JIDC | THE JOURNAL OF INFECTION IN DEVELOPING COUNTRIES

Original Article

Serologic evidence of silent Rift Valley fever virus infection among occupationally exposed persons in northern Nigeria

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Abstract

Introduction: Rift Valley fever (RVF) is a zoonotic disease caused by RVF virus (RVFV) and transmitted primarily by mosquitoes and contact with fluids and tissues of infected animals. First described in Kenya, it has spread to many African countries and beyond. In humans, it is sometimes misdiagnosed because the symptoms resemble those of influenza and/or malaria. Butchers, abattoir workers, and livestock keepers have the highest risk of infection.

Methodology: In this study, serum samples collected between February and September 2019 from 196 individuals comprising of butchers (n = 121), abattoir/slaughterhouse workers (n = 55), and livestock keepers (n = 20) in Benue, Sokoto, and Borno States of northern Nigeria were screened using a commercial ELISA that detected anti-RVFV IgM and IgG alike (i.e., without discrimination). Data from administered questionnaires and the ELISA results were statistically analyzed.

Results: Thirty-nine (19.9%) of the 196 samples were positive for RVFV antibodies. The distribution by states showed that 17.4% (8/46), 21.7% (15/69), and 19.8% (16/81) of samples from Benue, Sokoto, and Borno States were seropositive, respectively. Additionally, 21.5% (26/121) butchers, 16.4% (9/55) abattoir workers, and 20% (4/20) livestock keepers were seropositive.

Conclusions: These findings provide serological evidence for exposure of occupationally at-risk individuals in northern Nigeria to RVFV. The higher seropositivity obtained in Sokoto and Borno states could be due to contact of these individuals with infected animal blood/tissues, aborted fetuses, and unhindered transboundary movement of animals and animal products into these states which share international borders with Niger, Chad, and Cameroon where evidences of RVFV infections were recently reported.

Key words: Rift Valley fever virus; antibodies; occupationally exposed individuals; Nigeria.

J Infect Dev Ctries 2022; 16(5):881-888. doi:10.3855/jidc.15367

(Received 25 May 2021 – Accepted 23 January 2022)

Introduction

Rift Valley fever (RVF) is a zoonotic viral disease transmitted primarily by mosquitoes of the *Aedes* species. It presents a wide range of clinical manifestations, including abortion and signs associated with the digestive and respiratory systems in animals and influenza- and malaria-like symptoms in humans. Although it was first observed in 1912 and officially reported in 1930 around the Rift Valley area of Kenya [1,2], RVF has now spread to different parts of Africa and beyond [3] and is currently assuming a global dimension. The disease is caused by RVF virus (RVFV) which belongs to the order *Bunyavirales,* family *Phenuiviridae* and genus *Phlebovirus* [4].

The main means of transmission of RVFV to humans is through the bite of infected mosquitoes, contact with bodily fluids and tissues of infected animals, and through fomites especially those contaminated with aborted fetal materials [5-7]. Following infection, humans may develop influenza- or malaria-like symptoms including fever, malaise, headache, myalgia, arthralgia, and nausea. Although a majority of infections run asymptomatic courses with eventual complete recovery, a minority (1-2%) may degenerate to lethal hemorrhagic fever syndrome [8]. The clinical manifestations would, in this case, include severe ocular lesions possibly leading to permanent blindness, encephalitis, and severe hepatic pathologies, ultimately resulting in case fatality rates of about 10-20% [9-11] which, in exceptional cases, maybe up to 31% as reported in Niger Republic [12].

The human populations at highest risk of RVFV infection are the occupationally exposed individuals including livestock keepers and traders, veterinarians, butchers, abattoir/slaughter-house workers, and people living around ranches, grazing fields, and livestock markets [13-16]. Specifically, butchers and other abattoir workers who are regularly exposed to animal blood and tissues are at higher risk of exposure to the virus [17]. They easily get infected through cuts from knives when butchering and processing infected animals and through inhalation of aerosols generated during these processes. Some butchers slaughter and manually process large numbers of livestock carcasses without proper protective equipment [18,19].

In Nigeria, RVF was first reported in 1959 by Ferguson in Merino sheep imported from South Africa [20]. Afterwards, the virus was isolated by Lee (1979) from *Culicoides* midges and *Culex antennatus* mosquitoes [21]. Subsequent studies provided evidence of RVFV infection in both human and livestock populations in the country [13,15,16,22-28]. Specifically, Tomori [23] screened human blood samples, most of which were archived samples collected during an investigation of a suspected viral disease outbreak in 1969-1978 and reported 6.6% RVFV seropositivity, while Opayele *et al.* [13] screened livestock handlers in Ibadan, southwestern Nigeria in 2016-2017, and obtained 5.3% seropositivity. In addition, Bukbuk *et al.* [27] reported 14.1% RVFV seropositivity among hospital patients in Borno state, northern Nigeria. Among domestic animals, Olaleye *et al.* [24] reported 11.5% seropositivity. Moreover, Anejo-Okopi *et al.* [26] and Alhaji *et al*. [28] obtained similar seroprevalence rates of 11.0% and 11.3%, respectively among domestic ruminants slaughtered in Jos abattoir, north-central Nigeria, and cattle in nomadic pastoral communities of Niger state, north-central Nigeria. However, there has not been enough research done in the last two decades to establish the current status of the disease in the country, especially in the human population, nor have clinical outbreaks of the disease been reported. Meanwhile, there has been significant trans-border movement of livestock between Nigeria and neighboring countries, some of which have recorded intermittent outbreaks of RVF and/or its seroprevalence among both human and animal populations [12,29-36].

This study was therefore designed to investigate the level of exposure to RVFV of occupationally at-risk humans including butchers, abattoir/slaughterhouse workers, and livestock keepers in three states of northern Nigeria.

Methodology

Study design, locations, and population

The study was cross-sectional, and three states, representing two vegetation zones and three geo-political regions of northern Nigeria, namely: Benue (Guinea Savannah, North Central), Sokoto (Sudan Savannah, North West), and Borno (Sudan Savannah, North East), were purposively selected. Within each of the states, specific location(s) were also purposively selected as follows: Benue State - Oracle Farms (7º 42' 12.9" N, 8º 28' 4.0" E) and Wurukun slaughter slab (7º 44' 2.1" N, 8º 32' 50.1" E), Sokoto State - Sokoto abattoir (13º 04' 14.0" N, 5º 13' 26.0" E), and Borno State - Maiduguri abattoir (11º 51' 31.4" N, 13º 10' 42.7" E). The target populations were butchers, abattoir/slaughterhouse workers, and livestock keepers in the three states.

Sample and data collection

Blood samples (5 mL each) were collected between February and September 2019 from 196 consenting individuals from the three states as follows: Benue (n = 46), Sokoto (n = 69), and Borno (n = 81). The blood samples were allowed to clot and sera, separated into sterile cryovials, were stored temporarily at 20 ºC or -80 ºC before being transported to the National Veterinary Research Institute, Vom, Plateau State where they were stored at -80 ºC until analyzed. The study participants were also interviewed using a structured questionnaire at the time of blood sample collection. Apart from socio-demographic data, the questionnaire also contained information about their level of exposure to mosquito bites and frequency of experience of fever and malaria-like symptoms in the past few years. Other perceived risk factors and indicators of RVFV infection considered include participants' occupations, their highest level of education, presence (or otherwise) of domestic ruminants around their residences, and the responses of their malaria-like symptoms to anti-malarial treatments, and the outcomes of their malaria parasite tests.

RVFV competitive ELISA

Serological analysis of all serum samples was performed using a competitive ELISA (ID Screen® Rift Valley Fever Competition Multi-species ELISA, IDvet, Grabels, France), which detects antibodies (both IgG and IgM without discrimination) directed against the RVFV nucleoprotein in plasma or sera, according to the manufacturer's instructions. According to the manufacturer, validation tests for the ELISA showed that it has a sensitivity of 91-100% and specificity of

100%. The optical density (OD) value obtained for each sample, compared to the mean OD value of the negative controls, was used to calculate the Sample/Negative percentage (S/N%) based on the formula: $S/N_{sample} = OD_{sample} / OD_{negative\ control} \times 100$. Samples with S/N% values ≤ 40% and > 50% were considered positive and negative, respectively, while those with values between 40% and 50% were considered doubtful.

Data analysis

Data obtained from the completed questionnaires and ELISA results were analyzed using descriptive statistics, correlations, and chi-squared test to establish differences or associations between participants' variables and prevalence rates. The statistical package, SPSS version 16.0 for Windows (SPSS Inc., Chicago, IL, USA) and Microsoft Excel software were used for the analyses, and p values ≤ 0.05 were considered statistically significant.

Ethical approval

Ethical approval to undertake this study was obtained from the University of Ibadan/University College Hospital Ethics Committee (UI/EC/18/0681).

Results

Study participants

A total of 196 consenting individuals participated in this study from the three states. All of them were males, with age ranging from category "1-20 years" to "above 60 years". A total of 46, 69, and 81 participants were recruited from Benue, Sokoto, and Borno states, respectively (Table 1). Of the 46 participants from Benue state, 20 were livestock keepers, while the remaining 26 and all the other participants from other states were butchers and abattoir workers.

Seropositivity of RVFV among the participants

Out of a total of 196 serum samples collected from the three states, 39 (19.9%) were positive for RVFV antibodies while 157 (80.1%) were negative. Eight (17.4%) of 46, 15 (21.7%) of 69, and 16 (19.8%) of 81 serum samples collected from Benue, Sokoto, and Borno states, respectively were positive for antibodies against RVFV (Table 1).

Risk factor analysis

Seropositivity based on the occupation of the participants showed that 21.5% (26/121) of the butchers, 16.4% (9/55) of the abattoir/slaughterhouse workers, and 20% (4/20) of the livestock keepers had been exposed to RVFV (Table 2). A majority (n = 150, 76.5%) of them had ruminants around their residences, including 91 butchers, 40 abattoir/slaughterhouse workers, and 19 livestock keepers, while 46 did not have ruminants at home. All the 196 participants were males within age ranges of 1-20 years (n = 31), 21-40 years (n = 119), 41-60 years (n = 38) and > 60 years (n = 8). The prevalence based on age was highest (75%, 6/8) among the oldest category of participants i.e., > 60 years. Among other age categories, the seropositivity rates were 28.9% (11/38) and 18.5% (22/119) for ages 41-60 and 21-40 years, respectively while there were no positive cases among the 1-20 years age category (0/31). These results indicate a strong association between RVFV seropositivity and age of the participants, with a higher prevalence among the older participants (χ^2 = 24.6, p = 0.001) (Table 2). Additionally, 39 of the participants did not receive any formal education, while 9, 43, 84, and 21 acquired Islamic, primary, secondary, and tertiary education, respectively. Seropositivity based on the level of education of the participants showed that 25.6% (10/39), 22.2% (2/9), 20.9% (9/43), 16.7% (14/84), and

Table 1. Distribution of RVFV antibody positivity based on States and occupations of the participants.

State	Occupation of participants	No. of samples tested	No. positive	Percentage positivity
Benue	Butchers	17	3	17.6
	Abattoir Workers	9	1	11.1
	Livestock keepers	20	4	20.0
	Overall	46	8	17.4
Sokoto	Butchers	48	11	22.9
	Abattoir Workers	21	4	19.0
	Overall	69	15	21.7
Borno	Butchers	56	12	21.4
	Abattoir workers	25	4	16.0
	Overall	81	16	19.8
Overall	Butchers	121	26	21.5
	Abattoir workers	55	9	16.4
	Livestock keepers	20	4	20.0
	Overall	196	39	19.9

19% (4/21) of those who received no formal education, or Islamic, primary, secondary, and tertiary education had anti-RVFV antibodies, respectively. There was therefore no statistically significant correlation between participants' level of education and RVFV seropositivity ($p = 0.43$) (Table 2).

Whereas 94.4% (n = 185) of the participants had experienced varying frequencies of mosquito bites in the last few years, only few (n = 11, 5.6%) reported that they were not always bitten by mosquitoes; 104 (53.1%) experienced at least five mosquito bites daily, 39 (19.9%) experienced about 1-2 bites daily, and 42 (21.4%) experienced few bites weekly. There was however no statistically significant association between RVFV seropositivity rates and frequency of mosquito bites (Table 2).

Table 2. Multivariate analysis of the perceived risk factors and indicators of possible RVFV infection, and seropositivity.

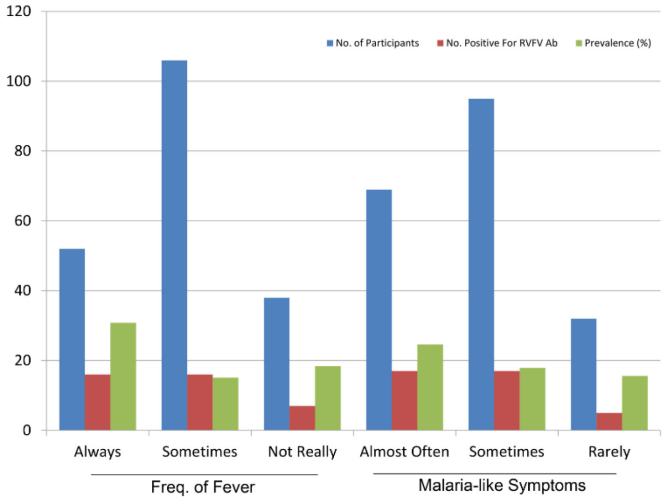
Variables	No. sampled	No. positive	Seropositivity (%)	Odds ratio	Chi-square	*p* value
State of sampling						
Benue State	46	8	17.4	1.056	0.056	0.813
Sokoto State	69	15	21.7			
Borno State	81	16	19.8			
Age of participants **						
1-20 years	31	0	0	1.988	24.597	0.001
21-40 years	119	22	18.5			
41-60 years	38	11	28.9			
Above 60 years	8	6	75			
Level of education						
No formal education	39	10	25.6	0.875	0.624	0.43
Islamic education	9	2	22.2			
Primary education	43	9	20.9			
Secondary education	84	14	16.7			
Tertiary education	21	4	19			
Occupation						
Butcher	121	26	21.5	0.871	0.261	0.61
Abattoir worker	55	9	16.4			
Livestock keeper	20	4	20			
Have animals at home						
Yes	150	31	20.7	0.808	0.243	0.622
No	46	8	17.4			
Frequency of mosquito bites						
> 5 times daily	104	20	19.2	0.999	0.001	0.994
1-2 times daily	39	9	23.1			
Few times weekly	42	8	19			
No mosquito bite	11	2	18.2			
Frequency of illness						
Almost often	62	15	24.2	0.802	0.625	0.429
Once in a while	108	19	17.6			
Rarely	26	5	19.2			
Fever involvement						
Always	52	16	30.8	0.639	2.756	0.097
Sometimes	106	16	15.1			
Not really	38	7	18.4			
Malaria-like symptoms						
Almost often	69	17	24.6	0.728	1.458	0.227
Once in a while	95	17	17.9			
Rarely	32	5	15.6			
Recovery following malaria treatment						
Yes	92	15	16.3	1.209	1.678	0.195
No	14	2	14.3			
Sometimes	52	13	25			
No treatment	38	9	23.7			
Outcome of malaria parasite tests**						
Usually positive	60	6	10	1.356	4.662	0.031
Usually negative	9	1	11.1			
Sometimes positive	8	5	62.5			
No malaria test	119	27	22.7			

**=*significant association exists at p ≤ 0.05*

Indicators of possible RVFV exposure

Most participants (n = 170, 86.7%) reported that they experienced intermittent illnesses over the time (ranging from the time of sampling to few years in the retrospective), which sometimes involved fevers in 158 (80.6%) of them. Development of malaria-like symptoms during such illnesses was reported to occur "almost often" and "once in a while" by 35.2% (n = 69) and 48.5% (n = 95) of the participants, respectively (Figure 1). Following such illnesses, 60.7% of them reported that they did not visit any healthcare facility for malaria parasite test (i.e., microscopic examination of thick and thin blood smears) but rather embarked on self-medication with anti-malarial drugs. Only 92 (46.9%) of the participants reported impressive recovery following such medications while the rest did not. Among the 60.7% (n = 119) that usually embarked on self-medication, 22.7% (n = 27) were seropositive for RVFV. Likewise, among those that usually tested for malaria before the commencement of treatment, 10% (6/60), 11.1% (1/9), and 62.5% (5/8) reported having 'usually positive', 'usually negative', and 'sometimes positive' results, respectively. There is therefore a strong correlation between the outcome of malaria tests and seropositivity (p = 0.031) (Table 2). The details of seropositivity based on indicators of possible RVFV infection among the participants is shown in Table 2. Although many of the factors considered showed positive association with RVFV seropositivity among the participants, it was only in few, i.e., "age" and "outcome of malaria test", that the associations were statistically significant (Table 2).

Figure 1. Association between RVFV seropositivity and experience of fever and malaria-like symptoms.



Discussion

Rift Valley fever is considered as one of the most important viral zoonoses in Africa [37], with domestic ruminants being the main hosts of the virus in West Africa. Human cases of the disease are mainly caused by virus exposure following abortion or slaughtering of viremic animals [38]. The focus of this study was to investigate the prevalence of antibodies against RVFV in occupationally exposed persons, including butchers, abattoir/slaughterhouse workers, and livestock keepers, in three states representing two vegetation zones of northern Nigeria. The detection of a 19.9% overall seropositivity of RVFV antibodies in these individuals indicates that approximately one-fifth of the human population sampled in the study area was seropositive for RVFV. This is quite instructive considering that RVF outbreak has not been previously reported in Nigeria, although virological and serological evidences of RVFV infection in humans and animals exist [13,15,16,22-28,39].

The ELISA technique used in this study detected both IgG and IgM antibodies, and thus did not differentiate between past and recent infections. These findings further confirm the possibility of continued occurrence of RVFV infections in the country despite the absence of outbreaks. This absence of RVFV outbreaks despite the existence of environmental and ecological factors that favor vector abundance such as unusually heavy rainfall with attendant flooding, and presence of pools of water that support mosquito breeding, may be due to poor disease surveillance and is consistent with the findings of Bolajoko and Babalobi in 2011 [40], who reported gross inadequacies in the disease surveillance and reporting systems in Oyo state, southwest Nigeria, a finding that may likely apply to the entire country.

The 19.9% RVFV seropositivity obtained in this study is higher than previously reported in human populations across the country: 6.6% reported in mostly archived samples collected from the general human population during an investigation of a suspected viral disease outbreak in 1969-1978 [23], 2.5% from a general population sampling conducted in different parts of the country [39], 14.8% in a diverse population including livestock farmers, butchers, wildlife rangers, health workers, and the general population (school teachers, office clerks and traders), from different ecological zones of the country in 1985-1989 [16], 14.1% in hospital patients in Borno state, northern Nigeria [27], and 5.3% among livestock handlers in Ibadan, southwestern Nigeria in 2016-2017 [13]. Worthy of note in the study of Olaleye *et al.* [16] is the

reporting of much higher seropositivity among the butchers, livestock farmers, and wildlife rangers compared to the general population. The higher RVFV seropositivity obtained in the present study compared to most of the previous ones suggests increased occurrence of RVFV infections in the country, but could also be due to the fact that the target populations of the current study were occupationally exposed individuals who are considered to be at higher risk of RVFV infection compared to the human populations tested in some of the previous studies.

Further, a comparison of the RVFV seropositivity rate obtained in this study with those from reports in other African countries shows that it is higher than the 3.3% reported in a large inter-epidemic serological survey of rural populations in Gabon [41], 4.0% among different occupational groups during an inter-epidemic period in Tanzania [42], and 15% among occupationally exposed populations in South Africa [43]. A further comparison of the results of this study with some others outside the African continent shows that it is higher than the 4.9% seroprevalence reported in a region-wide serological survey conducted in the urban and rural areas of Mersin province, Turkey [44], and the 0.75% IgG seropositivity among slaughterhouse workers and livestock handlers in seven non-epidemic regions of Saudi Arabia [45].

The higher RVFV seropositivity rates obtained in this study for the extreme northern States of Sokoto and Borno (21.7% and 19.8%, respectively) compared to Benue State (17.4%) (Table 1), may be related to the fact that they share international borders with neighboring countries that have previously reported evidences of RVFV infections, including Niger Republic, Republic of Chad and Cameroon [12,29-36]. It is known that these borders are porous and not properly monitored, thus allowing unhindered movement of domestic ruminants, some of which may be infected. This poses a great risk, not only for transborder transmission of RVF, but also for possible importation and spread of other transboundary and emerging diseases of zoonotic importance in Nigeria.

The RVFV seropositivity rates obtained in this study showed an increase with advancement in age of participants, with those older than 60 years having the highest seropositivity (75.0%), while the 1-20 years age group had no seropositive cases (Table 2). This positive association between age and seropositivity could be because participants in the older age groups had been exposed to RVFV-infected animals for longer periods of time. Although a majority (n = 185, 94.4%) of the participants in this study reported different levels of exposure to mosquito bites, there was no association between frequency of mosquito bites and RVFV seropositivity. This suggests that the main route of exposure of these individuals to RVFV infection may not be mosquito bites. This possibility is corroborated by the fact that about 89.8% of the participants were butchers and abattoir workers who regularly got exposed to blood, other bodily fluids, tissues and animal products, and aborted fetuses of slaughtered animals. Some of the risky practices they engaged in that could predispose them to RVFV infection include slaughtering, evisceration and processing of raw meat without personal protective equipment (PPE) such as gloves, aprons, goggles, and gumboots [19], as well as blowing air with their bare mouths into the subcutaneous tissue of small ruminants to enhance flaying, which is a common practice in many abattoirs in Nigeria. In addition, some of them were observed to have open wounds from knife cuts on their hands, which puts them at high risk of contracting RVFV from infected animal fluids and tissues. Indeed, slaughtering of infected animals has been reported to increase the risk of infection through inhalation of aerosols generated during the process, especially when traditional slaughtering methods are practiced [46,47]. Therefore, these behavioral practices, which are of public health significance, make the abattoirs and slaughterhouses high-risk environments that provide excellent prospects for transmission of disease agents from animals to humans.

About 83.7% (n = 164) of the participants reported that they had been having malaria-like illness sporadically in the past few years, with different frequencies of fever involvement in 80.6% (n = 158) of them. These findings were positively associated with RVFV seropositivity as participants with more frequent fevers and malaria symptoms had higher RVFV seroprevalence rates compared to other categories of participants (Figure 1, Table 2). It is possible that some RVF cases might have been misdiagnosed as malaria since RVF also presents with fever and malaria-like symptoms, thus contributing to the under-reporting of the disease in Nigeria. The consequent silent infections with the virus underscore the inadequacy of arboviral disease surveillance and reporting systems in the country.

Conclusions

The findings of this study provide evidence of RVFV infection among occupationally exposed individuals in Nigeria even though no clinical outbreak of the disease has previously been reported in the

country. There is need for continuous surveillance and reporting of RVF and/or RVF-like illnesses among animals and at-risk human populations such as butchers, abattoir workers, livestock keepers, pastoralists, and veterinarians in order to better define the epidemiology of the disease in Nigeria. Further, since there is currently no licensed RVF vaccine for use in humans, early detection and disease prevention measures including public health education aimed at achieving behavioral modifications among occupationally at-risk populations such as those involved in the current study, are recommended. According to de St. Maurice *et al.* [48], such educational programs should encourage increased hand hygiene and the use of PPE during care of sick animals and slaughtering, emphasize methods of reducing mosquito bites, and promote safe consumption of meats and dairy.

Acknowledgements

The authors thank the Management and Staff of the abattoirs in Maiduguri (Borno State) and Sokoto (Sokoto State), the slaughter slab at Wurukun, Makurdi (Benue State), as well as Oracle Farm (Benue State), from where samples were collected for this study.

Authors' Contributions

Conceptualization (AOO and DOO); Sample Collection, Laboratory and Data analyses (AOO and FCO); Original draft preparation (AOO); Review and editing (AOO and DOO). All authors read and approved the final version of the manuscript

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Conflict of interests: No conflict of interests is declared.