Medicon Agriculture & Environmental Sciences
Volume 9 Issue 2 August 2025
Article Type: Research Article
ISSN: 2972-2691

Distribution, Abundance and Diversity of Mosquito Species and Molecular Detection of Their Associated Arboviruses in Maiduguri, Nigeria

Ali Mohammed[1,2]*, Abdullahi Abubakar Biu[1], Marycelin Mandu Baba[3,4], Albert Wulari Mbaya[1] and Babagana Kachalla Kayeri[1]

[1]*Department of Veterinary Parasitology and Entomology, University of Maiduguri, Faculty of Veterinary Medicine, Maiduguri Nigeria*

[2]*Diagnostic Laboratory, Veterinary Teaching Hospital University of Maiduguri, Maiduguri, Nigeria*

[3]*Medical Laboratory Science, Faculty of Allied Sciences University of Maiduguri, Maiduguri, Nigeria*

[4]*WHO Polio Laboratory, University of Maiduguri Teaching Hospital, Nigeria*

***Corresponding Author:** Dr. Mohammed Ali (MVSc), Department of Veterinary Parasitology and Entomology, University of Maiduguri, Faculty of Veterinary Medicine, Nigeria.

Received: June 19, 2025; **Published:** August 14, 2025

Abstract

Mosquitoes are notorious arthropods, causing nuisance and serving as vectors to numerous diseases of Medical and Veterinary importance. Entomological surveys for mosquito and their infection status for arbovirus is critical for planning and deployment of proper mosquito control initiatives. Therefore, this study was carried out to investigate the species diversity, abundance and distribution of mosquitoes in Maiduguri, Borno State. Mosquitoes were collected using the Center for Disease Control (CDC) light trap, Developmental stage trap (DT), and Human Landing Collection (HLC) from July to October 2018 (peak rainy season), November 2018 to February 2019 (cold dry-harmmatan season) and February/March to May, 2019 (hot dry season). Morphological identification of the mosquitoes was done using standard keys. Mosquitoes were pooled according to genera and homogenates were propagated on Cell Line Culture. RT-PCR was used for the detection of *Flavivirus* group of arboviruses (Yellow fever virus, Dengue virus, West Nile virus and Zika virus) while Next Generation Sequencing (NGS) was also used for the amplification of virus genetic materials.

In total, 5335 adult mosquitoes were collected from all the 12 districts of Maiduguri belonging to three genera namely *Culex*, *Anopheles*, and *Aedes*. Of the 3 genera, *Culex* species 4154(77.9%) was the most abundant, compared with *Anopheles* species 947 (17.8%) while *Aedes* species 234 (4.4%) was the least. In mosquito trapping, the CDC traps had the highest catch of 2525 (47.3%) followed by DT with a total catch of 2185 (41%) while HLC had the lowest catch of 625 (11.7%) ($p<0.05$). The wet/rainy season had the highest distribution with 2499 (46.8%) for all the species of mosquitoes collected, followed by the cold-dry harmattan with 1500 (28.1%), while the hot-dry season had the lowest distribution with 1336 (25.1%). Across all seasons, mosquitoes consistently persist irrespective of species. The month of September had the highest distribution 688 (12.9%) of all mosquito species collected and May had the lowest distribution with 258 (4.8%). RT-PCR assay were negative for all *Flavivirus*. Next Generation Sequencing (NGS) using illumine Miseq revealed the presence of two important arboviruses namely *Culex Flavivirus* (CxFV) and Hubei partiti-like virus 22. The amplification of CxFV is the first report from mosquito species in nature from Nigeria. In conclusion, 3 genera of mosquito species were collected and identified in Maiduguri of which *Culex* was the most abundant and distributed.

Keywords: Mosquitoes; Arboviruses; Culex flavivirus; Nigeria

Abbreviations

CDC: Center for Disease Control.
BOSIEC: Borno State Independent Electoral Commission.
DNA: Deoxyribonucleic acid.
RNA: Ribonucleic Acid.
NCDC: Nigeria Center for Disease Control.
NAVRC: National Arbovirus and Vector Research Center.
BMLS: Borno State Ministry for Land and Survey.
FGN: Federal Government of Nigeria.
NPC: National Population Commission.
WHO: World Health Organization.
HLC: Human Landing Collection.
DT: Developmental Trap.
NGS: Next Generation Sequencing.
ELISA: Enzyme Linked Immonosorbent Assay.
RT-PCR: Real Time Polymerase Chain Reaction.
RVFV: Rift Valley Fever Virus.
DENV: Dengue Virus.
YFV: Yellow Fever Virus.
KOTV: Kotokan Virus.
BTV: Blue Tongue Virus.
WNV: West Nile Virus.
USUV: Usutu Virus.
AHSV: African Horse Sickness Virus.
CCHFV: Cremean Congo Hemorrhagic Fever Virus.
ASFV: African Swine Fever Virus.
PBS: Phosphate Buffer Solution.
FBS: Fetal Bovine Serum.
MEM: Minimum Essential Media.
CPE: Cyto-Pathic Effect.
B.P: Base Pair.
COI: Cytochrome Oxidase I.
ITS: Internal Transcriber Spacer.
RAPD: Random Amplified Polymorphic DNA.
RELP: Restriction Fragment Length Polymorphism.
SSCP: Single Strand Conformational Polymorphism.
ASA: Allele Specific Amplification.
MIB: Midgut Infectious Barrier.
MEB: Midgut Escape Barrier.
SGIB: Salivary Gland Infectious Barrier.

SGEB: Salivary Gland Escape Barrier.
BHK: Baby Hamster Kidney.
TAQ: Thermus aquaticus.
GDP: Gross Domestic Product.

Introduction

Emerging and re-emerging pathogens are a threat to global health security due to their consequences to human and animal health as well as devastation to economies. Across the world, mosquitoes are regarded as one of the major arthropod vectors of human infectious disease [6]. The distribution and abundance of mosquito species varies depending on the location [44;3]. In tropical regions of the world, season, climatic factor, geography of water supply and anthropogenic related factors have been associated with the abundance and distribution of mosquitoes [44]. Mosquitoes are known to transmit diseases and cause nuisance to humans and livestock, and some of the diseases transmitted could be parasitic, bacterial, protozoan and viral origin [7: 41]. Mosquitoe-borne diseases constantly pose a serious health threat, especially in the rural areas which are usually associated with poor sanitary conditions, agricultural practices, and irrigated agro-ecosystems thus, enhancing the diversity and density of mosquito populations [49].

Mosquitoes, lice, ticks, fleas and sandflies are known hematophagous arthropods that can transmits arthropod-borne viruses (arboviruses) to susceptible vertebrate hosts [12]. Of the over 700 known arboviruses, about 100 are known to cause infection ns in animals and humans [11]. Arboviral infections of medical importance includes flaviviral infections such as West Nile fever, dengue fever, Zika virus disease, yellow fever and Chikungunya fever virus. The latter is caused by an alphavirus and has been documented to be responsible for outbreaks in Africa, Asia, Europe and South Pacific [47]. Several factors affect the transmission dynamics of these viruses which may vary from viral genetics to vector competence as well as ecological interactions between hosts and vectors [49: 17]. In most parts of sub-Saharan Africa, *Aedes*, *Culex* and *Anopheles* spp., are responsible for transmitting mosquito-borne viral diseases as well as malaria [50:21]. Mosquitoes within the genera *Aedes* and *Culex* play an important role in the transmission of arboviruses. *Aedes aegypti* and *A. albopictus* are two species incriminated in the vast transmission of arboviruses of medical importance such as dengue virus, yellow Fever virus and Chikungunya virus [4]. Also, *Culex* species are principal vectors for numerous viruses including West Nile virus, Japanese encephalitis virus and many others [18]. On the other hand, *Anopheles* mosquitoes is the main vectors of malaria [13]. The co-circulation of these mosquito-borne viruses with other pathogens such as *Plasmodium* responsible for malaria poses some challenges in diagnosis and management [47]. Consequently, this has led to under-estimation of mosquito-borne viral infection as most febrile illness of unknown origin are regarded as malaria [27: 35]. The application of next-generation sequencing (NGS) has allowed unbiased detection of organism within a sample. This approach has powered pathogen detection for both diagnostic and surveillance applications [46].

Chikungunya (CHIK) is a viral infection caused by an Alphavirus (*Togaviridae*) transmitted between humans by *Aedes* mosquito, [10]. CHIK causes severe joint pain, however, the disease shares some clinical signs with dengue and can be misdiagnosed in areas where dengue is common, [13]. There are no vaccines or specific therapeutics since 2004, and chikungunya fever has reached epidemic proportions with considerable morbidity and suffering. On the other hand, Rift valley fever (RVF) is an emerging zoonotic disease affecting primarily domestic ruminants and also humans, it caused by a *Phlebovirus* (*Bunyaviridae*) transmitted primarily by *Aedes*, *Culex* and *Anopheles* mosquitoes [43]. Several genera and species of mosquito are able to act as vectors for transmission of the RVF virus, and the epidemics are more and more frequent in Africa and the Middle East, probably in relation with climatic changes, as well as intensified livestock trade, [25].

The study area (Maiduguri), being an urban area is characterized by anthropological activities which encourage the breeding of mosquitoes and consequently increase incidence of mosquito-borne diseases in the area. Due to their epidemiological importance, the knowledge of mosquito species in the study area and assessment of their potential as vectors of pathogens are crucial. However, there is paucity of survey on entomological monitoring of mosquitoes in Maiduguri and its environs, more over high prevalence of non-ma-

laria febrile illness transmitted by arthropods that are often mis-diagnosed and treated asymptomatically are being recorded in these regions of the study area, due to confusion stems from difficulty in clinical discrimination between different infectious pathogens, [8: 48: 40]. Therefore, study on the species composition, distribution, abundance and diversity, and their associated arboviruses in Maiduguri are necessary as information generated will be helpful in the design of appropriate control measures against the vector and arboviruses they possibly transmit. Therefore, this study was undertaken (i) to determine the abundance, distribution and diversity of mosquitoes in the study area (ii) to detect arboviruses harbored by mosquitoes identified in the study area.

Study area

This study was carried out in Maiduguri, the capital and largest urban city of Borno State, Northeastern Nigeria. Maiduguri is cosmopolitan in nature and comprises of three Local Governments out of the twenty-seven Local Governments that make up the State, i.e. Maiduguri Metropolitan, Jere and Konduga Local Government Areas. It lies between latitude 11^0 50'N and 13^0 09'N and Longitude $12^0$33'E and 15^0 50'E and has a land mass area of 75,540 square kilometers [9]. Borno State is semi-arid, with long dry season that is characterized by temperature variation between 34.4 °C and 37.8 °C from April to May. Relative humidity ranges between 5% for the months of December and January, and 45% for the months of June through August [15]. Annual rainfall ranges between 500 and 1000 mm per annum beginning from June to September in the North and May to October in the South [16]. The population of the State is about 4,151,193 people, consisting mostly of farmers, animal herders, fishermen, traders and civil servants, all sparsely distributed over the large land mass [37].

Study design, site description and sample collection

Convenient random sampling was adopted for the collection of mosquitoes from all study areas. From the selected twelve (12) wards from which samples were collected, a further five (5) units or sampling points were selected for each ward. In total, sixty (60) sites/units were used for sample collection throughout all the sampling areas. Adult mosquitoes were collected using Center for Disease Control (CDC) light traps, human landing collection and developmental traps for both endophilic (indoor) and exophilic (outdoor) mosquitoes. Adult mosquitoes were collected between July and October, 2018 (peak rainy season), November, 2018 and February, 2019 (cold dry-harmattan season) and February/March to May, 2019 (hot dry season). The CDC light trap was placed in each house hold, and in each of the rooms where sampling/collection is to be carried out, with the trap suspended from the roof very close to the bed net and about 2 meters above the floor. The collection was done twice weekly with the trap being switched on at 06:00 p.m. local time and off at 05:00 a.m. local time. Mosquitoes were retrieved from the traps early in the morning and transported to the laboratory. Also, breeding sites were visited and both the larval and pupal stages of mosquitoes were harvested and later reared in the Veterinary Parasitology and Entomology laboratory, University of Maiduguri, to their adult stages for further study. Additionally, human landing collection (HLC) was carried out around 5:00-7:00 hours a.m. and 4:00-6:00 p.m. in areas suspected to harbor *Aedes* species. Human baits were used with the trousers raised above the ankle to allow *Aedes* mosquito settled for blood feeding to be gently trapped and or collected into a container. All collected samples were placed into well labeled plastic vials, and stored at-80 °C for further studies.

Morphological Identification of Mosquitoes

Morphological identification was carried out in the Department of Zoology, Faculty of Life Sciences, Ahmadu Bello University (ABU) Zaria, Kaduna State. Adult mosquitoes were identified at the genus level on the basis of their morphological characteristics with the aid of a dissecting microscope (Model: Motic SMZ 140 series) at x 40 and guided by the taxonomic keys of Hopkins and Highton [22: 45].

Homogenization of Mosquitoes

After identification, mosquitoes were sorted and pooled (minimum of 50 mosquitoes per pool) by genus and collection sites. 1 - 2 mL diameter stainless steel beads were placed inside the Eppendorf tube with the pool of mosquitoes and 1.5 mls of Phosphate Buffer Solution (PBS) containing Gentamycin (10 µg/mL) was added to each tube. Samples were homogenize using Bullet blender for 3 min-

utes at 800 cycles/minutes, using a mixer-mill which shakes the tubes at high velocity and metal beads inside each tube to disrupts the mosquito tissue. The supernatant was then harvested by centrifugation in a refrigerated bench-top centrifuge at 3000 rpm for 30 minutes and stored in 2 mL Eppendorf tubes at -80 °C for testing until inoculated into Vero cells and Baby Hamster Kidney cells (BHK-21). All procedure was carried out under controlled temperature with reagents maintained at 4°C.

Cell culture inoculation

Virus isolation using cell line culture was evaluated at the World Health Organization (WHO) Polio Laboratory, University of Maiduguri Teaching Hospital. All homogenates from the mosquito pool supernatants were screened for viruses by cell culture inoculation using Vero cells and Baby Hamster Kidney (BHK-21). Exactly 200 µL of supernatant from each processed mosquito pool (about 20 pools) was inoculated into tissue culture flask with each tissue culture flasks representing each pool of mosquitoes. Each tissue flask contained a monolayer of Vero cell cultures in growth medium (Minimum Essential Medium (MEM) with Earles salts, with 5% Fetal Bovine Serum (FBS), 2% Glutamine, 100 units/ml of penicillin, 100 ug/ml streptomycin and 1 µL/ml of Fungizone) and BHK-21. Inoculated cultures were incubated at 37 °C for one hour to allow virus adsorption. 1 mL of Maintenance medium was then added (MEM with Earles salts with 5% FBS, 2% Glutamine, 100 units/ml of penicillin, 100 ug/ml streptomycin and 1 µL/ml of Fungizone). Cells were incubated at 37 °C and observed daily for cytopathic changes from 2nd day post-inoculation up to 14 days. Cell culture supernatants were harvested when cytopathic effect (CPE) involving 50% of the cell monolayer was observed. About 1-2 mL of media from virus-positive cell cultures was placed into labeled cryotubes and stored at -80°C.

Total Viral RNA Extraction and cDNA Synthesis

Molecular detection was conducted at the North-East Zonal Biotechnology Center of Excellence, University of Maiduguri. Viral RNA was extracted from pooled mosquito homogenate using the QIA® amp Viral RNA Mini kit (QIAGEN, Hilden, Germany) according to manufacturer's recommendations. Briefly, 140 µL of supernatant were added to 560 µL AVL buffer containing carrier RNA into a 1.5 mL micro-centrifuge tube and mixed by pulse-vortexing for 15 seconds followed by incubation at room temperature for 10 minutes. Protein precipitation was followed by adding 560 µL of absolute alcohol mixed by pulse-vortexing for 15 seconds. The lysate was then passed through a silicate column, and the column was washed twice with 750 µL of washing buffers AWl and AW2, respectively. Finally, RNAs were carefully eluted by 60 µL of buffer AVE equilibrated to room temperature and the extracted mosquito RNA were stored in -80 °C at the University of Maiduguri Teaching Hospital, World Health Organization (WHO) Polio Laboratory before amplification. To convert extracted RNA into cDNA, 4 µL of RNA template was combined into 16 µL of a master-mix containing 4 µL of 5 x Reaction buffer, 2 µL of Transcript Enzyme Mixture and l0 µL nuclease free water; the whole volume was brought up to 20 µL. The tube contents were mixed by vortexing for 15 seconds and incubated in the thermocycler at the following conditions: 25 °C for 10 minutes, 42 °C for 60 minutes, and the reaction was terminated at 85 °C for 5 minutes and 4 °C as hold temperature.

Arbovirus Detection by reverse transcription-PCR (RT-PCR) and Gel Electrophoresis

Detection of mosquito borne arboviruses by tissue culture was conducted as described by [5] and [39]. Briefly, the cDNA amplicons were used for RT-PCR amplification (Sigma-Aldrich^{R} Viral RNA Oligos) using the universal primer targeting *Flavivirus* genera of arboviruses, (Yellow fever virus, Dengue virus, West Nile virus and Zika virus). A total of 25 µL reaction mixture containing 12.5 µL of 2x dream Taq green PCR master mix (Thermo Scientific), 0.5 µL of both forward and reverse primers, 2 µL of cDNA and 9.5 µL nuclease free water were constituted into a 25 µL volume. The PCR cycling was performed as follows with initial denaturation step at 94 °C for 5 minutes, followed by 35 cycles of denaturation at 94 °C for 10 seconds, annealing temperature at 54 °C for 30 seconds each extension at 68 °C for 30 seconds. The reaction mixture in each PCR tube was then subjected to a final extension step at 68 °C for 5 minutes, and thermal profiles were performed on *Eppendorf master cycler nexus*. Electrophoresis of the amplified DNA products was done on a 1.5 2% Agarose gel in Tris-borate EDTA buffer stained with Gel Red. The PCR product bands were visualized by a UV transilluminator and recorded using a gel photo imaging system.

Next-Generation Sequencing and Bioinformatic Analysis

Virus isolates obtained from mosquitoes grown on BHK and Vero cell line were sequenced based on established unbiased protocol [30]. Briefly, RNA was extracted using QIAamp Viral RNA extraction kit (Qiagen, Hilden, Germany) according to manufacturer's instructions. Extracted RNA was turbo Dnased to remove contaminating DNA and cDNA synthesis was carried out. Sequencing libraries were made using illumina nextera XT kit. Next generation sequencing was carried out on illumina Miseq at African Centre of Excellence for Genomics of infectious Diseases (ACEGID). The sequences were further used for Basic Local Alignment Search Tool (BLAST) in National Center for Biotechnology Information (NCBI) database using the BLASTn with default setting for highly similar sequences deposited in the GenBank. Phylogenetic analysis was carried out to determine the evolutionary relationship of the sequences of the Hubei virus and *Culex flavivirus*. The Molecular Evolutionary Genetic Analysis (MEGA 6.06) was used, [46]. The sequences of the genes from this study were copied separately to notepad along with similar sequences of the respective genes from GenBank as determined from BLASTn search and then saved in FASTA format. Thus, Hubei virus (-3145.89) and *Culex flavivirus* (-25554.07) were imported into MEGA and subjected to multiple sequence alignment (MSA) using the cluster W algorithm for pair wise alignment. Genetic distance between pairs of sequence was calculated using maximum composite likelihood model UPGMA (Unweighted Pair Group Method with Arithmetic Mean).

Data Analysis

Collected samples were pooled and mosquito abundance was computed using simple percentile, mosquito distribution was evaluated using Chi-square correlation while the diversity of mosquito was computed using Simpson's diversity index $Di=l-\Sigma n\ (n-1)\ /\ N(N-1)$ on a scale of 0-1, where 0 is indicating uniformity and 1 is showing complete diversity. The cut-off point for diversity used was 0.20 to 0.39 for low diversity, 0.40 to 0.59 for moderate diversity and greater than 0.60 for high diversity. Where Di = is for diversity index, n = is the number of entities belonging to a particular type, and N = is the total number of entities in the data set. Two-way analysis of variance was used to compare the data between groups (species) and value $p<0.05$ was considered significant [17].

Results

Abundance and Diversity of Mosquitoes in some Maiduguri Communities

The results on the abundance and diversity of mosquito species collected from different locations within the study area are presented in table (2). A total of 5335 mosquitoes was collected comprising of three genera across the different locations within the study areas. Based on the sampled wards, *Culex* species had the highest abundance with 4154 (77.9%). There was a significant association between abundance and different wards where samples were collected ($p=0.0038$). *Culex* species had the highest abundance 330 (88.5%) in Abbaganaram ward and the least 282 (64.7%) in Zabarmari ward. There was a significant association between abundance and sampled wards ($p=0.0004$). Lastly, *Anopheles* species had the highest abundance of 133 (30.5%) in Zabarmari ward, with the least 29 (7.8%) in Abbaganaram ward (Table 2). There was a significant association between abundance and the sampled wards ($p=0.005$). The three species of mosquitoes; Aedes, *Anopheles* and *Culex* showed various degrees of uniformity in relation to Diversity index (Di) where *Aedes* (0.01), *Anopheles* (0.03) and *Culex* (0.61), all values less than one (1) were uniform. However, the diversity of mosquitoes within study wards has also shown uniformity as all values were less than one. Diversity within wards indicated that there was complete uniformity for all species while diversity within species also showed uniformity for the three species ($Di<1$).

Oligo Name	*Sequence (5'-3')*	*Length, base pair*	*Reference*
CFD2	GTGTCCCAGCCGGCGGTGCATCAGC	260	Smith *et al*., 2009
FS 778	AARGGHAGYMCDGCHATHTGGT	220	Testuro *et al*., 2012
MAMD	AACATGATGGGRAARAGRGARAA	230	Weidman *et al*., 2010

Table 1: DNA Primers and Probes used for RT-PCR *Flavivirus* Detection.

Wards	*Abundance (%)*			*Diversity Index (Di within wards)*	*Total abundance (%)*
	Aedes	*Culex*	*Anopheles*		
Abaganaram	14 (3.8)	330 (88.5)	29 (7.8)	0.21	373 (6.9)
Adam Kolo	12 (3.5)	290 (85.3)	38 (11.2)	0.26	340 (6.4)
Bolori	14 (3.9)	288 (80.0)	58 (16.1)	0.33	360 (6.7)
Bulunkutu	27 (6.2)	360 (82.4)	50 (11.4)	0.31	437 (8.2)
Custom	11 (2.1)	384 (73.1)	130 (24.8)	0.40	525 (9.8)
Dala	11 (3.0)	282 (77.7)	70 (19.3)	0.36	363 (6.8)
Gamboru	12 (2.3)	461 (87.8)	52 (9.9)	0.22	525 (9.8)
Gwange	44 (6.0)	536 (73.5)	149 (20.4)	0.41	729 (13.7)
Kullolori	29 (5.9)	364 (74.3)	97 (19.8)	0.41	490 (9.2)
Muna	23 (5.7)	306 (75.6)	76 (18.8)	0.39	405 (7.6)
Polo	16 (4.6)	271 (77.0)	65 (18.5)	0.37	352 (6.6)
Zabarmari	21 (4.8)	282 (64.7)	133 (30.5)	0.49	436 (8.2)
Total	234 (44)	4154 (77.9)	947 (17.8)		5335 (100.0)
Di	0.01	0.61	0.03		

Inference for Di = values less than 1 = complete uniformity.

values ≥ 1= complete diversity.

Table 2: Abundance and Diversity of Mosquitoes in Sampled Wards of Maiduguri Metropolis.

Seasonal Distribution of Mosquito Species amongst Wards in Maiduguri

The wet/rainy season had the highest distribution of 2499 (46.8%) for all species of mosquitoes collected, followed by the cold-dry harmattan with 1500 (28.1%), while the hot-dry season had the lowest distribution with 1336 (25.1%) (Figure 2). Across all seasons, Gwange ward consistently had the highest distribution of mosquitoes irrespective of species. Furthermore, in both wet/rainy season and cold-dry harmattan Adam Kolo had the lowest distribution of mosquitoes collected. Similarly, in the hot-dry season, Dala had the lowest distribution of mosquitoes collected. With respect to species of mosquitoes, *Culex* species was consistently the most highly distributed species of mosquitoes collected, *Anopheles* species were most highly distributed in the wet/rainy season in comparison to the cold-dry harmattan or hot dry season. Additionally, *Aedes* species appeared to have the highest distribution in the wet/rainy season compared to cold-dry harmattan and dry season.

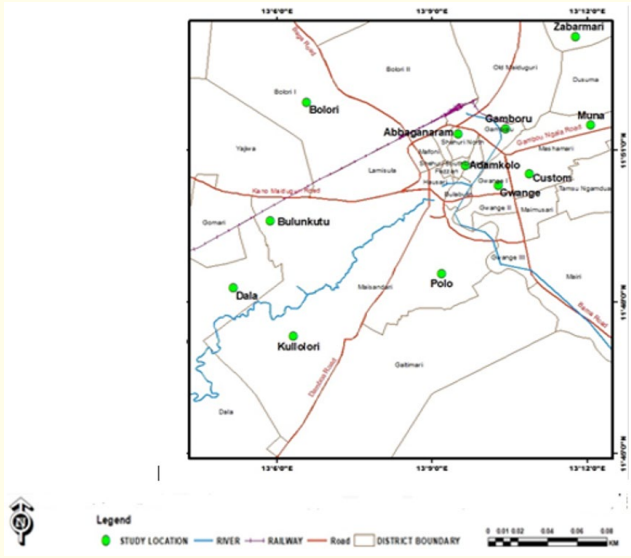
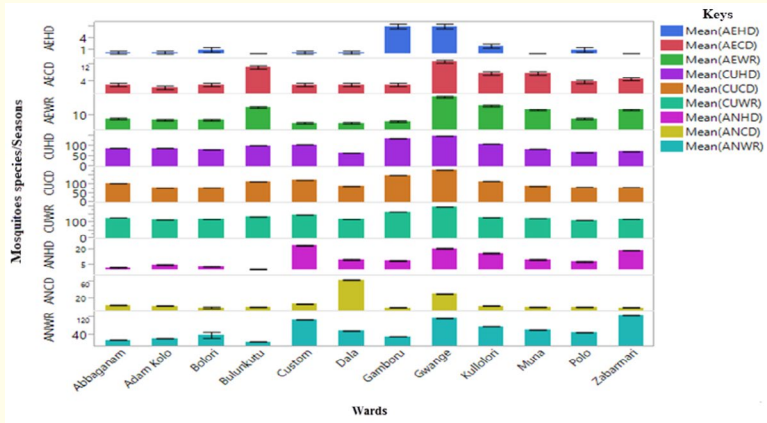


Figure 1: Map of the Sample Collection Sites in Green Color.



ANWR = Anopheles wet/rainy, ANCD = Anopheles cold dry, ANHD = Anopheles hot dry, CUWR = Culex wet/rainy, CUCD = Culex cold dry, CUHD = Culex hot dry, AEWR = Aedes wet/rainy, AECD = Aedes cold dry, AEHD = Aedes hot dry.

Figure 2: Seasonal Distribution of Mosquito Species amongst the Sampled Wards in Maiduguri.

Monthly Distribution of Mosquitoes in Maiduguri

The month of September had the highest distribution of 688 (12.9%) for all mosquito species collected, while May had the lowest distribution with 258 (4.8%) (Figure 3). Gwange ward had observed the highest distribution of mosquitoes collected within the 12 months of collection 729 (13.7%) followed by Custom and Gamboru wards with 525 (9.8%) each, while Adam Kolo had the lowest distribution 340 (6.4%). Irrespective of the month and location of collection *Culex* mosquitoes were most highly distributed compared to other species.

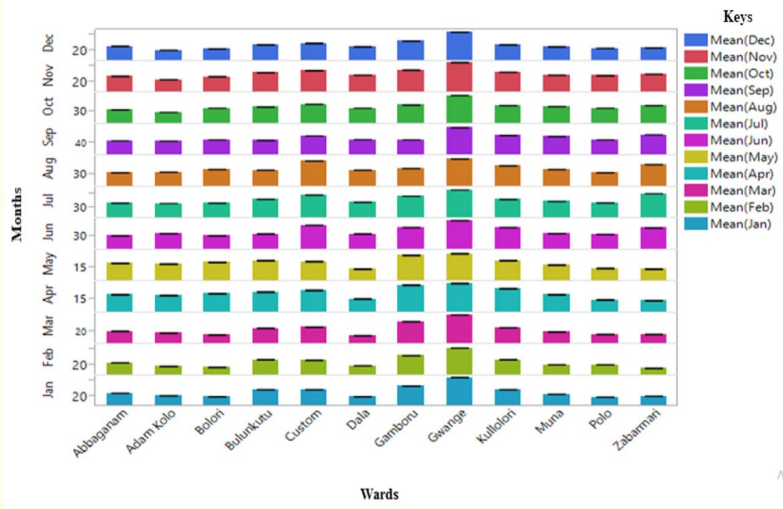


Figure 3: Monthly Distribution of Mosquitoes amongst Sampled Wards of Maiduguri.

Trapping Techniques and their Mosquito Catches in Maiduguri

Results of the catching performance of the different techniques employed in this study are presented in Table 3. the CDC had the highest catch of 2525 (47.3%), with 1674 (66.3%) for *Culex* species, 845 (33.5%) for *Anopheles* and 06 (0.24%) for *Aedes* in descending order ($p<0.05$). The human landing collection had a total catch of 625 (11.7%) with 384 (61.4%) for *Culex* species, 139 (22.3%) for *Aedes* and 102 (16.3%) for *Anopheles* ($p<0.05$). The developmental trap had a total catch of 2185 (41%), with 2069 (94.7%) for *Culex* species, 89 (4.07%) for *Aedes* and 0.00 (0%) for *Anopheles* ($p<0.05$). Thus, the best trap for *Culex* was DT, *Anopheles* was CDC and *Aedes* was HLC.

Trapping Technique	***Mosquitoes Catch [No (%)]***			***Total (%)***
	Culex	***Anopheles***	***Aedes***	
Center for Disease Control (CDC) light Trap	1674 (40.3)	845 (89.2)	06 (2.56%)	2525 (47.3)
Developmental trap	2096 (50.5)	0 (0.0%)	89 (38.0%)	2185 (41.0)
Human landing technique	384 (9.2)	102 (10.7%)	139 (59.4%)	625 (11.7)
Total (%)	4154 (77.9)	947 (17.8)	234 (4.39)	5335 (100.0)

Table 3: Trapping Techniques and their Mosquito Catches in Maiduguri.

Virus isolation and identification

Cytopathic Effects (CPE) principally cell liquefaction and aggregation was observed following inoculation of mosquito pool homogenate into BHK-21 and Vero-E16 Monolayer Cell cultures. Attempts was made to characterize those isolates showing CPE using PCR but no virus genome was amplified as confirmed using agarose gel with the absence of bands.

Viral characterization using NGS

Phylogenetic analysis of the sequences obtained from NGS indicates the presence of an unclassified RNA virus named previously as Hubei partiti-like virus 22 (Figure 4) and *Culex Flavivirus* (Figure 4). For the latter, the two isolates showed marked genetic diversity.

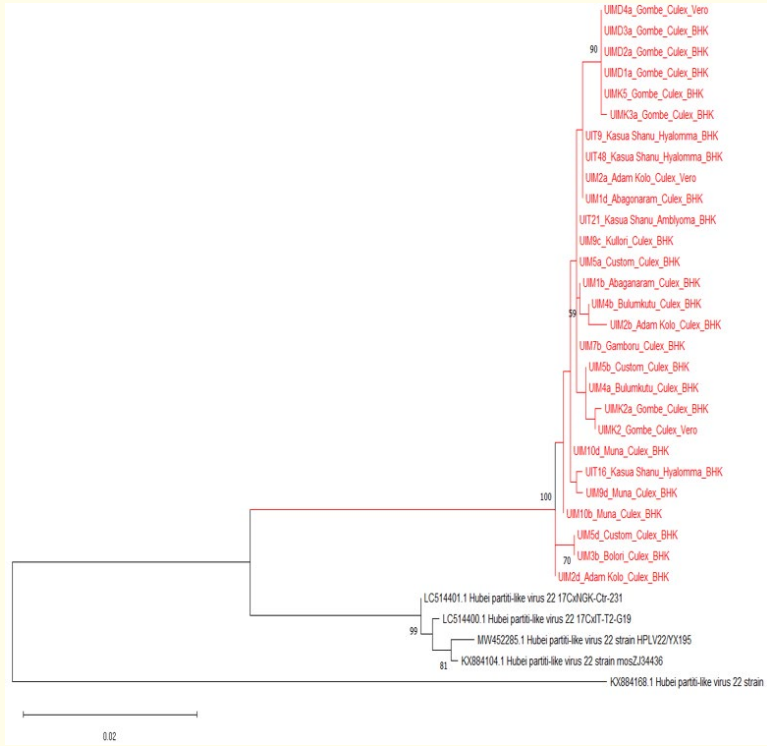


Figure 4: Phylogenetic Tree for Hubei_ partiti_ like virus 22 Showing 28 Closely related genomes.

Discussion

Geographical assessment of mosquito vectors and their role in virus transmission is an important aspect for the determination of high-risk areas within a locality where emergence and circulation of arthropod-borne viral diseases might occur. This study investigated mosquito abundance, distribution and diversity, and their role in arbovirus transmission.

In total, 5335 mosquitoes were collected across all the different sampling areas across the city of Maiduguri during the twelve-month period. This number is lower than over 8,000 collected by different researchers in Yobe [23] and Imo [31] states but higher than the numbers collected in Adamawa [48] and Taraba [24] states of Nigeria. Mosquito species found in Maiduguri where Anopheles, *Aedes* and *Culex*. The latter was the most distributed mosquito specie compared with *Anopheles* and *Aedes* species. This agrees with the work of [36]. who established that the strong presence of *Culex* mosquito can be considered as a biological marker of urbanization, because of their ability to breed in different types of waste water, as observed in other studies in Akure, Ondo State [2]. The distribution of mosquitoes between study wards is strongly connected with the availability of breeding water and moisture, as observed in Gwange ward 729 (13.7%) which had the highest distribution while Adam Kolo ward 340 (6.4%) was the least distributed. The distribution of mosquitoes in Gwange ward may be due to River Ngadabul; a natural body of flowing water that passed through the ward besides

other human made urbanization factors that favors the mosquito breeding. Furthermore, the presence of water and or moisture favors the breeding of mosquitoes as opined by [29].

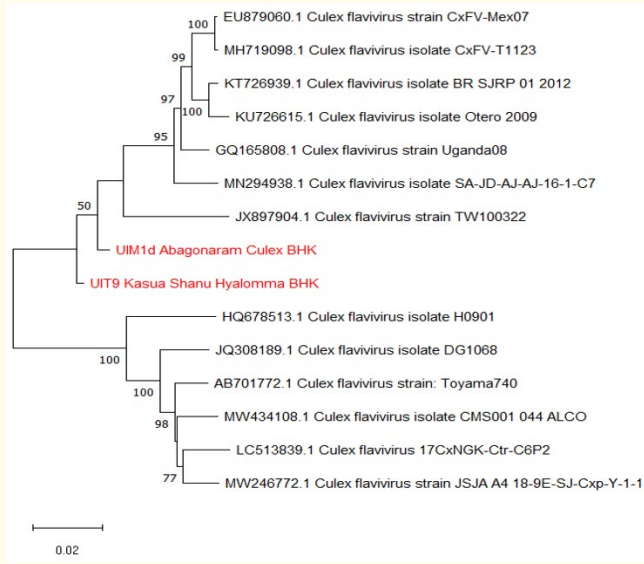


Figure 5: Phylogenetic Tree for Culex flavivirus showing two divergent genomes.

This study also showed that the three species of mosquitoes; *Aedes*, *Anopheles* and *Culex* had various degrees of uniformity in relation to their Diversity indices (Di), having values less than one (1). Also, the diversity of mosquitoes within the metro wards showed uniformity as all values are less than one. This observation has been supported by the work of [34]. where anthropological activities have shown positive influence in insect diversity. Also, [1]. have reported that diversity index is a quantitative measure of species richness in a particular area which is useful in epidemiological studies to postulate vectoral capacity and disease incidence or prevalence.

Based on seasonal variation, high abundance was observed during the wet-rainy season and with a significant variation amongst the wards examined. This agrees with [42]. And [38]. who reported that the abundance of mosquitoes between districts/wards is usually connected to variations in ecological terrain, soil type, vegetation cover and flood water which influences availability of breeding and resting sites. Besides increase in quantity of rainfall, other human activities like frequent water logging associated with urbanization such as dam construction, open drainage system, sewage and suck away chambers amongst others have contributed in amplifying the number of mosquito vectors [32: 20]. Furthermore, it was difficult to compare the results obtained from the catches using the different traps. This is largely due to different methodologies and sampling procedure applied [28]. According to [41]., there is the need for the use of a range of techniques to collect adult and immature mosquitoes in order to ensure accurate sampling due to variations in arthropod biodiversity in an environment as observed in Sao Paulo, Brazil. Minute differences in sleeping arrangements, availability of alternative hosts, humidity, temperatures, and wind speed and direction between the different days might introduce some sampling bias in the use of the different trapping techniques. On the whole, the different trapping methods all showed that *Culex* species was the most abundant of all catches.

Viruses isolated from Mosquito species in the present study were similar to the unclassified RNA viruses previously known as Hubei partiti-like virus 22 based on characterization and phylogeny, and the detected strains of *Culex flavivirus* are *flavivirus* species-specific. This is the first report on the isolation of *Culex flavivirus* from Nigeria and precisely from the city of Maiduguri. In Africa, it was

first reported in Uganda [13], and also in Japan and Indonesia [19], Guatemala [33] and Mexico [14]. The sequences detected in the present study have a boot-strap support of only 50% which differs from sister sub-clades from Brazil BR SJRP 01 2012 and Mexico CxFV-Mex07 that are 100% homogeny [8]. Thus, it appears that the strain reported in Maiduguri belonged to the group 1 genotype and is genetically divergent from other group 1 sequences obtained from other parts of Africa and North America. *Culex flavivirus* has a great diversity of reservoirs, and this could influence their phylogeny. The phylogenetic tree showed two clades; one of which is associated with Asia and the second one is mostly with American and African strains. However, the detected strains are phylogenetically related to the Brazil strain. In genetic terms, *Culex flavivirus* appears to be stable over many years in a habitat-dependent manner. The clustering of the Uganda isolates with the isolate obtained in the current study on the same group probably suggests and support the assertion advanced above [26].

Conclusion

This study has reported the distribution, abundance and diversity of mosquitoes collected across several wards within the Maiduguri metropolis. On the whole, three genera of mosquitoes were collected comprising of Aedes, *Anopheles* and Culex. Interestingly, their abundance, distribution and diversity had a correlation with seasons and wards of collection with *Culex* species been the most abundance while *Aedes* species was the least. Also, NGS revealed the existence of *Culex Flavivirus* similar to the unclassified Hubei partiti like virus 22.

References

1. Adelusi SM, Ada RT and Omudu EA. "Diversity and abundance of insect's species in Makurdi, Benue State, Nigeria". International Journal of New Technology and Research 4.6 (2018): 52-57.
2. Afolabi OJ., et al. "The effects of alteration of pH on the breeding characteristics of mosquitoes in phytotelmata in Ahmadu Bello University Zaria, Nigeria". International Journal of Bioscience 5.1 (2010): 32-36.
3. Afolabi OJ, Simon-Oke IA and Osomo BO. "Distribution, abundance and diversity of mosquitoes in Akure, Ondo State, Nigeria". Global Journal of Acarology, Parasitology and Vector Biology 1.1 (2013): 10-14.
4. Agarwal A, Parida M and Dash PK. "Impact of transmission cycles and vector competence on global expansion and emergence of arboviruses". Reviews in Medical Virology 27.5 (2017): e1941.
5. Armstrong PM., et al. "Detection of infectious virus from field-collected mosquitoes by Vero cell culture assay". JoVE (Journal of Visualized Experiments) 52 (2011): e2889.
6. Becker N., et al. "Mosquitoes and their control, 2nd edn". Kluwer Academic / Plenum Publishers, New York (2010).
7. Bennette KE and Olson KE. "Variation in vector competence for Dengue 2 virus among 24 collections of Aedes aegypti from Mexico and United States". American Journal of Tropical Medicine and Hygiene 67.1 (2002): 85-92.
8. Bittar C., et al. "Genome sequencing and genetic characterization of Culex flavirirus (CxFV) provides new information about its genotypes". Virology Journal 13.1 (2016): 158.
9. BOSIEC. Borno State Independent Electoral Commissions annual evaluation report to the Borno State Government, Musa Usman Secretariat Maiduguri, Borno State (2020).
10. Calisher CH and Childs JE. "Bats: Important reservoir hosts of emerging viruses". Clinical Microbiology Review 19.3 (2006): 531-545.
11. Chandler LJ. Arthropod-Borne Virus Information Exchange December 1997; Centers for Disease Control and Prevention (U.S.gov): Atlanta, GA, USA (1997).
12. Conway MJ, Colpitts TM and Fikrig E. "Role of the vector in arbovirus transmission". Annual Review of Virology 1 (2014): 71-88.
13. Cook S., et al. "Isolation of a novel species of flavivirus and a new strain of Culex flavivirus (Flaviviridae) from a natural mosquito population in Uganda". The Journal of General Virology 90.Pt 11 (2009): 2669-2678.
14. Farfan-Ale JA., et al. "Detection of flaviviruses and orthobunyaviruses in mosquitoes in the Yucatan Peninsula of Mexico in 2008".

Vector-Borne and Zoonotic Diseases 10.8 (2010): 777-783.

15. FGN. Federal Government of Nigeria. Encyclopedia Britannica, first edition published by Risafu & Company 2 (2019).
16. Gadzama UN., et al. "Effect of livestock, human host and aquatic habitat distribution on the abundance of Anopheles gambiae complex in Borno State Arid Zone of Nigeria". World Rural Observations 3.4 (2021): 71-77.
17. Gan VCH, and Leo YS. "Current epidemiology and clinical practice in arboviral infections-implications on blood supply in South-East Asia". ISBT Science Series 9.1 (2014): 262-267.
18. Gould E., et al. "Emerging arboviruses: why today?". One health 4 (2017): 1-13.
19. Hoshino K., et al. "Genetic characterization of a new insect flavivirus isolated from Culex pipiens mosquito in Japan". Virology 359.2 (2007): 405-414.
20. Hubálek Z, Rudolf I and Nowotny N. "Arboviruses pathogenic for domestic and wild animals". Advance Virus Research 89 (2014): 201-275.
21. Kamgang B., et al. "Different populations of Aedes aegypti and Aedes albopictus (Diptera: Culicidae) from Central Africa are susceptible to Zika virus infection". PLoS neglected tropical diseases 14.3 (2020): e0008163.
22. Koekemoer LL., et al. "A cocktail polymerase chain reaction assay to identify members of the Anopheles funestus (Diptera: Culicidae) group". The American Journal of Tropical Medicine and Hygiene 66.6 (2002): 804-811.
23. Lamidi TB. "Distribution and seasonal abundance of anopheline mosquito species in Nguru, Yobe State, North-Eastern Nigeria". Animal Research International 6.1 (2009): 949-952.
24. Lamidi TB, Elijah MI and Ireebanije FJ. "Prevalence of mosquito species and malaria transmission in three riverine communities in Bali district, Taraba State, Nigeria". IOSR Journal of Pharmacy and Biological Sciences 14.1 (2019): 61-65.
25. Lequime S and Lambrechts L. "Vertical transmission of arbovirus in mosquitoes: A historical perspective". Infectious Genetics and Evolution 28 (2016): 681-690.
26. Liang W., et al. "Distribution and phylogenetic analysis of Culex flavivirus in mosquitoes in China". Archives of Virology 160.9 (2015): 2259-2268.
27. Marchi S, Trombetta CM and Montomoli E. "Emerging and Re-Emerging Arboviral Diseases as a Global Health Problem". Public Health; Majumder, M.A.A., Ed.; IntechOpen: London, UK (2018): 25-46.
28. Mathenge EM., et al. "Comparative performance of the Mbita trap, CDC light trap and the human landing catch in the sampling of Anopheles arabiensis, An. funestus and culicine species in a rice irrigation in western Kenya". Malaria Journal 4.1 (2005): 1-6.
29. Mathew N., et al. "Larvicidal activity of Saraca indica, Nyctanthes arbortristis, and Clitoria ternatea extracts against three mosquito vector species". Parasitology Research 104.5 (2009): 1017-1025.
30. Matranga CB., et al. "Unbiased deep sequencing of RNA viruses from clinical samples". JoVE (Journal of Visualized Experiments) 113 (2016): e54117.
31. Mgbemena IC and Ebe T. "Distribution and occurrence of Mosquito species in the municipal areas of Imo State, Nigeria". Analele Universitatii din Oradea, Fascicula Biologie 19.2 (2012): 92-100.
32. MooreA DL and Kemp GE. "Bluetongue and related viruses in Ibadan, Nigeria: serologic studies of domesticated and wild animals". American journal of veterinary research (1974).
33. Morales-Betoulle ME., et al. "Culex flavivirus isolates from mosquitoes in Guatemala". Journal of Medical Entomology 45.6 (2008): 1187-1190.
34. Mokam DG, Djiéto-Lordon C and Bilong Bilong CF. "Patterns of species richness and diversity of insects associated with cucurbit fruits in the southern part of Cameroon". Journal of Insect Science 14.1 (2014): 248.
35. Ndhlovu M., et al. "Antibiotic prescribing practices for patients with fever in the transition from presumptive treatment of malaria to 'confirm and treat'in Zambia: a cross-sectional study". Tropical Medicine & International Health 20.12 (2015): 1696-1706.
36. Nguku PM., et al. "An investigation of a major outbreak of Rift Valley fever in Kenya: 2006-2007". American Journal of Tropical Medicine and Hygiene 5 (2010): 5-13.
37. NPC (National Population Commission). Nigerian Census Report (2016).

38. Nwoke BEB., et al. "Epidemiological characteristics of Bancroftian filariasis and the Nigerian environment". Journal of Public Health and Epidemiology 2.6 (2010): 113-117.
39. Oderinde BS. "Viral metagenomics investigation of patients with fever of unknown origin and potential insect vectors in Borno State, Nigeria". A PhD Thesis in Medical Microbiology and Parasitology submitted to the Post graduate School, University of Ilorin, Ilorin, Nigeria (2016): 94-103.
40. Ogwuche J., et al. "Arbovirus surveillance in pregnant women in north-central Nigeria, 2019-2022". Journal of Clinical Virology 169 (2023): 105616.
41. Olurunniyi OF. "Abundance and Diversity of Mosquito genera at Ilokun and Iras Communities Ado-Ekiti, Nigeria". Journal of Bio Innovation 5.3 (2016): 379-385.
42. Sang R., et al. "Rift Valley fever virus epidemic in Kenya, 2006/2007: the entomologic investigations". The American Journal of Tropical Medicine and Hygiene 83 (2010): 28-37.
43. Shaman J, Day JF and Stieglitz M. "St.Louis encephalitis virus in wild birds during the 1990 South Florida epidemic: the importance of grought, wetting conditions and the emergence of Culex nigripalpus to arboviral amplification and transmission". Medical and Veterinary Entomology 13 (2003): 265-273.
44. Simon-Oke IA and Olofintoye IK. "The effect of climatic factors on the distribution and abundance of mosquito vectors in Ekiti State". Journal of Biology, Agriculture and Healthcare 5.9 (2015): 142-146.
45. Snell AE. "Identification keys to larval and adult female mosquitoes (Diptera: Culicidae) of New Zealand". New Zealand Journal of Zoology 32.2 (2005): 99-110.
46. Temmam S., et al. "Viral metagenomics on animals as a tool for the detection of zoonoses prior to human infection?". International Journal of Molecular Sciences 15.6 (2014): 10377-10397.
47. Velu RM., et al. "Mosquito-Borne Viral Pathogens Detected in Zambia: A Systematic Review". Pathogens 10.8 (2021): 1007.
48. Wahedi JA., et al. "Diversity and relative density of larval mosquito species in Mubi metropolis, Adamawa state, Nigeria". International Journal of Mosquito Research 7.4 (2020): 21-25
49. Weaver SC and Reisen WK. "Present and future arboviral threats". Antiviral Research 85.2 (2010): 328-345.
50. Weetman D., et al. "Aedes mosquitoes and Aedes-borne arboviruses in Africa: current and future threats". International Journal of Environmental Research and Public Health 15.2 (2018): 220.

Volume 9 Issue 2 August 2025