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Chikungunya Virus Seroprevalence and Associated Factors among Hospital Attendees in Two States of Southwest Nigeria: A Preliminary Assessment

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ABSTRACT

Chikungunya virus (CHIKV) is a re-emerging pathogen causing long-term polyarthritis and encephalitis. In conducting a preliminary investigation, we hypothesized that there is no serologic evidence of CHIKV infection among attendees of selected hospitals in Lagos and Osun States, Nigeria. Sera from 304 consecutively selected participants were screened for CHIKV IgG and IgM using ELISA. Findings were analyzed vis-à-vis participants' demographic and clinical data. Over 90.0% of the participants had never heard of CHIKV despite the fact that a large proportion of them (88.8%) had secondary/tertiary education. Overall, 41.8% were positive for, at least, one antibody type (IgG or IgM), while about 16.0% of the participants had dual seropositivity (CHIKV IgG and IgM) with gender as associated factor (odds ratio [OR]: 2.8, $p = 0.03$). Prevalence rates were 31.8% and 38.4% for CHIKV IgG and IgM, respectively. Only hospital location (Osogbo) was associated with CHIKV IgG (OR: 2.2, $p = 0.009$), while gender alone was associated with CHIKV IgM (OR: 3.0, $p = 0.001$). Participants seropositive for CHIKV antibodies were mostly adults (18–59 yrs) belonging to the active work-force; five (22.7%) and three (20.0%) of the pregnant participants had CHIKV IgG and IgM, respectively. Detection of CHIKV IgM in some participants might make them potentially infectious to the newborn and mosquito vectors. Importantly, participants positive for either IgG or IgM had fever (72.8%, 67.2%) and general body pains (61.7%, 57.6%), respectively. This ELISA-based study revealed serologic evidence of CHIKV infection among hospital attendees in Lagos and Osun states with the group-specific prevalence rates being considerably high.

Abbreviations: Chikungunya virus (CHIKV); Chikungunya (CHIK); enzyme-linked immunosorbent assay (ELISA); immunoglobulin G or M (IgG/IgM); odds ratio (OR); non-structural proteins (nsP); hemagglutination inhibiting (HI); complement fixing (CF); neutralization test

KEYWORDS

Body pains; chikungunya virus; febrile condition; hospital attendees; IgG; IgM; prevalence; risk factor

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(NT); immunofluorescence assay (IFA); plaque reduction neutralization test (PRNT); confidence interval (CI); analysis of variance (ANOVA); body temperature (BT); Building Nigeria's Response to Climate Change (BNRCC).

Introduction

Chikungunya virus (CHIKV) is a mosquito-borne virus first isolated from the serum of a febrile human in Tanzania (formerly Tanganyika) in 1953 during an epidemic of dengue-like illness (Robinson, 1955; Ross, 1956). As observed in recent times for arboviruses, the causative agents of most important emerging infectious diseases responsible for significant global public health problems (Gubler, 2001), CHIKV has also been reported to have re-emerged as a zoonotic pathogen (Powers & Logue, 2007; Sam et al., 2015). The virus belongs to the Semliki Forest antigenic complex in the genus *Alphavirus*, family Togaviridae (Kuhn, 2013) and possesses a single-stranded RNA genome of positive polarity that encodes four non-structural proteins (nsP 1 to 4) at its 5′ end and three structural proteins C, E1 and E2 at the 3′ end. The glycoprotein E2 primarily used by the virus to mediate entry into susceptible host cells is also the immunodominant viral molecule that induces neutralizing antibodies (Kam et al., 2012a; Griffin, 2013; Lum et al., 2013). In Africa, CHIKV is vectored chiefly by *Aedes (Ae) aegypti and Ae. albopictus* although other species including *Ae. furcifer, Ae. vittatus, Ae. fulgens, Ae. luteocephalus, Ae. dalzieli, Ae. vigilax, Ae. camptorhynchites, Culex annulorostris* and *Mansonia uniformis* have also been implicated. In addition, Anopheles mosquitoes have occasionally been incriminated in CHIKV transmission (Jupp et al., 1981; Jupp & McIntosh, 1990). These mosquitoes, which are zoophilic and anthropophilic, are usually prevalent in rural and urban areas of Africa and are day-biting in nature (World Health Organization (WHO), 1997, 2016). Primates, including humans, become infected when bitten by CHIKV-infected and infectious mosquitoes, most commonly *Aedes spp*. Epidemiologically, the virus first detected in Tanzania has now spread to south-east Asia, India, Philippines, Indonesia, Europe and the Americas (Kuhn, 2013; Leparc-Goffart et al., 2014; Pan American Health Organization, 2015). A reason for this is the mutation (A266V) in the virus glycoprotein E1 that enabled it to adapt to *Ae. albopictus* (Tsetsarkin et al., 2014). This mosquito is silent but aggressive, active all-day long, has a lifespan longer than other mosquitoes (up to 8 weeks) and, in the last decades, has expanded to several areas previously known to be *Aedes*-free (Charrel et al., 2007).

Chikungunya virus replicates in infected host cells of susceptible humans with resultant self-limiting disease ranging from mild febrile to explosive flu-like illness (Chikungunya fever) and then to rash and severe, persistent polyarthritis in 1–12 days after infectious mosquito bite (Roques et al., 2015; Langsjoen et al., 2016). Other clinical symptoms/signs associated with CHIK fever include headache, back pain, myalgia and arthralgia (Hochedez et al., 2006; Saxena et al., 2006). Skin involvement may be present in about 40–50% of cases (Brighton et al., 1983). The attack rates can be as high as 70.0%, and in rare cases, severe neurological manifestations and multi-organ failure may occur; the fatality rate is mostly unknown but it was estimated to be in the region of 1:1,000 (Roques et al., 2015).

Infected humans specifically respond to CHIKV infection by producing virus-specific antibodies that bind to and eventually eliminate the virus from the body. Whereas the antibodies can be hemagglutination inhibiting (HI), complement fixing (CF) or neutralizing (NT), their isotypes are usually immunoglobulins M and G. Serosurveys for CHIKV virus have been reported in many countries using serologic methods such as HI, CF, immunofluorescence assay (IFA), plaque reduction neutralization test (PRNT) and enzyme-linked immunosorbent assay (ELISA) (Adesina & Odelola, 1991; Baba et al., 2013; Caglioti et al., 2013; Vourc'h et al., 2014). Importantly, in Nigeria, Adesina and Odelola (1991) used HI tests to screen human and animal sera and reported CHIKV seroprevalence of 14.3% and 2.3%, respectively, while Baba et al. (2013) used PRNT, a more specific diagnostic technique, and obtained 50.0% prevalence of CHIKV neutralizing antibodies in humans.

Recent large outbreaks of CHIK fever occurring globally have made the virus an "International Focus Issue" (Roques et al., 2015). Moreover, since CHIK fever syndrome clinically resembles that of dengue fever, many cases of the former are misdiagnosed as dengue fever (Omarjee et al., 2014). Hence, as a re-emerging virus with potential for causing morbidity and mortality (Campion et al., 2015; Higgs & Vanlandingham, 2015), there is a need to determine whether the virus actively circulates among humans in southwest Nigeria with a view to generating data that will aid formulation of prevention and control policies before the disease assumes epidemic proportions. This preliminary ELISA-based investigation was therefore undertaken to screen hospital attendees in Lagos state and in Ede and Osogbo, Osun state for anti-CHIKV IgG and IgM antibodies.

Materials and methods

Study area/location

The study was conducted in Lagos and Osun states, southwest Nigeria (Figure 1) between November, 2015 and September, 2016. With a coastline of approximately 180 km, Lagos state lies

Figure 1. Map of Nigeria showing the study area (A = Lagos state, B = Osun state).

between latitude N6.465° and longitude E3.406° and has coastal wetlands as well as upland rainforest as dominant ecozones. The vegetation cover is typically a mosaic of mangrove swamps, freshwater swamps, secondary forest, farmland and fallow land, while the soils are mostly deep and poorly drained. Its climate is wet equatorial and influenced by nearness to the equator and the Gulf of Guinea. Lagos state enjoys rainy season with two peaks: May to July and September to October, with the former being the heaviest. Floods characterize the peaks due to the poor surface drainage systems of the coastal lowlands. The mean annual rainfall ranges from 1,567.2 mm in the north-western part of the state to 1,750 mm in the mainland areas, while the temperature is generally consistently high, with a mean monthly maximum of about 30°C (Iwugo et al., 2003; Building Nigeria's Response to Climate Change (BNRCC) project, 2012).

Osun state lies between latitude 7.5876° N and longitude 4.5624° E. It covers a total land area of about 8,602 square kilometers and is located between 300 and 600 m above sea level with a largely gentle and undulating landscape. Average rainfall in Osun state ranges from 1,125 mm in the derived savannah to 1,475 mm in the rainforest belt. The average annual temperature ranges from 27.2°C in the month of June to 39.0°C in December, while soil types in the state mostly contain a high proportion of clay and sand (Sofoluwe et al., 2011). Though a landlocked state, it is blessed with many rivers and streams which serve the water needs of the people. Osogbo, the capital city, lies on coordinates 7°46′ N, 4°34′ East, is easily accessible from any part of the state due to its central location and is also the commercial and industrial hub of Osun state. Ede town lies along the Osun River and is located in the guinea savannah zone. The people engage in farming and other commercial activities.

Study population and design

This is a cross-sectional, hospital-based study with participants selected from patients visiting seven different healthcare facilities in Lagos (one hospital) and Osun states (two hospitals in Osogbo and four hospitals in Ede). They were largely resident in Lekki, Ajah and Victoria Island in Lagos state as well as Ede and Osogbo in Osun state.

The study was approved by the Human Research Ethics Committee, College of Health Sciences, Osun State University, Osogbo. Medical personnel in each hospital clearly explained the objectives/benefits of the study to the patients and only those who consented by completing and endorsing filled-in questionnaires were consecutively recruited. With the assistance of the hospital staff, demographic and clinical data were obtained and documented. Inclusion criteria were consenting attendee (male or female aged 10 years and above) with one or a combination of the following clinical signs/symptoms: fever, headache, skin rash, back pain, muscle pain, joint pain or general body pain, while exclusion criteria were unwillingness to participate in the study and being less than 10 years of age.

Based on a 50.0% prevalence rate of CHIKV antibody from a previous study in Nigeria (Baba et al., 2013), a sample size of 424 humans (385 plus 10% attrition) was obtained using the prescribed formula (WHO, 2004; Niang et al., 2006). However, for logistics reason, 304 serum samples were randomly selected and used for this study.

Blood collection and serum preparation

About 5 ml of blood was aseptically collected by venepuncture from consecutively selected and consenting participants by the attending nurses. Serum was separated from each

clotted blood sample by centrifugation at 3,000 rpm for 15 minutes. The serum was then collected and stored in appropriately labeled cryovials at −20°C until tested.

Serology

The sera were screened for IgG and IgM antibodies against CHIKV antigens using CHIKjj *Detect*TM ELISA kits (InBios, Seattle, WA, USA) according to the manufacturer's instructions. Results obtained were interpreted according to guidelines contained in the kit protocol. The IgM ELISA has 100% sensitivity and specificity (95% confidence interval: 88–100%) according to a CDC evaluation (Johnson et al., 2016).

Data analysis

The results of the study were presented with descriptive statistics: mean and proportions ± 95% confidence interval (CI). Inferential statistics such as t-test, ANOVA, chi-square tests, and binary logistic regression were used as appropriate 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), was used for the analyses, and p values ≤ 0.05 were considered statistically significant in a two-tailed hypothesis.

Results

Demographic/clinical profile of participants

The study participants were majorly from Osun State (n = 256, 84.2%) and their age ranged from 10 to 78 years (yrs) (mean: 31.3 ± 1.6 yrs). They were categorized into three age-groups (Table 1) and were significantly different (p = 0.001) in mean age. There were 199 females (11–78 yrs) and 105 males (10–75 yrs) with the two genders being comparable (p = 0.99) in mean age. Participants with tertiary education were 159 (16–78 yrs), 111 had secondary education (11–70 yrs), while those with primary education were 28 in number (10–63 yrs) and six had no formal education (42–62 yrs). Only those with tertiary and primary education were similar (p = 0.35) in mean age. Married participants were 184 (16–78 yrs), while singles were 120 (10–41 yrs); 25 of the married participants were pregnant (21–42 yrs, mean: 28.64 ± 1.95 yrs). Information regarding knowledge of CHIKV and its mode of spread is shown in Table 1. Out of the 282 participants that had never heard of CHIKV, 99 and 155 had secondary and tertiary education, respectively. It was observed that participants with general body pains were significantly older (p = 0.03) in age.

Clinically, 155 and 160 of the participants reported having headaches and general body pains, respectively (Table 1), while 58 had skin rash. The mean body temperature (BT) of some participants (n = 274) was measured and it ranged from 34.0–40.0°C (mean: 37.7 ± 0.09°C). Of these, 185 had fever/"febrile illness" (BT = 37.5–40.0°C; mean: 38.1 ± 0.07°C), while the remaining 89 had mean BT of 36.9 ± 0.23°C (range 34.0–37.4°C). The group with "febrile illness" had significantly higher (p = 0.001) BT. Except for differences in proportions of "yes" and "no" for headache (p = 0.73) and general body pains (p = 0.36), comparisons of other proportions showed significant differences (Table 1).

Table 1. Demographic and clinical profiles of study participants.

Variable	No. of participants	Mean ± 95% CI	Proportion (%) ± 95% CI	*p* value
Gender				
Male	105		34.5 ± 5.3	0.0001
Female	199		65.5 ± 5.4	
Age (years)				
10–17	29	16.52 ± 0.28	9.5 ± 3.3	0.001
18–59	253	30.16 ± 1.27	83.2 ± 4.2	
60–78	22	64.95 ± 2.28	7.2 ± 2.9	
Location				
Lagos	48		15.8 ± 4.7	0.001
Osun	256		84.2 ± 4.1	
Educational status				
Illiterate	6		2.0 ± 1.6	0.001
Primary	28		9.2 ± 3.2	
Secondary	111		36.5 ± 5.4	
Tertiary	159		52.3 ± 5.6	
Marital status				
Single	120		39.5 ± 5.5	0.001
Married	184		60.5 ± 5.5	
Ever heard of CHIKV?				
Yes	22		7.2 ± 2.9	0.0005
No	282		92.8 ± 2.9	
If "Yes", do you know it is transmitted by mosquitoes?				
Yes	4		18.2 ± 16.1	0.003
No	18		81.8 ± 16.1	
Presently having headache?				
Yes	155		51.0 ± 5.6	0.73
No	149		49.0 ± 5.6	
Presently having skin rash?				
Yes	58		19.1 ± 4.4	0.0005
No	246		80.9 ± 4.4	
Presently having general body pains?				
Yes	160		52.6 ± 5.6	0.36
No	144		47.4 ± 5.6	
Body temperature (°C)*				
≤ 37.4	89	36.9 ± 0.23	32.5 ± 5.6	0.0005
37.5–40.0	185	38.1 ± 0.07	67.5 ± 5.5	

* = Body temperature of only 274 participants was measured.

Serology

The 304 study participants were grouped as shown in Figure 2. Of the 132 tested for CHIKV IgG alone, 50 (37.9%) were positive, while 13 of 49 participants (26.5%) tested for CHIKV IgM alone were seropositive. Out of 123 participants tested for both CHIKV IgG and IgM, 11 were

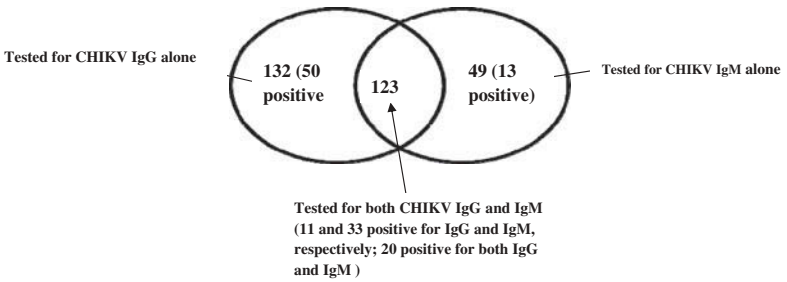

Figure 2. Grouping of the participants tested for CHIKV IgG and IgM with corresponding ELISA results.

positive for only IgG, 33 for only IgM and 20 for both IgG and IgM (dual positivity). Based on these results, the participants positive for at least one of the two anti-CHIKV antibodies were 127, giving a prevalence rate of 41.8% (127/304). Overall, 255 participants were screened for CHIKV IgG and 172 for CHIKV IgM (Figure 2). The corresponding seroprevalence rates for these groups were 31.8% (81/255) and 38.4% (66/172).

Out of a subset of the participants (n = 123) tested for CHIKV IgG and IgM (Figure 2, Table 2), 20 were positive for both antibodies giving dual anti-CHIKV antibody prevalence rate of 16.3%. Most (17) of these 20 participants were adults (17.0–50.0 yrs, mean: 27.6 ± 4.6 yrs), and 11 were males.

Eighteen of them had a minimum of secondary school education, and 12 were married. Ten of these 20 participants were febrile (37.5–39.0°C, mean: 38.3 ± 0.42°C), while 7, 7 and 13 of them had headache, skin rash and general body pains, respectively. In addition, 16 of them had never heard of CHIKV nor did they know that it was transmitted by infected mosquitoes. Regarding the dual anti-CHIKV seropositivity, other group-specific prevalence rates are shown in Table 2. Male participants had significantly higher ($p = 0.03$) dual positivity compared to the females.

Regarding anti-CHIKV IgG seropositivity, the prevalence rate for female participants was higher (though not significantly [$p = 0.62$]) than that of males, while for anti-CHIKV IgM seropositivity, the males had significantly higher ($p = 0.001$) prevalence rate. Other group-specific CHIKV IgG and IgM prevalence rates are shown in Table 3.

Table 2. Dual anti-CHIKV antibody (IgG and IgM) positivity among participants.

Variable	No. tested	No. positive (% ± 95% CI)	Odds ratio	*P* value
Gender				
Male	42	11 (26.2 ± 13.3)	2.8	0.03
Female	81	9 (11.1 ± 6.8)		
Age (years)				
10–17	11	3 (27.3 ± 26.3)	1.9	0.37
18–59	103	17 (16.5 ± 7.2)		
60–78	9	0		
Location				
Lagos	22	2 (9.1 ± 12)	0.46	0.32
Osun	101	18 (17.8 ± 10.4)		
Educational status				
Illiterate	1	0	1.2	0.84
Primary	11	2 (18.2 ± 22.8)	1.1	0.85
Secondary	41	7 (17.1 ± 11.5)		
Tertiary	70	11 (15.7 ± 8.5)		
Marital status				
Single	48	8 (16.7 ± 10.6)	1.1	0.92
Married	75	12 (16.0 ± 8.3)		
Presently having headache?				
Yes	60	7 (11.7 ± 8.2)	2.0	0.18
No	63	13 (20.6 ± 10.0)		
Presently having skin rash?				
Yes	26	7 (26.9 ± 17.0)	2.4	0.10
No	97	13 (13.4 ± 6.8)		
Presently having general body pains				
Yes	70	13 (18.6 ± 9.1)	1.5	0.44
No	53	7 (13.2 ± 9.1)		
Body temperature (°C)				0.94
≤ 37.4	36	6 (16.7 ± 12.2)	1.0	
37.5–40.0	87	14 (16.1 ± 7.7)		

Table 3. Single anti-CHIKV antibody (IgG or IgM) positivity among the study participants.

	Anti-CHIKV IgG				Anti-CHIKV IgM			
Variables	No. tested	No. positive (% ± 95% CI)	Odds ratio	*P* Value	No. tested	No. positive (% ± 95% CI)	Odds ratio	*P* value
Gender								
Male	81	24 (29.6 ± 9.9)	1.2 (0.6–2.1)	0.62	66	36 (54.5 ± 12.0)	3.0	0.001
Female	174	57 (32.8 ± 7.0)			106	30 (28.3 ± 8.6)		
Age (years)								
10–17	24	10 (41.7 ± 19.8)	1.6 (0.7 – 3.9)	0.20	16	7 (43.8 ± 24.4)	2.1	0.39
18–59	211	64 (30.3 ± 6.2)	1.2 (0.5 – 3.2)	0.67	145	56 (38.6 ± 7.9)	1.7	0.46
60–78	20	7 (35.0 ± 20.9)			11	3 (27.3 ± 26.3)		
Location								
Lagos	42	12 (28.6 ± 13.7)	0.83 (0.4–1.7)	0.63	28	11 (39.3 ± 18.1)	1.0 (0.5–2.4)	0.91
Osun	213	69 (32.4 ± 6.3)			144	55 (38.2 ± 7.9)		
Educational status								
Illiterate	5	1 (20.0 ± 35.1)		0.66	2	0		0.18
Primary	20	6 (30.0 ± 20.1)	1.7 (0.2–18.7)	0.77	19	10 (52.6 ± 22.4)	2.0 (0.7–5.4)	0.69
Secondary	93	24 (25.8 ± 8.9)	2.2 (0.3–21.1)	0.46	59	23 (39.0 ± 12.5)	1.1 (0.6–2.2)	
Tertiary	137	50 36.5 ± 8.1)			92	33 (35.9 ± 9.8)		
Marital status								
Single	100	30 (30.0 ± 9.0)	1.1 (0.7–2.0)	0.63	68	22 (32.4 ± 11.2)		
Married	155	51 (32.9 ± 7.4)			104	44 (42.3 ± 9.5)	1.5 (0.8–2.9)	0.19
Ever heard of CHIKV?								
Yes	60	18 (30.0 ± 11.6)	1.1 (0.6–2.1	0.74	12	7 (58.3 ± 27.9)	2.5 (0.7–8.1)	0.13
No	195	63 (32.3 ± 6.6)			132	48 (36.4 ± 8.2)		
If "Yes", do you know it is transmitted by mosquitoes?								
Yes	4	1 (25.0 ± 42.4)	Invalid	Invalid	3	1 (33.3 ± 53.3)		
No	14	5 (35.7 ± 25.1)			9	6 (66.7 ± 30.8)	Invalid	Invalid
Presently having headache?								
Yes	130	42 (32.3 ± 8.0)	1.1 (0.6–1.8)	0.85	85	34 (40.0 ± 10.4)	1.1 (0.6–2.1)	0.66
No	125	39 (31.2 ± 8.1)			87	32 (36.8 ± 10.2)		
Presently having skin rash?								
Yes	51	19 (37.3 ± 13.3)	1.4 (0.7–2.6)	0.35	33	17 (51.5 ± 17.0)	2.0 (0.9–4.2)	0.08
No	204	62 (30.4 ± 6.3)			139	49 (35.3 ± 8.0)		
Presently having general body pains?								
Yes	137	50 (36.5 ± 8.1)	1.6 (0.9–2.8)	0.08	93	38 (40.9 ± 10.0)	1.3 (0.7–2.3)	0.47
No	118	31 (26.3 ± 8.0)			79	28 (35.4 ± 10.5)		
Body temperature (°C)								
≤ 37.4	72	22 (30.6 ± 10.7)	1.1 (0.6–2.0)	0.80	53	19 (35.8 ± 12.9)		0.84
37.5–40.0	183	59 (32.2 ± 6.7)			104	39 (37.5 ± 9.3)	1.1 (0.5–2.1)	

It was observed that 185 participants had fever (temperature ≥ 37.5°C), of which 104 reported general body pains but 81 had no body pains. Of the former, 36.3% (33/91) tested positive for CHIKV IgG, while 36.1% (22/61) were positive for CHIKV IgM. Among the latter (81 febrile participants without general body pains), 23.9% (16/67) and 39.5% (17/43) were positive for CHIKV IgG and IgM, respectively. These results indicated that some of the febrile participants with or without general body pains were positive for either CHIKV IgG or IgM. It is also noteworthy that more of the participants positive for either IgG or IgM had fever (72.8% and 67.2%) and general body pains (61.7% and 57.6%), respectively (Table 3).

Anti-CHIKV seropositivity of pregnant women

Twenty-five pregnant women were involved in this study, but only 22 were tested for CHIKV IgG. Of this, five (22.7%) were positive. Out of the 15 screened for CHIKV IgM, three (20.0%) were positive, while only one (8.3%) of the 12 tested for both CHIKV IgG and IgM had dual antibody positivity. Thirteen of the pregnant women had fever (37.6–38.2°C, mean: 37.9 ± 0.1°C). Of these, one had CHIKV IgG, while one of the six febrile pregnant women tested for CHIKV IgM was positive. Only one of the pregnant women had ever heard of CHIKV and knew that it was transmitted by mosquitoes.

Discussion

This study was carried out to investigate exposure of humans in Lagos and Osun states, Nigeria to CHIKV and to identify any associated risk factors. Hospital attendees were studied in order to increase the likelihood of detecting exposure of humans to the virus since individuals with febrile illness and or general body pains (especially headaches, joint and muscle pains) are more likely to seek healthcare. A similar hospital-based study revealed CHIKV infection among adults having neurologic manifestations in Guayaquil, Ecuador (Acevedo et al., 2017).

Analysis of demographic profile of the participants revealed that the proportion of females was significantly higher than that of males, a finding that is consistent with previous reports that women and girls exhibit greater health-seeking behaviors (Uneke et al., 2005; Renault et al., 2007). It was observed that the three age groups significantly varied in mean age with those in the 18–59 yr age bracket having the largest proportion which was significantly higher than either of the other two age groups. A plausible reason for this is the fact that these 18–59-yr-old participants constitute the active work-force which stays more outdoors where they engage in various occupations or leisure activities. Results of analysis of data obtained on the educational status of the participants with a view to assessing their level of awareness regarding CHIK fever revealed that majority of them had tertiary education. However, about 93% of the participants reported that they had never heard of CHIKV, while about 82% of those that had heard of the virus before the study never knew that it was spread by mosquitoes. These observations generally showed that the study participants had very low level of enlightenment about CHIKV or its mode of spread.

The overall anti-CHIKV antibody (i.e., CHIKV IgM and IgG positivity) prevalence rate of 41.8% was considerably high compared to previous seroprevalence rates of

14.3% and 11.0% obtained for the disease in Nigeria using hemagglutination inhibition and rapid diagnostic tests, respectively (Adesina & Odelola, 1991; Ayorinde et al., 2016) although Baba et al. (2013) reported a higher seroprevalence of 50.0% using plaque reduction neutralization test. The disparity in the seroprevalence rates obtained may be attributed to differences in sensitivity of the various tests used. However, despite the existence of cross-reactivity between CHIKV and other alphaviruses in the Semliki Forest antigenic complex such as O'nyong-nyong and Semliki Forest viruses (Blackburn et al., 1995; Tappe et al., 2014), this ELISA-based study has revealed evidence of human exposure to CHIKV in Lagos and Osun states of Nigeria.

The detection of dual positivity (i.e., CHIKV IgG and IgM) in 20 (16.3%) of the participants indicates that these individuals were actually exposed to the virus through the bites of infected mosquitoes. Although the male participants were smaller in number than their female counterparts, they recorded significantly higher dual seropositivity (Table 2) with about three times higher likelihood of being so. This finding might be due to the fact that males are generally more likely to be outdoors than females in the study locations and other parts of southwest Nigeria. Thus, only gender had significant association with dual seropositivity among the 123 individuals tested. Additionally, the observation that 17 of the 20 participants with dual CHIKV antibody positivity were adults with 18 of them having a minimum of secondary school education further establishes the possibility of exposure to the virus as this group was more likely to be outside during the day for work-related activities that could have exposed them to the day-biting mosquito vectors of the disease. Ten of these 20 individuals also had fever, while thirteen reported general body pains which are suggestive of CHIKV disease (Chopra et al., 2008). These findings corroborate the reports of Panning et al. (2008) and Kam et al. (2012a, 2012b) that CHIKV IgG and IgM are produced during acute infection.

With respect to single CHIKV antibody positivity, overall IgG and IgM prevalence rates of 31.8% and 38.4%, respectively, were observed. These rates might be considered high in our environment where recent report on CHIKV is sparse. The detection of CHIKV IgG suggests long-time exposure and protection, while the presence of IgM suggests primary or ongoing infection as at the time of sampling (Mond et al., 1995; Kam et al., 2012a, 2012b). Although the female participants tested for CHIKV IgG were more than twice the number of males, there was no significant difference in their CHIKV IgG prevalence rates, thereby indicating comparable exposure of both genders to the virus. On the contrary, however, the smaller male population had significantly higher CHIKV IgM prevalence rate (Table 3). Possible reasons for this observation are not readily discernible. Further, while no significant differences in CHIKV IgG and IgM seropositivity were observed with regard to age, most of the individuals positive for both antibodies were in the 18–59 years age group (Table 3). Hence, since this group represents the active work-force of any community/country, it can be inferred that CHIKV-mediated illness/polyarthritis may lead to low productivity as previously reported (Ahmed et al., 2015; Long & Heise, 2015). Also, while no other variable had significant association with either CHIKV IgG or IgM, it is noteworthy that most of the participants positive for either antibody had minimum of secondary school education although majority of them were still ignorant of CHIKV and its mode of spread (Table 3). Additionally, more of the seropositive (CHIKV IgG or IgM) participants were married, febrile and had general body pains (Table 3).

The observation that some of the participants with normal body temperature and those who responded "no" to having headache and skin rash were positive for either antibody shows that CHIKV-infected persons can be asymptomatic, thus constituting a potential source of infection to susceptible humans, especially if they are IgM-positive (Table 3). Additionally, this study has shown that some of the febrile participants with or without general body pains were positive for both CHIKV IgG and IgM. Since this category of patients is rarely tested for flaviviral or alphaviral infections in Nigeria, we suggest inclusion of CHIKV infection in differential diagnosis of febrile conditions, with or without general body pains, in Nigeria. Moreover, since CHIKV reportedly causes encephalitis (Chandak et al., 2009; Nelson et al., 2014; Acevedo et al., 2017), it should also be considered in the diagnosis of neurologic diseases (encephalitis).

The detection of anti-CHIKV IgG in five (22.7%) of the 22 pregnant women involved in this study is an indication of prior natural exposure to the virus since there is currently no vaccine against the disease (WHO, 2016). Moreover, the finding that three of the pregnant women tested had CHIKV IgM coupled with the fact that one of them had fever suggests ongoing or recent infection with the virus and possibility of vertical transmission. It has been reported that vertical transmission of CHIKV occurs if a pregnant woman is infected shortly before birth (Ramful et al., 2007; Gerardin et al., 2014) and that newborns delivered to pregnant women with acute CHIKV infection shortly before birth eventually developed CHIKV-mediated nervous infection (Fritel et al., 2010). Since information regarding age of pregnancy was not obtained from these women (a limitation of the study), we are unable to make any clear statement on the possibility of *in utero* infection of babies that would have been delivered by these CHIKV IgM-positive pregnant women.

Conclusion

This ELISA-based preliminary assessment revealed that hospital attendees in the study locations in Lagos and Osun states had anti-CHIKV antibodies and that the seroprevalence rates were relatively high. Hospital location and gender were the only factors associated with CHIKV IgG and IgM seropositivity, respectively. The participants had very low level of enlightenment about CHIKV and its mode of transmission, while the asymptomatic CHIKV IgM-positive participants were most likely infectious to mosquitoes and, by extension, to susceptible individuals. Additionally, few pregnant women had serologic evidence of ongoing CHIKV infection. Though this study has revealed exposure of some hospital attendees to CHIKV, the true burden of the disease in the study area remains unknown as the country is endemic for other arboviruses such as dengue, West Nile, yellow fever and O'nyong-nyong viruses which were not screened for in this study. We recommend inclusion of CHIKV in differential diagnosis of febrile conditions with or without general body pains and neurologic disease in Nigeria.

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Ethics approval

The study was approved by the Human Research Ethics Committee, College of Health Sciences, Osun State University, Osogbo. The questionnaire forms used to obtain demographic and clinical data were endorsed by participants as a mark of consent to participate in the study following explanation of the details to each participant by selected healthcare personnel in each hospital.

Declaration of interest

The authors report no conflicts of interest.

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