

Sero-molecular surveillance and determinants of chikungunya virus infection among patients with myo-arthritogenic illnesses in Zaria, northwest Nigeria

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**Serological and molecular detection and determinants of chikungunya virus infection
among patients with myo-arthritogenic illnesses in Zaria, northwest Nigeria**

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ABSTRACT

Background:

Chikungunya (CHIK) is a neglected and globally re-emerging mosquito-borne viral infection, often characterised by myoarthritogenic disorders that can lead to significant long-term reductions in human productivity. This study determined the seroprevalence of anti-CHIKV antibodies, the frequency of CHIKV RNA detection, risk determinants of CHIKV-associated myo-arthritis illnesses, and knowledge and practices regarding CHIKV among patients attending Ahmadu Bello University Teaching Hospital (ABUTH), Zaria, northwestern Nigeria.

Materials and Methods:

Blood samples were collected from 250 non-malaria patients with myo-arthritis illnesses over a three-week period. The blood samples were processed and analysed for anti-CHIKV IgM and IgG using enzyme-linked immunosorbent assays, while the CHIKV RNA was detected by RT-qPCR. Pretested structured questionnaires were used to collate epidemiological data from patients. Multiple regression statistics were used to identify factors associated with CHIK-associated myo-arthritis illnesses.

Results:

Of the 250 participants with arthritis illnesses, 28% (70/250) knew about CHIKV. Of these, the majority (48.6%, 34/70) learnt about it from newspapers/print media. The use of long clothing at least 1-3 times per week during all outdoor activities was significantly associated with CHIKV knowledge ($p=0.0035$). CHIKV RNA was detected in 13.6% (34/250) participants, of which 2.8% (7/250) were co-positive for anti-CHIKV IgM and IgG. The seroprevalence of anti-CHIKV IgM was 10% (25/250), and of anti-CHIKV IgG, 12% (30/250). Residence close to bushes or dumpsites, ≤ 100 meters from residential houses, was significantly associated with anti-CHIKV IgM seropositivity (AOR=3.60, $p=0.012$). A lower practice score was significantly associated with increased odds of anti-CHIKV IgM seropositivity (AOR=1.91, $p=0.002$).

Conclusion:

These data indicate active CHIKV transmission and its role as a significant etiological agent of myo-arthritis illness in the study area. Collectively, these emphasise the necessity for improved public awareness of CHIKV, integrated surveillance and differential laboratory-based diagnosis, especially in patients with high fever and arthritis symptoms in Nigeria and countries with immigrants from CHIKV endemic countries.

Keywords: Chikungunya, Nigeria, seroprevalence, RT-qPCR, myo-arthritis illnesses, CHIKV

1.0.INTRODUCTION

Chikungunya (CHIK) is considered a neglected tropical disease (NTD) because most CHIK virus infections occur in resource-limited countries, where healthcare infrastructure is limited, and research funding is scarce ¹. CHIKV, an alphavirus of the *Togaviridae* family. CHIKV has a positive-sense single-stranded RNA of about 11.8 kb, and two open reading frames encoding the non-structural (nsP1, nsP2, nsP3, nsP4) and structural polyproteins (C, E3, E2, 6K, E1) ². Based on sequence analysis of the E1 gene of CHIKV, 4 genotypes (lineages) have been identified: the West African, East-Central-South African, Asian, and Indian Ocean lineages ³. The E1 gene of CHIKV is the primary target for PCR and molecular detection because it is a highly conserved structural gene essential for viral entry, making it both reliable for detection and critical for tracking its phylogenetic diversity ².

The word ‘chikungunya’ was used by certain Tanzanians to describe the severe joint pains that affected patients’ posture, where it was first reported during an explosive outbreak in 1952 ⁴. CHIK is arthritogenic and one of the most endemic arboviral infections in sub-Saharan Africa, with a pooled estimated IgG seroprevalence of 23.7% ⁵. Moreover, an estimated global CHIK annual burden of 14.4 million, with 0.96 million disability-adjusted life years in 103 countries ^{6 8}. The resurgence of CHIKV in 2024-2025 has been driven by intensified globalization, international travel, and climate-driven expansion of *Aedes* mosquito habitats ⁶.

Aedes aegypti and *A. albopictus* mosquitoes, which bite during the day, are present in 167 countries and facilitate the transmission of CHIKV to humans through the urban transmission cycle, in which the virus cycles between domestic mosquitoes and humans ^{7, 8}. Additionally, they help maintain the virus during inter-epidemic periods via the sylvatic transmission cycle, where the virus cycles between vectors and wild animals in the forest ⁸.

Following inoculation by an infected mosquito, CHIKV enters the bloodstream through the skin, resulting in high viremia ^{8, 9}. It then spreads to target tissues, mainly fibroblasts and myofibers ^{8, 9}. Unlike the dengue virus (DENV), CHIKV causes symptoms in most infected individuals ⁸. After an incubation of 2 to 4 days (range 1 to 14), the acute phase begins with sudden fever above 39°C, often followed by musculoskeletal pain ⁸. CHIKV infection of musculoskeletal progenitor cells and induction of skeletal muscle necrosis may explain CHIKV-induced myopathies and suggest muscle as a site of viral persistence ⁹. Additionally, CHIKV-associated arthralgia is typically symmetric and affects distal synovial joints ⁹.

The knowledge, attitude, and practice of individuals and communities are critical for preventing CHIKV infection. Valneva developed IXCHIQ, the world's first licensed chikungunya vaccine, which was supplied to help manage outbreaks like the one on France's Island of La Réunion and used in Brazil ¹⁰. Other vaccine candidates, such as VIMKUNYA (PXVX0317), MV-CHIK, BBV87, mRNA-1388 (VAL-181388) and ChAdOx1 Chik continue to undergo clinical testing and evaluation ¹¹. Moreover, vector control and personal protective measures are key preventive measures against the transmission of CHIKV ¹². In a 2020 meta-analysis and systematic review, we found pooled prevalences of 26.7% and 3.8% for anti-CHIKV IgM and detectable RNA, respectively, among febrile patients in Nigeria ¹³. Subsequently, Baba *et al* and Nyamngee *et al* separately reported CHIKV IgM and RNA in 24.3% and 3.6% of febrile patients in northern Nigeria, respectively ^{14, 15}. Moreover, a recent nationwide study demonstrated the presence of CHIKV in several *Aedes* species in Nigeria ¹⁶. Despite the direct association of CHIKV-associated myo-arthritogenic disorders that cause significant long-term reductions in human productivity ¹², there is a paucity of studies investigating the link between CHIKV infection and these clinical and public health entities in Nigeria. Consequently, this study aims to determine the seroprevalence of anti-CHIKV antibodies, the frequency of CHIKV RNA detection, risk determinants of CHIKV-associated

myo-arthritogenic illnesses, and knowledge and practices regarding CHIKV among patients attending Ahmadu Bello University Teaching Hospital (ABUTH), Zaria, northwestern Nigeria.

2.0. MATERIALS AND METHODS

2.1. Study Area

The study was conducted at ABUTH, Shika, Zaria, Kaduna State, Nigeria (latitudes 11°7'N–11°24'N, longitudes 7°31'E–7°50'E; elevation 650–700 m). ABUTH is a 1000-bed tertiary hospital serving Northwest Nigeria and neighbouring countries. Zaria has an estimated population of 712,688 and features a savannah ecology with thick shrubs. The area experiences a mean annual rainfall of about 1090 mm, mostly from May to September, with peak rainfall in August and lowest in November. Temperatures range from 30 °C to 37 °C, with the highest in March. Rainfall influences groundwater and, combined with water-filled potholes during the rainy season, creates suitable breeding sites for mosquitoes.

2.2. Ethical consideration

This study and its protocols underwent thorough review and received approval (NHREC/10/12/2015, supplementary material 1) from the Ethical Research Committee of ABUTH, Shika, Nigeria, ensuring compliance with institutional and national ethical standards. Prior to their enrolment, all participants were fully informed about the study's objectives, procedures, potential risks, and benefits, and provided their written informed consent voluntarily. The collection of samples and data adhered strictly to established ethical guidelines, following the principles set forth in the Declaration of Helsinki, as revised in 2024. Throughout the study, participant confidentiality and privacy were rigorously maintained, and all procedures were conducted in a manner that prioritized the safety, dignity, and rights of the individuals involved.

2.3. Study design and population

This hospital-based cross-sectional study took place from 2021 to 2025. Specifically, participants' enrolment took place from March to August 2021. During this period, questionnaires were administered and blood samples collected. The serum samples were extracted in two aliquots for each patient and preserved at -80°C for pending laboratory analyses. The first aliquot was used for the enzyme-linked immunosorbent assay (ELISA) test, which took place in February 2023, while the molecular analyses of CHIKV were performed on the second aliquot of serum samples in January 2025.

It involved patients with febrile and musculoskeletal illnesses attending the rheumatology, medical wards, emergency and general outpatient clinics of ABUTH. Potential participants were screened, identified and enrolled by physicians, rheumatologists and nurses from various wards and clinics of the hospital. Only adults aged ≥ 18 years were enrolled, and myo-arthritis illness was diagnosed based on medical history and physical exams by family physicians and rheumatologists. Aside from clinical symptomology of patients, subsequent imaging and routine laboratory tests were not included for diagnosis of myo-arthritis illness. For this study, myo-arthritis illness refers to conditions affecting both the muscles (*myo*) and the joints (*arthro*), such as inflammatory myopathies (like polymyositis or dermatomyositis) or systemic rheumatic diseases. These included progressive muscle weakness, joint and muscle pain, severe fatigue, and swelling.

2.4. Sampling method

Probabilistic patient selection (random sampling) was used to give every individual in the target group an equal or known chance of being chosen. The target population was identified at ABUTH during the study period using the set criteria through assessment of symmetrical or asymmetrical muscle weakness, joint swelling, tenderness, or characteristic rashes. Probabilistic selection was applied by assigning a unique computer-generated random number

to each name on the final clean list and using a random number generator to select participants. Only patients with non-missing data were included in the selection process.

2.5. Sample size and participants' selection criteria

Given that the present study is cross-sectional, the minimum sample size was determined using the CHIKV prevalence of 13.1% reported by Kolawole *et al*¹⁷. Accordingly, the minimum sample size was calculated using Cochran's sample size formula as follows:

$$\text{Number of participants (n)} = \frac{Z \times Z (1 - P) p}{e \times e}$$

Where n is the sample size, Z is the Z-score (1.96) for a 95% confidence interval, p is the prevalence of CHIKV (0.131)¹⁷ from a previous study, and e is the margin of error (0.05) at 95% CI.

$$n = \frac{1.96 \times 1.96 (1 - 0.131) 0.131}{0.05 \times 0.05} = 175$$

The required minimum sample size was 175. However, 250 patients were enrolled.

The participants were selected randomly to avoid bias. Criteria for selecting participants for this study included individuals without malaria parasitaemia (for at least 1 month), HIV seronegative, and hepatitis B and C seronegative, based on clinical information provided by the participants. All participants were screened for malaria parasitaemia using rapid diagnostic test strips (SD Bioline Malaria Ag P.f, histidine-rich protein (HRP-2) 2, Gyeonggi-do, Republic of Korea). Moreover, individuals with a history of chronic disorders such as osteoporosis, diabetes mellitus, obesity and clinical hypertension, which could cause musculoskeletal pains and pro-inflammatory conditions, were excluded. The rationale for excluding these conditions is to minimize or avoid other potential causes of myo-arthritis illness aside from CHIKV in the study participants.

2.6. Data collection

After patients passed all the selection criteria, pretested interviewer-based questionnaires were used to obtain socio-demographic information (gender, age, educational level, occupation, and residential area). We developed and validated a KAP (Knowledge, Attitude, and Practice) questionnaire for CHIKV by defining the study scope through a literature review and drafting items using a 5-point Likert scale. We assessed questionnaire content validity via expert panels, conducted a pilot test, and ran reliability tests.

Questions on knowledge variables (such as type of infection, symptoms, route of transmission, and sources of information on CHIKV), attitude (such as perceived severity of CHIK, personal vulnerability to CHIKV, responsibility for control against CHIKV, confidence in prevention against mosquito bites, and concern over CHIKV outbreaks) and practice (frequency or adherence using options like 1 = Rarely/Never, 2 = Sometimes, and 3 = Always/Often) by all the participants.

2.7. Sample collection and processing

Five millilitres of venous blood were collected from all the participants and centrifuged at 3000 revolutions per minute for 10 minutes to obtain serum samples. Samples were processed in batches within 4 hours of collection. Extracted sera were stored at -80 °C after adding aprotinin to prevent endogenous enzymes from breaking down fragile biochemicals during collection, processing, and storage, as this preserves sample integrity for accurate diagnostic and research assays.

2.8. Laboratory analyses

Of the 372 patients initially screened for this study, 32.8% (n=122) had malaria antigenemia, leaving 250 eligible malaria-seronegative participants who were eventually enrolled for this study.

2.8.1. Detection of chikungunya virus IgM & IgG antibodies

All serum samples were investigated for the presence of anti-CHIKV IgM (ESR148M) and IgG (ESR148G) using the Institut Virion/Serion ELISA kits (Institut Virion/Serion, Würzburg, Germany) according to the manufacturer's instructions. The anti-CHIKV IgM uses μ -capture ELISA technology to improve IgM specificity, and the microtiter wells contain recombinant CHIKV-like particles (VLPs) containing structural proteins E1, E2, and capsid. It provides high specificity (>99%) and a reported sensitivity of 91.7% and is designed for the diagnosis of acute infections. The Anti-CHKV indirect ELISA offers excellent diagnostic efficiency for detecting chronic infection or past exposure and is often used for the differential diagnosis of VLPs, achieving >99% sensitivity and >99% specificity. The ELISA kits are equipped with an internal negative control and standard sera, which consist of human serum prepared in a protein-containing phosphate buffer. Based on the kit manufacturer, these components have been specifically tested and confirmed to be negative for the presence of antibodies against HIV (anti-HIV antibodies), Hepatitis B virus surface antigen (HBs-Ag), and antibodies against Hepatitis C virus (anti-HCV antibodies). This ensures the assay's reliability and accuracy by providing baseline references that exclude cross-reactivity and false-positive results caused by these viral markers. The inclusion of such controls and standards is critical for validating the specificity and sensitivity of the ELISA tests in detecting target antibodies in clinical or research samples. Interpretation of results from optical density (OD) measurements of CHIKV antibodies at 405nm was based on the manufacturer's instructions.

After passing all the internal control measures provided by the kit manufacturer, SERION ® recommended the interpretation of anti-CHIKV ELISA results as follows:

- a. Seropositive: OD > 0.49 for IgM and OD > 0.18 for IgG
- b. Seronegative: OD < 0.34 for IgM and OD < 0.13 for IgG
- c. Borderline: Between 0.34 and 0.47 for IgM; between 0.13 and 0.18 for IgG

Borderline (or equivocal) ELISA results for anti-CHIKV IgM and IgG were classified as a zone of uncertainty where antibody levels are near the assay cut-off (neither definitively positive nor negative). These results are not used to confirm an infection and require a follow-up convalescent blood sample or PCR.

2.8.2. Chikungunya Virus RNA Detection

For viral RNA extraction, 28 µl of each serum sample was dispensed into an Eppendorf tube containing 112 µl of extraction fluid, resulting in a total mixture volume of 140 µl (1:4 ratio). The extraction was performed using the Qiagen QIAamp Viral RNA mini spin column kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Following the binding and washing steps, RNA was eluted from the spin columns in a final volume of 60 µl of elution buffer (Qiagen, Hilden, Germany). Serum samples that tested positive or borderline for CHIKV-specific IgM and IgG antibodies were stored at -80 °C prior to RNA extraction.

Reverse transcription-quantitative polymerase chain reaction (qRT-qPCR) was conducted using the TaqMan (probe-based) protocol to detect and quantify CHIKV RNA on a QuantStudio real-time PCR system (Applied Biosystems) using the following mix preparation: 12.5 µL of 2× reaction master mix was combined with 0.5 µL of enzyme/RT mix, 0.5 µL forward and 0.5 µL reverse primers (final concentration 0.4 µM each), 0.3 µL probe (0.2 µM), 5 µL of extracted viral RNA template, and nuclease-free water was added to bring the entire mixture to 25 µL. A CHIKV E1 marker (strain 37997, recombinant protein from HEK293 cells) positive control. The assay targeted a 208-base-pair fragment of the CHIKV E1 gene, corresponding to genome positions 10387 to 10595. The oligonucleotide primers and a probe targeting the CHIKV E1 gene were used as previously described by Pastorino *et al* ¹⁸. Specifically, the forward primer CHIKV-E1-F (5'-AAGCTYCGCGTCCTTTACCAAG-3'), the reverse primer CHIKV-E1-R (5'-CCAAATTGTCCYGGTCTTCCT-3'), and the probe (5'-CCAATGTCYTCMGCCTGGACACCTTT-3') were utilized.

The PCR conditions were based on those previously described by Masrinoul *et al*¹⁹ as follows: reverse transcription at 50°C for 30 min; enzyme inactivation at 95°C for 15 min; 44 cycles of denaturation at 95°C for 20 sec, annealing at 55°C for 30 sec, and extension at 72°C for 30 sec; followed by a final extension at 65°C for 5 sec. Viral RNA quantification used cycle threshold (CT) values compared to a standard synthetic RNA, with viral genome copies correlated to PFU/ml. A cut-off value of $Ct \leq 37$ was chosen (values from 10 to 36 were considered positive). Though not necessary, a second check on the size of the CHIKV E1 gene after a positive RT-qPCR was done (Supplementary Figure S1). The amplicons from samples and the positive control were run on a 2% agarose gel with Tris-acetate-EDTA buffer. Each amplicon was mixed with 5 µl loading dye before loading. The gel was run at 100 V for 30 minutes and visualized using a UV Invitrogen Safe Imager (BioRad Gel Doc).

2.9. Statistical Analysis

Data were reported in frequencies and percentages for categorical variables. Tables and charts were plotted, and the association between categorical variables (such as knowledge, attitude and practice regarding CHIKV, risk factors, and socio-demographic variables of participants) and CHIKV prevalence was assessed at 95% confidence intervals (CIs).

Association levels were assessed for knowledge of CHIKV and practice using the Chi-square test (Monte Carlo) and Fisher's exact test. To determine factors independently linked to binary outcomes, a multivariate logistic regression analysis was conducted. To adjust for confounders, we selected candidate confounders through bivariate screening, chose a regression framework, validated model fit and performed multicollinearity checks. Generalized linear mixed models were used to estimate adjusted odds ratios after bivariate and chi-square analyses to adjust for confounders in CHIKV seroprevalence. Multicollinearity was assessed when predictor variables were highly correlated by examining correlation matrices among predictors. All

analyses used R 4.2.2 with complete case analysis, and no model assumption violations were detected. Tests were two-tailed, and *p*-values below 0.05 indicated statistical significance.

3.0. RESULTS

Of the 250 participants with arthritogenic illnesses, 56.8% were male, and 43.2% were female. The majority were between 20 and 40 years old (49.6%). Most participants reside in rural areas (42.4%) and had only a primary education (43.2%) (Supplementary table S1). Only 70 (28%) knew about CHIK. Of those who knew CHIK, the majority (48.6%) learnt about it from newspapers/print media, and social media (27.1%). Only 55.7% knew about the mode of CHIKV transmission being mosquito bites, while 70% know about the availability of the CHIKV vaccine (Table 1). Data on participants' knowledge of CHIKV symptoms showed that 41.1% and 30% recognised joint and muscle pains as attributes of the infection, respectively. However, all 70 (100%) participants knew that fever is a sign of CHIKV infection (Table 1). Ninety participants (36%) indicated that they never wear long trousers, shirts, or shirts covering all their limbs (Table 1). However, 19.6% wear these clothing styles daily. About 65.2% of the participants indicated they never used indoor insecticide spray, while 14% applied insecticides daily in the houses. About 71.2% of the participants store water in open containers within their houses. Similarly, 71.2% live near artificial water collectors, while 75.5% of participants reside near bushes ≤ 100 meters from their houses (Table 1).

Table 1. Knowledge, attitudes and preventive practices against mosquito bites among participants and chikungunya

Question	Response	n (%)
Knowledge		
Ever heard of CHIK?	Yes	70 (28)
	No	101 (40.4)
	No response	79 (31.6)
Source of information (n=70)	Newspapers / Print media	34 (48.6)
	Social media	19 (27.1)
	Healthcare facility	10 (14.3)
	Friends / Family	7 (10)
Do you know about the mosquito transmission of CHIKV? (n=70)	Yes	39 (55.7)

	No	35 (44.3)
Do you know about the CHIKV vaccine? (n=70)	Yes	50 (70)
	No	20 (30)
Recognizes signs and symptoms (n=70)	Joint pain	29 (41.1)
	Fever	70 (100)
	Muscle pain	21 (30)
	Stomach ache	3 (4.3)
Attitudes and Preventive Practice		
Wears long trousers, shirts and skirts covering all the limbs	Never	90 (36)
	1-3 times per week	41 (16.4)
	4-5 times per week	66 (26.4)
	Daily	49 (19.6)
	No response	4 (1.6)
Uses indoor insecticide spray	Never	163 (65.2)
	1-3 times per week	28 (11.2)
	4-5 times per week	21 (8.4)
	Daily	35 (14)
	No response	3 (1.2)
Stores water in open containers within the house	Yes	178 (71.2)
	No	63 (25.2)
	No response	9 (3.6)
Reside near artificial water collectors	Yes	178 (71.2)
	No	53 (21.2)
	No response	19 (7.6)
Reside near bushes or dumpsites $\leq$ 100meters from your houses	Yes	189 (75.6)
	No	61 (24.4)

The use of long trousers, shirts and skirts at least 1-3 times per week in all outdoor activities was significantly associated with the knowledge of CHIKV ($p=0.0035$) and its vaccine ($p=0.0144$). Moreover, Daily storage of water in open containers within the house and the presence of bushes or dumpsites $\leq$ 100 meters from participants' residential houses were significantly related to CHIKV knowledge and the time of CHIKV mosquito bites ($p < 0.05$) (Table 2).

Table 2: Associations between knowledge, attitude variables and preventive practices against mosquito bites and CHIKV infection

Knowledge	Practice	Test	<i>p</i> value
Have you ever heard of CHIKV	Use of indoor insecticide sprays at least 1-3 times per week	Chi-square (Monte Carlo)	0.5419

Do you know about the mosquito transmission of CHIKV?	Use of indoor insecticide sprays at least 1-3 times per week	Chi-square (Monte Carlo)	0.2014
Do you know about the CHIKV vaccine?	Use of indoor insecticide sprays at least 1-3 times per week	Chi-square (Monte Carlo)	0.7113
Do you know the time of a CHIK mosquito bite?	Use of indoor insecticide sprays at least 1-3 times per week	Chi-square (Monte Carlo)	0.351
Have you ever heard of CHIKV	Use of long trousers, shirts and skirts at least 1-3 times per week in all outdoor activities	Chi-square (Monte Carlo)	0.1043
Do you know about the mosquito transmission of CHIKV?	Use of long trousers, shirts and skirts at least 1-3 times per week in all outdoor activities	Chi-square (Monte Carlo)	0.3088
Do you know about the CHIKV vaccine?	Use of long trousers, shirts and skirts at least 1-3 times per week in all outdoor activities	Chi-square (Monte Carlo)	0.0144*
Do you know the time of a CHIK mosquito bite?	Use of long trousers, shirts and skirts at least 1-3 times per week in all outdoor activities	Chi-square (Monte Carlo)	0.2331
Have you ever heard of CHIKV	Daily storage of water proximal to rooms or the house entrance (≤ 100 meters)	Fisher's Exact	0.0035*
Do you know about the mosquito transmission of CHIKV?	Daily storage of water proximal to rooms or the house entrance (≤ 10 meters)	Fisher's Exact	0.1728
Do you know about the CHIKV vaccine?	Daily storage of water in open containers within the house	Fisher's Exact	0.0125*
Do you know the time of a CHIK mosquito bite?	Daily storage of water in open containers within the house	Fisher's Exact	0.5342
Have you ever heard of CHIKV	Presence of bushes or dumpsites ≤ 100 meters from your houses	Fisher's Exact	0.3137
Do you know about the mosquito transmission of CHIKV?	Presence of bushes or dumpsites ≤ 100 meters from your houses	Fisher's Exact	0.273
Do you know about the CHIKV vaccine?	Presence of bushes or dumpsites ≤ 100 meters from your houses	Fisher's Exact	0.0011*
Do you know the time of a CHIKV mosquito bite?	Presence of bushes or dumpsites ≤ 100 meters from your houses	Fisher's Exact	0.022*

Knowledge of mosquito transmission of CHIKV positively correlated with the use of long trousers and shirts ($r=0.16$), while knowledge of CHIKV strongly correlated with knowledge of mosquito transmission of CHIKV ($r=0.37$) (Figure 1).

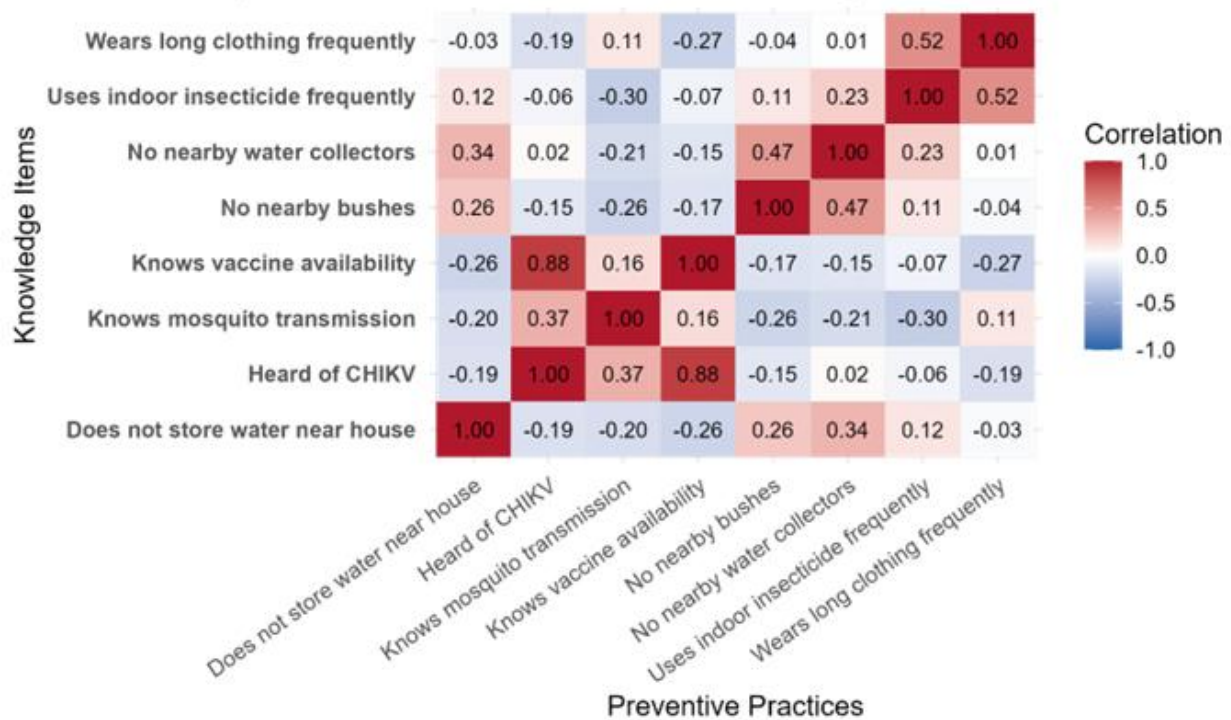


Figure 1: Polychoric correlation between Knowledge and prevention practices

The time range for the myoarthritogenic syndromes among the patients was 3 to 10 days, and the prevalence of CHIKV infections was 13.6% (n=34) by RT-qPCR, of which 2.8% (7/250) were co-positive for anti-CHIKV IgM and IgG (Figure 2). The seroprevalence of recent CHIKV infection (IgM) was 10% (25/250), while anti-CHIKV IgG was 12% (30/250) (Figure 2).

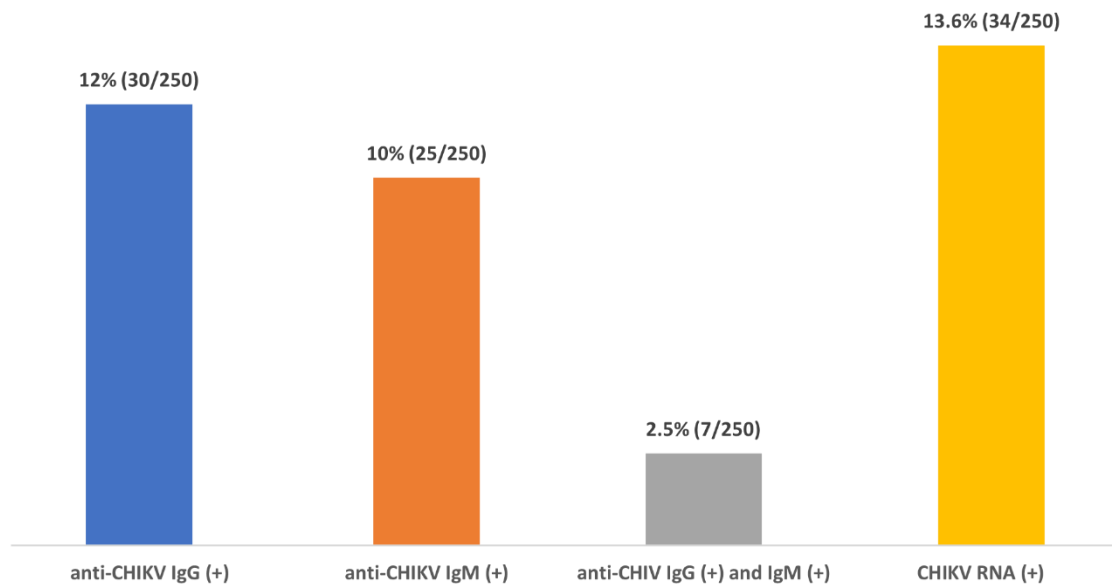


Fig 2. Prevalence of CHIKV RNA detection and antibody among patients with myo-arthritogenic illnesses

After adjusting for potential confounders, residence in urban areas (AOR=0.61) was associated with lower odds of anti-CHIKV IgM compared to rural residence. Moreover, higher monthly income earners (41–80 USD) had lower odds (AOR=0.61) of anti-CHIKV IgM compared to those with lower income (<20 USD). Females showed a trend toward higher odds (AOR = 2.64) than males (Table 3).

Table 3. Multivariate logistic regression analysis of sociodemographic variables associated with anti-CHIKV seropositivity

Category	Anti-CHIK IgM			Anti-CHIKV IgG			Anti-CHIKV IgM+IgG		
	n (%)	AOR (95% CI)	p value	n (%)	AOR (95% CI)	p value	n (%)	AOR (95% CI)	p value
Biological sex									
Male	12 (8.5)	1	—	18 (12.7)	1	—	2 (1.4)	1	—
Female	13 (12)	2.64 (0.84–9.06)	0.105	12 (11.1)	1.04 (0.35–3.08)	0.950	5 (4.6)	17.62 (1.18–1502.13)	0.107
Age (years)									
20–40	14 (11.3)	1	—	15 (12.1)	1	—	4 (3.2)	1	—
<20	3 (7.3)	0.69 (0.12–2.97)	0.644	5 (12.2)	1.36 (0.34–4.75)	0.643	1 (2.4)	0.35 (0–7.68)	0.587
>40	8 (9.4)	1.02 (0.31–3.14)	0.968	10 (11.8)	0.84 (0.29–2.32)	0.744	2 (2.4)	0.64 (0.06–4.85)	0.675
Residence									
Rural	10 (9.4)	1	—	18 (17.0)	1	—	3 (2.8)	1	—
Suburb	11 (12.5)	1.62 (0.53–5.16)	0.398	8 (9.1)	0.42 (0.14–1.17)	0.104	2 (2.3)	0.91 (0.08–9.60)	0.932
Urban	4 (7.1)	0.61 (0.12–2.66)	0.518	4 (7.1)	0.30 (0.07–1.08)	0.080	2 (3.6)	1.10 (0.08–12.82)	0.941
Education level									
Non-formal	6 (12.77)	1	—	8 (17.02)	1	—	2 (4.26)	1	—
Primary	8 (7.41)	0.49 (0.12–1.93)	0.307	13 (12.04)	0.82 (0.25–2.63)	0.729	2 (1.85)	0.31 (0.02–4.04)	0.373
Secondary	9 (11.25)	1.34 (0.27–6.46)	0.712	7 (8.75)	0.56 (0.12–2.45)	0.445	3 (3.75)	1.69 (0.09–25.83)	0.705
Tertiary	2 (15.38)	1.24 (0.11–10.78)	0.850	2 (15.38)	1.26 (0.13–9.21)	0.827	2 (15.38)	0 (NA–1.69e ⁺¹⁶⁹)	0.997
Occupation status									
Farmer	4 (9.76)	1	—	7 (17.07)	1	—	1 (2.44)	1	—
Driver	5 (18.52)	1.97 (0.44–9.46)	0.376	3 (11.11)	0.64 (0.12–2.79)	0.571	2 (7.41)	2.05 (0.12–57.23)	0.616
Housewife	6 (8.11)	0.41 (0.07–2.57)	0.335	9 (12.16)	1.13 (0.24–5.31)	0.879	3 (4.05)	0.17 (0–9.24)	0.384
Civil servant	5 (7.69)	0.60 (0.10–3.86)	0.581	6 (9.23)	0.76 (0.16–3.57)	0.728	1 (1.54)	0.05 (0–4.13)	0.201
Unemployed	5 (13.89)	1.08 (0.20–6.02)	0.924	5 (13.89)	1.43 (0.32–6.19)	0.630	0 (0)	0 (NA–1.27e ⁺¹¹⁹)	0.995
Monthly income									
<20 USD	13 (14.13)	1	—	12 (13.04)	1	—	2 (2.17)	1	—
20–40 USD	8 (11.59)	0.90 (0.29–2.67)	0.851	8 (11.59)	1.61 (0.54–4.72)	0.382	3 (4.35)	1.89 (0.24–17.47)	0.541
41–80 USD	4 (8.51)	0.61 (0.12–2.59)	0.519	7 (14.89)	3.15 (0.86–11.42)	0.079	7 (14.89)	1.91 (0.13–23.75)	0.609
>80 USD	0 (0)	0 (0–5.28e ⁺¹⁹)	0.991	3 (17.65)	3.89 (0.62–21.33)	0.123	0 (0)	0 (NA–1.85e ⁺¹¹⁹)	0.997

Table 4. Risk determinants of anti-CHIKV seropositivity among patients with arthritogenic illnesses

Category	Anti-CHIK IgM			Anti-CHIKV IgG			Anti-CHIKV IgM+IgG		
	n (%)	AOR (95% CI)	p value	n (%)	AOR (95% CI)	p value	n (%)	AOR (95% CI)	p value
Household Density									
<5 people	13 (14.13)	1	—	12 (13.04)	1	—	2 (2.17)	1	—
5–8 people	8 (11.59)	0.63 (0.20–1.77)	0.397	8 (11.59)	1.67 (0.68–4.08)	0.257	3 (4.35)	0.85 (0.11–4.69)	0.862
≥ 9 people	4 (8.51)	1.62 (0.38–5.85)	0.478	7 (14.89)	0.50 (0.07–2.09)	0.395	2 (4.26)	0 (NA–7.16e ⁺¹²⁰)	0.995
Knowledge of Mosquito Bite Time									
Day	17 (8.99)	1	—	20 (10.58)	1	—	6 (3.17)	1	—
Night	6 (17.14)	0.99 (0.31–3.14)	0.992	9 (25.71)	0.64 (0.21–1.82)	0.412	1 (2.86)	1.78 (0.28–11.76)	0.531
Both	1 (20)	0.54 (0.17–1.67)	0.289	0 (0)	0.70 (0.26–1.83)	0.467	0 (0)	0.18 (0.01–1.72)	0.178
Uses indoor insecticide spray									
Never	13 (7.98)	1	—	19 (11.66)	1	—	19 (11.66)	1	—
1–3 times per week	3 (10.71)	1.23 (0.24–4.93)	0.779	3 (10.71)	0.49 (0.07–2.22)	0.408	0 (0)	0 (NA–5.14e ⁺¹³⁰)	0.996
4–5 times per week	2 (9.52)	0.75 (0.10–3.62)	0.746	3 (14.29)	0.99 (0.20–3.73)	0.983	0 (0)	0 (NA–4.24e ⁺¹⁴⁴)	0.996
Daily	7 (20)	2.41 (0.61–9.51)	0.205	5 (14.29)	1.00 (0.22–4.15)	0.999	2 (5.71)	2.15 (0.10–49.67)	0.637
Do you store water in open containers within the house?									
No	7 (6.67)	1	—	8 (7.62)	1	—	2 (1.9)	1	—
Yes	18 (13.64)	1.26 (0.46–3.29)	0.645	22 (16.67)	1.27 (0.49–3.13)	0.607	5 (3.79)	2.35 (0.38–13.63)	0.335
Do you reside ≤ 100 meters of bushes or dumpsites?									
No	14 (7.41)	1	—	25 (13.23)	1	—	5 (2.65)	1	—
Yes	11 (18.64)	3.60 (1.32–10.02)	0.012	5 (8.47)	0.49 (0.14–1.40)	0.210	2 (3.39)	2.15 (0.22–15.72)	0.462
Do you frequently wear long clothing?									
No	4 (4.4)	1	—	6 (6.59)	1	—	1 (1.1)	1	—
Rarely	5 (11.9)	1.51 (0.32–7.32)	0.600	5 (11.9)	2.66 (0.62–11.48)	0.181	1 (2.38)	1.66 (0.05–58.29)	0.760
Sometimes	8 (11.94)	2.81 (0.78–11.82)	0.127	11 (16.42)	2.59 (0.87–8.39)	0.094	3 (4.48)	5.36 (0.60–118.47)	0.170
Always	8 (16)	2.97 (0.62–15.43)	0.177	8 (16)	2.27 (0.51–10.16)	0.279	2 (4)	2.51 (0.09–107.54)	0.602

Residences close to bushes or dumpsites ≤ 100 meters from houses were significantly associated with anti-CHIKV IgM seroprevalence (OR= 3.60, 95% CI: 1.32–10.02, $p=0.012$) (Table 4). Regarding participants' occupations, civil servants had the lowest odds (AOR=0.76) of anti-CHIKV IgG seropositivity (Table 3). The analysis in Table 3 involved fitting separate adjusted logistic regression models for CHIKV IgG and IgM seropositivity as binary outcomes. For each outcome, both the knowledge score and practice score were included as continuous predictors in the same model to adjust for their mutual effects. Regression coefficients were estimated using maximum likelihood, then exponentiated to obtain AORs with 95% Wald confidence intervals. Regarding IgG seropositivity, the knowledge score showed a borderline non-significant inverse association (AOR=0.666, 95% CI: 0.413–1.020, $p=0.076$), while the practice score was not significantly associated (AOR=0.905, 95% CI: 0.554–1.398, $p=0.668$). For IgM seropositivity, the practice score had a significant positive association (AOR=1.905, 95% CI: 1.255–2.885, $p=0.002$), whereas the knowledge score showed no significant effect (AOR=0.902, 95% CI: 0.568–1.385, $p=0.649$) (Table 5).

Table 5: Adjusted logistic regression analysis of the association between Knowledge/Practice scores and CHIKV IgG/IgM seropositivity.

Outcome	Factor	AOR	95% CI	<i>p</i> value
CHIKV IgG	(Intercept)	0.237063	0.113445 to 0.474395	0.00007
CHIKV IgG	Knowledge score	0.666216	0.41279 to 1.019881	0.07604
CHIKV IgG	Practice score	0.904591	0.553531 to 1.398428	0.66849
CHIKV IgM	(Intercept)	0.070144	0.02867 to 0.156721	0.00000
CHIKV IgM	Knowledge score	0.902471	0.568399 to 1.385279	0.64872
CHIKV IgM	Practice score	1.905482	1.255353 to 2.884996	0.00217

4.0. DISCUSSIONS

CHIKV infections frequently cause incapacitating musculoskeletal disease, with chronic, often permanent, destruction of connective tissue leading to significant long-term productivity losses in the working population²⁰. A meta-analysis estimated a pooled prevalence of 40.2% for

CHIKV infection among patients with chronic arthritis ²¹. With the upsurge in cases of musculoskeletal illnesses among the young working population in Nigeria, one would wonder if the clinical conditions are connected with CHIKV ²². A pooled prevalence of anti-CHIKV IgG was estimated from 5 studies to be 44.9% in Nigeria ⁵. However, the true prevalence of CHIK may be misestimated due to inadequate surveillance, diagnostic capacity, and high rates of overlap and co-infection with other arboviruses ²³ and difficulty in distinguishing it from rheumatoid arthritis ^{24, 25}.

Very few participants in this study (28%) knew about CHIK, and a little over half knew its transmission was through mosquito bites, highlighting low public awareness and gaps in professional-based health education. This is not unexpected, as even most healthcare workers (68.8%) in a Nigerian city indicated that they never heard of arboviral infections ²⁶. The low level of CHIKV knowledge among the respondents in the present study is significantly lower than in previous studies in low- and middle-income countries (LMICs), where 67.3%, 92.5%, and 97% of respondents indicated having heard of CHIKV in Ethiopia, Bangladesh, and Sudan, respectively ^{27, 28, 29}. However, most respondents who knew CHIK learnt about it through print and social media, highlighting the relevance of these sources of information, which could be explored by local government policymakers to improve media campaigns focused on source reduction, symptom recognition, and proactive community engagement education about CHIKV and other reemerging arboviral diseases ³⁰.

To our knowledge, this is the first study in Nigeria to focus on CHIKV detection in patients with arthritogenic illnesses. Most previous studies were mainly on febrile patients without musculoskeletal symptoms and pregnant women. In the present study, we found a 10% seroprevalence of anti-CHIKV IgM and 13.6% of CHIKV RNA among patients with arthritogenic illnesses. The anti-CHIKV IgM obtained in the present study is closely similar to the 9% reported by Muhammad *et al* ³¹, but higher than the values recorded across all six

geopolitical zones in Nigeria (2.5 to 6.5%)^{31, 32, 33, 34}. However, higher anti-CHIKV IgM prevalence was reported in Nigeria (11.8 to 38.4%)^{35, 36, 37}. Conversely, the prevalence of anti-CHIKV IgG observed in the present study (12%) was similar to that reported by Kolawole *et al.* (13.1%)¹⁷ but significantly lower than that reported in many previous studies in Nigeria and other LMICs^{5, 35, 36, 37, 38}. The diversity in CHIKV seroprevalence across these studies is mainly due to differences in laboratory methods, study participants, epidemiological factors, and host-related factors³⁹. For instance, studies that used rapid diagnostic tests reported very low prevalences of anti-CHIKV IgM because many commercial RDTs exhibit low sensitivity, often falling below 50% for IgM detection in the acute phase of CHIKV infection⁴⁰. While anti-CHIKV IgG indicates prior infection, it does not rule out primary infection, in which both IgM and IgG are present after seroconversion⁴¹, as observed in 2.5% of our study participants. It is important to note that potential serological cross-reactivity between CHIKV and other alphaviruses or co-circulating arboviruses, such as O'nyong'nyong, Sindbis, and dengue virus, can cause false-positive IgM and IgG ELISA results due to shared, highly conserved structural surface glycoproteins like E1 and E2⁴². Moreover, there is a possibility of co-infection of CHIKV with other arboviruses among the patients⁴³.

Remarkably, CHIKV RNA detection was superior to anti-CHIKV IgM testing for confirming active infection in the present study, as nucleic acid amplification assays detect the virus during the first 7-8 days of symptom onset, whereas IgM enzyme immunoassays are typically not consistently detectable⁴⁴. Consequently, RT-qPCR provides accurate output for early confirmation of CHIKV and for distinguishing it from co-circulating arboviruses⁴⁵.

Findings from the present study indicate that individuals with higher knowledge of the virus are more likely to engage in protective behaviours, such as consistently wearing appropriate clothing during outdoor activities, which are significantly associated with higher knowledge

of CHIKV. This could be a relevant preventive strategy for travellers from low- or zero-CHIKV endemic regions to LMICs with high prevalence ⁴⁶.

From this study, we found that storage of water in open containers and the presence of vegetation or dump sites within 100 meters from homes are significantly associated with mosquito breeding and CHIKV seropositivity. These findings are consistent with previous reports indicating that proxies for environmental changes, such as pollution and ecological drivers, play an important role in driving the risk of CHIKV infection ^{47,48}. In our current study, a lower practice score, indicating poor preventive measures such as inadequate repellent application or poor environmental sanitation, was significantly associated with higher odds of anti-CHIKV IgM seropositivity. A small number of positive CHIKV marker outcomes were obtained, which is why there were very few statistically significant associations between the CHIKV markers and the variables studied.

A limitation of this study is its inability to differentiate the types of arthritogenic illness in patients and to perform viral sequencing of circulating strains. Genotyping of CHIKV is a critical surveillance tool that identifies circulating lineages, which directly correlate with the virus's virulence, transmission efficiency, and potential to cause severe, large-scale outbreaks. We excluded patients who were seronegative for IgG and IgM for PCR due to funding constraints in most low- and middle-income countries like ours. Notwithstanding, we acknowledge that testing both PCR and serology ensures better diagnosis. Taken together, the inability to identify alternative causes of musculoskeletal illnesses in patients who are CHIKV-negative could limit the generalizability of our findings.

5.0. CONCLUSIONS

A considerable number of patients with arthritogenic illness had active CHIKV, indicating the active transmission of CHIKV in the study area. Because CHIKV symptoms are often misdiagnosed as malaria, dengue and other tropical infections, enhanced laboratory capacity,

specifically PCR for acute detection, is crucial. Improved awareness is needed to recognize symptoms and reduce exposure, as the disease is largely managed by controlling *Aedes* mosquito populations. In high-endemic regions, there is a need to include CHIKV in national Integrated Disease Surveillance and Response systems to better track CHIKV hotspots and potentially control the likelihood of future outbreaks in the general population.

Integrating CHIKV into Nigeria's health policies is vital because overlapping symptoms and co-infections with malaria lead to widespread misdiagnosis. Policy frameworks must shift from isolated disease control to integrated febrile and musculoskeletal illness surveillance, combining vector tracking, dual-testing (serological and molecular) protocols, and unified community interventions.

Consent for publication

Not applicable

Availability of data and materials

The tables, figures, and supplementary materials presented all the data generated from this study. However, further requests could be made through the corresponding author.

Competing interests

None declared by the authors.

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Authors contribution

I.N.A: Conceptualization, Methodology, Validation, Software, Formal analysis, Data curation, Visualization, Writing – original draft, Writing – review & editing. A.O.A., H.A.A., K.U., Y.U., A.E.A., A.U.A., S.M., M.M.: Methodology, Validation, Formal analysis, Writing – original draft, Writing – review & editing; A.O.A., I.N.A.: Supervision, Project administration.

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