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ARTICLE

Prevalence of Rift Valley Fever Virus in Febrile Malaria Patients using Serological and Molecular-based Evidence

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Abstract

Rift Valley fever virus (RVFV) is a zoonotic virus classified as category A priority pathogen. Rift Valley fever (RVF) has been poorly investigated in Nigeria with the infection among Nigerians last reported in 1996. Two hundred (200) febrile subjects with symptoms of malaria attending local hospitals in Ilorin, Nigeria were investigated for malaria, malaria positive subjects were investigated for the presence of RVF. Malaria screening was done using *Carestart*™ malaria HRP2(pf), while RVF antibodies were tested for using anti-RVF IgM ELISA. Molecular identification of the viral genome was carried out using RNA extraction (QIAGEN) and quantitative Polymerase Chain Reaction (qPCR). Of the 200 subjects tested for malaria infection, 93 (46.5%) were positive, while 20 (21.5%) of the 93 subjects were seropositive for RVF. RVF virus genome was found in 5 (25%) of the 20 positive subjects. The high prevalence of RVF among malaria positive subjects show that there is a risk of a RVF outbreak if its prevalence remains unchecked.

Keywords: Rift valley fever; Malaria; Epidemiology; Risk factors.

1.0 Introduction

Rift Valley fever (RVF) is a disease caused a by mosquito-borne virus that was first identified in the Great Rift Valley of Kenya (Martin *et al.*, 2008; WHO, 2010). Humans become infected with RVF when they are bitten by mosquitoes abhorring the virus or come in contact with tissues of animals already infected with the virus (Schmaljohn *et al.*, 2007). In majority of the cases of RVF, humans usually develop mild febrile illnesses which are usually self-limiting, but in some cases could progress to encephalitis and hemorrhagic fever (Schmaljohn *et al.*, 2007).

Rift Valley fever virus (RVFV) belongs to the family Bunyaviridae, and genus *Phlebovirus* (Pepin *et al.*, 2010). It is an enveloped negative sense RNA virus of about 90-110 nanometers in diameters with three segments large (L), medium (M), and small (S) (Pepin *et al.*, 2010). RVFV is a virus of great public health concern, and was classified as a biothreat agent by National Institute of Allergy and Infectious Diseases (NIAID) which could also cause significant loss in livestock when they infect them (Schmaljohn *et al.*, 2007; Freiberg *et al.*, 2008).

Although no documentation exist on human to human transmission of the RVFV, animal to human transmission is often seen. Apart from direct bite from the vectors (mosquitoes) transmitting the virus, individuals working in slaughter houses, farmers and herders are also at high risk of acquiring RVFV (WHO, 2010).

Malaria still remains one of the deadliest diseases in Nigeria which is characterized by chills and fever (Afolabi *et al.*, 2015). The prevalence and transmission of malaria is largely influenced by factors associated with the vector, human host and environment (WHO, 2016). Despite the fact that RVF and malaria share different species of mosquitoes as vectors for the propagation of diseases in humans, similar factors encourage the co-occurrence of these vectors. High risk factors which predispose individuals to RVF include; exposure to mosquito bites, method of storing water, proximity to bushes, method of preventing mosquito bites, contact with animal fluids, and sheltering of animals in homes, there is a need to look out for the prevalence of RVF in populations where malaria is endemic.

Although no outbreak of RVF has occurred in Nigeria, animals such as sheep, cattle and goats have been shown to have significant antibodies titers against RVF (Ezeifeka *et al.*, 1982). While evidence of the presence of the virus among humans in Nigeria was last shown in 1996 by Olaleye et al. majority of individuals presenting with febrile ilnesses are usually treated for malaria. Thus this study was aimed at providing a molecular and serological evidence for the prevalence of RVF among high risk individuals in Ilorin, Kwara state.

2.0 Materials And Methods

2.1 Study Site/Design

This study was carried out in Ilorin, Kwara State. Ilorin is the state capital city of Kwara state and located on (8.4799° N, 4.5418° E). The state is made up of about 2.5 million people and has a land mass of 32,500 square kilometre (KSG, 2017). The settlement settings of the study site were both rural and nucleated. This study was a hospital based cross-sectional study of febrile malaria patients attending General Hospital Ilorin, and Ajibola Memorial Hospital Oke Oyi Ilorin, Kwara state, Nigeria. Patients that presented symptoms similar to malaria and typhoid fever were recruited for the study for a period of 6 months between April 4th– September 5th, 2016

2.2 Study Population/Sampling Technique

The study population consisted of patients that presented with symptoms of malaria (headache, shivering, fever, nvomiting, joint pain, jaundice) and typhoid fever in referral government hospitals in Ilorin, Kwara state. Two hundred febrile patients were screened for malaria after an informed consent form was dully filled and signed. Subjects who were confirmed to have malaria were then screened for Rift Valley fever.

Ethical Consideration Approval for the study was obtained from the Ethical Review Board of Ministry of Health, Ilorin after which informed consent was obtained from subjects and/or parents and guardians.

2.3 Sample Collection

Blood samples were obtained via acupuncture from the subjects. The blood samples (2 ml) were collected into EDTA bottles and then centrifuged at 3,000 rpm for 5 min. The serum was collected into a sterile eppendorf bottle using pasteur pipette and then stored at -20 ^{o}C in the refridgerator for further assay.

2.4 Statistical Analysis

Self-administered closed ended questionnaires were used to generate information on the socio-demographic characteristics and predisposing risk factors of the study population. All data generated from the study was checked manually for errors in filling responses. Descriptive statistics such as mean, frequency, standard deviation, percentage and graph were used in the discussion of the results, in order to give a lucid representation of the data analysed. The interaction between the prevalence of RVF and associated risk factors were tested using χ^2 (Chi-Square) test at 5% confidence interval. P value <0.05 was regarded as statistically significant.

2.5 Serological Analysis for Malaria Parasite.

Several subjects were tested using *Carestart*^{TM} malaria HRP2 (pf), following the manufacturer's procedures. Five (5) ml of blood sample was collected using a micropipette, the blood sample was then added into a well designated "S" on the test devise. Two (2) drops of 60 ml assay buffer solution was then added into the second well designated "A" well on the test device as above. The set up was allowed to rest on the laboratory bench for 20 min before the test result was read.

2.6 Test For RVF Antibodies

The test for RVF antibodies was carried out using IgM Enzyme linked Immunosorbent Assay method (WKEA Med Supplies, China: WH-1822) which has a sensitivity and specificity of about 98%. The colour intensity which is proportional to the amount of Rift Valley Fever virus antibody present in the sample were measured with a microwell reader at 450nm, according to the manufacturers (WKEA Med Supplies, China) instructions. For test validity, the average for positive control wells $\geq$ 1.00; the average for negative control well is $\leq$ 0.10, read at 450/630-700 nm, average negative control was 0.047, the critical (CUT OFF) was

regarded as the average negative control well + 0.15. Samples were regarded as positive for RVF IgM when the OD ≥ CUT OFF (≥0.197).

2.7 Molecular Identification of RVFV

Molecular studies were done to confirm the actual presence of RVFV genes in the blood samples of subjects positive for RVF IgM. RNA extraction was done using RNeasy minikit (QIAGEN, Maryland 20874, USA). The process was carried out as follows. RVF Virus primer (RF1 CCAAATGACTACCAGTCAGC and RF2 CCTGACCCATTAGCATG selected to amplify a portion of the Gn glycoprotein of the virus) (Grobbelaar et al., 2011) were contained in 2 ml tubes which were centrifuged for 10 secs to dissolve the primers from the walls of their tube. A 100 µm stock solution was prepared by adding PCR grade water of 303.8 µL to RVF-R2 CCTGACCCATTAGCCATG. A working solution was then prepared by extracting 2 µL from each stock solution into a new 2 ml tube containing 98 uL of PCR grade water. The PCR reaction mixture contained 12.5 µL of Maxima SYBR Green qPCR master mix (2.5 mM, $MgCl_2$, dNTP, dUNTP, maxima care start Taq DNA polymerase, SYBR Green Idye), 0.3 µM each primers, 2 µL of the extracted RNA and 4.5 µL of PCR grade water to make a final volume of 25 µL. The PCR mixture was introduced into a thermocycler (icycler) (Bio-Rad, USA) programmed to incubate for 60 min at 37 ^{o}C and 5 min at 95 ^{o}C to allow cDNA production and initial denaturation and then proceed with 40 cycles of denaturation (95 ^{o}C for 30 sec), primer annealing (65 ^{o}C for 30 sec), primer extension (72 ^{o}C for 7 min) and the amplicon maintained at 4 ^{o}C.

3.0 Results

A total of 200 febrile subjects were tested for malaria parasite infection, out of which 93 (46.5%) were positive. The 93 subjects positive for malaria parasite infection were then tested for rift valley fever out of which 20 (21.5%) were positive for RVF. Of the 20 subjects positive for RVF, 5 showed the presence of RVFV genome (Figure 1).

The largest proportion of the subjects surveyed 74 (37%) were young adults within the age bracket of 20-39 years. Table 1 shows other demographic characteristics of the subjects including; gender, marital status, level of education and religion. Results further revealed that considering the socio-demographic factors of the subjects, only occupation influenced the prevalence of RVF statistically (p value = 0.02) (Table 2). Results showed that the major factors which influenced the prevalence of malaria among the subjects included methods used in preventing mosquito bites (Table 3). Table 4 shows some of the risk factors which either influenced or did not influence the prevalence of RVF among the subjects. Some of the influencing factors include; recent malaria infection, methods of preventing mosquito bites and methods of storing water.

4.0 Discussion

This study provides for the first time since 1996 molecular and serological evidence for the prevalence of RVF in a Nigerian community. It also shows the prevalence of RVF in people with malaria infection. It also describes some of the socio-demographic characteristics of the participating respondents as well as risk factors associated with RVF and malaria co-infection.

Table 1: Socio-Demographic Characteristics of the Study Population

Socio-demographic characteristics	Study population (%)
Age	
5-9 years (Children)	36 (18.0)
10-19 years (Puberty)	47 (23.5)
20-39 years (Young adults)	74 (37.0)
40-50 years (Adults)	43 (21.5)
Gender	
Male	80 (40.0)
Female	120 (60.0)
Marital status	
Married	82 (41.0)
Single	109 (54.5)
Divorced	9 (4.5)
Level of Education	
Informal (illiterates)	53 (26.5)
Informal (literates)	55 (27.5)
Formal	51 (25.5)
Others	41 (40.0)
Religion	
Christians	83 (41.5)
Muslims	102 (51.0)
Other	15 (7.5)

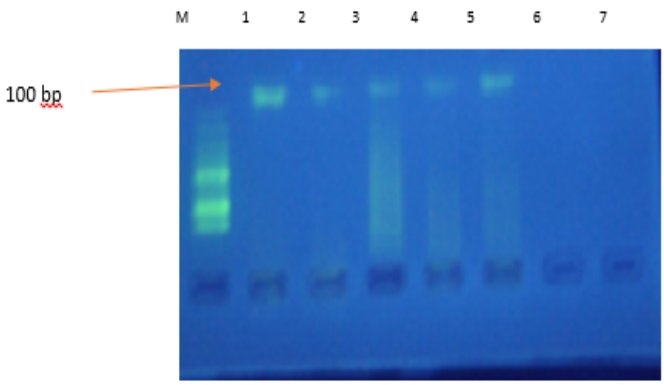

Figure 1: *Rift Valley fever RT-qPCR (1 kb Plus DNA Ladder, 0.9% Agarose gel Stained with ethidium bromide)*

Table 2: Relationship between the prevalence of RVF and the Socio-demographic characters of the study population

Risk factor	Positive (%)	χ^2 (P value)
Age		2.545 (0.467)
5-9 years (Children)	4 (4.3)	
10-19 years (Puberty)	2 (2.2)	
20-39 years (Young adults)	9 (9.7)	
40-50 years (Adults)	5 (5.4)	
Gender		0.508 (0.476)
Male	10 (10.8)	
Female	10 (10.8)	
Marital status		4.155 (0.125)
Married	7 (7.5)	
Single	11 (11.8)	
Divorced	2 (2.2)	
Level of Education		2.439 (0.503)
Informal (illiterates)	4 (4.3)	
Informal (literates)	4 (4.3)	
Formal	7 (7.5)	
others	5 (5.4)	
Subjects occupation		11.636 (0.02)
Civil servant	9 (9.7)	
Farming	2 (2.2)	
Artisan	2 (2.2)	
Business	2 (2.2)	
Others	5 (5.4)	

Table 3: Probable risk factors contributing to the prevalence of RVF among the respondents

Risk factor	Positive (%)	χ^2 (P value)
Method of storing water		0.543 (0.909)
Local water pots	2 (1.0)	
Bowls without cover	13 (6.5)	
Bowls with cover	34 (17.0)	
Water tanks	25 (12.5)	
Frequency of contact with bushes/forest		0.895 (0.639)
Weekly	25 (12.5)	
Monthly	41 (20.5)	
Seldom	27 (13.5)	
Method of preventing mosquito bites		8.69 (0.034)
Use of Insecticide treated nets	37 (18.5)	
Insecticide spray	6 (3.0)	
Repellant ointment	8 (4.0)	
Cover cloth	42 (21.0)	
Presence of trees around residence		4.543 (0.084)
Yes	52 (26.0)	
No	41 (20.5)	

The result of this study showed an overall RVF prevalence rate of 21.51% subjects with malaria using RVF-IgM ELISA. In Kenya, Ochieng *et al.* reported that in the year 2007, the national seropositivity of antibody to Rift Valley Fever (RVF) virus was 4.5%. In Nigeria, the first and most recent study on RVF infection in humans by Olaleye *et al.* (1996) showed a prevalence of RVF infection of 14.8% by Haemagglutination Inhibition (HI) of which 84.6% demonstrated neutralizing antibodies. The prevalence rate in this study was largely dependent on the season of the year which this study was conducted. The study spanned through April to Septmber 2016, which is the period of heavy rainfall accompanied with flooding which favors the prevalence of the hatching dynamic of the vector (Martin *et al.*, 2008).

In this study the highest proportion of the respondents were adults of age group 20 – 40 years. This is as a result of the fact that adults belonging to this age group are the agile working class that spend most of their time outside their homes either farming, hunting or engaged in other activities. This is in contrast with Olaleye et al. (1996) who reported respondents of 40 years and above.

In this study the highest proportion of subjects testing positive for malaria infection were within the age range of 20 -39 years, while children within 5-9 years ranked next. This could be due to the number of hours these group of people spend outdoors which correspondingly increases their level of exposure to mosquito bites. More subjects with recent mosquito bites were shown to have malaria, compared to those who have not had mosquito bites recently ($p = 0.033$). Furthermore, subjects method of preventing mosquito bites played a significant role in the acquisition of malaria. The use of cloths to cover the body against mosquitoes which is not effective is quite common in Nigeria. Although the study of Elijah *et al.* (2014) showed that the method used by individuals in preventing mosquito bites influences the rate of malaria prevalence, the study of Thomas *et al.* (2015) showed that mosquito bite prevention tools did not affect the prevalence of *Plasmodium* infection.

Results from this study showed that there was no statistical significance between the age of subjects and RVF/malaria co-infection ($p = 0.467$). This is in contrast with the result of Ochieng et al., 2015 where they reported in Kenya that the elderly (50–64 years) had higher odds of exposure to RVF than the younger group (15–29 years). Contrary to the study of Labeaud *et al.* (2010) where they reported that male sex is considered to be more at risk of exposure to RVF, sex was not considered a factor in the acquisition of RVF in this study. In the study of the prevalence of RVF in Kenya, lack of education was significantly associated with RVF seropositivity (Ochieng *et al.*, 2015) but this was not the case in this study as literacy did not contribute statistically to the epidemiology of RVF in the study area.

Table 4: Other Probable risk factors contributing to the prevalence of RVF among the respondents

Risk factor	Positive (%)	χ^2 (P value)
Recent malaria infection		3.863 (0.049)
Yes	8 (8.6)	
No	12 (12.9)	
Frequent contact with blood of ruminants		1.429 (0.232)
Yes	2 (2.2)	
No	18 (19.4)	
Methods of preventing mosquito bites		27.38 (0.001)
Insecticide treated net	2 (2.2)	
Insecticide spray	5 (5.4)	
Repellant ointment	5 (5.4)	
Cover cloth	8 (8.6)	
Trees and bushes around residence		1.893 (0.169)
Yes	11 (11.8)	
No	9 (9.7)	
Method for storing water		8.062 (0.045)
Local water pots	2 (2.2)	
Bowls without cover	3 (3.2)	
Bowls with cover	12 (12.9)	
Water tanks	3 (3.2)	
Meals per day		8.091 (0.044)
One	0 (0)	
Two	8 (8.6)	
Three	9 (9.7)	
Irregular	3 (3.2)	

From this study, it was observed that subjects with recent malaria infection were at a higher risk of having RVF and malaria co-infection ($p = 0.049$). This is because presence of an infection is a major impediment to cellular and humoral immunity of any individual, this brings about a fall in the antibody titre which should combat disease pathogen such as bacteria, protozoans and even viruses. As a result, invasion by these disease pathogens is not far-fetched.

Mosquitoes have been shown to be a major vector for RVFV (WHO, 2010), hence preventing mosquito bites could help in reducing the risk of acquiring RVF through the vector. This study showed that there was significant relationship between RVF and malaria co-infection and subjects method of preventing mosquito bites ($p = 0.001$). Hence, this study proved that method of preventing mosquito bite is a risk factor for the co-infection of RVF and malaria.

Also the subjects methods of storing water played a significant role RVF prevalence. It is a common practice to store water in small containers in and outside of homes in Nigeria (Ayanda, 2009), these storage bowls are sometimes left uncovered which could result in the presence of breeding grounds for mosquitoes. In the last report of RVF in humans in Nigeria, Olaleye *et al.* (1996) showed that RVF virus was prevalent in farmers of the Sudan savannah zone who stored water in small dams constructed in many areas at the beginning of the wet season. Also, McIntosh and Jupp (1981) stated that the method of water storage, especially saving water in dams enhances breeding of mosquito and consequently the spread of RVF virus.

Although constant contact with animals, especially those infected with RVF, increases the risk of acquisition of RVF (WHO, 2010), in this study subjects contact with livestock did not constitute a significant risk factor in the acquisition of RVF. This is owing to the fact that the indigenous residents in this study areas do not really engage in rearing livestock such as cattle, sheep, etc that are reservoirs for RVFV and when they do, they practice semi-intensive and/or extensive form of livestock rearing which requires little or no care for the animals. The study of Himeidan *et al.* (2014) reported that contact with infected animals such as consumption or handling products from sick animals, touching an aborted animal foetus or being herdsperson are risk factors to RVF infection. Similarly, Animal fetus, have been shown to constitute an important factor in the transmission of RVF during interepidemic and epidemic periods.

The consistency of taking meals among the subjects which could after the nutritional intake of the subjects, influenced the prevalence of RVF among the subjects. Cellular and humoral immunity are impaired by malnutrition. Hence, the risk of infection is high in individuals with an inbalance nutritional in-take.

5.0 Conclusion

The seroprevalence of 21.51% obtained in this study shows that RVF is prevalent in Ilorin, Kwara State. If this prevalence remains unchecked, there could be an outbreak of RVF associated viral hemorrhagic fever with its associated health challenges such as fever, liver dysfunction, jaundice, bleeding from nose and gums which is usually associated with high mortality.

Conflict of Interest

Authors declare no conflict of interest

Authors Contribution

Conception: OMK and AOA
Design: OMK, AOA and JIO
Execution: AOA and JIO
Interpretation: OMK, AOA and JIO
Writing the paper: AOA and JIO

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