



Seroprevalence and molecular detection of Rift Valley fever virus in ruminants from households in Kano State, Nigeria

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Abstract

Rift Valley fever Virus (RVFV) is a mosquito-borne virus belonging to the genus *Phlebovirus*, causing Rift Valley Fever (RVF) in both humans and animals. RVF is a significant transboundary disease predominantly infecting domestic ruminants and humans. This study employed a cross-sectional design to assess the seroprevalence and molecular detection rate of RVFV in household ruminants in Kano State, Nigeria. Blood was sampled from 184 ruminants (84 goats, 61 sheep and 39 cattle) across four Local Government Areas in the state (Rano, Makoda, Wudil and Nassarawa). Competitive Enzyme-Linked Immunosorbent Assay (C-ELISA) was employed to determine the Seroprevalence of RVFV infection in the study, followed by reverse transcriptase-polymerase chain reaction (RT-PCR) analysis of ELISA-positive samples to detect viral RNA. Among the 184 samples, 11 (6%) were seropositive: 6 cattle (15.4%), 2 sheep (3.3%), and 3 goats (3.6%), with a significant seroprevalence in cattle ($p = 0.02$). RT-PCR detected RVFV in 2 out of 11 seropositive samples (18%). This study may be the first to report serological and molecular detection of RVFV in household ruminants in Kano State, affirming virus circulation in the state. The presence of the virus in household ruminants poses a potential public health threat, especially in communities where close contact with animals is common. Therefore, strengthening the surveillance system, improving public health enlightenment, and coordinating One Health strategies are recommended to prevent potential outbreaks and reduce the likelihood of human infection in this region.

Keywords: Kano-Nigeria, Molecular detection, Rift valley fever, Ruminants, Seroprevalence

Introduction

Rift Valley fever (RVF) is a mosquito-borne viral disease affecting humans and animals. It infects ruminants mainly, causing abortion in adult females, while the younger ones suffer haemorrhagic fever and acute mortality (Wilson, 1994). The enveloped

virus is a negative-sense, single-stranded RNA genome (Swanepoel & Coetzer, 2004; Maclachlan & Dubovi, 2010) and has a diameter of 80 to 120 nm. The tripartite genome of Rift Valley fever virus (RVFV) is approximately 11.9 kb in length and comprises

three segments: Large (L), Medium (M), and Small (S). The L segment encodes the L protein, which functions as the viral RNA-dependent RNA polymerase (Giorgi *et al.*, 1991). The M segment is responsible for encoding the envelope glycoproteins Gn and Gc, as well as 78 kDa and 14 kDa non-structural proteins. The S segment, utilizing an ambisense coding strategy, encodes both the nucleocapsid protein and a non-structural protein (Giorgi *et al.*, 1991).

The primary mode of human infection is through direct exposure to the infected tissues or blood of sick animals, particularly during animal births, slaughtering, or carcass disposal (Peyre *et al.*, 2015; Warimwe *et al.*, 2016). Transmission can also occur through cuts or wounds from contaminated knives, inhaling infected aerosols, or consuming raw animal products (Balkhy & Memish, 2003; LaBeaud *et al.*, 2010). Regular monitoring for RVFV is insufficient in Nigeria and many African countries, leading to underreporting and misdiagnosis of cases (Adamu *et al.*, 2020). Additionally, unrestricted movement of animals and individuals from neighbouring RVF-affected nations into the country poses the threat of introducing the virus (Adamu *et al.*, 2020).

Outbreaks of RVF have been documented in East Africa, South Africa, Central Africa, Madagascar, and the Comoros. Additionally, some West African countries, including Mauritania, Burkina Faso, Mali, Niger, and Senegal, have noted RVF cases in both animals and humans (Weaver & Reisen, 2010; FAO, 2012; El Mamy *et al.*, 2014; WHO, 2016). Although Nigeria has no outbreak records of RVF, serological studies in humans and livestock detected RVFV antibodies. Reported seroprevalence rates range from 0.7% to 14.8% (Tomori, 1980; Ezeifeka *et al.*, 1982; Olaleye *et al.*, 1996; Adeyeye *et al.*, 2011; Opayele *et al.*, 2019). Despite this evidence of exposure, clinical cases of RVF have not been observed in indigenous livestock. However, Ferguson (1959) documented a fatal RVF infection in a Merino sheep imported from South Africa, and Lee (1970) later isolated three strains of the virus from *Culicoides* and *Culex antennatus* in the country.

In Kano State, there is limited information on the prevalence of RVF among household ruminants. Recognizing its presence is essential for assessing the disease's impact and associated risks. This study aims to establish baseline data on RVF in the region, thereby increasing public health awareness among herdsmen, veterinarians, medical professionals, and relevant authorities. The findings will support the

development of effective prevention and control strategies. Specifically, the study reports the presence of RVF infection in household ruminants in Kano State, Nigeria, and examines how factors such as age, sex, breed, and location of the sampled animals influence its distribution.

Materials and Methods

Study area

Kano State is Northern Nigeria's (Figure 1) commercial centre and the second largest city after Lagos in the country. The sampling locations within the state are shown in Figure 2.

According to the 2006 census, Kano State has the largest population in Nigeria, with approximately 9,383,682 people (Ibrahim *et al.*, 2022). Kano State is within Latitude 10° 3' N to 12° 3' North of the Equator and Longitude 7° 35' E to 9° 20' East of the Prime Meridian (Abdullahi *et al.*, 2023).

Kano State has two main seasons (dry and wet), with annual rainfall ranging from approximately 800 mm to 1100 mm. It also has an average high temperature of 38.2°C and low temperatures of 23.6°C (Nabegu, 2014), with the hottest month being April and the coolest month being December (Weather Atlas, 2020).

Kano State has three administrative divisions known as the Agricultural Development Programme (ADP) zones - Zone I, II, and III, with each comprising of its respective Local Government Areas (LGAs) and headquarters. Kano is the ADP Zone I headquarters, Danbatta is the Zone II headquarters, and Gaya is for Zone III (Ibrahim *et al.*, 2022).

Sample size determination

The sample size was obtained using the formula of Thrusfield's *et al.* (2017) and the prevalence estimated by Olaleye *et al.* (1996).

$$n = \frac{Z^2 \times p(1-p)}{d^2}$$

n = required sample size

Z = Z-value for confidence level (1.96 for 95%)

p = expected prevalence; the prevalence of 11.5% was used (Olaleye *et al.*, 1996)

d = desired absolute precision (0.05)

$$n = \frac{1.96^2 \times 0.115(1-0.115)}{0.05^2}$$

n = 156.39

For the study, 184 samples were used to minimise sampling error.

Ethical approval

The study was approved by Ahmadu Bello University Committee on Animal Use and Care, with approval number ABUCAUC/2023/158, which was granted on 30th November, 2023.

Study design

A cross-sectional study was conducted. A multistage clustered sampling was utilized in collecting data from the agricultural zones of the state. In the first stage, one LGA from Zones I and II, and two LGAs from Zone III were chosen using simple random sampling. Rano LGA was chosen from Zone I, Makoda LGA from Zone II, and Wudil and Nasarawa LGAs from Zone III. In the second stage, one ward was randomly selected from each of the chosen LGAs. Dawaki Kofar Fada village from Rano LGA, Wudil from Wudil LGA, Danmarke from Makoda LGA, and Kawo from Nasarawa. In the third stage, households with domestic ruminants were selected using convenience sampling based on animal owner compliance. Ward heads served as informants and guides.

The sample size was divided proportionately among the sampled animals as shown in Table 1. Samples that were positive by ELISA were further subjected to RT-PCR.

Sample collection

The samples were collected from February to March 2024. In the course of sampling, the age, sex, location and breed of each sampled animal were also recorded. Phenotypic/external characteristics were used for species and breed classification. The ages of the animals were approximated through dentition and history, when available. Blood samples (5ml) were collected from the cattle, goats and sheep via

jugular venipuncture using a sterile syringe and needle. The samples were transferred into labelled

sterile sample bottles without anticoagulants, which were then transported in a standard transport box

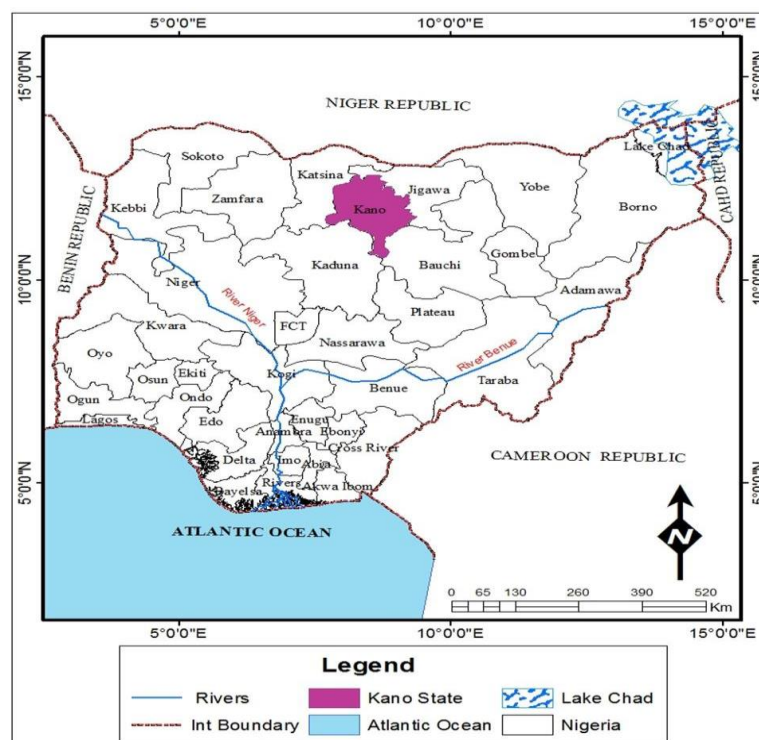


Figure 1: Kano State in Nigeria (Source: GIS Lab Department of Department of Geography, ABU, Zaria Using ArcGIS 10.8)

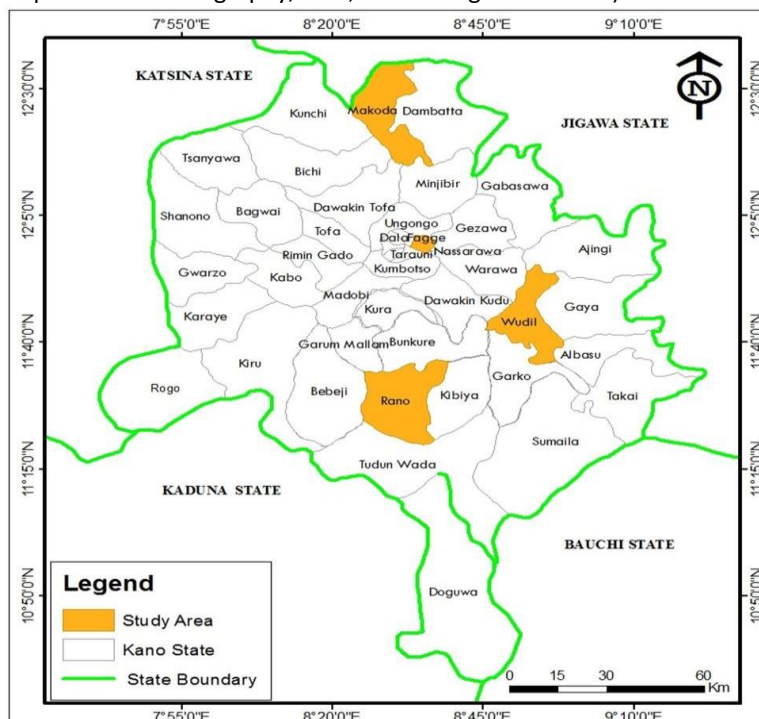


Figure 2: Kano State showing the sampling locations (Source: GIS Lab Department of Department of Geography, ABU, Zaria Using ArcGIS 10.8)

Table 1: Distribution of serum samples from cattle, sheep, and goats among the three agricultural zones of Kano State

Agricultural zones	LGA	Ward sampled	Sample size	Number of animal species sampled (%)		
				Cattle	Sheep	Goats
Zone I	Rano	Dawaki Kofar Fada	71	16	27	28
Zone II	Makoda	Danmarke	44	7	15	22
Zone III	Wudil	Wudil	44	14	11	19
	Nasarawa	Kawo	25	2	8	15
Total			184	39 (21.2)	61 (33.2)	84 (45.6)

LGA- Local Government Area

containing ice packs to the Laboratory (Viral Zoonosis Laboratory of the Department of Veterinary Public Health and Preventive Medicine, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria). Sera were later separated from the blood by centrifuging at 3000 rpm and gently siphoned into new labelled sample bottles, which were stored at -20°C until analysed.

Laboratory analysis

Serological analysis using capture IgM and IgG Enzyme-Linked Immunosorbent Assay (ELISA) Serological identification of RVFV infection was achieved using the Competitive Enzyme-Linked Immunosorbent Assay (C-ELISA) technique. The ID screen® Rift Valley Fever Competitive Multi-Species ELISA kit (ID VET, Montpellier, France) was employed in detecting antibodies (IgG and IgM) specific to RVFV following the manufacturer's instructions. The diagnostic ELISA kit specifically detects antibodies (IgM and IgG without distinguishing between them) targeting the RVFV nucleoprotein (NP) in serum samples. The samples were interpreted as positive when they had a competition percentage of <40%, doubtful as 40-50%, and negative as >50%. However, in our study, all doubtful samples were interpreted as negative.

The competition percentage (S/N %) for each of the samples was derived using the equation below:

$$S/N\% = \frac{OD \text{ sample} \times 100}{ODNC}$$

OD = each sample's Optical Density

ODNC = Optical Density for the Negative Control.

RVFV RNA extraction

RVFV genomic RNA extraction was carried out on the RVF IgM and IgG-positive sera samples.

This procedure was achieved by utilizing the ELK BIOTECHNOLOGY® Viral RNA Mini Kit (Wuhan, China) in accordance with the manufacturer's guidelines.

The obtained RNA samples were then labelled and preserved at -20°C until use.

Reverse transcription polymerase chain reaction (RT-PCR)

One-step RT-PCR was conducted on an iQ5 PCR machine (Bio-Rad, USA) by utilizing TransScript® One-Step RT-PCR SuperMix (TransGen Biotech®, Beijing, China) following the manufacturer's guidelines.

The reaction mixture was set up as described by Jupp *et al.* (2000) in a single tube by the addition of 0.4 µl of TransScript® One-Step Enzyme Mix (containing reverse transcriptase and RNase inhibitor) and 2×TS One-Step Reaction Mix (containing Taq DNA polymerase, optimized dual-cation buffer, dNTPs, a PCR enhancer and a PCR stabilizer). About 0.4 µl of the forward primer FD1 (5'-CCAAATGACTACCGTCAGC-3') and the reverse primer RVF E (5'-CCTGACCCATTAGCATG-3') were used to synthesize and amplify the 571-bp region of the Gn glycoprotein gene of the virus, which corresponds to the viral complementary DNA sequence of the M segment of the ZH501 RVF virus isolate (Grobbelaar *et al.*, 2011). The total reaction volume was brought to 20 µl by adding 4 µl of target RNA and 4.8 µl of RNase-free water.

The PCR thermal profile begins with a reverse transcription for 10 min at 45°C, after which an initial denaturation for 5 min at 94°C follows. This was succeeded by 40 cycles of denaturation for 30 s at 94°C, annealing at 50°C for 30 s, and extension for 30 s at 72°C, and concluded with a final extension for 5 min at 72°C.

Electrophoresis was conducted using a 1% agarose gel in 1 × TAE buffer for 40 minutes at 100 V. The gel was stained with 1 µl of ethidium bromide solution, and RNase-free water served as a negative control. The 100-bp DNA ladder (GelPilot, QIAGEN) was used to size the amplicons, which were visualized on a BioRad™ transilluminator.

Data entry and analyses

The laboratory analysis data were entered into Microsoft Excel and analyzed by utilizing the Statistical Package for Social Sciences (SPSS) version 20.0 software (Armonk, NY, USA). The chi-square test was used to assess associations between categorical variables, while Fisher's exact test was applied when expected cell counts were small. Statistical significance was set at $p < 0.05$ (95% confidence level)

Results

A total of 184 ruminants (39 cattle, 61 sheep, and 84 goats) were analysed for IgG and IgM-specific RVFV antibodies. The results showed 11 positive samples: 6 from cattle, 2 from sheep and 3 from goats. Consequently, the overall seroprevalence was 6% (11/184), as presented in Table 2. The seroprevalence among the animal species was 15.4% (6/39), 3.3% (2/61), and 3.6% (3/84) in cattle, sheep, and goats,

respectively. These rates differed significantly ($p = 0.02$) among these groups (Table 2).

The females had a seroprevalence rate of 2.8% ($n = 108$), and the males had 10.5% ($n = 76$) (Table 2).

The sampled ruminants >3 years had a higher seroprevalence of 7.7% (4/52) than those between 1-3 years (5.3%, 7/132). Rano LGA had the highest prevalence of 9.9% (7/71), followed by Wudil LGA at 9.1% (4/44) and none from Makoda LGA and Nasarawa LGA (Table 2). There were no significant differences in seroprevalence rates based on sex, breed, age, or location of the sampled animals. The Sokoto Gudali breed of cattle had the highest seroprevalence of 20% (1/5), followed by the Rahaji breed at 18.2% (4/22). Only the Yankasa breed and Red Sokoto breed were positive, with a seroprevalence of 3.4% (2/58) and 4.3% (3/70) among the breeds of sheep and goats sampled, respectively (Table 3).

Table 2: Seroprevalence of RVFV in Household Ruminants of Kano State

		Number of samples	Number (%) positive	χ^2	P value
Species	Bovine	39	6(15.4)	0.02	0.02*
	Ovine	61	2(3.3)		
	Caprine	84	3(3.6)		
Sex	Male	76	8(10.5)	0.03	0.053
	Female	108	3(2.8)		
Age	1-3	132	7(5.3)	0.53	0.50
	> 3	52	4(7.7)		
Location	Rano	71	7(9.9)	0.07	0.06
	Makoda	44	0		
	Wudil	44	4(9.1)		
	Nasarawa	25	0		
	Total	184	11(6%)		

* Statistically significant. RVFV- Rift Valley Fever Virus

Table 3: Seroprevalence of RVFV among the various animal breeds

	Breed of ruminants	Number of samples	Number (%) positive	χ^2	P value
Bovine	Rahaji	22	4(18.2)	0.73	0.83
	Sokoto gudali	5	1(20)		
	Bunaji	6	0		
	Cross	6	1 (16.7)		
Ovine	Yankasa	58	2(3.4)	0.95	1.00
	Uda	2	0		
	Cross	1	0		
Caprine	Red Sokoto	70	3(4.3)	0.43	1.00
	Kano Brown	14	0		
	Total	184			

RVFV- Rift Valley Fever Virus

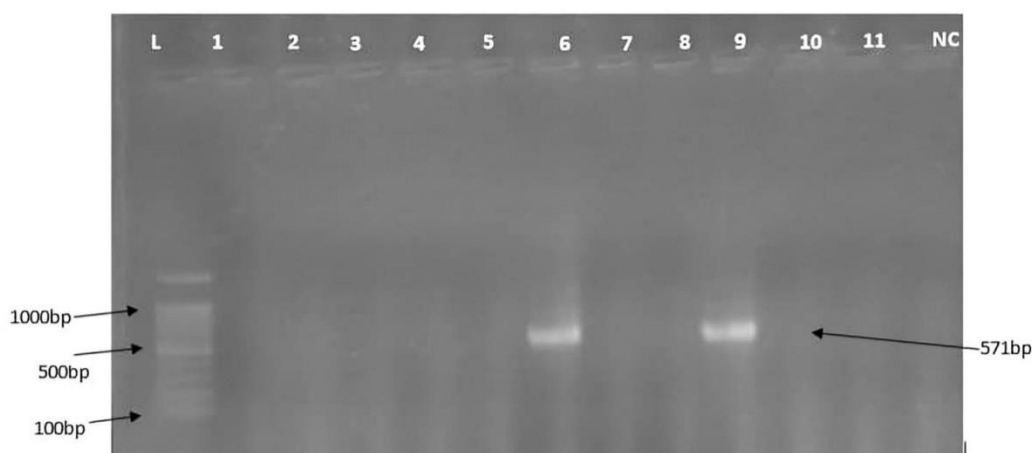


Plate I: Agarose Gel Electrophoresis Showing the 571bp RT-PCR Amplicons of RVFV from Ruminants in Kano State, Nigeria. L= Molecular weight marker; Bovine=Lanes 1,4,5,6,8,10; Ovine= Lanes 3,11; Caprine= Lanes 2,7,9; NC=Negative control

RT-PCR analysis of the 11 RVFV IgM- and IgG-positive serum samples revealed that only 2 (18%) amplified the targeted 571 bp fragment, as visualized by gel electrophoresis (Plate I). The positive samples were from a Rahaji breed of cattle (<3 years old) in Wudil and a Red Sokoto breed of goat (<3 years old) in Rano.

Discussion

This study identified RVFV antibodies (IgG and IgM) in goats, sheep, and cattle raised in households in some selected LGAs across Kano State, Nigeria, indicating the circulation of RVF virus among livestock in the region. The overall seroprevalence of RVFV in this study is consistent with previous studies in Nigeria that reported rates between 0.7% and 14.8% in both humans and animals (Tomori, 1980; Ezeifeka *et al.*, 1982; Olaleye *et al.*, 1996; Adeyeye *et al.*, 2011; Opayele *et al.*, 2019). Variations in the seroprevalence may be attributed to differences in detection methods, seasonal factors, and sampling locations.

Although sheep are generally the most vulnerable to RVFV infection (Mariner, 2018), this study revealed the highest seroprevalence in cattle at 15.4%, followed by goats at 3.6% and sheep at 3.3%. Mosquito feeding preferences may influence this discrepancy, as mosquitoes are more likely to bite larger hosts like cattle (World, 2017). Additionally, cattle are usually kept for longer periods than sheep and goats, leading to greater exposure to mosquito bites. Again, their frequent use as draught animals on

farms or in fields with high mosquito populations significantly increases their infection risk. Anejo-Okopi *et al.* (2020) reported similar findings, noting a higher prevalence in cattle compared to other ruminants. However, earlier studies from Southern Nigeria observed lower rates (Opaleye *et al.*, 2019), possibly due to varying detection methods, as their studies only identified IgM, while in this study, both IgM and IgG were detected.

This study did not identify any significant association between the occurrence of RVF antibodies and inherent characteristics such as breed, age, sex, or location, aligning with findings by Alhaji *et al.* (2020). Similarly, Ahmed *et al.* (2011) and Sumaye *et al.* (2013) found that the sex of the animals has no significant effect on the seroprevalence of RVFV. However, other studies observed a relationship between RVFV seropositivity and age, as reported by Jeanmaire *et al.* (2011), DiNardo *et al.* (2014), and Opayele *et al.* (2019).

RT-PCR detected RVFV in 18% of the IgG and IgM-positive samples, a lower rate than the 39% reported by Maquart *et al.* (2014). Other Nigerian studies found a higher detection rate in ELISA-negative samples (Oragwa *et al.*, 2023), while Chengula *et al.* (2014) in Tanzania reported that all ELISA-positive samples were RT-PCR negative. These findings show the value of RT-PCR as a diagnostic tool for detecting endemic RVFV circulation, particularly in Nigeria, where seroconversion may take place without visible clinical signs (Oragwa *et al.*, 2023).

The reliability of serological tests is not influenced by the host's immune response, which can be affected by factors such as age, overall health, nutrition, and state of the immune system (Maquart *et al.*, 2014), but also by the diagnostic parameters of the assay itself, including sensitivity and specificity, which may vary depending on the number, type, and origin of the samples tested. Additionally, the RT-PCR assay's sensitivity can be influenced by sample type, method of nucleic acid extraction, and thermal profile used (Garcia *et al.*, 2001).

Although Rift Valley fever (RVF) antibodies have been detected in Nigeria, no significant outbreaks have occurred. This study revealed the undetected RVFV circulation among all species, age groups, and sexes of sampled domestic ruminants, indicating the possibility of mild or asymptomatic infections due to insufficient active surveillance. Similar trends have been observed in Uganda (Magona *et al.*, 2013), Mozambique (Blomström *et al.*, 2016), Kenya (Nanyingi *et al.*, 2017), Cameroon (Zeller *et al.*, 1995), and Tanzania (Sumaye *et al.*, 2013).

Compared to methods like virus neutralization and haemagglutination-inhibition assays, the ELISA used in this study offers greater sensitivity for detecting immunological responses to RVFV infection or vaccination (Paweska *et al.*, 2003). Reverse transcription PCR (RT-PCR) remains one of the most sensitive and specific methods for the detection and quantification of Rift Valley fever virus, although it is increasingly complemented by other molecular and serological techniques (Lapa *et al.*, 2024; Afshar *et al.*, 2025).

This research took place in the dry season (February to March), suggesting that RVFV transmission might continue throughout the year, similar to earlier reports indicating that the spread of RVF occurrence is not limited to a particular season in Nigeria (Alhaji *et al.*, 2018; Opaleye *et al.*, 2019). Other studies from the Central African Republic and Nigeria reported that transmission occurred more frequently during the rainy months due to increased mosquito abundance (Nakoune *et al.*, 2016; Clark *et al.*, 2018; Olaleye *et al.*, 1996). It is important to interpret this discrepancy with caution, as this study measured both IgM and IgG without differentiation and was restricted to the dry season.

This study provides the first report of serological evidence and molecular detection of Rift Valley fever virus (RVFV) among household ruminants in selected areas of Kano State, Nigeria. The overall seroprevalence of 6% and the RT-PCR detection of RVFV RNA in 18% of seropositive samples point to the

unnoticed circulation of RVFV among apparently healthy livestock, particularly cattle, which has the highest seroprevalence, and they may pose a great risk of exposure to the public and animals. The presence of the virus in household ruminants poses a potential public health threat, especially in communities where close contact with animals is common. Therefore, strengthening the surveillance system, improving public health enlightenment, and coordinating One Health strategies are recommended to prevent potential outbreaks and reduce the likelihood of human infection.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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