RESEARCH ARTICLE

Sokoto Journal of Veterinary Sciences

(P-ISSN 1595-093X: E-ISSN 2315-6201)

http://dx.doi.org/10.4314/sokjvs.v22i4.7

Igah *et al*./*Sokoto Journal of Veterinary Sciences*, **22**(4): 283 - 290.

Detection of Crimean-Congo haemorrhagic fever virus circulating in ticks and cattle in Plateau and Kaduna States, Nigeria

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Publication History:
Received: 02-05-2024
Revised: 02-11-2024
Accepted: 10-11-2024

Abstract

Crimean-Congo haemorrhagic fever (CCHF) poses a significant threat to human health in Nigeria. This study provide-s an updated assessment of the prevalence and distribution of Crimean-Congo haemorrhagic fever virus (CCHFV) in cattle and tick vectors in North Central Nigeria. We investigated 333 cattle from Plateau and Kaduna States in Northern Nigeria using molecular and quantitative modelling techniques. Screening for CCHF antibody was performed using a commercial enzyme-linked immunosorbent assay. The study estimated a seroprevalence of 67.00% for IgG antibodies of CCHFV. Additionally, the virus was detected in *Rhipicephalus (Boophilus) decoloratus*, which serves as a reservoir host. Among the 41 pools of ticks tested for CCHF, only one pool out of the 35 *Rhipicephalus (Boophilus*) ticks (2.40%) tested positive for the virus. This positive pool's RT-qPCR cycle threshold (CT) value was 31.88. The detection of CCHFV in both cattle (the amplifying host) and ticks (the reservoir vector) underscores the need for active surveillance. Appropriate tick control mechanisms should be established to prevent disease spread to humans. Education and awareness among human and animal health workers are essential to prevent nosocomial outbreaks. This research contributes valuable insights to our understanding of CCHF dynamics in Nigeria and informs strategies for disease prevention and control.

Keywords: Crimean-Congo Haemorrhagic Fever Virus, Cattle, ELISA, Nigeria,Ticks, Virus detection

Introduction

Crimean-Congo haemorrhagic fever (CCHF) is a tick-borne viral zoonotic disease characterized by fever and haemorrhagic signs. It is caused by the Crimean-Congo haemorrhagic fever virus (CCHFV), which belongs to the genus Orthonairovirus of the family Nairoviridae and order Bunyavirales (Garrison *et al*., 2020). The case fatality rate for the disease can be up to 30% or higher (Hawman & Feldmann, 2023). Although it can be fatal in humans, it is asymptomatic in animals, allowing for its continuous and unnoticed maintenance/spread in nature in an enzootic tick-animal cycle, posing a major public health risk due to its high pathogenicity in humans (Papa *et al*., 2017; Mendoza *et al*., 2018). The incubation period varies significantly depending on the mode of acquisition, ranging from 1-3 days (maximum 9 days) following a tick bite, to 5-6 days (maximum 13 days) after contact with viraemic blood and tissues (Tavana, 2006). The virus causes systemic infections in humans and other animals, circulating in the blood and tissue fluids (Shahhosseini *et al*., 2021). Aside from the enzootic cycle of maintenance of the virus in nature, the virus

is also maintained in ticks in both the transovarian and transtadial cycles (Estrada-Peña *et al*., 2011). The distribution of the virus reflects the geographical distribution of the vector, mainly ticks of the genus *Hyalomma* but also of the *Rhipicephalus* and *Dermacentor* genera (Boulanger *et al*., 2019). Transmission to human is mainly through the bite of an infected tick, particularly *Hyalomma* spp., which has been recognized as the primary vector (Hawman & Feldmann, 2023). Climate change, bird migration aiding transportation of ticks, and human activities may interact to change tick distribution. This could lead to introducing the virus to new areas, where it previously didn't exist thereby becoming a serious cause for concern (Gale *et al*., 2012). The virus can also spread through direct contact with infected animal blood or tissue fluid, human-to-human contact, and close contact with animals, posing an occupational risk to veterinarians, slaughterhouse workers, and livestock farmers (Akuffo *et al*., 2016; Aydin *et al*., 2020; Patel *et al*., 2023). Infection can also occur through the crushing of infected ticks on skin with compromised integrity and via inhalation of contaminated aerosol. There is evidence of maternal-to-child transmission occurring horizontally (Saijo *et al*., 2004; Pshenichnaya *et al*., 2017). Due to the higher viral load during transmission, CCHFV infections acquired through nosocomial infections have a higher mortality rate than through tick bites (Patel *et al*., 2023). It is reported that the virus remains stable at temperatures below 60^{0}C but is destroyed by autoclaving (Tezer & Polat, 2015).

CCHFV is endemic in Africa, Asia, and Eastern Europe but has more recently emerged in southwestern Europe (Espunyes *et al*., 2021). In Africa, there have been reports of the disease in several countries including Mauritania, Mali, Burkina Faso, Senegal, Niger, Nigeria, Sudan, Democratic Republic of the Congo, Kenya, South Africa, Egypt, Chad, Uganda and Tanzania (Temur *et al*., 2021). In Nigeria, as in most developing countries, febrile or haemorrhagic diseases such as malaria, typhoid and Lassa fever are endemic thereby allowing subclinical or sporadic CCHFV infections to go undiagnosed and unreported due to misdiagnosis and limited knowledge of the disease (Nordstrand *et al*., 2007; Goutier *et al*., 2013). A study conducted in Nigeria over five decades ago revealed the isolation and circulation of 34 strains of the virus among wild and domesticated animals, including the isolation of the virus in arthropod vectors such as ticks and biting midges (Causey *et al*., 1970). Another study conducted in Northern Nigeria, reported a sero-prevalence of 25.7% to CCHFV antibodies (Umoh *et al*., 1983). Recent sero-surveillance studies conducted in livestock (Oluwayelu *et al*., 2020) and humans (Bukbuk *et al*., 2016) have confirmed the circulation of the virus in Nigeria. Though a few reports have confirmed the circulation of the virus in livestock in some parts of Nigeria, none of these studies have evaluated the presence of the CCHF virus in tick vectors. This study was therefore aimed to update the status of CCHFV in Nigerian cattle and tick vectors to fill the gap in knowledge.

Figure 1: Study area showing cattle sampling sites for ticks and plasma

Materials and Methods

Study area

The study was conducted in Plateau and Kaduna States of Nigeria (Figure 1). Plateau State is located between latitude $9^{0}14'6.3''$ N and longitude $9^{0}43'23.76''$E, while Kaduna State is located between Latitude $10^{0}36'33.5484''$N and longitude $7^{0}25'46.2144''$E. Plateau State is situated in one of Nigeria's highest altitudes giving it a near-temperate climate with temperatures ranging between 13 and 22°C. Kaduna, have a warmer climate with an annual average temperature of 34°C.

Ethics approval

Approval for this study was obtained from the Animal Ethics Committee of the National Veterinary Research Institute (NVRI) with Ref. no. AEC/02/112/22.

Sample size and study design

Using the formula described by Thrusfield *et al*.(2007), the sample size was estimated based on a prevalence of 30.4 % obtained by Dzikwi-Emennaa *et al*. (2022). A total of 333 extensively managed cattle were randomly selected between August and December 2021 from cattle herds in Kaduna and Plateau States using a multistage probability sampling method.

Data collection

Plasma samples

The sample collection process involved using a syringe and needle to collect 3-5 millilitres of blood from each animal. The blood was then carefully deposited into vacutainer bottles containing EDTA. These samples were meticulously transported to the Infectious and transboundary animal disease Laboratory of the National Veterinary Research Institute in Vom, Nigeria under cold chain conditions. Upon arrival, the samples were centrifuged at 2000 rpm for 5 minutes using a refrigerated centrifuge. The resulting individual plasma was carefully transferred into labelled cryovials and stored at a temperature of -20°C for subsequent analysis and research purposes.

Tick samples, pooling and homogenization

The whole body of each animal from which blood was drawn was examined for ticks. The ticks were collected alive into separate vials, marked with collection points, and taken to the Entomology Laboratory of the National Veterinary Research Institute, Vom, Plateau State where they were morphologically identified under a stereomicroscope using standard keys according to the procedures of Walker *et al*. (2003) and afterwards preserved at -80°C until required for further studies.

The collected tick specimens were pooled by genus and sampling location. Each pool was homogenized in PBS in a sterile mortar and pestle. The homogenized sample was centrifuged in a refrigerated centrifuge at 3000 rpm for 10 min. The supernatant was decanted into cryovials and properly labelled before storage at -80°C pending the extraction procedure.

Serology

To estimate the seroprevalence, ID Screen® CCHF double antigen multi-species ELISA kit (IDvet, France) was used, which had a sensitivity of 98.9% (95% CI 96.8% - 99.8%) and a specificity of 100% (95% CI 99.8% - 100%)(Sas *et al*., 2018a). The assay was conducted on the plasma to detect specific antibodies for the CCHFV nucleoprotein according to the manufacturer's recommended protocol. Samples showing S/P (sample to positive ratio) percentage (S/P %) greater than 30% were considered positive while those less than or equal to 30% were considered negative.

Molecular testing

Viral RNA was extracted from the homogenised tick pools (ticks were put together according to species and LGA of collection to make each pool) using the QIAamp Viral RNA Kit (Qiagen, Valencia, CA) according to the manufacturer's recommendation. The RNA was stored at -80^{0}C until utilised. RT-qPCR was performed to amplify a fragment of the CCHFV gene as previously described (Sas *et al*., 2018b). The RT-qPCR was performed in a 25µl reaction volume containing nuclease-free water of 4.9µl; 2X PCR buffer 12.5µl; 2. forward primer (CCHF-deg- 5'-CAAGGGGKACCAAGAAAATGAARAAGGC -3') reverse primer (CCHF-deg- 5'-GCMACAGGGATTGTYCCAAAGCAGAC-3') of 1.0µl (20µm) each, CCHF Probe -1 and -2, 0.2µl (5µm) each, Enzyme mix 0.2µl and RNA template 5µl. The reaction was carried out on a Rotor-Gene Q thermal cycler (Qiagen).

Data analysis

The collected data were organized in a spreadsheet using Microsoft Excel and subsequently imported into R software (version 3.5.1, 2024) for analysis, utilizing the epitools package (version 0.5-10.1) (R-Core Team, 2024). Risk factors, such as locations and species, were expressed in terms of frequencies and percentages. Proportions were accompanied by Clopper-Pearson intervals, and the chi-square test was employed to assess the equality of proportions. To explore the factors linked to CCHFV seropositivity, multinomial logistic regression was applied. The strength of the association between categorical variables was conveyed through adjusted odds ratios (aOR) along with a 95% confidence interval (CI). A significance level of 5% was established as significant for the study.

Results

Figure 2 shows the distribution of the species of ticks for this study. A total of 1,470 ticks were picked from the animals with four species of ticks morphologically identified to parasitize cattle in the sampled location namely, *Rhipicephalus (Boophilus) decoloratus* (n = 508, 34.56%), *Hyalomma truncatum* (n = 484, 32.93%), *Amblyomma variegatum* (n = 361, 24.56%) and *Rhipicephalus sanguineus* (n = 117, 7.96%).

Table 1 illustrates the distribution of CCHFV across the various Local Government Areas (LGAs) in Plateau and Kaduna States. Among Plateau State's LGAs, cattle from Barkin Ladi LGA exhibited the highest seropositivity compared to others. The prevalence of CCHFV antibodies was notably high in cattle from Barkin Ladi (40; 78.43%), Jos North (36; 61%), Riyom (22; 56.41%), Bokkos (21; 55.26%), Jos South (19; 50%) LGAs in Plateau, and also elevated in Kaduna's LGAs, with Kubau (30; 83.33%) showing the highest positive response to CCHFV antibodies, followed by Sabon Gari (49; 80.33%) and Zaria (6; 54.55%). The confidence interval indicates considerable variation. Significant differences in CCHFV distribution were observed within the two studied states in Nigeria.

Table 2 shows the distribution of tick species on cattle from Plateau and Kaduna States, Nigeria. Higher positive response of cattle was observed for *Rhipicephalus (Boophilus)* (508) than other species. The odd ratio and confidence interval for the species were similar which range from 1.016 – 1.020 while the confidence interval did not show much differences (CI: 1.012- 1.024). As shown in Table 3, the overall seroprevalence recorded for CCHFV in the study locations was 67%. Of the 333 plasma samples screened for CCHFV IgG antibody, 22 samples tested from Plateau State had a 61.3% seroprevalence, while 108 tested samples from Kaduna State had a 78.7% seroprevalence. At the local government areas (LGA) level, the following seroprevalences were recorded in Plateau State; Bokkos (55.26%), Barkin Ladi (78.43%), Jos South (50.00%), Riyom (56.41%) and Jos North (61.02%). In Kaduna State, 3 LGAs namely, Sabon gari, Zaria and Kubau had a seroprevalence of 80.33%, 54.55% and 83.33% respectively. All 1,470 ticks

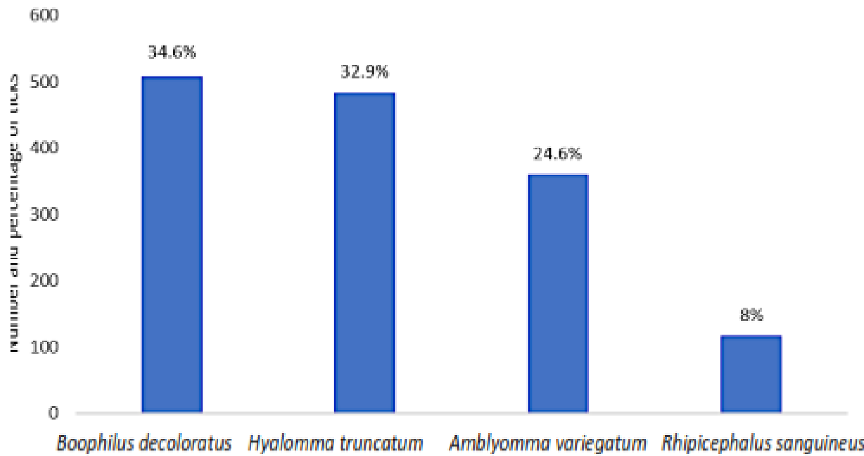

Figure 2: Distribution of tick species on cattle with antibodies to Crimean-Congo haemorrhagic fever virus

Table 1: Distribution of CCHFV antibodies in cattle in Plateau and Kaduna State, Nigeria

Locations	n	Positive	%	95% CI	p-value	aOR (95% CI)
Seropositivity	333	223	66.96	18.91, 15.81		
Plateau						
Bokkos	38	21	55.26	13.82,8.17	<.0001	0.99(0.92,1.07)
Barkinladi	51	40	78.43	24.24,16.76		1.11(1.04,1.18)
Jos south	38	19	50.00	12.71,7.29		0.97(0.89,1.06)
Riyom	39	22	56.41	14.38,8.62		Ref
Jos north	59	36	61.02	22.06,14.94		1.09(1.02,1.15)
Kaduna						
Sabon Gari	61	49	80.33	29.10,20.90	<.0001	1.50(1.07,2.09)
Kubau	36	30	83.33	18.79,12.21		1.41(1.01,1.97)
Zaria	11	6	54.55	5.46,1.54		Ref

95% CI based on Clopper-Pearson intervals. Ref: Reference category. aOR-Adjusted Odd ratio

Table 2: Distribution of species of ticks in cattle from Plateau and Kaduna State

Genera	n	95% CI	P-value	aOR (95% CI)
Rhipicephalus	117	65.21,52.79	<.0001	Ref
Amblyomma	361	191.80,170.20		1.016 (1.012,1.020)
Boophilus	508	267.30,241.70		1.020 (1.016,1.024)
Hyalomma	484	254.99,230.01		1.019 (1.016,1.023)

95% CI based on Clopper-Pearson intervals. Ref: Reference category. aOR-Adjusted Odd ratio

Table 3: Positivity of cattle and ticks to CCHFV by ELISA and RT-qPCR respectively

		Positive by ELISA (%)	No. of Ticks pooled				Positive pool by RT-qPCR			
LGA	N		A	B	H	R	A	B	H	R
Plateau										
Bokkos	38	21(55.26%)	17	104	41	4	0	0	0	0
Barkin ladi	51	40(78.43%)	29	145	35	6	0	0	0	0
Jos south	38	19(50.00%)	48	57	40	68	0	0	0	0
Riyom	39	22(56.41%)	122	51	95	27	0	0	0	0
Jos North	59	36(61.02%)	76	46	22	0	0	0	0	0
Kaduna										
Sabon Gari	61	49(80.33%)	5	35	8	8	0	35	0	0
Zaria	11	6(54.55%)	0	0	0	0	0	0	0	0
Kubau	36	30(83.33%)	64	71	243	4	0	0	0	0
Total	333	223(67%)	361	508	484	117	0	35	0	0

Keys: A – *Amblyomma*, B – *Boophilus,* H – *Hyalomma,* R – *Rhipicephalus*

were pooled according to species and LGA of collection to make a total of 41 pools as shown in Table 4. Of the 41 pools of ticks tested for CCHF, only one pool of 35 *Rhipicephalus (Boophilus)* ticks (2.4%) was positive for CCHF virus with TR-qPCR cycle threshold (CT) value of 31.88.

Table 4: Pooled Ticks and Positivity to CCHFV by RTqPCR

Location	Tick type (Spp.)	Number Tested	Pools	RT-qPCR Positivity
Jos South	*Rhipicephalus (Boophilus)*	164	4	-
	Amblyomma	48	2	-
	Rhipicephalus	63	2	-
	Hyalomma	40	2	-
Jos North	*Rhipicephalus (Boophilus)*	46	1	-
	Amblyomma	38	1	-
	Rhipicephalus	0	0	-
	Hyalomma	22	1	-
Barkin Ladi	*Rhipicephalus (Boophilus)*	233	4	-
	Amblyomma	46	2	-
	Rhipicephalus	4	1	-
	Hyalomma	195	4	-
Riyom	*Rhipicephalus (Boophilus)*	96	2	-
	Amblyomma	77	2	-
	Rhipicephalus	27	1	-
	Hyalomma	95	2	-
Bokkos	*Rhipicephalus (Boophilus)*	35	1	-
	Amblyomma	0	0	-
	Rhipicephalus	0	0	-
	Hyalomma	0	0	-
Sabon Gari	*Rhipicephalus (Boophilus)*	35	1	+
	Amblyomma	5	1	-
	Rhipicephalus	8	1	-
	Hyalomma	8	1	-
Kubau	*Rhipicephalus (Boophilus)*	36	1	-
	Amblyomma	64	2	-
	Rhipicephalus	4	1	-
	Hyalomma	81	1	-

Key: + = Positive, – = Negative

Discussion

This study shows the detection of CCHFV in cattle (amplifying host) and ticks (reservoir vector). This detection completes the requirement for the possible spread of arboviral infection. In addition, it confirms the existence and silent amplification of CCHFV in Plateau and Kaduna States with possible spill-over to humans, posing a severe public health threat. The high seroprevalence of in this work corroborates the report of Msimang *et al*. (2021) in South Africa who reported a higher seroprevalence of 74.2% in cattle, but unlike South

Africa, no clinical case of CCHF has been reported in Nigeria despite several research findings revealing enzootic circulation of CCHFV and the occupational exposure to the virus by individuals at-risk. The detection of IgG antibody to CCHFV in cattle in this study, with unreported human cases corroborates an earlier report by Christova *et al*. (2018) in Bulgaria, who reported a high seroprevalence for CCHFV in ruminants without corresponding reports of human cases. A previous study by Oluwayelu *et al*. (2020) shows a seroprevalence of 24% for IgG antibodies to CCHFV. However, this is lower compared to the findings in this study. This may be due to the small sample size (50 cattle) used by Oluwayelu *et al*. (2020) that spread over 4 states in Nigeria. In contrast, the present study covers 2 states and is widely distributed across 8 LGAs. The difference in seroprevalence found in the two Nigerian studies may also be attributed to the use of different diagnostic tests, which could have varying levels of accuracy and sensitivity. Although the sampling by Oluwayelu *et al*. (2020) concentrated along states sharing international borders with other countries, it is worthy to note that the Fulani pastoralist practice an extensive movement of cattle across the country which is partly responsible for the transboundary spread of CCHFV.

The detection of CCHFV in tick species other than *Hyalomma* spp. may not new as the virus has been detected in other tick spp. in several parts of the world. In Iran, the results of a study carried out showed that the soft tick, *Ornithodoros* spp., was infected with the CCHF virus in addition to detecting it in *Hyalomma* spp. and *Rhipicephalus* spp. (Telmadarraiy *et al*., 2010). It is important to note that the detection of CCHFV in blood-fed engorged ticks only shows the circulation of the virus in a location but does not confirm the tick as a vector or reservoir for CCHFV (Gargili *et al*., 2017).

CCHF is a zoonotic arboviral disease capable of causing a deleterious public health challenge in at-risk populations such as livestock farmers and slaughterhouse/abattoir workers. This implies that unsuspecting human/animal health care personnel at the study location may be infected with a nosocomial pathogen if appropriate personal protective equipment (PPE) is not utilized when handling or treating infected animals or individuals. Indeed, this highlights the pattern of outbreaks likely to occur in Nigeria unless a preventive approach is undertaken to curb the virus's rapidly growing enzootic tick-animal-tick cycle.

Despite studies revealing the presence of CCHFV in Nigeria as far back as 1970, clinical cases of CCHF are rarely reported. In the study locations, no official clinical cases of CCHF have been diagnosed or reported in humans despite high seroprevalence and detection in the tick vector, which may be because of the failure to detect sporadic and subclinical infections of CCHF due to misdiagnosis and confusion with other endemic febrile illnesses such as malaria, hepatitis, and Lassa fever. The diagnosis of CCHF in humans depends on the patient’s clinical symptoms and patients’ history, but this diagnostic method cannot be used to differentiate CCHF from other viral haemorrhagic diseases (Garrison *et al*., 2007) which makes a suspicion of CCHF unlikely. The results of this study, coupled with reports by Bukbuk *et al*. (2016) who demonstrated the circulation of antibodies to CCHFV among hospitalized patients (who were admitted for other ailments) in Borno State, is an indication of the likely existence of human exposure and infection in Nigeria. This calls for the inclusion of this disease as a differential in all cases of febrile haemorrhagic conditions presented to healthcare facilities to ensure prompt diagnosis and administration of an effective management regimen. Metagenomic sequencing of the virus from the single positive pool did not yield the desired result possibly due to the high cycle threshold or sample quality.

In conclusion, this study detected a high seroprevalence of antibodies to CCHFV in Plaeau and Kaduna States, Nigeria and reports the detection of the virus in *Rhipicephalus (Boophilus) decoloratus* which acts as a reservoir. This is the first report of the virus being detected in *Rhipicephalus (Boophilus)* spp. in Nigeria and detection in ticks since 1970. This study highlights the need to establish appropriate tick control mechanisms to prevent the spread of the disease to humans. Also, the enlightenment of human and animal health workers, about the infection, prevention, and control methods is needed to prevent nosocomial outbreaks. Further work needs to be conducted to detect the role of haematophagus flies in transmitting the disease especially in areas where CCHFV was detected in cattle but not in ticks.

Acknowledgements

The authors would like to thank the Animal Health Component of Regional Disease Surveillance Enhancement (REDISSE) and the Department of Veterinary and Pest Control Services, Abuja for the reagents support. The authors also wish to express their appreciation for the support of Dr OM Akinsola and James Kube Budaye.

Funding
No funding was received.

Conflict of Interest
The authors declare that there is no conflict of interest.

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