

Detection of Chikungunya Virus among Febrile Patients in Lagos

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Abstract: Chikungunya virus (CHIKV) is an alphavirus transmitted by culicine mosquitoes and causes Chikungunya fever, a febrile illness usually with concomitant rash and arthralgia. Having spread to over 40 countries worldwide and classified as a class B priority pathogen, CHIKV is an important threat to public health but has poor surveillance as with other viral haemorrhagic fevers (VHF) in Nigeria. The epidemiology of CHIKV in Nigeria is vague, thus, this study was designed to determine the current prevalence of CHIKV infection among febrile patients in Lagos. This was a cross-sectional study in which samples were collected from April to July 2018 at two General hospitals in Lagos. A total of 130 blood samples of febrile patients aged 1-60 years including 56 (43.1%) males and 74 (56.9%) females were collected and RNA was extracted from the serum samples. The extracted RNA was amplified using one-step RT-PCR with the specific CHIKV primers. The resulting amplicons (427bp) were run on 2% agarose gel and viewed on gel imager for the presence of CHIKV. A total of 9 out of 130 (6.9%) serum samples were positive for CHIKV RNA. The positive samples consist of 4 out of 56 (7.1%) males and 5 of 74 (6.8%) females ($P>0.05$). The age group 11-20 years had the highest prevalence of 14.3% whereas age groups 41-50 years and 51-60 years were both CHIKV RNA negative. This study shows a plausible circulation of CHIKV among febrile patients in Lagos with a prevalence of 6.9%. It also revealed that CHIKV, which is not generally suspected or diagnosed by physicians, may have contributed to the burden of febrile cases in Lagos.

Keywords: Chikungunya Virus, Viral Hemorrhagic Fever, Prevalence, Febrile

INTRODUCTION

Chikungunya virus (CHIKV) is a positive sense single-stranded RNA virus of the *Togaviridae* family with a capsid diameter of 60-70nm and a phospholipid envelop (WHO, 2009). There are four distinct CHIKV genotypes to date. The West African and the East-Central-South African (ECSA) genotypes are mostly enzootic in Africa and are located at the basal position in the phylogeny of CHIKV (Rodrigues *et al.*, 2016); the Asian genotype is mainly found in Southeast Asia whereas the latest Indian Ocean lineage spread from the Comoros Islands in 2004 and caused a large outbreak in India and Southeast Asia in 2005-2008 (Volk *et al.*, 2010; Rodrigues *et al.*, 2016).

The viral genome which is approximately 12 kb consists of two open reading frames and encodes two polyproteins that are cleaved by viral and cellular proteases into five structural proteins (C, E3, E2, 6K, and E1) and four non-structural proteins (n1, P2, P3, and P4) (Powers *et al.*, 2001; Khan *et al.*, 2002; Issac *et al.*, 2014). Experiments with animal cells show that CHIKV primarily targets fibroblast cells and may also gain entry into monocytes and kupffer cells of the liver (Voss *et al.*, 2010). The structural glycoproteins E1 and E2 play important roles in viral replication (Deeba *et al.*, 2016). Whereas the E1 is responsible for membrane fusion, the E2 is essential for the virus entry into the cell through endocytosis (Wahid *et al.*, 2017).

Chikungunya virus is transmitted by culicine mosquitoes (*Aedes aegypti*, *Aedes albopictus* and *Aedes polynesiensis*) and causes Chikungunya fever, a febrile illness usually presenting with high fever, headache, myalgia, arthralgia, polyarthralgia, hemorrhage, and rash (Reiter *et al.*, 2006; Lo Presti *et al.*, 2014; Wahid *et al.*, 2017).

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Having spread to over 60 countries worldwide (WHO, 2017) and classified as a class B priority pathogen (NIAID, 2018), CHIKV is an important threat to public health but has poor surveillance as with other viral haemorrhagic fevers (VHF) in Nigeria. The pattern of spread of Chikungunya across the world is rather unique, having the potential to emerge and re-emerge, considerably infecting a population and then remaining undetected for years (Deeba *et al.*, 2016). The first outbreak of CHIKV epidemic in Nigeria occurred in 1969 (Moore *et al.*, 1974). After a period of quiescence, numerous African countries have faced re-emergence of this viral infection resulting in outbreaks in Senegal, Democratic Republic of Congo, Kenya, Gabon, Cameroon, and Republic of Congo between 1996 and 2011 (Thonnon *et al.*, 1999; Pastorino *et al.*, 2004; Pialoux *et al.*, 2007; Gould *et al.*, 2008; Kelvin, 2011; Hertz *et al.*, 2012; Caron *et al.*, 2012; Bacci *et al.*, 2015). A confirmed case of CHIKV may be either epidemiological or laboratory confirmation through virus isolation, RT-PCR, or ELISA (Rodrigues *et al.*, 2016). It is difficult to detect and differentiate an acute infection and a recent past infection as the serum IgM and IgG are not really sensitive (4-22%) for CHIKV acute infection, but become very sensitive (reaching 80%) after one week (Dash *et al.*, 2011; Sanyaolu *et al.*, 2016). However, Polymerase Chain Reaction (RT-PCR) can detect the viral RNA during the acute phase of CHIKV infection with high viral counts generally lasting 4-6 days after the onset of illness, thereby making RT-PCR a very useful diagnosis within the first 7 days (WHO, 2015; Sanyaolu *et al.*, 2016). A number of broad-spectrum antiviral drugs including ribavirin used in combination with interferon-alpha (IFN- α), have shown potent inhibitory effects on *in vitro* replication of CHIKV (Briolant *et al.*, 2004). However, the effects of these drugs have not been characterized using *in vivo* models of CHIKV infection (Tharmarajah *et al.*, 2017).

Currently, there is no licensed vaccine against CHIKV, though several vaccine platforms are being developed, including chimeric, live attenuated, DNA, subunit and VLPs (Ahola *et al.*, 2015).

CHIKV infection has similar clinical presentations with malaria which is endemic in Nigeria, and often result in clinically indistinguishable febrile syndromes. Current estimates suggest that more than 70-94% of febrile illnesses are likely to be misdiagnosed and treated as malaria in Nigeria (Ayukekong, 2014; Oyibo *et al.*, 2016; Nwokolo *et al.*, 2017). These underscore the desperate need for proper understanding and documentation of the epidemiology of CHIKV and other viral infections presenting with febrile illnesses. Therefore, the aim of this study was to determine the prevalence of CHIKV infection among febrile patients in Lagos, Nigeria.

MATERIALS AND METHODS

Study Area and Patient Population

This cross-sectional study was conducted in Alimosho and Orile-Agege local government areas of Lagos state, Nigeria. Whereas Orile-Agege (6°37'19"N 3°19'33"E) is a multi-ethnic community of 650,000 people in which the Yorubas are predominant with the presence of a sparse population of non-Yoruba speaking people, Alimosho (6°36'38"N 3°17'45"E) is the largest local government in Lagos state with above 1.2 million inhabitants as at 2006, mostly of Yoruba ethnic group (Lagos State Government Abstract of Local Government Statistics, 2016). The samples were collected from patients presenting with fever (>37.5°C) at the pediatrics, general medicine and gynecology units of Alimosho and Orile-Agege General Hospitals. These hospitals are the major public hospitals in these communities and open to everyone. Accessibility and availability of resident physicians were major requirements used for the selection of these hospitals.

Ethical Approval

The ethical approval for this research was obtained from the Institutional Review Board of Nigerian Institute of Medical Research (IRB /18/009) and the Lagos State Government Health Service Commission (LSHSC/2222/VOL.IVB/283). Procedures were followed in accordance with the ethical standards of these committees and with the Helsinki Declaration of 1975, revised in 2000. All results were delinked from patient identifiers and anonymized.

Eligibility/Exclusion Criteria

The study excluded children less than 1-year-old and those patients who were unable or unwilling to give written consent.

Inclusion Criteria, Sample Collection and Processing

Informed consent was obtained from participants. Further criteria for selection included age above 1-year-old with symptoms of fever ($>37.5^{\circ}\text{C}$) as well as with more than or equal to one of the following symptoms: headache, myalgia, arthralgia, hemorrhage, and rash. Five millilitres of

whole blood samples were collected from one hundred and thirty patients who met our study criteria, over a period of 5 months from April to August 2018 using EDTA-containing bottles. Plasma was separated from each sample in the field, stored at -20°C and transported in a cold chain to the Department of Microbiology, the University of Lagos where they were stored until analyzed.

PCR amplification and Detection

Viral RNA was extracted from samples using E.Z.N.A (Omega Bio-tek, Inc. USA) viral RNA kit following manufacturer's instructions. Chikungunya virus E2 gene was amplified using reverse transcription polymerase chain reaction (RT-PCR) as previously described in Pfeffer *et al.* (2002). Briefly, reverse transcription was done using genus specific primer cChk-4 while both primers listed in Table 1 were used for the subsequent PCR producing amplicons of approximately 472 bp. The target region was detected using agarose gel electrophoresis.

Table 1: Oligonucleotide primers used for the detection of Chikungunya

Name	Orientation	Location	Sequence (5'-3')	Reference
Chik-1	Forward	3746-3765	TAATGCTGAACTCGGGGACC	Pfeffer <i>et al.</i> , 2002
cChik-4	Reverse	3962-3983	ACCTGCCACACCCACCATCGAC	Pfeffer <i>et al.</i> , 2002

Statistical Analysis

The statistical analyses were performed using SPSS version 20. Categorical variables were compared using chi-square tests as applicable. Significance was set at $P < 0.05$.

RESULTS

A total of one hundred and thirty (130) consecutive outpatients with fever comprising 4 (56.9%) female and 56 (43.1%) male participants enrolled in this study. Nine (6.9%) of the 130 serum samples were positive for CHIKV RNA (Figure 1). The

positive samples comprise 4 (7.1%) of male patients while 5 (6.8%) was recorded in female ($P > 0.05$) as shown in Table 2. The age range of participants was 1-60 years with a mean age of 13.31 years. Out of the total 9 positive samples, 5 (5.7%) were found in the age group of 1-10 years while the remaining 4 positives were distributed within age group 11-40 years. However, age groups 41-50 years and 51-60 years were all CHIKV RNA negative by RT-PCR (Figure 2)

Table 2: Sex distribution of the Chikungunya virus prevalence

Sex	Negative (%)	Positive (%)	Total (%)
Male	52 (40.0)	4 (7.1)	56 (43.1)
Female	69 (53.1)	5 (6.8)	74 (56.9)
Total	121 (93.1)	9 (6.9)	130 (100.0)

 $\chi^2 = 3.84$

P > 0.05

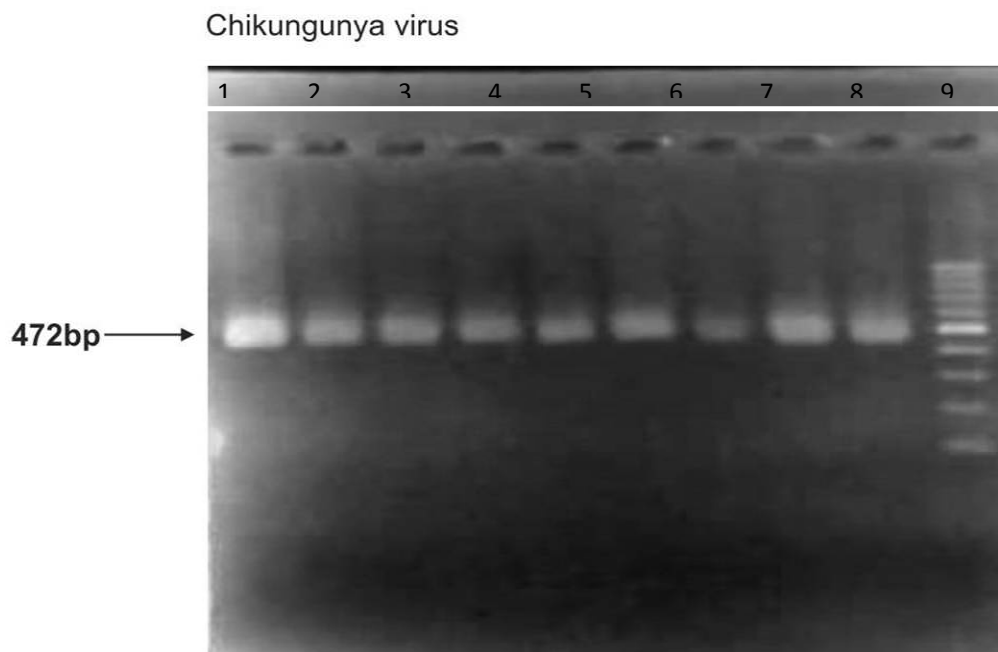


Figure 1: Agarose gel photograph of Chikungunya virus E2 gene. Expected base pair size shown is 472 bp. Wells 1-8 represents positive samples; Well 9 represent 100bp ladder

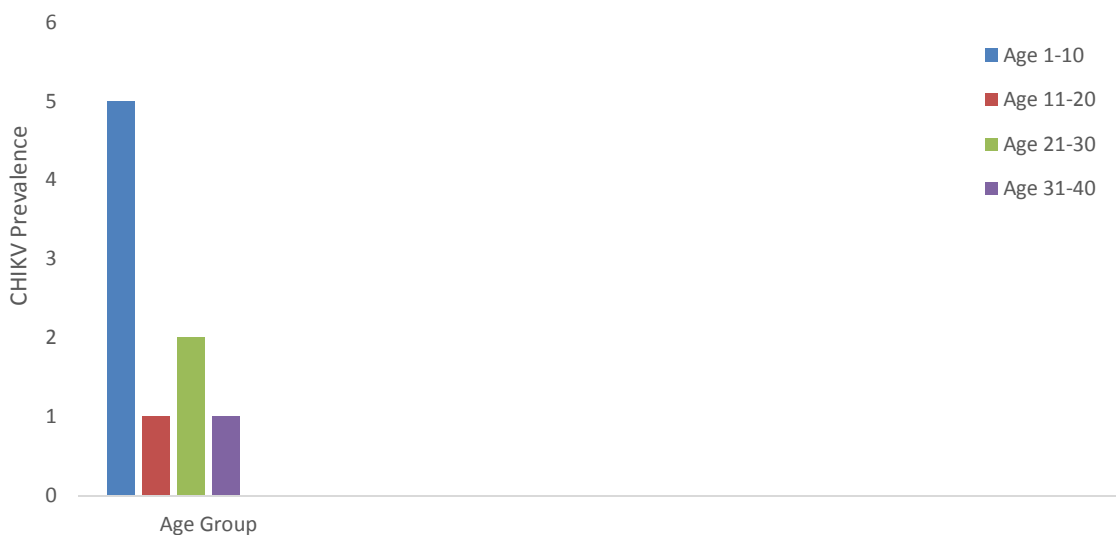


Figure 2: Distribution of CHIKV infection among the age groups

DISCUSSION

In malaria-endemic regions like Nigeria, febrile illnesses are most likely to be misdiagnosed and treated as malaria (Ayukekbong, 2014; Oyibo *et al.*, 2016; Nwokolo *et al.*, 2017). CHIKV is one of the viral aetiologies of fever that has been neglected by researchers over the years. Since the first report of the 1969 outbreak by Moore *et al.* (1974), there had been a paucity of information on the epidemiology of CHIKV in Nigeria until a few recent studies appeared. This may either be due to the neglect of the pathogen by researchers or the characteristic quiescence of the virus. It has been established that CHIKV has the potential to emerge and re-emerge, considerably infecting a population and then remaining undetected for years (Deeba *et al.*, 2016).

We detected CHIKV RNA in 9 (6.9%) of the febrile patients implying current circulation of CHIKV in the urban city of Lagos, Nigeria. This is quite significant, considering that Adesina *et al.* (2017) has also recently reported a CHIKV RNA prevalence of 1.8% in a study that investigated the involvement of four arboviruses in febrile conditions of patients in Ile-Ife, Nigeria. This indicates that CHIKV has been circulating in the Nigerian populace, however, the disparity may be attributed to the differences in study sites and sampling period. Another study that aimed to determine the seroprevalence and prevalence of acute infections of Dengue and CHIKV among participants presenting with malaria-like symptoms in Tanzania, reported a 4.2% acute cases CHIKV by RT-PCR (Kajeguka *et al.*, 2016). Our report shows a significantly higher prevalence of acute CHIKV infection than the recent contemporaneous systematic review and meta-analysis in which the crude overall CHIKV RNA prevalence has been demonstrated to be 2.8% in Africa (Simo *et al.*, 2019).

Our study is somewhat different from some CHIKV studies in Nigeria and Africa which

have investigated and reported seroprevalence of antibodies against the virus. Evidence however shows that it is difficult to detect and differentiate an acute infection and a recent past infection as the serum IgM and IgG are not detectable early enough for CHIKV acute infection, but become very sensitive (reaching 80%) after one week (Dash *et al.*, 2011; Sanyaolu *et al.*, 2016). In a recent study of the prevalence of CHIKV among febrile patients in Maiduguri, Akinola *et al.* (2017) reported the presence of antibodies in 10.7% of participants (n=370), however, 6.5% tested positive to IgM only. Kajeguka *et al.* (2016) also identified IgM and IgG seroprevalence of 12.6% and 5.6% respectively. However, the prevalence of CHIKV IgM and IgG in Africa has been shown by meta-analysis to be 9.7% and 16.4% respectively (Simo *et al.*, 2019).

There was no significant difference between the prevalence of CHIKV infection among males and females in our study ($\chi^2 = 3.84$; $p > 0.05$), although the rate of infection among males, 4 (7.1%), was slightly higher than females, 5 (6.8%). Our findings also show that out of the 9 CHIKV RNA positive samples, 5 were children within the age range of 1-10 years whereas 2 were found in the age group 21-30 years and 1 each was from the age groups 11-20 years and 31-40 years. This is in some way different from the distribution observed in a similar study in which CHIKV RNA was distributed among the age groups 11-20 years, 21-30 years, and 51-60 years (Adesina *et al.*, 2017). This could be as a result of differences in the social behavior of people living in rural areas and urban cities in Nigeria. Kajeguka *et al.* (2016) also reported no difference between the prevalence of CHIKV IgM antibodies among children and adults, but a significantly higher IgG positivity among the adults (5.9%) compared to children (3.0%) in Tanzania. This study has shown evidence of CHIKV circulation in Lagos using RT-PCR which is

also reputed with the ability to detect the viral RNA during the acute phase of infection, thereby making it a very useful diagnosis within the first 7 days (WHO, 2017; Sanyaolu *et al.*, 2016). CHIKV infection may have contributed alongside other viral aetiologies of fever, to the wrong diagnosis and treatment of many febrile cases in Lagos which are generally treated as malaria and or typhoid fever. CHIKV

should, therefore, be included in laboratory investigations of patients with febrile illness. However, we are aware that this study was limited in its inability to confirm acute CHIKV infection among the febrile patients and sequencing to know which lineage of CHIKV was detected. Subsequent studies would include ELISA IgM and IgG.

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