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Chikungunya Virus Infections Unmasked from Suspected Malaria Febrile Patients in Northeastern Nigeria

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Abstract **Background**: Chikungunya is an emerging global threat because of the unprecedented magnitude of its spread and highly debilitating disease. **Objective:** This study was informed by the need to use serological methods to unmask the chikungunya virus (CHIKV) from suspected malaria patients. **Methodology:** The ELISA IgM and $PRNT_{90}$ (nAb) were used to assess the presence of CHIKV antibodies. **Findings:** Of 530 patients, 129 (24.3%) had CHIKV IgM, 199 (37.5%) neutralizing antibody (nAb) and 23 (4.3%) IgM + nAb. Only 4.3% of the patients and 43.9% of the IgM negatives had CHIKV nAb indicating acute and past infections respectively. Of 200 patients from Adamawa State, 6.5%, 42.5%, and 3.5% were CHIKV IgM, nAb, and IgM + nAb respectively. In Borno State, 47.0% were IgM, 49.0% nAb and 4.5% were IgM+ nAb . Of 130 samples from Bauchi State, 72.3% had IgM, 16 (12.3%) nAb, and 5.4% IgM + nAb. CHIKV infections in Bauchi and Borno were significantly higher than in Adamawa but residents of the three states are at risk. This study also detected co-infections between CHIKV and flaviviruses at varied degrees: dengue viruses (DENV) (17.7%), Zika virus (ZIKV) (1.5%, West Nile virus (WNV) (4.0%), and yellow fever virus (YFV) (1.3%). The type of settlement, gender, age, and yellow fever vaccination status of the patients were not significantly associated with CHIKV nAb. However, patients aged >60 years were more likely to have experienced CHIKV infections than younger age groups. CHIKV nAb and samples collected 1-7 days after the onset of symptoms were significantly different from those within 7-10 days. CHIKV nAb among recipients of anti-malaria anti/antibiotics treatments and those untreated were significantly associated. **Conclusion:** The persistent misdiagnosis of CHIKV infections poses a global public health threat in the phase of climate change if unchecked. Misuse of antibiotics/antimalaria could lead to antimicrobial resistance

Keywords: *Chikungunya virus, malaria, ELISA IgM, neutralizing antibody, febrile illness, Nigeria*

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Introduction

Chikungunya, an arboviral disease caused by the chikungunya virus (CHIKV), was first described in Tanzania in 1952 [1] and it later caused outbreaks in Asia and Africa. In 2004, CHIKV spread to India and several islands in the Indian Ocean, causing major outbreaks that affected more than 1 million people and has now spread to non-endemic regions including the Americas and Europe [2]. The word "chikungunya" was derived from the Tanzanian language 'Kimakonde' which means 'to become contorted' [1]. CHIKV is a single-stranded positive RNA virus that belongs to the *Alphavirus* genus and family *Togaviridae*. It is transmitted by Aedes in urban and sylvatic cycles [3,4]. The symptoms of CHIKV infections range from non-specific but malaria-like (fever, headache, muscle pain, joint swelling, or rash) to more debilitating musculoskeletal symptoms [4]. The atypical symptoms of malaria and arbovirus infections at the initial phase often facilitate misdiagnosis [5]. This situation is further worsened by the lack of access to appropriate diagnostic facilities for differential diagnosis of febrile illness and co-infections [6,7,8]. Interestingly, CHIKV was first reported in Nigeria in 1964 and most recent studies [8,9,10,11] in Nigeria used non-species-specific ELISA-based techniques according to previous reports [11,12]. IgM tests detect CHIKV with a 100% accuracy rate for samples taken more than 6 days of symptom onset

[6,7,13]. In the CDC algorithm, PRNT is required to confirm a positive IgM test in diagnosing CHIKV disease because of its high specificity [11,14,15]. This study aimed at using a more species-specific serological method to unmask CHIKV infections from suspected malaria patients.

Materials and Methods

Study Area

Nigeria is a West African country with a population of more than 200 million and covers an area of 923,769 square kilometers (356,669 square mi). North-eastern Nigeria is one of the six geopolitical zones and comprises Adamawa, Bauchi, Borno, Gombe, Taraba and Yobe states. The simple random sampling technique was used to select three health institutions: Federal Medical Centre (FMC), Adamawa state, Abubakar Tafawa Balewa University Teaching Hospital (ATBUTH), Bauchi State and Specialist Hospital, Borno State (Figure 1).

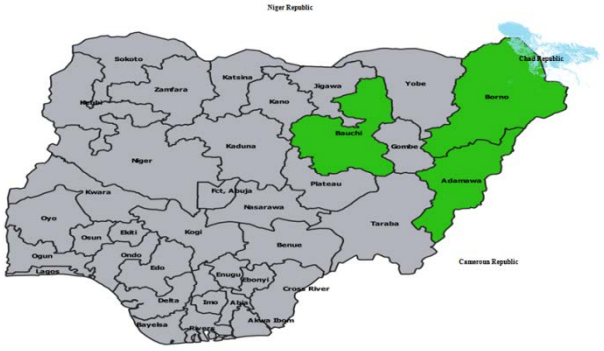

Figure 1. Map of Nigeria with indicated states and collection sites (green)

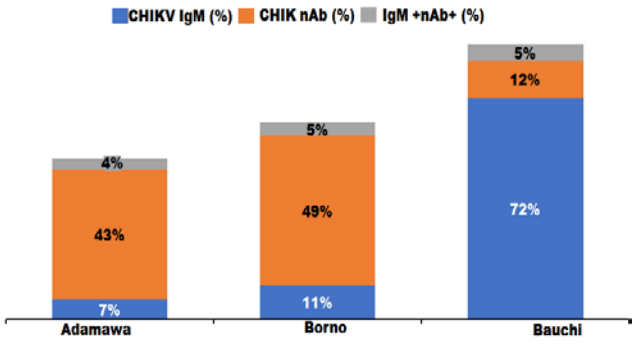

Figure 2. Distribution of Chikungunya virus antibodies in three northeastern states, Nigeria

Population and sample collection

A total of 538 malaria-suspected patients who visited the selected hospitals to request malaria tests were recruited. The socio-demographic characteristics of the study participants were collected using a structured questionnaire. The study was conducted between April and August 2018. The serum was collected into sterile cryovials and stored at -20°C until transported to the virology laboratory at, the University of Maiduguri Teaching Hospital (UMTH). Each of the 530 serum samples was tested for CHIKV, DENV, YFV, ZIKV and WNV by $PRNT_{90}$ while ELISA IgM was used for CHIKV only.

Qualitative CHIKV IgM ELISA (Diagnostic Automation/Cortez Diagnostics, Inc., Accu Diag TM Chikungunya IgM ELISA Kit USA, code: 8113-25, specificity of 100% and sensitivity of 90.9%). The assay was performed according to the manufacturer's instructions.

Plaque reduction neutralization test (PRNT)

The PRNT was performed as previously described for the Yellow fever virus (17). Each serum sample was inactivated at 56°C for 30 minutes and stored at -80 °C. Vero E6 cells were grown in Eagle's Minimum Essential Medium (EMEM) supplemented with 10% fetal bovine serum, 2 mM glutamine, 2% Penicillin/Streptomycin, 1 % HEPES and 2.5 % sodium bicarbonate. The cells were seeded in 24-well plates at a density of 1 x 10^5 cells/ well and incubated at 37°C for 24-48 hours or until the confluency reached 70-80%. Each serum sample was diluted 1:8 with in-house diluent (IHD) prepared in PBS containing Penicillin/Streptomycin (PS), Gentamycin and Fungizone supplements. PS preparation involved dissolving 1gm Penicillin G (1X 10^6 units) and 1 gm Streptomycin sulfate (10µg/ml) in 100 ml of PBS to obtain the stock PS. Then 10 ml of the stock PS was added to 35 ml of PBS. 500µl of Gentamycin (80mg/2ml) diluted 1/100 and 0.02% of Fungizone (2.5µg/ml) were added to the 35 ml of PBS to make the IHD. About 100µl CHIK virus stock (obtained from Molecular Virology Laboratory, International Centre for Genetic Engineering and Biotechnology- ICGEB, Trieste, Italy) at a concentration of 100 PFU/ml was added to 100µl of the diluted serum and incubated at 37 °C for 1 hour. Each serum dilution–virus mixture was prepared in duplicate and three controls including a virus dose control (100 PFU virus plus cells and diluent), a cell control (diluent and cells only), and serum control (serum plus diluent and cells) were tested simultaneously. Each serum was tested against CHIKV, zika virus, dengue viruses, West Nile virus, and yellow fever virus. After incubation at 37°C for 1 hour, the 50 µl virus-serum mixture was added to Vero cells and incubated for 1 hour at 37°C. The plates were rocked gently every 15 minutes for uniform distribution of the inoculum. Different concentrations of overlay medium (Carboxyl methylcellulose (CMC) salt (Low viscosity) (Sigma) was tested to determine the best consistency. The overlay medium consisted of an equal volume of 10%, 12%, and 14 %% CMC with EMEM supplemented with 4% fetal bovine serum (FBS) (the resultant concentration of the FBS became 2% while that of CMC was 5%, 6% and 7% respectively). The best consistency was 6%. 1 ml of the 6% CMC (overlay medium) was added to each of the 24 wells and incubated at 37°C for 3-7 days. After removing the overlay medium, 500µl of formaldehyde solution (37.0-40.0%) diluted in 1:10 with PBS was added to each well for 30 minutes and stained with 500 ul of 0.5% crystal violet (Sigma) for 20-30 minutes after removing the formaldehyde. The percentage of plaque reduction by the specific antibody in the serum was calculated using the formula: 100-(Number of plaques in sample/Number of plaques in control) x 100. The validity

of the test was determined by the virus control (cells + medium + virus) having a minimum of 50 plaques and cell control (Cell + medium) and serum control (Cells + serum + medium) with no plaques at all. The PRNT titer was defined as the reciprocal of the test serum dilution that reduced the number of plaques by 90% ($PRNT_{90}$) [17,18].

Statistical Analysis

The data was analyzed using Statistical Product and Service Solutions (SPSS), Version 25.0, from IBM SPSS. Age groups and occupations were compared using the Pearson $\chi 2$ or Fisher's exact test for categorical variables when the expected count was less than 5. Binary Logistic Regression in SPSS was used to obtain odds ratio and confidence intervals for different associations. The fitness of the model to correctly predict the PRNT results by the independent variables was tested and found significant using the Omnibus test of model coefficient and insignificant with the Hosmer and Lemeshow test. Overall, the percentage accuracy in the classification was 87% with 100% specificity but 0% sensitivity.

Results

CHIKV infections in three northeastern states of Nigeria

Of 530 patients tested, 129 (24.3%) had CHIKV IgM , 199 (37.5%) nAb and 23 (4.3%) IgM + nAb. Of 401 IgM negative patients, 176 (43.9%) had CHIKV nAb in the three states (Fig. 2). Of 200 patients from Adamawa State, 13 (6.5%), 85 (42.5%) and 7(3.5%) were CHIKV IgM, nAb and IgM+nAb respectively. Of 200 patients from Borno State, 94 (47.0%) were IgM and 98 (49.0%) nAb while 7(4.5%) had both IgM +nAb. Of 130 samples from Bauchi State, their CHIKV antibodies were 94 (72.3%) IgM, and 16 (12.3%) nAb as well as 7 (5.4%) IgM +nAb (Supplementary table). CHIKV nAb in Bauchi (χ^2= 48.905, df=1, p = 0.001, OR= 0.228, Cl=0.139-0.373) and Borno (χ^2= 16.262, df=1, p = 0.001, OR=1.559, CL=1.259-1.931) were significantly higher than Adamawa (χ^2= 4.867, df=1, p = 0.032, OR-1.289Cl= 1.031-1.612) (Table 2).

Distribution of CHIKV infections among rural and urban dwellers

Of the 530 patients studied, 80 (15.1%) and 380 (71.7%) were rural and urban dwellers respectively. However, 70 (13.2%) did not disclose their settlement status. Of 380 urban dwellers, 96 (25.3%) had CHIKV IgM, 138 (12.1%) nAb and 1 (3.2%) IgM + nAb. Similarly, 17(21.3%, 31(20.2%) and 19 (1.4%) rural dwellers had CHIKV IgM and nAb and IgM+nAb respectively. Of 70 undisclosed settlement status, 16 (22.9%) were IgM, 30 (13.5%) nAb, and 3 (4.1%) IgM + nAb (Table 1). The type of settlement and CHIKV nAb were not significantly different (χ^2=0.486, df=1, p=0.486, OR=1.112 Cl=0.846-1.463) (Table 2).

Table 1. The distribution of Chikungunya virus antibodies among urban and rural dwellers

Settlement	Total Tested	No. Positive		
		CHIKV IgM (%)	CHIKV nAb (%)	IgM +nAb + (%)
Rural	80	17 (21.3)	31 (20.2)	1 (3.2)
Urban	380	96 (25.3)	138 (12.1)	19 (1.4)
Not disclosed	70	16 (22.9)	30 (13.5)	3 (4.1)
Total	**530**	**129 (24.3)**	**199 (18.7)**	**23 (4.3)**

Age distribution of CHIKV virus antibodies among febrile patients in northeastern, Nigeria

Among patients aged 1-14 years , 16 (26.6%), 25 (38.5%) and 1(1.5%) had CHIKV IgM, nAb and IgM +nAb respectively. Within patients aged 15-29years, 39 (20.4%) were IgM, 71(37.2%) nAb and 8 (4.2%) IgM +nAb. For 30-45 age groups, 55 (28.2%), 75(38.5%) and 12 (6.2%) were IgM, nAb and IgM +nAb respectively. Of patients aged 46-60 years, 15 (25.4%) had IgM, 18 (30.5%) nAb) and 1 (1.7%) IgM +nAb. Among patients aged 60 years and above, 4 (20.0%) IgM, 10 (50.0%) nAb, and 1(5.0%) IgM nAb were obtained (Table 3). Although, the ages of the patients were not significantly associated with CHIKV nAb (χ^2=1.244, df=1, p=0.348), patients aged >60 years were more likely to have experienced CHIV infections than younger age groups studied (OR=1.623, Cl= 0.688-3.830) (Table 2).

Gender and occupational distribution of CHIKV infections

Of 244 males tested, 62 (25.4%), 94 (38.5%) and 14 (5.7%) had CHIKV IgM, nAb and IgM +nAb respectively. Among 280 females, 67 (23.9%) were IgM, 105(37.5%) nAb and 9(3.2%) IgM+nAb. CHIKV antibody was high among students, housewives and civil servants but least among retirees (Supplementary file). Gender and occupation were not significantly associated with CHIKV nAb (P> 0.05) (Table 2).

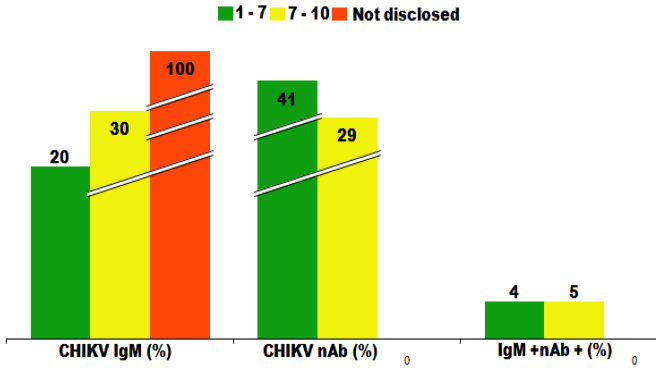

Figure 3. Chikungunya virus antibodies at different intervals between the onset of symptoms and sample collection

Distribution of CHIKV Infection Rates According to the Intervals Within Which Samples Were Collected After the Onset of Symptoms

Of 387 samples collected 1-7 days after the onset of symptoms,79 (20.4%), 160 (41.3%), and 17 (4.4%) were CHIKV IgM, nAb and IgM +nAb respectively. Among 133 samples collected within 7-10 days, 30 (30.1%) were IgM, 39 (29.3%) nAb and 6 (4.5%) IgM +nAb (Figure 3). CHIKV nAb and samples collected 1-7 days after the onset of symptoms were significantly higher (χ^2=8.546, df=1, p = 0.004) than 7-10 (OR=1.170, Cl=1.058-1.294) (Table 2).

Distribution of CHIKV Infections Among Patients who Received Antimalaria/Antibiotic Treatment Before Performing Laboratory Tests for Malaria Parasites

Of 315 patients who received antimalaria/antibiotics treatments, 41(13.0%) and 131 (41.6%) had CHIKV IgM and nAb respectively. Of 204 untreated patients, 77 (37.7%) had CHIKVIgM and 67(32.8%) nAb (Figure 4). CHIKV was significantly associated with antibiotics/antimalaria treatments [χ^2= 4.988, df=1 p=0.026, OR=1.177, Cl=1.023-1.353] (Table 2).

Co-infections Between CHIKV and Some Flaviviruses

Of 199 samples with CHIK nAb, 17.7%, 1.5%, 4.0% and 1.3% also neutralized 90-100% infectivity of DENV, ZIKV, WNV and YFV respectively (Supplementary file).

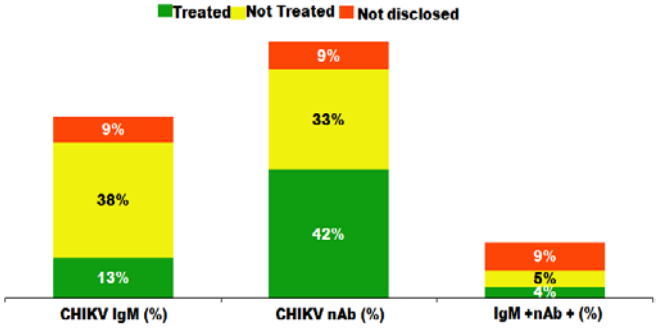

Figure 4. Distribution of West Nile virus antibodies among patients who were treated with antibiotics/antimalaria

Table 2. Logistic Regression tables for all the independent variables

Variables	β	Standard Error(β)	P-value (β)	χ^2-value	P-value (χ^2)	Odd Ratio (OR)	C.I Lower	C.I Upper
State								
Adamawa	0.409	0.186	0.028	4.867	0.032	1.289	1.031	1.612
Bauchi	-1.831	0.286	<0.001	48.905	<0.001	0.228	0.139	0.373
Borno	0.743	0.186	<0.001	16.262	<0.001	1.559	1.259	1.931
Sex								
Male	-0.082	0.181	0.651	0.205	0.653	1.045	0.864	1.263
Female	-	-	-	-	-	0.963	0.817	1.135
Settlement								
Rural	0.150	0.198	0.447	0.578	0.447	1.112	0.846	1.463
Urban	-	-	-	-	-	0.957	0.854	1.073
Interval								
1-7days	-0.624	0.215	0.004	8.546	0.002	1.170	1.058	1.294
7-10days	-	-	-	-	-	0.627	0.453	0.867
Treatment								
Treated	-0.420	0.189	0.026	4.988	0.026	1.177	1.023	1.353
Untreated	-	-	-	-	-	0.773	0.613	0.975
YF-Vaccination								
Vaccinated	-0.113	0.209	0.587	0.295	0.601	1.089	0.801	1.482
Unvaccinated	-	-	-	-	-	0.973	0.879	1.077
Age (Yrs.)								
1-14	-0.016	0.273	0.952	0.004	0.527	1.014	0.636	1.619
15-29	0.024	0.188	0.900	0.016	0.488	0.985	0.778	1.247
30-45	-0.063	0.187	0.736	0.114	0.403	1.040	0.827	1.309
46-60	0.323	0.300	0.283	1.161	0.176	0.749	0.441	1.273
>60	-0.504	0.457	0.269	1.244	0.188	1.623	0.688	3.830
Occupation	-	-	-	12.387	0.089	-	-	-

Yellow Fever Vaccination Status of Patients and the Distribution of CHIKV Antibodies

The YF vaccination status in Adamawa state was higher at 53.5% than in Bauchi at 6.2%. and Borno with 7.5% (Supplementary file). In the three states, only 130 (24.5%) patients received YF vaccination as against the majority (75.5%) who did not. Among the vaccinated, 18 (13.8%) had CHIKV IgM and 51(39.2%) with nAb. Among the unvaccinated, 111(27.8%) and 148 (37.0%) had CHIKV IgM and nAb respectively (Table 4). CHIKV nAb was not significantly associated with the YF vaccination status

[χ^2= 0.295, df=1, p=0.601, OR=1.089, Cl=0.801-1.482] (Table 2).

Table 3. Age distribution of Chikungunya virus antibodies among malaria-suspected patients in three northeastern states in Nigeria

Age group (years)	Total tested	No. positive		
		CHIKV IgM+ (%)	CHIKV nAb (PRNT) (%)	CHIKV IgM+/PRNT+ (%)
1-14	65	16 (26.6)	25 (38.5)	1 (1.5)
15-29	191	39 (20.4)	71(37.2)	8 (4.2)
30-45	195	55 (28.2)	75 (38.5)	12 (6.2)
46-60	59	15 (25.4)	18 (30.5)	1(1.7)
>60	20	4 (20.0)	10 (50.0)	1 (5.0)
Total	530	129 (24.3)	199 (37.5)	23 (4.3)

Table 4. Chikungunya virus antibodies and Yellow Fever vaccination status of the patients

Yellow Vaccination status	Total Tested	No. Positive		
		CHIKV IgM (%)	CHIKV nAb (%)	IgM +nAb (%)
Vaccination	130 (24.5)	18(13.8)	51 (39.2)	6 (4.6)
No vaccination	400 (75.5)	111 (27.8)	148 (37.0)	17 (4.3)
Total	530	129 (24.3)	199 (37.5)	23 (4.3)

Discussion

CHIKV infections can be confirmed serologically by the presence of virus-specific IgM and or neutralizing antibodies (nAb) in a single serum sample during the acute and/or convalescent stage of infection [1,19,20]. PRNT-positive results serve as a mark of protection against reinfection [11,12,15,21,22], quantify the antibody and show its effectiveness on the virus [18]. The use of a higher stringent endpoint titer of 90% in PRNT provides greater species specificity than low titres [18]. In this study, only diluted sera with a plaque count of ≤ 5 against the control well of 40-50 was considered positive.

The ability of 4.5% of IgM positives to neutralize 90-100% of CHIKV infectivity, indicated recent infections in consistence with previous reports [19,23]. A higher number of patients (37.5%) with nAb indicated both recent and past CHIKV infections. CHIKV infections in the three states were significantly different (Figure 2, Table 2) as they differ in terms of vegetation, climatic conditions and enzootic interaction which impact vector competence and transmission of arboviruses [20,24,25]. The seroprevalence rates of 24.3 % CHIKV IgM and 37.5% nAb in this study were slightly lower than 26.7% IgM and 46.1% nAb in a previous study in Nigeria [26]. The difference could be attributed to the source of data, study periods and areas covered. CHIKV IgM of 6.5% and 11.0% were obtained in Borno State in 2017 [25] and this study (2018) respectively. The difference in the seroprevalence rates could be due to the type and source of the ELISA kits used. Furthermore, CHIKV IgM of 5.8% obtained in Kogi State, northcentral Nigeria [8] was significantly lower than what was obtained in Bauchi (72.3%) and Borno (11.0%) states in this study. We speculate that different climatic conditions and vegetations of Kogi, Bauchi and Borno are contributory factors.

In this study, CHIKV nAb and settlements, gender as well as the occupation of the patients were not significantly different. Probably in Nigeria, the mosquito vector does not discriminate between settlements, gender, and occupation in its quest for a blood meal. Although the age of the patients and CHIKV nAb were not significantly associated, patients aged ≤ 60 years were more likely to have experienced CHIKV infections than the younger age groups (Table 2 and Table 3). Our findings corroborated with other studies in Nigeria [25], India [27] Singapore [28] and Malaysia [29], but contrasted with studies in India [30] and Tanzania [31]. We speculate that the degenerated immune functions and sedentary lifestyles of the elderly population could be a risk factor for CHIKV infections.

We observed that CHIKV nAb obtained from samples collected between 1-7 days were significantly different from those within 7-10 (Figure 3,Table 2). The timing of sample collection and the purpose of the testing influences the choice of the assay for a better diagnostic outcome. The presence of CHIKV nAb and recipients of antibiotic/antimalaria treatments were significantly different (Figure 4, Table 2). The heavy burden of malaria/typhoid in Nigeria necessitates the frequent use of antibiotics/antimalaria. Possibly, many misdiagnosed CHIKV patients must have been treated with these drugs. It is not clear whether these drugs actually inhibit the progression of CHIKV infections or not. However, the ability of doxycycline to strongly inhibit the replication of CHIKV in Vero cell line has been demonstrated [32]. Further studies are necessary to determine the effects of these drugs on CHIKV. Importantly, irrational use of these drugs could contribute to the global antimicrobial resistance burden.

This study detected co-infections between CHIKV and flaviviruses at varied degree: DENV (17.7%), Zika virus (ZIKV) (1.5%, WNV (4.0%), and YFV (1.3%) (Supplementary file) in agreement with other studies (33–35). Aside from individual host being coinfected with more than one virus at different times, the same mosquito vector is capable of transmitting four viruses (CHIKV, DENV, YFV and ZIKV) simultaneously [36]. The capability of CHIKV-nAb to neutralize flaviviruses is a mark of protection against reinfection by the co-infected viruses in agreement with previous reports [22,37]. CHIKV nAb was not significantly associated with the YF vaccination status probably because both viruses belong to different virus families: CHIKV and YFV belong to Alphaviridae and Flaviviridae respectively.

The limitations of this study include our inability to perform multiple PRNT on various dilutions of CHIKV-positive sera and co-infecting flaviviruses due to an insufficient quantity of samples. Additionally, the high cost of the reagents prevented us from performing more useful analysis such as qRTPCR. We are unable to conclude that there was no cross- reactivity with other alphaviruses although the utilization of $PRNT_{90}$ may limit this potential.

Conclusion

ELISA IgM and PRNT were used to unmask CHIKV infections from febrile patients suspected of malaria in Adamawa, Bauchi and Borno states at different prevalence rates. The species specificity of $PRNT_{90}$ allowed the discrimination between infections with CHIKV and Flaviviruses. Both acute and past infections were detected. Treatment with anti-malaria /antibiotics and CHIKV nAb were significantly associated. Samples collected within 1-7 days are likely to yield more nAb than 7-10 days. The elderly (≥ 60 years) patients were more likely to have experienced more CHIKV infections than the younger ones. Co-infections of CHIKV and some Flaviviruses have been detected. Yellow Fever vaccination status, gender, settlements and CHIKV nAb were not significantly different.

This study has highlighted the prevalence of CHIKV being commonly masked by malaria which is also hyperendemic in Nigeria. Lack of access to appropriate diagnostics for CHIKV as opposed to malaria limits its detection in the country. The study also demonstrated the risk areas to be considered for the future management of the CHIKV infections. However, additional work is required to support the findings of this study with the view to providing more detailed evidence base information for improved public health with respect to CHIKV infections and disease prevention strategies.

Authors' Contributions

(1) MMB : the conception, design of the study, drafting of the article and final approval of the version

(2) **BSO** and **BM** : acquisition of data and editing of the article

(3) **YSJ :** analysis and interpretation of data and editing of the article

(4) **KI :** Map design, data analysis and editing of the article

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Conflict of interest: The authors declare that the study was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest. This does not alter our adherence to journal's policies on sharing data and materials.

Research Ethics

The research ethical clearance approval letter was obtained from the Research and Ethics Committee of ATBUTH (ref ATBUTH/ADM/42/Vol.1, dated 25 May 2017), State Specialist Hospital (SSH), Maiduguri, Borno State (ref no. SSH/GEN/641/Vol.1 dated 14 February 2018) and Federal Medical Centre, Yola (ref no FMC/YO/001/Vol.1 dated May 2018). The approved consent form was given to each participant to read, endorse and return if they agreed to be recruited for the study. Only those with endorsed forms were recruited for the study. Parents of children below five years endorsed the forms on behalf of their children.

Abbreviations

WHO-World Health Organization, FMC: Federal Medical Center, ATBUTH- Abubakar Tafawa Balewa University of Maiduguri Teaching Hospital, Bauchi, SSH-Specialist State Hospital, UMTH- University of Maiduguri Teaching Hospital, nAb- Neutralizing antibody, PRNT- Plaque reduction neutralizing antibody test, IgM-Immunoglobulin M antibody, IgG-Immunoglobulin G antibody, ELISA-Enzyme-Linked Immunosorbent assay, EMEM- Earle's Minimum Essential Medium, In-house Dilution, ICGEB- International Center for Genetic Engineering and Biotechnology, DENV- Dengue virus,

YFV- Yellow Fever Virus, WNV- West Nile virus, CHKV- Chikungunya virus, IDP- Internally Displaced persons.

References

[1] WHO. Chikungunya. 2020 [cited 2022 Oct 5]. Available from: https://www.who.int/news-room/fact-sheets/detail/chikungunya

[2] Zavala-Colon, M. and Gonzalez-Sanchez,J. History and Geographic Distribution of Chikungunya Virus. Chikungunya Virus - A Growing Global Public Health Threat. IntechOpen [Internet]. 2021; Available from.

[3] Wong, K. Z., Chu, J.J.H. The interplay of viral and host factors in Chikungunya virus infection: targets for antiviral strategies. Viruses [Internet]. 2018;10(6):294. Available from: https://www.mdpi.com/1999-4915/10/6/294/htm

[4] European Centre for Disease Prevention and Control. Chikungunya - Annual Epidemiological Report 2016 [2014 data] [Internet]. 2016 [cited 2023 Feb 18]. Available from: https://www.ecdc.europa.eu/en/publications-data/chikungunya-annual-epidemiological-report-2016-2014-data. Acessed on 20 January 2023.

[5] Baba, M, Logue, C. H., Oderinde, B., Abdulmaleek, H. Williams, J., Lewis, J., et al. Evidence of arbovirus co-infection in suspected febrile malaria and typhoid patients in Nigeria. J Infect Dev Ctries. 2013 Jan 15;7(01):051–9.

[6] Natrajan, M. S., Rojas, A. W.J. Beyond Fever and Pain: Diagnostic Methods for Chikungunya Virus. J Clin Microbiol. 2019;57(6):e00350-19.

[7] Asaga, M. P., Airiohuodion, P.E., Yako, A. .B. Makpo, J. K.Kreger A. The Seroprevalence and Hidden Burden of Chikungunya Endemicity and Malaria Mono- and Coinfection in Nigeria. Int J Environ Res Public Heal. 2022;19(8896).

[8] Omatola, C., Onoja, B. A., Fassan, P. K., Osaruyi, S. A., Iyeh, M., Samuel, M. A., Haruna, P.U. Seroprevalence of chikungunya virus infection in five hospitals within Anyigba, Kogi State of Nigeria. Braz J Infect Dis [Internet]. 2020;24(1):1–6. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9392021/

[9] Udeze, A. O., Odebisi-Omokanye, B.M., Onoja, A.B., Daodu, D.M. Olasomi, O. J. Screening of Immunoglobulin G Antibodies Against Chikungunya Virus Among Urban Population in Ilorin Nigeria. Niger Vet J. 2019;40(3):227 – 238.

[10] Olayinka, M., Olajiga, O. E,. Adesoye, A. P. E., Abiodun, J., Adeyemi, E. O., Adeyefa, I. A., Aderibigbe, S. A., Wasiu, O., Adebimpe, O. O., Opaleye, Sule, W.H., Oluwayelu, D.O. Chikungunya Virus Seroprevalence and Associated Factors among Hospital Attendees in Two States of Southwest Nigeria: A Preliminary Assessment. A J Mol Cell Immunol. 2017;

[11] Johnson, B. W., Russell, B. J., Goodman, C. H. Laboratory Diagnosis of Chikungunya Virus Infections and Commercial Sources for Diagnostic Assays. J Infect Dis. 2016;15(214(suppl 5)):S471-S474.

[12] Jacobsen, S., Patel , P., Schmidt-Chanasit, J., Leparc-Goffart, I., Teichmann , A., Zeller H., Niedrig, M. External quality assessment studies for laboratory performance of molecular and serological diagnosis of Chikungunya virus infection. J Clin Virol. 2016;76(55–65).

[13] Andrew, A., Navien, T. N,, Yeoh, T. S., Citartan, M., Mangantig, E., Sum. M.S.H., Ch'ng, E. S., Tang, T.H. Diagnostic accuracy of serological tests for the diagnosis of Chikungunya virus infection: A systematic review and meta-analysis. PLoS Negl Trop Dis. 2022;16(2):e0010152.

[14] Azami, N., Moi, M. L., Takasaki, T. Neutralization Assay for Chikungunya Virus Infection: Plaque Reduction Neutralization Test. Methods Mol Biol. 2016;1426:273-82.

[15] Chua, C. L, Sam, I. C., Chiam, C.W., Chan, Y.F. The neutralizing role of IgM during early Chikungunya virus infection. 2017;12(2):e0171989.

[16] Population Total. Nigeria [Internet]. Wikipaedia. 2023 [cited 2023 May 13]. Available from: https://simple.wikipedia.org/wiki/Nigeria. Accessed on 20 January 2023

[17] Baba, M. M., Yahaya, K. M., Ezra, E. M., Adamu, M., Kulloma, B.M., Ikusemoran, M., Momoh, J. P., Oderinde, B. S.Assessment of immunity against Yellow Fever virus infections in northeastern Nigeria using three serological assays. J Med Virol. 2021;93(8):4856–64.

[18] World Health Organization. Guidelines for plaque reduction neutralization testing of human antibodies to Dengue viruses. World Health Organization [Internet]. 2007 [cited 2022 Apr 12]. Available from: https://apps.who.int/iris/handle/10665/69687. Accessed on 20 January 2023.

[19] Kam, Y.W., Pok, K. Y., Eng, K. E, Tan, L. K., Kaur, S., Lee, W.W.L, Leo, Y. S., Ng, L. C., Ng, L.F.P. Sero-prevalence and cross-reactivity of chikungunya virus specific anti-E2EP3 antibodies in arbovirus-infected patients. LoS Negl Trop Dis. 2015;

[20] Bovay, A., Nassiri, S., Maby–El Hajjami, H., Marcos Mondéjar, P., Akondy, . RS., Ahmed, R., Lawson, B., Speiser, D. E., Marraco, F. S. A. Minimal immune response to booster vaccination against Yellow Fever associated with pre-existing antibodies. Vaccine. 2020;38(9).

[21] Natrajana, M.S., Rojas, A. Waggoner, J.J. Beyond Fever and Pain: Diagnostic Methods for Chikungunya Virus. J Clin Microbiol. 2019;57(10).

[22] Srikiatkhachorn, A., Alera, M. T., Lago, C. B., Tac-An, I. A., Villa, D., Fernandez, S., Thaisomboonsuk, B., Klungthong, C., Levy, J.W., Velasco, J. M., Roque, V.G. Jr., Nisalak, A., Macareo, L.R., Yoon I. K. Resolution of a Chikungunya Outbreak in a Prospective Cohort, Cebu, Philippines, 2012-2014. Emerg Infect Dis. 2016;22(10):1852–4.

[23] Ribeiro, M..RC, Khouri, R., Sousa, P. S, Branco , MRFC, Batista RFL, Costa, E. P. F., Alves , M.T.S.S.B., Amaral, G. A., Borges, M.C.R., Takahasi, E. H.M., Gomes, L.N., Mendes, A. K.T., Cavalcante, T. B., Costa, L.C., Félix ,A. C., Souza, N.C.S., Silva, A.A.M. Plaque Reduction Neutralization Test (PRNT) in the Congenital Zika Syndrome: Positivity and Associations with Laboratory, Clinical, and Imaging Characteristics. Viruses. 2020 Oct 31;12(11):1244.

[24] Chepkorir, E., Lutomiah, J., Mutisya, J., Mulwa, F., Limbaso, K., Orindi,B., Ng'ang'a, Z., Sang, R. Vector competence of Aedes aegypti populations from Kilifi and Nairobi for dengue 2 virus and the influence of temperature. Parasit Vectors. 2014;7(1):435.

[25] Akinola, M. T., EL-Yuguda, A. D., Bukbuk, D. N., & Baba SS (2017). Prevalence of IgG and IgM antibodies to Chikungunya virus among outpatients with febrile illness attending University of Maiduguri Teaching Hospital, Maiduguri, Borno State, Nigeria. African J Microbiol Res. 2017;11(7):306-311.

[26] Abdullahi, I. N., Akande, A. O., Muhammed, Y., Rogo, L.D., Oderinde, B.S. Prevalence Pattern of Chikungunya Virus Infection in Nigeria: A Four Decade Systematic Review and Meta-analysis. Pathog Glob Heal. 2020;114(3):111–6.

[27] Patil, S.S., Patil, S. R., Durgawale, P. M. , Patil, A. G. A study of the outbreak of Chikungunya fever. J Clin Diagn Res. 2013;7(6)::1059-62.

[28] Ang, L. W., Kam, Y.W., Lin, C., Krishnan, P.U., Tay, J., Ng, L.C., James, L., Lee, V.J.M., Goh, K.T., Lisa, F.P. N., Raymond, T.P.L. Seroprevalence of antibodies against chikungunya virus in Singapore resident adult population. PLoS Negl Trop Dis [Internet]. 2017;11(12):e0006163. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5760101/

[29] Azami, N.A.M., Salleh, S.A., Shah, S.A., Neoh, H.M., Othman, Z., Zakaria, S. Z. S., Jama , R. Emergence of chikungunya seropositivity in healthy Malaysian adults residing in outbreak-free locations: Chikungunya seroprevalence results from the Malaysian Cohort. BMC Infect Dis [Internet]. 2013;13(67):1–9. Available from: file:///C:/Users/DIRECTOR/Downloads/1471-2334-13-67 (1).pdf

[30] Kawle, A. P., Nayak, A.R., Bhullar, S.S., Borka,r S.R., Patankar, S.D., Daginawala, H.F., Singh, L.R., Kashyap, R.S. Seroprevalence and clinical manifestations of Chikungunya virus infection in rural areas of Chandrapur, Maharashtra, India. 2017;54(1):35–43. Available from: https://www.jvbd.org/temp/JVectorBorneDis54135-4250531_114825.pdf

[31] Hertz, J.T., Munishi, O. M., Ooi, E.E., Howe, S., Lim, W.Y., Chow, A., Morrissey, A.B., Bartlett, J.A, Onyango, J.J., Maro, V.P., Kinabo, G. D., Saganda, W., Gubler, D.J., Crump, J.A. Chikungunya and dengue fever among hospitalized febrile patients in northern Tanzania. Am J Trop Med Hyg. 2012;86(1):171-7.

[32] Rothan, H. A., Bahrani, H., Mohamed, Z., Teoh, T.C., Shankar, E.M., Rahman, N. A., Yusof, R.A. combination of doxycycline and ribavirin alleviated chikungunya infection. PLoS One. 2015;10(5):e0126360.

[33] Mota, M. L., Marinho, R. S. S., Duro, R, L.S., Hunter, J., de Menezes, I. R. A., de Lima Silva, J.M.F. L., Pereira, G.L.T, Sabino, E. C., Grumach, A., Diaz, R. S. D., Lucena, M.S. L., Komninakis, S.V. Serological and molecular epidemiology of the Dengue, Zika and Chikungunya viruses in a risk area in Brazil. BMC Infect Dis. 2021;21(1).

[34] Kaur, M., Singh, K., Sidhu, S. K., Devi, P., Kaur, M., Soneja, S. S.N. Coinfection of chikungunya and dengue viruses: A serological study from North Western region of Punjab, India. J Lab Physicians. 2016;10(4):443–7.

[35] Soghaie, r M. A., Abdelgadir, D. M, Abdelkhalig, S.M., Kafi, H., Zarroug, I.M.A., Sall, A.A., Eldegai, M.H., Elageb, R.M., Osma, M. M., Khogali, H. Evidence of pre-existing active Zika virus circulation in Sudan prior to 2012. BMC Res Notes. 2018 Dec 19;11(1):906.

[36] Goertz, G. P., Vogels, C.B.F., Geertsema, C., Koenraadt, S., Pijlman, G. P.. Mosquito coinfection with Zika and chikungunya virus allows simultaneous transmission without affecting vector competence of Aedes aegypti. PLoS Negl Trop Dis. 2017;11(6):e0005654.

[37] Jin, J., Montoya, J G G, Sun, S. Y., Goldsmith, C.S., Toole, E. T. O., Ackerman, L., Carlson, L. A., Weaver, S.C., Chiu, W, Simmons, G. Neutralizing Antibodies Inhibit Chikungunya Virus Budding at the Plasma Membrane. Cell Host Microbe. 2018;24:24, 417–428.