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Spatio-temporal distribution of *Aedes aegypti* oviposition activity in Kano metropolis north-Western Nigeria

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Abstract
Aedes aegypti are vectors of serious and debilitating arbovirus diseases that affect humans as well as domestic and wild animals worldwide. Center for Disease Control oviposition traps were used (January - December, 2019) to monitor oviposition in four sampling entities within Kano Metropolis. No oviposition activity was observed throughout the dry season with 0.00% Ovitrap Index across all locations. Oviposition activity of the mosquitoes was moderately distributed (50%) during the rainy season. Gardens had the highest oviposition activity during the rainy season (OI = 50±35%, $p>0.05$). Chi-square of the seasonal mean OI revealed that *Aedes aegypti* oviposition depends significantly ($p<0.05$) on season of the year with rainy season having OI of 31±9% and 0.00±0.00% for dry season. This study underscores the relevance of entomological surveillance through establishing the presence, seasonal population dynamics and identifying hot spot areas of *Aedes aegypti* in Kano Metropolis.

Keywords: *Aedes aegypti*, vector, entomological surveillance, Kano, distribution

1. Introduction
Worldwide, mosquitoes are the focus of entomological research because of their importance as vectors of a wide range of debilitating viral and parasitic diseases affecting both humans and animals [1]. More than half of the world's population lives under the risk of becoming infected by mosquitoes that carry the causative agents of diseases such as malaria, yellow fever, dengue, Chikungunya, West Nile, Japanese encephalitis and lymphatic filariasis [2, 3].
Members of the *Aedes* genus are known vectors for numerous viral infections. The two most prominent species are *Aedes aegypti* and *Aedes albopictus* which transmit Zika virus and other viruses that cause dengue fever, yellow fever, West Nile fever, Chikungunya, and Eastern equine encephalitis, as well as many other arbovirus diseases [4]. Some studies conducted in Nigeria revealed that the major *Aedes* mosquito species include: *Ae. aegypti*, *Ae. albopictus*, *Ae. africanus*, *Ae. luteocephalus*, *Ae. simpsoni* complex, *Ae. vittatus* [5, 6, 7].
There has been re-emergence of yellow fever confirmed cases in Kano and other parts of Nigeria [8]. Studies have advocated that numbers and statistics should be the basis upon which funders and politicians make their decisions regarding public health safeguarding, for without quality public health data, interventions may be misguided and wasteful [9]. Organized and continuous surveillance will not only give Nigeria a data base of the *Aedes* species but will help in policy formulation and planning a targeted vector control strategy.
Aedes species normally breed in containers, jars, transient water collections, tree cavities, leaf axils, bamboo stumps, rock pools, tin cans, coconut shells, discarded vehicle tyres, broken earthen and ceramic wares, among others [10]. These breeding habits keep them in close proximity with man, facilitating disease transmission. They lay their eggs just above the water level. As a result, water waves or increase in water volume leads to hatching of viable eggs [7].
Aedes aegypti is one of the most efficient mosquito vectors for arboviruses that causes; Dengue fever, Chikungunya, Yellow fever, West Nile, and several other arboviral diseases [11]. The presence of *Aedes* as well as diseases associated with these mosquitoes in Kano state has

been established [12]. However, little data is available for their spatial and seasonal population dynamics within the metropolis. This study provides data on the distribution of oviposition activities and its seasonal occurrence by *Aedes aegypti* within the Kano metropolis urban landscape, which is vital in making informed decisions during state and regional arbovirus diseases control interventions.

2. Materials and Methods

2.1 Study area

Kano metropolis, Kano State, Nigeria, lies between latitudes 11 º5' N and 12 º47' N, longitude 8º 22' E and 8º 39' E with elevation of 472m above mean sea level. Kano metropolis is bordered by Madobi and Tofa LGA to the South West, Gezawa LGA to the East, Dawakin Kudu LGA to the South East, and Minjibir LGA on the North East. The study area is made up of eight (8) LGAs; Dala, Fagge, Gwale, Kano Municipal, Nassarawa, Tarauni and parts of Ungogo and Kumbotso. Kano metropolis is the second largest city in Nigeria after Lagos. The climate of the area is influenced by the movement of the two air masses, the maritime air masses originating over Atlantic Ocean and the dry air masses coming from the Sahara Desert. Consequently, the area is characterized by rainy season (May - September) which is characterized by south western maritime winds that carry warm and humid air; the dry season (October – April) is characterized by the tropical dry continental wind (Harmattan) from north. The average temperature is a bit hot, even during the cool harmattan period the minimum temperature hardly falls below 11 ºC, whilst the monthly average temperature is not less than 20 ºC, whereas during the hot season usually Mid – March to Mid-May, the maximum temperature reading may be as high as 40 ºC.

2.2 Sampling sites

Samples were collected from four (4) different entities; houses, schools, health facilities, and gardens.

Houses: Three hundred and eighty- five (385) houses were sampled based on Cochran's sample size formula. Three (3) LGAs (Gwale, Kano Municipal and Kumbotso) were randomly selected to draw the samples. Considering the heterogeneity of the LGAs, multistage stratified random sampling was employed to collect data from the sample population. Proportionate random sampling of houses based on the population of each of the representative LGAs was adopted.

Schools: Five (5) schools were selected using convenience sampling. These schools include;

1. Gandun Albasa Model Primary School
2. Government Secondary School Goron Dutse
3. Government Arabic College Gwale
4. Government Junior Secondary School Kabuga
5. Government Girls Arabic Secondary School Kofar Naisa

Health Facilities: Two (2) Hospitals were included in this study based on convenience. These include;

1. Aminu Kano Teaching Hospital
2. Kano State Emergency Relief and Rehabilitation Agency

Gardens: Two gardens were conveniently sampled and include

1. Botanical Garden, Bayero University Kano
2. Ecological Garden, Bayero University Kano

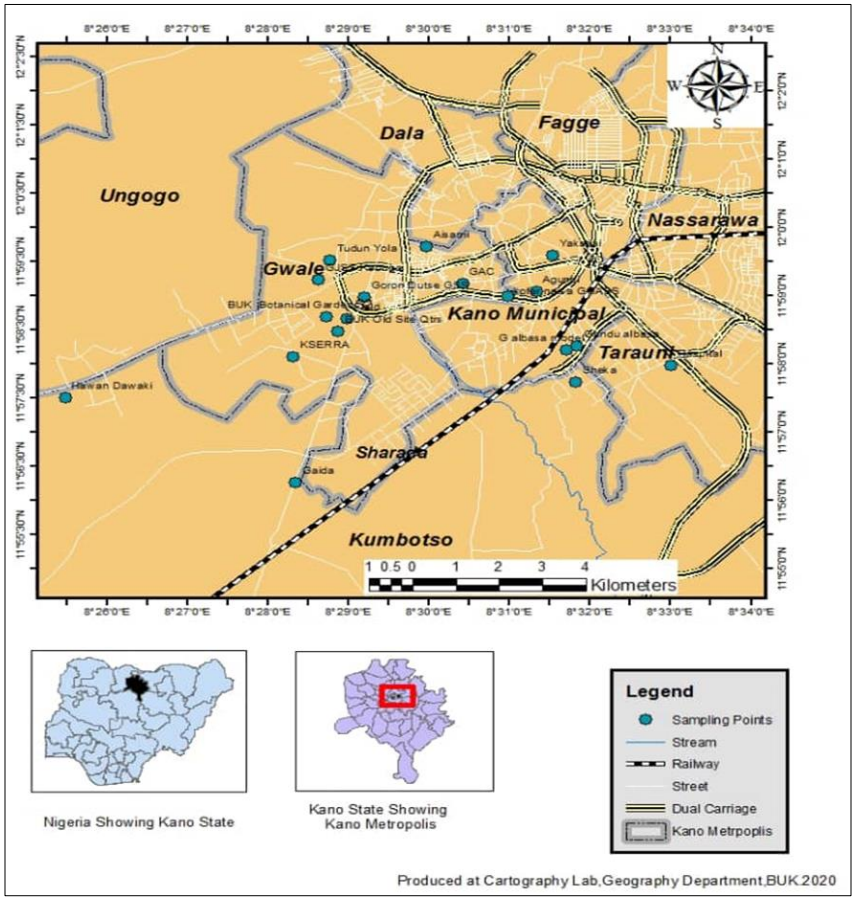

Fig 1: Map of Kano Metropolis with the study area and sampling sites

2.3 Ovi trapping and *Aedes* Rearing

The CDC oviposition traps were placed in the randomly selected sampling sites in Kano metropolitan and allowed to stay for two days. Eggs were collected and transferred into rearing pans (30 by 12.5 cm) in the laboratory to await emergence of larvae. The larvae were fed with 0.5 g of Quaker Oats per 500 larvae until they pupated [13]. The pupae were transferred into 500 ml plastic cups after which they were placed in a 30 cm^3 mosquito rearing cage for the emergence of the adults. The adults were euthanized with Ethyl acetate prior to identification, using the method of [14]. This served as an alternative to freezing which could not be relied upon due to electricity issues.

2.4 Determination of Ovitrap Indices

The Ovitrap Index (OI) was calculated as,

$$OI = \frac{Number\ of\ infested\ traps\ with\ eggs\ or\ larvae}{Total\ number\ of\ traps\ placed} \times 100$$

2.5 Morphological Identification

The recovered eggs and larvae were reared to adult under laboratory conditions. The mosquitoes were morphologically identified at the adult stage using the taxonomy key [15] with the aid of a 2.0 Unimake digital USB microscope.

2.6 Molecular Identification

Thirty percent (30%) of the morphologically identified mosquitoes were each placed in Eppendorf tubes and enplaned. Polymerase Chain Reaction (PCR) was conducted based on the protocol of Beebe *et al*. (2007).

DNA Extraction

DNA was extracted from the adult mosquitoes using rapid DNA isolation procedure that involved grinding a small part of the mosquito in 100litre of TE and immediately boiling it for 5 mins. One microliter of either extraction was used in the PCR reaction.

PCR Amplification and Analysis

All PCR amplifications were carried out in a 48-well, 0.2-ml PCR microtiter plate. The final 25 µl PCR mixture contained 1 µl of the template DNA, 0.4 µM each primer, 1.25 mM MgCl2, 1.5 mM each dNTP, 1X Taq reaction buffer and 1 U of TaqDNA polymerase. Primers used for amplification of the internal transcribed spacer region 1 (ITS1) were: forward primer: ITS1A, 5- CCT TTG TAC ACA CCG CCC GTC G, and reverse primer: ITS1B, 5-ATG TGT CCT GCA GTT CAC A. The cycling regime was 94 ºC for 4 min and then 35 cycles of 94 ºC for 30s, 51 ºC for 40s, and 72 ºC for 30s. PCR products were size separated on a 1.2% agarose gel to confirm product size (5µl). Restriction analysis was carried out in a 0.5-ml microfuge tube containing 5 µl of PCR product and 5 µl of 2X RsaI buffer (premade stock) containing 1Ð2 U of RsaI enzyme per reaction). The mixture was incubated at 37 ºC for 2hrs and then size separated on a 3.0% agarose gel at 100V for 30min. Finally, the gel was stained with 5 g/ml ethidium bromide for 15min and viewed at 312 nm.

2.7 Determination of Distribution Pattern

The following formula $C = \frac{N}{n} \times 100$ was used.

Where, C = Distribution pattern n = Number of sites positive for the occurrence of mosquitoes, N = Total number of sites studied.

When C = 0 – 20% the distribution pattern is sporadic, C = 20.1 – 40% the distribution pattern is infrequent, C = 40.1 – 60% the distribution pattern is moderate C = 60.1 – 80% the distribution pattern is frequent and C = 80.1-100% the distribution pattern is constant [16].

2.8 Data Analyses

Variation of the mean oviposition across sampling entities was determined using One-way Analysis of Variance. Descriptive analysis was used to determine means, and a Post hoc test (Duncan) was used to determine the multiple comparisons of means across sites and seasons. Chi-square test of the seasonal OI to test independence of the variable means was also used.

3. Results

With the exception of three sampling sites, namely Bayero university old site staff quarters of Gwale LGA Gandun Albasa of Kano Municipal and Hawan Dawaki of Kumbotso LGA which yielded *Culex* mosquitoes, all the surveyed houses in the dry season were found to be mosquito-negative, while the rainy season *Aedes* survey was quite productive as seen in Table 1. Five (5) out of Nine (9) sampled sites were found to be *Aedes* positive, BUK old site and Tudun Yola of Gwale LGA; Gaida, Hawan Dawaki and Sheka of Kumbotso LGA. Kano Municipal was found to be *Aedes* negative; as shown in table 1 below.

Collections from around schools indicated that all schools were *Aedes* negative during the dry season, while two (2) out of the five (5) sampled schools were found to be *Aedes* positive during the rainy season. The *Aedes* positive schools were GAC Gwale and GSS Goron Dutse. Significant difference (p<0.05) was observed in sites and seasonal oviposition activity for the selected schools

Out of the two surveyed Health Facilities, only Kano State Emergency Relief and Rehabilitation Center was found to be *Aedes* positive during the rainy season. Both facilities were found to be uniformly *Aedes* negative during the dry season. A remarkable significance between sites and seasons, with AKTH recording a 0% *Aedes* mean and KSERRA recording 48% was observed.

Aedes negative status was recorded for both sampled gardens during the dry season. Only Botanical Garden, Bayero University Kano yielded positive *Aedes* results during the rainy season. A remarkable significance (P<0.05) was observed between the oviposition sites and seasons as shown in Table 2.

During dry season, zero (0) *Aedes* ovitrap index was recorded for all the sampled houses as described in Table 3. For the raining season, however, 66% was recorded for houses in Gwale LGA (0% for Goron Dutse, 16% for BUK Old Site and 50% for Tudun Yola), 0% for Kano Municipal and 25% for Kumbotso (14% for Gaida, 4.5% for Hawan Dawaki and 6.8% for Sheka)

Throughout the dry season, *Aedes* ovitrap indices for all sample schools was calculated as zero (0). rainy season calculations were also the same except for GAC Gwale (66%) and GSS Goron Dutse (50%).

Table 1: Seasonal variation of *Aedes* occurrence across sampling entities

Entity	Local Government	Site	*Aedes* Seasonal Status	
			Dry	Rainy
Houses	Gwale	BUK Old Site	-	+
		Goron Dutse	-	-
		Tudun Yola	-	+
	Kano Municipal	Dan Agundi	-	-
		Gandun Albasa	-	-
		Yakasai	-	-
	Kumbotso	Gaida	-	+
		Hawan Dawaki	-	+
		Sheka	-	+
Schools	Gwale	GAC Gwale	-	+
		GGSS Jambulo	-	-
		GJSS Kabuga	-	-
		GSS Goron Dutse	-	+
	Kano Municipal	Gandun Albasa Model	-	-
Health Facility	Gwale	KSERRA	-	+
	Kano Municipal	AKTH	-	-
Garden	Gwale	Botanical Garden BUK	-	+
		Ecological Garden BUK	-	-

Note: +: Presence of mosquitoes -: Absence of Mosquitoes

Table 2: Seasonal comparison of *Aedes* OI across different study sites

Entity	Site	Season		
		Mean±SE	Dry	Rainy
Houses	BUK old site Goron Dutse Tudun Yola Interaction × Site × Season	0.40±1.67^{a} 0.00±0.00^{b} 0.54±1.15^{a} p.value 0.003**	0.00±0.00^{b}	0.63±1.62^{a}
	Dan Agundi Kundila Yakasai Interaction×Site×Season	0.00±0.00^{b} 0.00±0.00^{b} 0.00±0.00^{b} p.value 0.000	0.00±0.00^{b}	0.00±0.00^{b}
	Gaida Hawan Dawaki Sheka Interaction × Site × Season	0.32±1.33^{a} 0.03±0.18^{a} 0.26±1.45^{a} p.value 0.213	0.00±0.00^{b}	0.40b±1.59^{a}
School	GAC Gwale GJSS Kabuga GGSS Jambulo GSS Goron Dutse Gandun Albasa Model Interaction × Site × Season	1.0±1.47^{ab} 0.00±0.00^{b} 0.00±0.00^{b} 2.16±4.01^{a} 0.00±0.00^{b} p.value 0.017*	0.00±0.00^{b}	1.58±3.02^{a}
Health facility	AKTH KSERRA Interaction × Site × Season	0.00±0.00^{b} 0.48±0.68a p.value 1.45e-09 ***	0.00±0.00^{b}	0.50±0.68^{a}
Garden	BUK Botanical Ecological Garden Interaction × Site × Season	1.0±1.01^{a} 0.00±0.00^{b} p.value 2e-16 ***	0.00±0.00^{b}	1.0 ±1.01^{a}

Note: Mean followed by different superscript are significant at $p<0.05$ using Duncan test
*: Degree of Significance

Dry and rainy season *Aedes* ovitrap indices for AKTH were found to be zero (0). For KSERRA, even though the ovitrap index for dry season was found to be zero (0), a remarkable ovitrap index of 83% was recorded for rainy season.

The only positive *Aedes* ovitrap index recorded for gardens was for BUK Botanical Garden during the rainy season (100%). While BUK Ecological Garden recorded 0% as seen in table 3.

Table 3: Rainy season mean ovitrap index of *Aedes aegypti* across sampling entities

Sampling entities	Mean ovitrap index (%)±SE
Houses	10±5
Schools	23±14
Hospitals	41±29
Gardens	50±35

*Note: SE, Standard Error. P>0.05

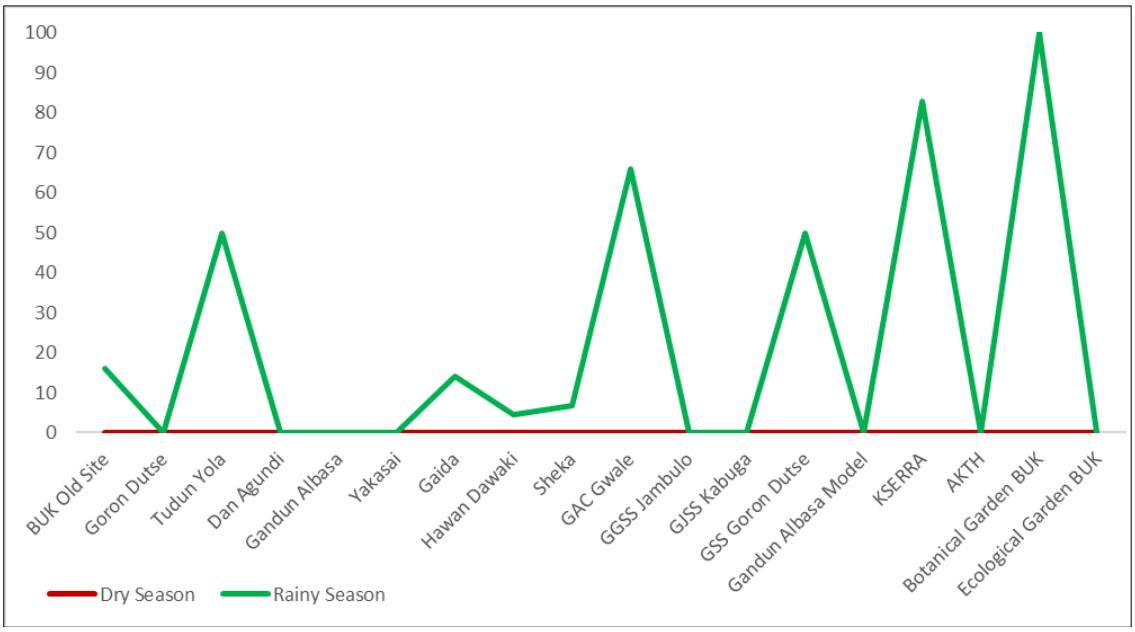

Fig 2: Ovi trap seasonal distribution pattern and occurrence of *Aedes aegypti* across the study area.

4. Discussion

Morphological and molecular identification of the *Aedes* in this study confirms to be *Aedes aegypti*. *Aedes aegypti* positive traps indicated that ovitraps suit the oviposition preference of the Female *Aedes aegypti* of breeding in black containers such as cans and discarded vehicle tyres. This is in conformity with previous findings [17] of *Aedes aegypti* preference of breeding in artificial containers. Further to this, more positives ovitraps were found during the rainy season than dry season, in fact there was no positive ovitrap throughout the dry season. This sheds light on the absence of *Aedes aegypti* during dry seasons which could be because there was no enough water to favor unlimited suitable habitats that leads to massive *Aedes aegypti* production, abundance and distribution. Population density of the Yellow Fever vectors is strongly influenced by rainfall [18]. Furthermore, *Culex* positive traps in some areas where there was no *Aedes* and the total absence of *Culex* species in the traps where *Aedes* thrive could mean that *Aedes aegypti* are highly competitive species.

Results compiled in Table 1 showed that not all the sampled LGAs yielded positive *Aedes aegypti* results. The absence of *Aedes aegypti* in the entire sampled sites of Kano Municipal highlights that geographic locations have a role to play in mosquito presence. This agrees with previous previous [19] which observed that mosquito distribution and abundance are related to population, land use and human activities. The presence of *Aedes* in houses is in line with earlier findings [20] which indicated that *Aedes aegypti* prefer human habitations as they provide resting and host-seeking possibilities. However, the high OI during the rainy season in botanical gardens and non for ecological garden where human activities occur only during day time requires further investigation. The result also showed that only 40% of sampled schools yielded positive *Aedes aegypti*. The presence of *Aedes aegypti* in 50% of sampled Health Facilities and Gardens were also respectively depicted. The spatial distribution and abundance of *Aedes aegypti* are related to the effects of anthropogenic changes in the environment [21]. Combination of factors such as temperature, dissolved oxygen, relative humidity, conductivity and anthropogenic related factors such as opened drainage system contribute to the increasing abundance of mosquitoes [22]. Statistical analysis of mean seasonal OI showed significant difference (p<0.05) between, season and *Aedes aegypti* presence and abundance.

Interestingly, it was observed during the course of this study that both immature and adult stages demonstrated negative phototaxis. This agrees earlier studies that described the behavior of *Aedes aegypti*, as possessing strong negative phototropism and extreme sensitiveness to vibrations and to light [23]. This feature can be considered in management strategies. Serendipitously, all the *Aedes aegypti* mosquitoes encountered in this study were discovered to be autogenous, yielding up to second filial generation progeny. Dating back to the nineteenth century there has been reported autogeny in *Ae. aegypti* populations from East Africa, with the highest frequency recorded in Uganda [24]. This finding can also be backed by a study that found 3.2% of females in a population of *Ae. aegypti* from Kenya to be autogenous, concluding that a suitable genotype and favorable environment like temperature, good nutrition in larval stages and feeding on higher concentrations of sugar solution during the adult stage play a major role for *Ae. aegypti* mosquitoes to be able to lay eggs without a blood meal [25]. Although the ideal conditions for the expression of this rare trait in these mosquitoes are still unknown, delving into the issue may help alleviate the existing conflict between Vector Controllers and Animal Rights Activists.

All the obtained Positive Ovitraps yielded an index than exceeds the 1.8% Container Index proposed as *Aedes* threshold values in rainy season [26]. Similarly, the ovitrap indices shows 0% presence of *Aedes* during dry season. Varying ovitrap Indices also suggest that location affects presence and abundance. The 'moderate' distribution pattern found in this study which is in contrast with the 'sporadic' pattern previously discovered in other studies [27] could indicate that Kano metropolis is high risk area for yellow fever or dengue outbreak.

5. Conclusion

With no oviposition activity across all the study locations and moderate abundance during the rainy season, this study concludes that abundance and distribution of *Aedes aegypti* in Kano metropolis is influenced by season. Therefore, control at individual, local and regional levels targeting *Aedes aegypti* should consider focusing on rainy season. Study on the vectorial capacity of this species is recommended.

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