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Undetected circulation of major arboviruses in West Sudan: urging for institutionalizing multisectoral one health strategy for the preparedness, prevention, and control of zoonotic arboviral diseases

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Abstract

Objectives Arboviruses pose a significant global health challenge. This study investigated the seroprevalence of major human arboviral infections, including yellow fever (YFV), dengue (DENV), Crimean-Congo hemorrhagic fever (CCHF), Rift Valley fever (RVF), West Nile virus (WNV), and chikungunya (CHIK), in Darfur region from September to December 2018. ELISA-IgM was used to detect antibodies. RT–PCR was used to differentiate YFV infection from vaccine-immuno-response in IgM samples.

Results A total of 152 blood samples were collected, with 123 (80.9%) from males and 29 (19.1%) from females. The participants were grouped by age: 50 (32.9%) were under 20 years, 96 (63.2%) were aged 20–45 years, and 6 (3.9%) were over 45 years. The seroprevalence rates for YFV, DENV, and CHIKV were 68 (44.7%), 23 (15.1%), and 5 (3.3%), respectively. There were 11 molecularly-confirmed YFV cases (7.2%). Among these, 3/11 were positive for DENV-IgM, and 1/11 was positive for CHIKV-IgM. Among the 68 YFV-positive individuals, 15 (22.1%) had been exposed to DENV, and 2 (2.9%) had been exposed to CHIKV. Co-exposure to DENV and CHIKV was detected in 3 (1.9%) patients, while 2 (1.3%) patients had triple exposure to YFV, CHIKV, or DENV. No exposure to CCHF, RVFV, or WNV was detected.

Keywords Pandemic preparedness, prevention, and response, Global health security, Health policy, Mosquito-borne diseases, Vector-borne diseases, Emerging zoonotic diseases, Africa

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Introduction

The global public health risk of arthropod-borne viral diseases (arboviruses) is rapidly increasing, with these viruses expanding their geographical distribution at an alarming rate [1]. This exponential rise in arboviral infections is driven by several risk factors, including globalization and unplanned urbanization [2]; increased international travel and trade [3, 4]; and environmental, ecological, and human practices that create favourable conditions for the breeding of competent arbovirus vectors and their increased contact with humans and animals [5, 6]. Furthermore, climate change and armed conflicts are additional risk factors contributing to the rapid spread and prevalence of arboviral diseases [7–9].

In Sudan, several major arboviruses are endemic, including yellow fever virus (YFV), dengue fever virus (DENV), Rift Valley fever virus (RVFV), Crimean-Congo hemorrhagic fever virus (CCHFV), West Nile virus (WNV), chikungunya virus (CHIKV), and Zika virus (ZIKV) [7]. DENV is widespread across the country, whereas CHIKV and ZIKV are prevalent in Eastern Sudan, whereas YFV and WNV are prevalent in West, and RVFV and CCHFV are prevalent in Central Sudan [7]. Recently, DENV has emerged in Darfur along with CCHF and WNV [4, 10]. Additionally, human arboviruses are becoming increasingly common in Sudan [11].

The recent increase in human population movements, including refugees, internally displaced persons (IDPs), war returnees, and humanitarian responders in Darfur region, has brought more than peace to the war-torn to the communities in western Sudan [12, 13]. Previously, Darfur area was characterized by a humanitarian crisis of the post-conflict environment, with a health system that had yet to recover from the war that began in 2003 [14, 15]. Most of the 13.4 million people in Darfur are IDPs, refugees, or war returnees living in humanitarian crisis settings [4]. The prolonged armed conflict in Darfur region severely disrupted the socioeconomic structure of local communities, altered the environment, and transformed the area into a humanitarian crisis zone [4, 10, 15, 16]. These significant changes in social structure and living conditions combined with climate change have facilitated the emergence of several infectious diseases, particularly vector-borne viral and parasitic diseases, including DENV, CCHF, YF, WNV, and malaria [11, 17–20].

The living conditions in refugee camps—characterized by a lack of stable water supply, high population density, increased exposure to infective vectors, and ineffective vector control—have favoured the introduction and establishment of competent vectors of arboviruses such as CHIKV, DENV, RVF, YFV, and WNV, mainly including *Aedes albopictus* and *Anopheles stephensi* [21–25]. The limited resources and laboratory capacity in the region make the detection of arboviral infections particularly challenging, often leading to misdiagnosis of malaria on the basis of its clinical presentation [16, 26–30]. This delay in diagnosis and interventions is commonly resulting in the development of arboviral diseases outbreaks [31, 32]. In this report, we present the results of serological and molecular analyses of secondary data collected during an epidemic of febrile illness in Darfur region.

Main text

Methods

Study design and study area

This retrospective cross-sectional study analysed secondary data collected by the national routine surveillance system during an investigation of an epidemic of febrile illness in Darfur region. Darfur region, covering 493,180 km^2 of desert and semidesert, is divided into 5 states: East, West, South, North, and Central Darfur.

Sample collection

Blood samples were collected during a survey investigating a febrile illness epidemic that occurred in late 2018. Blood samples were collected from febrile patients at outpatient clinics within refugee and IDP camps. These patients, who tested negative for malaria, had experienced febrile illness within the previous 2–3 weeks. Sera were separated from the blood samples and stored at -20 °C until they were shipped to the National Public Health Laboratory for further analysis. All the data were anonymised, and personal identifiers were removed to ensure confidentiality.

Laboratory testing

Serum samples were tested for antibodies against DENV, RVFV, CCHFV, YFV, and CHIKV via commercially available IgM capture ELISA kits following the manufacturer's instructions (Panbio, Inverness Medical Innovations Australia Pty Ltd., Brisbane, Australia). Additionally, an RT–PCR confirmatory test was performed on samples positive for YFV-IgM via the RealStar® yellow fever RT–PCR Kit (Altona Diagnostics GmbH, Hamburg, Germany) to reduce the bias of false-positive results due to previous YFV vaccination campaigns in the region. A Ct value for the YFV-specific target was ≤40. Samples with a Ct value above 40 were considered negative.

Statistical analysis

The data were analysed, and the frequencies of the variables were calculated via the Statistical Package for the Social Sciences (SPSS v20). Frequencies were calculated for variables, including age group, sex, and laboratory test results.

Table 1 Shows the demographics and lab results of our research participants:

Characteristics		State					Total (%)
		Central Darfur	East Darfur	North Darfur	South Darfur	West Darfur	
Gender	Female	6 (20%)	6 (100%)	4 (11.4%)	7 (13.5%)	6 (20.7%)	29 (19.1%)
	Male	24 (80%)	0 (0%)	31 (88.6%)	45 (86.5%)	23 (79.3%)	123 (80.9%)
Age Group	< 20 years	11 (36.7%)	4 (66.7%)	7 (20%)	15 (28.8%)	13 (44.8%)	50 (32.9%)
	20–45 years	19 (63.3%)	2 (33.3%)	26 (74.3%)	35 (67.3%)	14 (48.3%)	96 (63.2%)
	> 45 years	0 (0%)	0 (0%)	2 (5.7%)	2 (3.8%)	2 (6.9%)	6 (3.9%)
YFV_IgM	Positive	14 (46.7%)	4 (66.7%)	10 (28.6%)	21 (40.4%)	19 (65.5%)	68 (44.7%)
	Negative	16 (53.3%)	2 (33.3%)	25 (71.4%)	31 (59.6%)	10 (34.5%)	84 (55.3%)
YFV_rtPCR	Positive	2 (6.7%)	1 (16.7%)	2 (5.7%)	1 (1.9%)	5 (17.2%)	11 (7.2%)
	Negative	28 (93.3%)	5 (83.3%)	33 (94.3%)	51 (98.1%)	24 (82.8%)	141 (92.8%)
CHIKV_IgM	Positive	1 (3.3%)	0 (0%)	0 (0%)	4 (7.7%)	0 (0%)	5 (3.3%)
	Negative	29 (96.7%)	6 (100%)	35 (100%)	48 (92.3%)	29 (100%)	147 (96.7%)
DENV_IgM	Positive	4 (13.3%)	0 (0%)	4 (11.4%)	8 (15.4%)	7 (24.1%)	23 (15.1%)
	Negative	26 (86.7%)	6 (100%)	31 (88.6%)	44 (84.6%)	22 (75.9%)	129 (84.9%)
Total number of positive samples (%)		30 (20%)	6 (4%)	35 (23%)	52 (34%)	29 (19%)	152 (100%)

Table 2 Co-exposure to yellow fever, Dengue, and Chikungunya viruses

Virus infections/exposure	YFV-RT-PCR	YFV-IgM	CHIKV-IgM	DENV-IgM	Positive all IgM	Negative all IgM
YFV-RT-PCR	11	6	1	3	-	-
YFV-IgM	6	68	2	15	-	-
CHIKV-IgM	1	2	5	3	-	-
DENV-IgM	3	15	3	23	-	-
Positive all IgM	-	-	-	-	2	-
Negative all IgM	-	-	-	-	-	0

Results

Blood samples were collected from 152 febrile patients who tested negative for malaria parasites through microscopic examination. Samples were collected through a passive surveillance; therefore, they are distributed unevenly among 4 states according to patients' presentation at healthcare facilities in North, South, West, and Central Darfur states with only 6 samples (4%) obtained from East Darfur state. The majority of the patients, 123 (80.9%), were males. One-third of all patients were children younger than 20 years of age, 96 (63.2%) were between 20 and 45 years, and only 6 (3.9%) were older than 45 years (Table 1).

Serological and molecular analyses of the serum samples revealed widespread exposure to YFV across all Darfur states, with 68 (44.7%) of the samples indicating previous exposure to YFV, primarily from South and West Darfur states: 21 (40.4%) and 19 (65.5%), respectively. The RT−PCR assay confirmed 11 recent YFV infections (Table 1). Additionally, five participants had previously been exposed to CHIKV, with cases originating from southern and central Darfur states (Table 1). The seroprevalence of DENV1 and 3 antibodies was 15.1%, with 23 positive individuals detected (14 were serotype 1 and 9 serotype 3) across four of the five states, except East Darfur (Table 1). No prior exposure to RVFV, WNV, or CCHFV was detected during this study.

Interestingly, co-exposure to two or more of these viruses was notably prevalent. Among the patients with recent YFV infections, three patients were also positive for DENV-IgM, and one was positive for CHIKV-IgM. Among the 68 individuals with YFV antibodies, 15 individuals had also been exposed to DENV, and two had been exposed to CHIKV. Additionally, co-exposure to both DENV and CHIKV was detected in three participants. Notably, two cases of triple exposure to YFV, CHIKV, and DENV were identified through IgM testing (Table 2).

Geospatial analysis of seroprevalence by state revealed that populations in Central Darfur and South Darfur were exposed to YFV, DENV, and CHIKV, whereas populations in West Darfur and North Darfur were exposed to YFV and DENV. In contrast, only exposure to YFV was detected in East Darfur state (Fig. 1).

Discussion

The findings of this study are alarming, as they present the first report of the cocirculation of YFV, CHIKV, and DENV in the Greater Darfur region. The high seroprevalence of YFV antibodies was anticipated because of the previous vaccination campaign following the 2012 YFV epidemic [17]. However, the detection of YFV cases via RT−PCR suggests that the virus is still actively circulating in the area. This persistence of YFV could be attributed

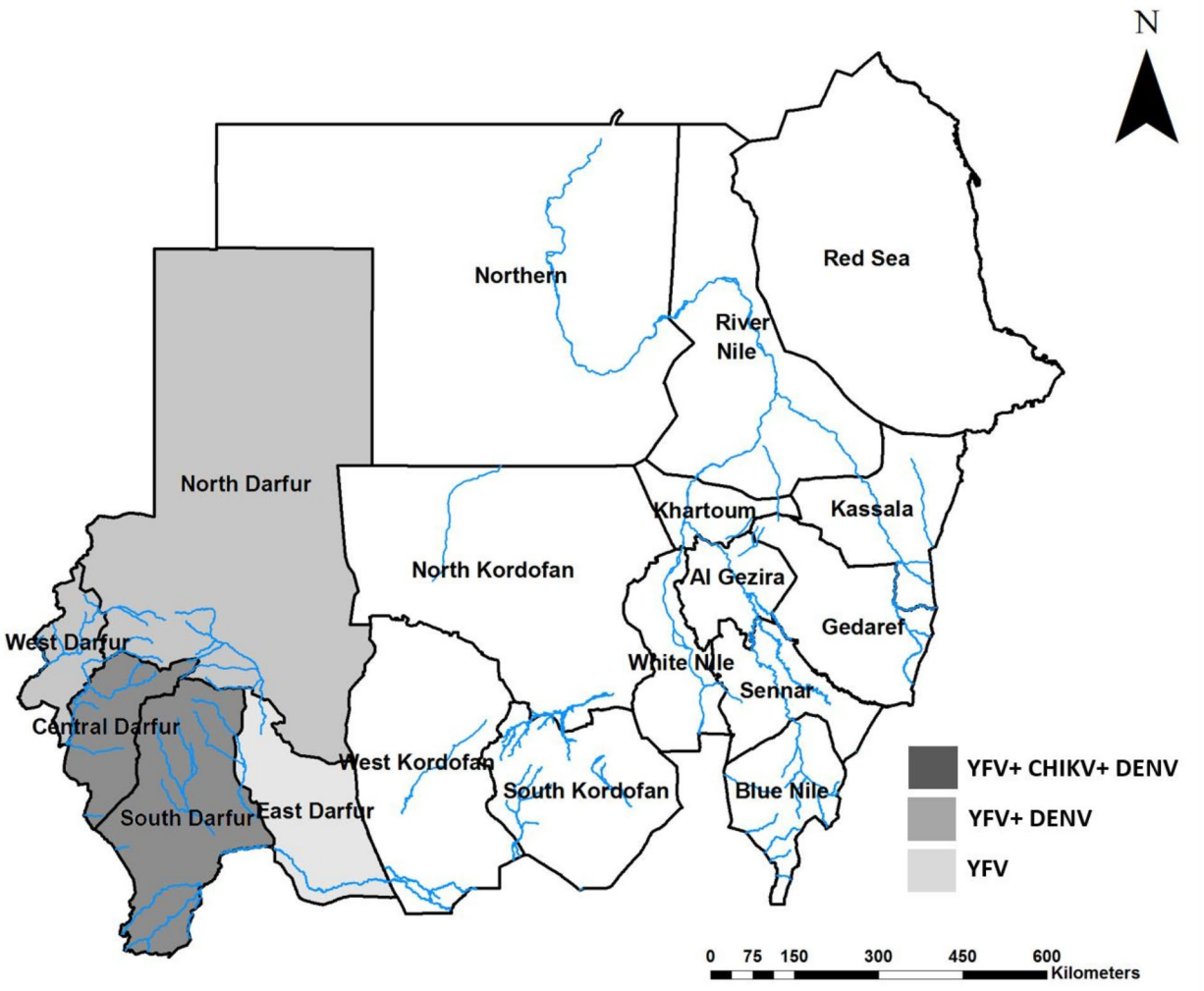

Fig. 1 Sudan map highlight the seroprevalence of YFV, DENV, and CHIKV in the greater Darfur region

to the significant cross-border movement between vaccinated and nonvaccine-susceptible areas due to conflict and political instability, which may have reduced vaccination coverage among residents [15, 33]. Alternatively, YFV might be maintained within populations of nonhuman primates [34].

DENV serotype 1 and 3emerged in the area in 2014/2015, starting in North Darfur, and soon after, an epidemic spread across Central, North, South, and West Darfur states, during which the cocirculation of WNV and CCHFV has also been documented [4, 10]. Additionally, a massive outbreak of CHIKV fever occurred in eastern Sudan between May 2018 and March 2019, with over 47,000 cases reported [7]. The region's heightened population dynamics, cross-border movement, trade, and influx of refugees contribute to its status as a hotspot for multiple vector-borne diseases, leading to annual outbreaks [35–38]. The low seroprevalence of CHIKV antibodies detected in this study suggests either that exposure to CHIKV might have occurred in East Sudan (with CHIKV-positive individuals being returnees) or there is an unrecognized local transmission of CHIKV in the area [39, 40]. The latter hypothesis is more likely, given the detection of major arboviral disease vector in the region; *Ae. albopictus* [21, 41–43].

The emergence and re-emergence of arboviral diseases pose a significant public health challenge, particularly in resource-limited settings with relatively weak health systems, where clinicians often rely heavily on clinical diagnosis [4, 10]. This reliance has resulted in numerous arboviral infections being misdiagnosed and treated as malaria [4, 10, 29]. The lack of publicly available up-to-date information on circulating diseases further limits the quality and capacity of healthcare services, as clinical diagnosis is heavily influenced by a clinician's awareness of endemic diseases, as well as the health and travel history of patients [7, 44]. Also, coinfections with other parasitic infections, such as malaria, which are

also characterized by fever, further complicates diagnosis. Without adequate laboratory diagnostic capacity to detect concurrent viral infections, especially when signs and symptoms become more severe and complicated, accurate diagnosis becomes even more challenging [27, 28, 45]. Additionally, the absence of a robust arboviral disease surveillance system significantly increases the risk of future epidemics [7]. This situation is further exacerbated by the currently ongoing war, therefore, health, humanitarian, and development stakeholders in Sudan and the neighbouring countries should collaboratively invest in strengthening the Global Health Security and Pandemic Preparedness and Prevention in East Africa, particularly that highly fatal diseases are outbreaking in the area [46–51].

Strengthening diagnostic capacity across national and regional laboratories is essential for accurate and rapid diagnosis, particularly in high-risk areas like refugee and IDP camps [20, 52, 53]. Implementing standardized data collection and reporting systems will enable timely detection and response to outbreaks [54]. Building local capacity through ongoing training for healthcare workers, entomologists, and public health professionals is necessary, alongside community engagement to raise awareness about prevention and early detection [47]. Continuous monitoring and evaluation are key to ensuring the system's sustainability and effectiveness in controlling arboviral diseases [54].

In countries endemic for multiple infectious diseases such as Sudan, unless the use of molecular and serological diagnostic tools was integrated into the routine healthcare practices, the circulation of these infections will remain undetected until they develop into outbreaks, as symptoms can be similar and microscopic tests cannot detect viral infections [55–57]. Therefore, the country would benefit greatly from rebuilding its health system, with a focus on primary healthcare, by improving diagnostic capacity, surveillance, and reporting systems [12, 44, 54]. Moreover, adopting a One Health approach would be instrumental in the prevention, early detection, and effective response to outbreaks of arboviral diseases [31, 47, 58–61]. The need for this approach is underscored by the increasing frequency of epidemics and epizootics of zoonotic arboviral diseases such as RVF, especially given the recent spatiotemporal changes in disease transmission [8, 9, 62].

In the absence of effective and sensitive surveillance systems for the early detection of arboviral disease outbreaks, these outbreaks will escalate into global threats. We, therefore, emphasize the urgent need for a nationwide surveillance system for arboviral diseases in Sudan [7–9, 19, 58]. While the current local capacity is limited due to resource constraints, international support for building local health capacity and preparedness for the early detection and containment of arboviral epidemics is worth the global investment to avoid the emergence of larger multi-country pandemics. We also urge local and international health partners to support efforts in increasing local diagnostic capacity, surveillance, reporting, prevention and control of arboviruses. Special attention should also be given to the health of displaced persons living in overcrowded refugee and IDP camps.

Limitations

- A major limitation of this study is the lack of participants travel history, entomological and disease vector-related data, to help understanding vector composition, their role in disease transmission, and their susceptibility to current vector control measures.
- Full integration of molecular and genomics analysis would have strengthening the study and the evidence generated including strains of the viruses and their dynamics.

Abbreviations

Arboviruses	Arthropod-borne viral diseases
YFV	Yellow fever virus
DENV	Dengue fever virus
RVFV	Rift Valley fever virus
RVFV	Rift Valley fever virus
CCHF	Crimean–Congo hemorrhagic fever
CCHFV	Crimean–Congo hemorrhagic fever virus
WNV	West Nile virus
CHIKV	Chikungunya virus
ZIKV	Zika virus

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Author contributions

Conceptualization, design, and investigation: AA, AE; Data acquisition: AA, AE; Formal analysis and interpretation: NSM, AE, and AA; Editing and writing: AA, NSM, CMM, EES, AOM, and AE; Original draft preparation: NSM and AA. Manuscript revision: AA, NSM, and CMM. All the authors have read and approved the final manuscript.

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Data availability

All the data generated or analysed during this study are included in this published article.

Declarations

Ethical approval and consent to participate

Ethical approval and requirement for informed consent were waived because of the use exclusive use of secondary data that was collected by the routine programmatic interventions by the Federal Ministry of Health.

Consent for publication

Not applicable.

Competing interests
The authors declare no competing interests.

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