NIGERIAN VETERINARY JOURNAL
ISSN 0331-3026
Nig. Vet. J., September 2019 Vol 40 (3): 227 – 238.
https://dx.doi.org/10.4314/nvj.v40i3.7 **ORIGINAL ARTICLE**

Screening of Immunoglobulin G Antibodies Against Chikungunya Virus Among Urban Population in Ilorin Nigeria

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SUMMARY

Chikungunya virus (CHIKV) is a mosquito-borne viral disease which is becoming a serious global public health problem. The principal vector in many parts of Africa is *Aedes species*. There are recent reports of CHIKV importation into Europe, Asia and America by travelers returning from west and central Africa. Yet, there is scanty information from the guinea savannah region of Nigeria. This study determined previous exposure to CHIKV in the urban population. It is a cross-sectional study involving 89 participants enrolled from three hospitals in Ilorin, Kwara State. A qualitative Chikungunya Enzyme Linked Immunoassay kit was used to detect IgG antibodies. Data was analyzed using SPSS version 22. Statistically significant level was $p \leq 0.05$. Out of the study participants, 24.7% were previously exposed to CHIKV. Age group 31-40years had highest proportion while children under 10years had least IgG level. In this study, we found Chikungunya to be endemic in Ilorin Nigeria. There is need for sustained surveillance, to determine spatio-temporal epidemiology of CHIKV. Efforts should be poised to strengthen vector control measures.

Key words: Chikungunya virus, Immunoglobulin G, Guinea Savannah, Ilorin, Urban

INTRODUCTION

Chikungunya virus (CHIKV) is an RNA virus that belongs to Family: Togaviridae; genus: alphavirus (Strauss and Strauss, 1994). Chikungunya epidemic was first observed from 1952-1953 in East Africa and isolation of the virus was carried out for the first time from serum of a febrile patient in the Tanganyika area (now Tanzania) in 1953 (Lumsden, 1955; Robinson, 1955; Ross, 1956). Between 1960s and 1980s, the virus was isolated repeatedly from numerous countries in central and southern Africa as well as in Senegal and Nigeria in western Africa (Halstead *et al.,* 1969). It is a zoonotic virus that is maintained by interaction between non-human primates and Aedes mosquitoes in the forest cycle. The principal vector *A. aegypti* has spread throughout many parts of Nigeria (Onoja *et al.,* 2016). It poses serious threat to human, as populations increase and expand geographically, facilitating contact with wildlife, disturbing their ecosystem for more agricultural activity to meet increased food demand (Bean *et al.*, 2013). Symptoms of CHIKV infection are nausea, myalgia, fever, headache, vomiting, arthralgia and rash (Powers *et al*., 2000) which are similar to clinical signs in people with dengue fever. Since CHIKV circulates in regions where dengue virus is equally endemic, many febrile infections are often misdiagnosed; hence incidence of Chikungunya is higher than what is reported especially in developing countries (Carey, 1971). Reports of Chikungunya has been made in more than 40 countries around the world. Several outbreaks have been reported in China, Indian subcontinent, Central Africa and South-East Asia. CHIKV transmission has expanded to the Caribbean, Americas, countries in Europe and the Pacific where it was brought by infected travelers returning from endemic countries or regions (Weaver, 2014; Nhan and Musso, 2015; Aubry *et al*., 2015). First case of autochthonous infection in Italy occurred in 2007, with over 200 people affected (Mavalankar *et al.,* 2007; Watson, 2007; Liumbruno *et al.,* 2008; Angelini *et al*., 2008; Vazeille *et al.,* 2008). In 2008, the United States National Institute of Allergy and Infectious Diseases (NIAID) listed it as a Category C pathogen of priority (Staples *et al*., 2009). Epidemics of Chikungunya re-emerged and were documented in the Democratic Republic of Congo area of Kinshasa from 1999–2000 (Pastorino *et al.,* 2004), Indonesia from 2001–2003 (Laras *et al.,* 2005) and the Indian Ocean islands of Mayotte, Mauritius and the La Réunion from 2005–2006 (Saxena *et al.,* 2006). In West Africa, epidemics have been reported in Senegal (Diallo *et al*., 1999), Ivory Coast (Thonnon *et al*., 1999). Also, large outbreaks were reported in East Africa and Comoros from 2004–2005 (Powers and Logue, 2007). During the early dry season of 1972, arbovirus surveillance was carried out in Saki, a rain forest region in Oyo State Nigeria and 24% Complement Fixing CHIKV antibody was reported (Fagbami, 1978). Recently, 11% active Chikungunya was reported among people in the rainforest region (Ayorinde *et al.,* 2015). However, there is scanty epidemiological information on CHIKV in the guinea Savannah region (north-central) of Nigeria. This study was designed to determine prevalence of past CHIKV infection among urban dwellers in Kwara State Nigeria.

MATERIALS AND METHODS

Study Design and Sample Collection

This study is a cross-sectional study carried out among 89 participants randomly selected from the General Hospital, Civil Service Hospital and Cottage Hospital in Ilorin, Kwara State Nigeria. It is located in the guinea savannah vegetation zone, which breeds different species of mosquitoes (Okogun *et al*., 2005). Sample size (N) was determined using the formula $N = Z\alpha^2pq/d^2$ (Thiberville *et al.,* 2013), where Zα = standard normal deviate set at 1.96, corresponding to 95% confidence interval (95% CI); p = proportion in the target population estimated to have a variable characteristic = 41.8% (0.418) from similar study in Nigeria (Ayorinde *et al.,* 2015); q = 1 - p = 58.2% (0.582); and d = degree of precision set at 0.05 (95% CI). Three milliliter of whole blood was aseptically collected by venipuncture into anticoagulant free tube from each consenting participant. This was allowed to stay at room temperature and centrifuged at 3000rpm for 5minutes in order to separate sera from clotted blood. Sera was subsequently collected into eppendorf tubes and shipped to the Arbovirus Research Laboratory in Department of Virology, College of Medicine, University of Ibadan, Nigeria where screening was done. Ethical approval MOH/KS/EU/777/275 was obtained for this study from Kwara State Ministry of Health and the study conformed to Ethics of Human subjects use in research following the Helsinki Declaration. Informed consent was obtained from adult participants, while parental assent was obtained from young persons under 18years of age. Inclusion criteria was visiting out-patients testing negative for malaria parasite examination and widal test. Infants, people who were positive for malaria, typhoid fever or HIV were excluded from the study.

Serology

Qualitative Competitive Chikungunya IgG ELISA kit was obtained from Cortez Diagnostics Inc. California, USA. The immunological assay detects human IgG antibodies by targeting CHIKV E2/E1 proteins. Upon taken delivery of kit, ready-to-use CHIKV antigen was immediately stored at -20^{o}C while other reagents where held at +4^{o}C until assay was to be carried out. Positive control, cut-off and negative control were assayed in duplicates. Samples and controls were diluted in ratio 1:100. Optical Density (OD) was visualized using single wavelength 450nm. Immune Serum Ratio (ISR) was calculated for each sample as mean ratio of OD_{450} value for test sample against mean OD_{450} value of Cut-Off control. ISR ≥1.0 was considered positive while ISR <1.0 OD was negative according to the manufacturers' interpretation.

Data Analysis

Data were analyzed using IBM Statistical Package for Social Sciences, version 22. Pearson Chi square and Exact Fishers test was used to test for associations between variables. Descriptive data were presented in tables and $p<0.05$ was considered level of statistical significance.

RESULTS

Out of 89 participants screened, 22 (24.7%) were positive. Out of this number, 16 (25.8%) are females and 6 (22.2%) males. There was no significant difference in gender association between males and females (p = 0.719). A 28 year old female had the highest Immune Serum Ratio (ISR) of 8.162, followed by a 31year old male with 8.140 ISR. Age groups 31-40years had the highest proportion of people with CHIKV IgG. Those under 10years of age were least exposed (Figure I). There was no significant difference between ISR across age of participants (p = 0.267). There was no significant association observed between marital status and ISR (TABLE 1). Participants with tertiary education had higher proportion of CHIKV IgG while those in preschool were not previously exposed in this study (TABLE II). Civil servants and participants who engaged in business activities had equal proportion of exposure to CHIKV (TABLE III). There was no significant association between CHIKV IgG and blood groups (TABLE IV). Those who always used mosquito net and those who never used mosquito nets were equally exposed to CHIKV (TABLE V). There was no significant difference observed between those who used indoor spray and those who did not use it (p=0.319).

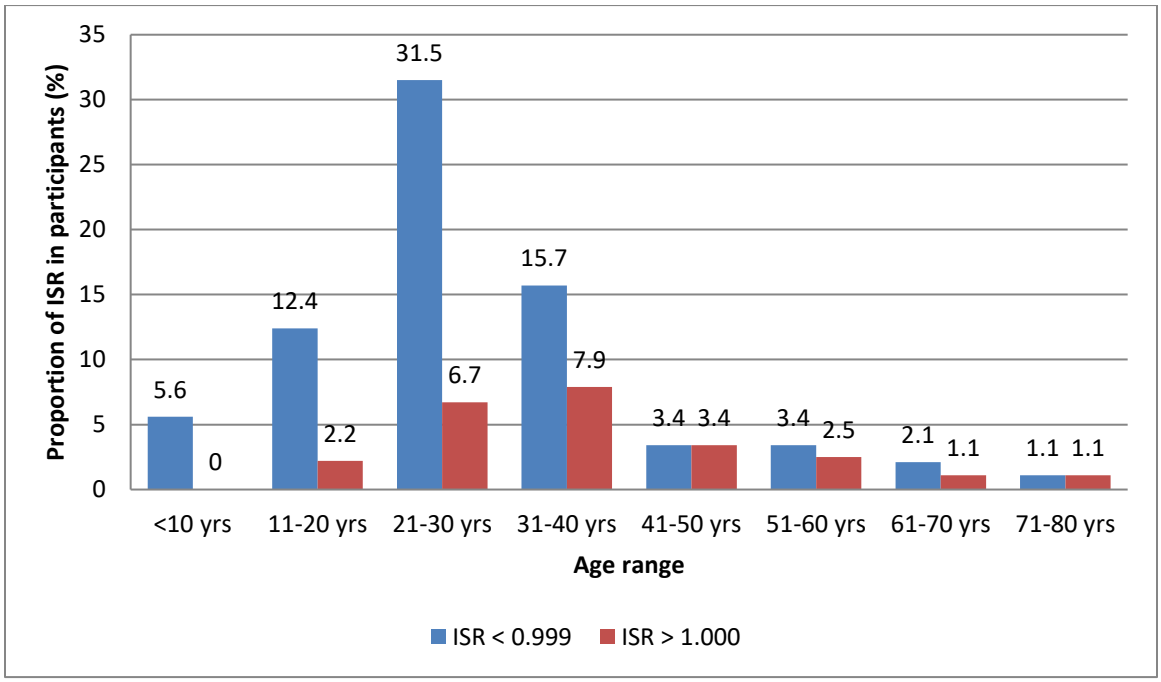

Figure I: Distribution of immune serum ratio across age of participants

TABLE I. Distribution of immune serum ratio according to marital status of participants

	Immune Serum Ratio			
Marital status	**<0.9999**	**>1.000**	**Total**	**p value**
Married	42 (47.2%)	15 (16.9%)	57 (64.0%)	
Single	24 (26.9%)	6 (6.7%)	30 (33.7%)	0.57
Widowed	1 (1.12%)	1 (1.12%)	2 (2.25%)	
Total	67 (75.3%)	22 (24.7%)	89 (100.0%)	

TABLE II. Immune serum ratio based on educational status of participants

	Immune Serum Ratio			
Education Status	**<0.999**	**>1.000**	**Total**	**p value**
No Formal Education	11 (12.3%)	2 (2.2%)	13 (14.6%)	
Preschool	1 (1.1%)	0 (0.0%)	1 (1.1%)	
Primary Education	3 (3.4%)	2 (2.2%)	5 (5.6%)	
Secondary Education	29 (32.6%)	7 (7.9%)	36 (40.4%)	0.515
Tertiary Education	23 (25.8%)	11 (12.3%)	34 (38.2%)	
Total	67 (75.3%)	22 (24.7%)	89 (100.0%)	

TABLE III. Occupation of participants and distribution of immune serum ratio

	Immune Serum Ratio			
Occupation	**<0.999**	**>1.000**	**Total**	**p value**
Artisan	3 (3.4%)	3 (3.4%)	6 (6.7%)	
Business	29 (32.6%)	7 (7.9%)	36 (40.4%)	
Civil Servant	8 (8.9%)	7 (7.9%)	15 (16.9%)	0.151
Student	22 (24.7%)	4 (4.5%)	26 (29.2%)	
Unemployed	5 (5.6%)	1 (1.1%)	6 (6.7%)	
Total	67 (75.3%)	22 (24.7%)	89 (100.0%)	

TABLE IV. Blood group and immune serum ratio of participants

	Immune Serum Ratio			
Blood group	**<0.999**	**>1.000**	**Total**	**p value**
AA	22 (24.7%)	10 (11.2%)	32 (35.9%)	
AS	8 (8.9%)	2 (2.2%	10 (11.2%)	
Not indicated	37 (41.6%)	10 (11.2%)	47 (52.8%)	0.562
Total	67 (75.3%)	22 (24.7%	89 (100.0%)	

TABLE V. Distribution of immune serum ratio and some environmental determinants

	Immune Serum Ratio			
Environmental determinants	**<0.999**	**>1.000**	**Total**	**p-value**
Use of mosquito net				
No	49 (55.1%)	11 (12.4%)	60 (67.4%)	
Occasionally	0 (0.0%	1 (1.1%)	1 (1.1%)	0.046
Always	18 (20.2%	10 (11.2%)	28 (31.5%)	
Use of indoor spray				
No	36 (40.4%)	10 (11.2%)	46 (51.7%)	
Occasionally	3 (3.4%)	3 (3.4%)	6 (6.7%)	0.319
Always	28 (31.5%)	9 (10.1%)	37 (41.6%)	
Proximity to stagnant water				
No	61 (68.5%)	21 (23.6%)	82 (92.1%)	0.505
Yes	6 (6.7%)	1 (1.1%)	7 (7.9%)	
Proximity to bush				
No	59 (66.3%)	20 (22.5%)	79 (88.8%)	
Yes	8 (8.9%)	2 (2.2%)	10 (11.2%)	0.713
Total	67 (75.3%)	22 (24.7%)	89 (100.0%)	

DISCUSSION

This observational survey highlights previous exposure to CHIKV infection in urban populations within the guinea Savannah region of Nigeria. Prevalence rate of 24.7% observed in this study is higher than 4.1% reported in the arid north located in Sahel Savanah (Akinola *et al*., 2017) and lower than 31.4% reported in the rain forest southern region (Olajiga *et al*., 2017). In the arid part of Nigeria the climate is harsh and relatively high temperature does not favour preponderance of vectors hence the lower prevalence observed. Prevalence observed in guinea savannah area is slightly lower than in rain forest region because there are scanty trees, and reduced wildlife compared to rain forest region. The increased humidity and vegetation cover provides breeding ground for Aedes mosquitoes. Areas rich in wildlife such as rain forest region are thought to be hotspots for zoonotic diseases such as Chikungunya (Allen *et al*., 2017). However, risk of transmission in guinea Savannah region is higher than in arid northern parts of Nigeria hence it serves as an effective transmission zone for people on transit. Several studies that focused on predicting chikungunya disease persistence identified age (Scilte *et al*., 2013; Geradin *et al*., 2013; Essakjee *et al*., 2013) and sex (Essackjee *et al*., 2013) as major determinants of Chikungunya. But this present study found no significant association between gender and age (Figure I).

There was no association between marital status and exposure to CHIKV (TABLE I). Since mosquitoes are not selective in their biting preferences, chances of infection are the same for married partners because they live in same house, most of the time. Children are likely to be exposed in a similar manner, if they are within the same house. This is because *Aedes aegypti* which is a major vector culprit is an indoor mosquito. Vector is infected once females take in viremic blood from infected individual. After successful extrinsic incubation period, vector is infectious and capable of transmitting CHIKV through bites of mosquitoes (Watts *et al*. 1987). Adult *Aedes species* like to rest on clothes and walls inside homes, where females take blood meals as they exclusively bite human hosts (Scott *et al.*, 1993, 2000).

Participants undergoing secondary and tertiary education had more exposure to CHIKV as shown in Table II. This is because they are mobile and likely to have acquired infection while commuting or trekking from their residences to school. It is a significant finding, which reinforces abundance of vectors. Ilorin climate is not arid, and therefore provides relative tropical environment for them to thrive. Businessmen and civil servants were equally exposed to CHIKV in this study (as shown in TABLE III). This is because vector bites everyone not minding their occupation. Although occupation does not matter with CHIKV exposure in this study, some occupations such as lumberjacks and farmers who frequently encroach into vector-wildlife-ecosystems are predisposed to acquiring the infection. Although participants with blood group AA were more exposed (TABLE IV) there is no need to entertain fears of being more predisposed to CHIKV infection when compared to other blood groups. This is because CHIKV IgG mediates

complete clearance of virus from cardiovascular system (Prince *et al*., 2015). Table V shows exposure of participants is irrespective of whether or not they are in close proximity to stagnant water, bush or if they use bed nets, whereas in other studies these environmental determinants have been shown to facilitate vector breeding (Dutta *et al*., 2011; Onyeneho, 2013; Tither, 2014). One reason that can be advanced for this is that the present study did not assay for active infection, which would have given a closer epidemiological link. Another study is needed to inform active CHIKV infection and determine incidence rates in Ilorin.

CONCLUSION

We have established in this present study that people in the guinea savannah are exposed to CHIKV. This study shows that competent vectors are prevalent in the region. This is worrisome because Ilorin is a transit point for people who traverse the north and southern parts of Nigeria. Further studies are needed to determine incidence rate of active Chikungunya infection in the guinea savannah region and to identify circulating clades, in order to know the extent of CHIKV transmission. This will inform strains to be incorporated in future vaccines for Africa. Additionally, this study will provide valuable information for chronic disease development, as patients with preceding Chikungunya disease might have higher chances of developing severe long-term disease associated with decreased long-term quality of life (Elsinga *et al*., 2018). Preventive measures and vector control efforts should be strengthened in the guinea savannah region to eradicate vectors.

Acknowledgments

We thank Kwara State Ministry of Health for giving the approval to carry out this study. We also acknowledge the management of General Hospital, Civil Service Hospital and Cottage Hospital Ilorin, Kwara State for granting our permission to enroll participants in their facility and the patients who participated.

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