activities in the surroundings (Table 1). Of the 581 samples, we detected anti-CCHFV antibodies in 2/352 (0.6%) in the PLWH cohort and 4/229 (1.75%) in the cohort of blood donors. The prevalence of anti-CCHFV antibodies according to each variable is presented in Table 2. Anti-CCHFV antibody positivity tended to be associated with male gender, living in a peri-urban biotope, and/or livestock or agricultural activities in the surroundings in both cohorts.

4. Discussion

We found a CCHFV seroprevalence of 0.6% among PLWH, all in peri-urban areas close to livestock or agriculture. This rate is slightly lower than that of 1.1% previously reported among participants living in southern Congo but the authors did not report the CCHFV seropositivity in Brazzaville in the late 1980s (Gonzalez et al., 1989). In addition, previous studies reported the circulation of hemorrhagic fever viruses in the human population in the Republic of Congo and in neighboring countries, such as the Central African Republic and Democratic Republic of Congo (Gonzalez et al., 1989; Grard et al., 2011; Meunier et al., 1987; Talani et al., 1999). Our results on the prevalence of anti-CCHFV antibodies are similar to those reported in the Central African population in 1987, which was 1.4% (Meunier et al., 1987). Our study thus indicates the potential introduction or circulation of CCHFV after the 1980s in Brazzaville or the detection of previously infected individuals in Brazzaville. Moreover, seroprevalence could be underestimated in this particular population due to an altered immune response. It is noteworthy that the seroprevalence of HIV has been estimated to be 2.4% in Brazzaville and 3.2% at the national level (Gokaba, 2020; Malonga et al., 2021). Thus, it may be informative to study CCHFV seroprevalence among non-HIV infected people to avoid an underestimation of CCHFV seroprevalence.

Among blood donors at the Gabriel TOURÉ hospital in Bamako, CCHFV seroprevalence was 1.75%, with half of seropositive individuals living in peri-urban areas near livestock or agriculture activities and others with potent exposure factors (working as a butcher or with frequent extra-urban travel). As seroprevalence is indicative of prior exposure to CCHV, our results corroborate those of Baumann et al. (2019), who detected an acute CCHFV infection in two pediatric patients hospitalized at the Gabriel TOURÉ Hospital in 2017 (Baumann et al., 2019) and a report by the International Society for Infectious Disease in 2020 concerning a new outbreak of CCHF due to human-animal contact in a village in the Mopti region (central Mali), resulting in the death of seven people and approximately 20 cases of human-to-human transmission (International Society for Infectious Diseases, 2020). Phylogenetic analysis showed a close relationship with a strain from Mauritania and another detected in ticks taken only 25 km from Bamako (Zivcec et al., 2014). These results may indicate a large underestimation of the circulation of CCHFV in Mali, with possible exposure of people living in Bamako. Description of CCHFV seropositivity within these populations in peri-urban and urban areas without the description of an acute CCHF outbreak could be attributed to the emergence of sporadic CCHFV cases without sustained transmission near these cities or to undetected CCHF in the countryside. In recent years, the epidemiology of CCHFV has been modified due to climate change and other anthropogenic changes, such as the density or movement of the human population, stockbreeding activities, and human-to-animal contacts that could modify the biology and environmental dynamics of the CCHFV vector (Estrada-Peña et al., 2010; Vescio et al., 2012). In addition, Brazzaville borders the Democratic Republic of Congo, where a CCHF outbreak was described (Casals, 1969), implying the need to strengthen active surveillance activities for hemorrhagic fever diseases. In addition, Mali has a similar ecology as Burkina Faso, Niger, Mauritania, and Nigeria, which have reported sporadic cases of CCHFV infection in cattle and humans, suggesting a more widespread CCHFV endemic region than expected or that the diagnosis of new cases of CCHF in Mali and countries of the West African sub-region is likely due to improved diagnostic capabilities rather than ecological changes related to the vector and human behavior, as indicated by Temur et al. (2021). Regardless of the reason, our study has strengthened the necessary collaboration of the countries, both within and outside their borders, to diagnose additional cases and better estimate the epidemiology of CCHF in this region.

However, one limitation of this study is the use of a commercial assay for detection of anti-CCHFV antibodies. Despite previous publications demonstrating a very good sensitivity and specificity (Grech-Angelini et al., 2020; Mertens et al., 2015; Sas et al., 2018), as well as the absence of cross-reactivity against the Hazara virus or the Dugbe virus, viruses related to the *Orthonairovirus* genus (Grech-Angelini et al., 2020), we cannot exclude cross-reactivities with other orthonairoviruses. Nevertheless, our results argues for further studies to decipher the landscape of *Orthonairovirus* epidemiology in these countries.

Table 2
Seroprevalence of anti-CCHFV antibodies in the cohorts from Brazzaville, Congo and Bamako, Mali.

	PLWHIV Brazzaville		Blood donors Bamako	
	Positive sera (N)	CCHFV Prevalence (%)	Positive sera (N)	CCHFV Prevalence (%)
All	2/352	0.6	4/229	1.8
Gender				
Male	1/97	1	3/133	2.3
Female	1/255	0.4	1/96	1
Educational level				
Uneducated*	0/8		3/99	3
Primary	1/98	1	0/60	
Secondary	1/177	0.6	1/42	2
Superior	0/69		0/28	
Prof. economic sectors				
Primary	0/5		0/28	
Secondary	0/30		1/34	3
Tertiary	0/193		2/87	2
Other**	2/124	1.6	1/80	1
Healthcare workers				
Yes	0/10		0/6	
No	0/342		0/223	
Biotope				
Urban	0/287		2/134	2
Peri-Urban	2/56	4	2/67	3
Rural	0/9		0/28	
Livestock/ Agricultural areas residency				
Yes	2/9	22	2/107	2
No	0/343		2/122	2

Prof.: professional, *Uneducated: people who did not attend primary school, **Other: unemployed or students. Statistical comparisons were performed using the Chi-squared test for categorical variables and the Mann-Whitney *U test* for continuous variables.

5. Conclusions

Despite a low seroprevalence of CCHFV in both cohorts, our results suggest that CCHFV circulates in the peri-urban areas of the capitals of the Republic of Congo and Mali, associated with previously described CCHFV risk-factors (Nasirian, 2019). Further studies incorporating entomological, veterinary, environmental, and human health components are needed to better assess the spatial distribution of CCFHV and unravel the possible transmission chains that occur at the animal-to-human interface in these two countries.

Ethical approval

This study was approved by the Health Sciences Research Ethics Committee under the approval number 222 / MRSIT / IRSSA / CERSSA.

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CRediT authorship contribution statement

Gervillien Arnold Malonga: Data curation, Resources, Investigation, Formal analysis, Visualization, Validation, Software, Writing – original draft. **Almoustapha Issiaka Maiga:** Conceptualization, Methodology, Supervision, Project administration, Funding acquisition, Data curation, Resources, Writing – review & editing. **Dimitry Moudiongui Mboungou Malanda:** Data curation, Resources. **Mahamadou Saliou:** . **Juthèce Private Malanda-Kiminou:** Data curation, Resources. **Oumar Dolo:** Data curation, Resources. **Anicet Luc Magloire Boumba:** Data curation, Resources. **Alhassane Ba:** Data curation, Resources. **Robert Murphy:** Writing – review & editing. **Jean Félix Peko:** Writing – review & editing. **Anne-Geneviève Marcelin:** Conceptualization, Methodology, Supervision, Project administration, Funding acquisition, Writing – review & editing. **Vincent Calvez:** Conceptualization, Methodology, Supervision, Project administration, Funding acquisition. **Stéphane Marot:** Conceptualization, Methodology, Supervision, Project administration, Funding acquisition, Investigation, Formal analysis, Visualization, Validation, Software, Writing – original draft.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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