Parasite Epidemiology and Control 14 (2021) e00213

Contents lists available at ScienceDirect

Parasite Epidemiology and Control

journal homepage: www.elsevier.com/locate/parepi

Relative abundance and molecular identification of *Culex pipiens* complex (Diptera: Culicidae), in Kura Local Government Area, North-western Nigeria

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ARTICLE INFO

Article history:
Received 23 December 2020
Received in revised form 7 April 2021
Accepted 28 April 2021

Keywords:
Aedes
Anophilines
Culex
Culicoides
Multiplex PCR
Nigeria

ABSTRACT

Culex species are the most widespread mosquito species across the world and are known to be highly opportunistic, feeding on humans and livestock. They are known to acquire the potential to transmit zoonotic diseases, including Rift Valley Fever (RVF). However, despite their public health significance, they remain understudied in North-western Nigeria, compared to Anophelines. This study was therefore aimed at determining the relative abundance and Multiplex polymerase chain reaction (Multiplex PCR) identification of members of the *Culex pipiens* complex, in Kura Local Government Area (LGA), North-western, Nigeria. Adult mosquitoes were collected using Center for Disease Control (CDC) miniature light traps from August to October 2019. Mosquitoes were identified using morphological identification keys. Members of the *Culex pipiens* complex were further identified using Multiplex PCR to assess the presence of sibling species. A total of 413 mosquitoes, belonging to 3 genera, *Culex*, *Anopheles* and *Aedes* were collected. Of this figure, 120 *Culex* spp. females were collected. Homes with livestock had the highest occurrence of mosquitoes, 123 (61.19%) compared to those without livestock, 78 (38.81%). There was no statistical difference among the two (2) categories of homes ($P \geq 0.005$). *Culicoides* spp. were the most common with 130 collected (65.38%). Again, homes with livestock had the highest occurrence, 85 whilst homes without livestock had 45 of the other flies caught. Multiplex- PCR revealed no expected bands for *Cx. quinquefasciatus* and *Cx. pipiens* from the DNA obtained from field collected mosquitoes as confirmed by using genomic DNA of an insectary *Culex quinquefasciatus* as control. *Cx.* spp. is presently regarded as a biting nuisance having no significant epidemiological importance. Efforts at its control should be intensified before it is too late. This study provides useful information on the occurrence and multiplex PCR of *Culex* spp in Kura Local Government Area, North-western Nigeria. These results have implications for the control of *Culex* spp. mosquito populations and the spread of human, livestock and avian diseases.

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https://doi.org/10.1016/j.parepi.2021.e00213

1. Introduction

Culex mosquitoes constitute a wide range of mosquitoes generally involved in the transmission of mosquito- borne arboviruses including; Sindbis virus, West Nile Virus, Equine encephalitis, St Louis, Oro-pouche fever, Avian malaria, Lymphatic filariasis and Rift valley fever (RVF) (Linthicum et al., 2016; Bellone and Anna-Bella, 2020). One of the most important groups in the *Culex* genus is *Culex pipiens* complex which comprises six members: *Cx. quinquifasciatus* Say, *Cx. pallens* Coquillet, *Cx. australicus* Dobrotworsky and Drummond, *Cx. pipiens* Linneaus, *Cx. globocoxitus* Dobrotworsky and *Cx. molestus* Forskll (Zittra et al., 2019). Members of the *Culex pipiens* complex are globally distributed important disease vectors (Nchoutpouen et al., 2019). Species of the *Culex pipiens* complex particularly *Cx. quinquefasciatus* are predominant in the urban environment notably in Africa including Nigeria where suitable environmental conditions created by rapid unplanned urbanization is contributing to their proliferation (Brown et al., 2008). Of the arboviral diseases, Rift Valley Fever Virus (RVFV) is transmitted by mosquitoes between animals and humans, particularly belonging to the *Aedes*, *Culex* and *Anopheles* genera (Seufi and Galal, 2010). Although there is no recent report on RVF outbreak in Nigeria, numerous studies revealed the occurrence of RVFV in Jigawa and Katsina (Adamu et al., 2020), Oyo (Opayele et al., 2018), Niger (Alhaji et al., 2018), Lagos and Borno (Olaleye et al., 1996), Kaduna and Sokoto States (Ezeifeka et al., 1982). Vector identification and control is quintessential to the success of the eradication of the RVF and other arboviral diseases. Therefore, the strategy of mosquito control is highly dependent on the vector competence of the *Culex pipiens* complex vector (Fall et al., 2014). However, this vector competence has not been evaluated, since it is quite difficult to distinguish *Cx. pipiens, Cx. pallens, Cx. antennatus* and *Cx. quinquefasciatus* morphologically (Shaikevich et al., 2016). Previous studies revealed the significance of molecular techniques associated with more precision and reliability than older morphological differentiation methods of species complex including multiplex polymerase reaction (Shahhosseini et al., 2018; Kayedi et al., 2020; Adugna et al., 2020; Kirby et al., 2008).

Knowledge on the particular species and siblings of these potential arboviral vectors is quintessential in the design of effective control strategies. There has been no published data on the relative abundance and molecular identification of *Culex pipiens* complex, in North-western Nigeria. The aim of this study therefore was to investigate the relative abundance and molecularly identufy *Culex pipiens* complex, the potential vectors of Rift valley fever (RVF), Lymphatic filariasis and West Nile Virus (WNV) in Kura LGA, North-western Nigeria.

2. Materials and methods

2.1. Study area

Kano State is located at latitude 11°30 U N and longitude 8°30 U E with a land area of 20,760 km^2 (Adamu and Bassey, 2010). Its vegetation falls mostly within the Sudan Savanna Zone (Plate 1). Annual average rainfall in the area ranges from 884 to 1200 mm (from north to south of the state) which is characterized by one peak period (mono-modal), usually attained in August (Tanko and Momale, 2013). The rainy season is from May to October. The temperature is, on average, warm to hot throughout the year at about 25 ± 7 °C (Olofin and Tanko, 2002). The economy of the state is predominantly agricultural production with over 75% of the population engaged in farming. Livestock are also being raised especially cattle, sheep and goats (Haruna and Murtala, 2005).

2.2. Mosquito collection

Adult Mosquitoes were caught for three consecutive nights monthly (August–October 2019) during a visit Imawa village, North-western Nigeria using the CDC light trap (Model 512, J.W. Hock Ltd., Gainesville, Florida, USA).

2.2.1. Description of catch method in this study

Each trap was suspended from the roof about 1.8 m above the floor and/or the tree near the livestock pens. Collections were made between 18.00 h- 6.00 am in each of the homes with and homes without livestock selected as mosquito collection sites according to the method used by Obenauer et al. (Obenauer et al., 2013). The traps were modified with carbon-dioxide (CO2) and chemical lure according to the methods described in Obenauer et al. (Obenauer et al., 2013).

2.2.2. Preservation and morphological identification

All mosquitoes harvested in the traps were preserved in EDTA bottles in 70% alcohol and transported to the Entomology Laboratory, Department of Parasitology and Entomology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria. Adult Mosquitoes were sorted according to their genera; *Anopheles*, *Aedes* and *Culex* spp. using keys described by Becker et al. (Becker et al., 2012) and, analysed with the aid of stereomicroscope. Female mosquitoes belonging to *Culex pipiens* complex were stored on silica in Eppendorf tubes and transported to the National Arbovirus and Vector Research Center (NAVRC), Enugu State, Nigeria for further molecular analysis.

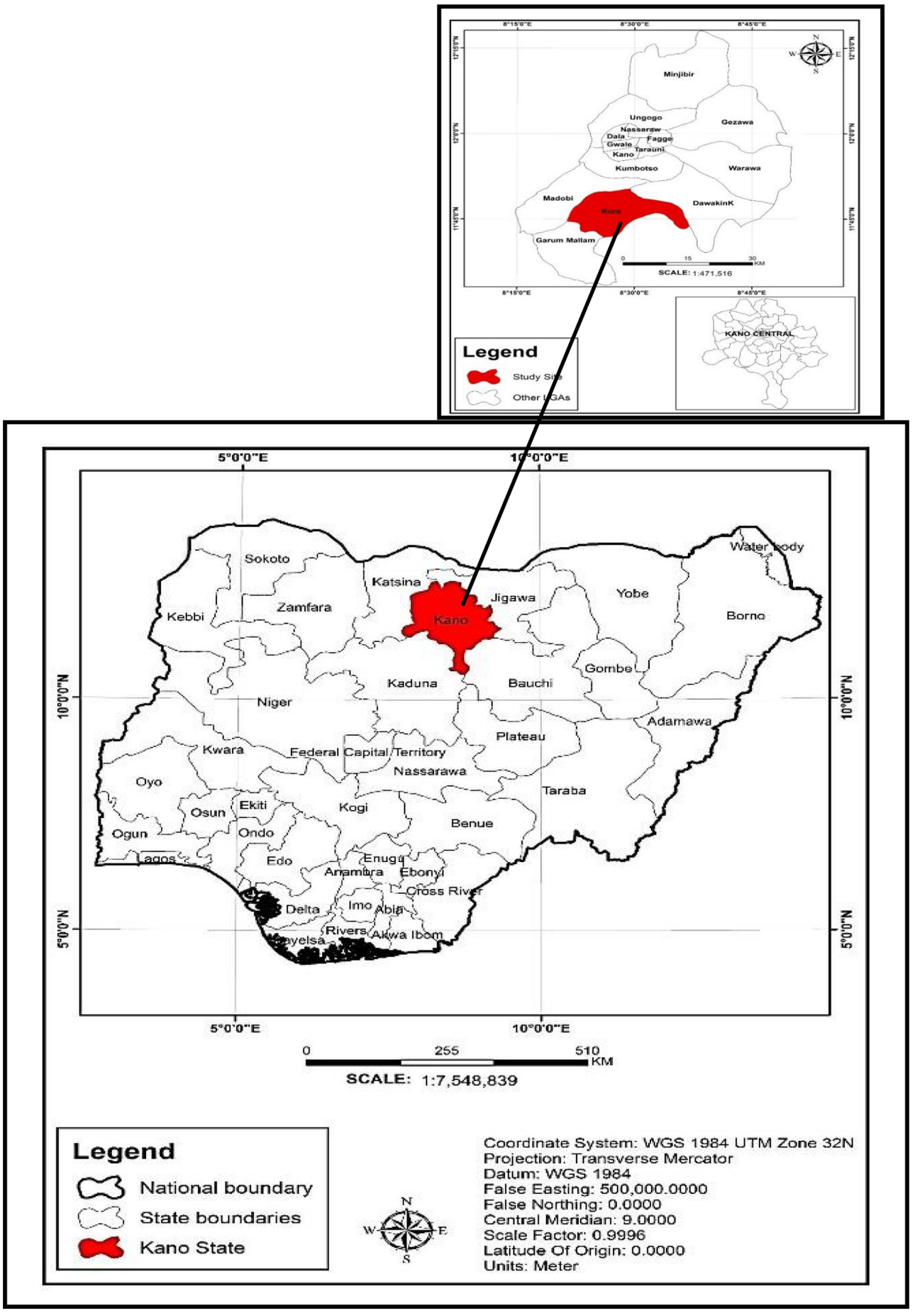

Plate 1. Map of Nigeria showing the geographical location of Kano State (A). Map of Kano State showing the study site: Kura Local Government Area (B).

2.3. Molecular identification of Culex pipiens complex

2.3.1. Isolation of genomic DNA

The extraction of Genomic DNA from the female *Culex* spp. of mosquitoes from was carried out using ZymoResearch DNA kit extraction (Cat. D 6015, ZymoResearch, California, CA, USA). The concentration and purity of the isolated DNA was analysed using a Nanodrop™ 1000 Spectrophotometer.

2.3.2. Multiplex polymerase chain reaction (PCR)

Molecular identification of *Culex pipiens* complex was conducted using the locus *ACE2 gene* of the mosquito through multiplex polymerase chain reaction as described by Smith and Fonseca (Smith and Fonseca, 2004).

Previously designed primers ACEpip for *Culex pipiens* (5′-GGA AAC AAC GAC GTA TGT ACT-3)′, ACEquin for *Culex quinquefasciatus* - (5′-CCTTCTTGAATGG CTG TGGCA-3); and the reverse primers B1246s (5′-TGGAGCCTCCTCTTCACGGC-3) (Nchoutpouen et al., 2019; Motayo et al., 2016). Multiplex PCR was performed in a 50 μl volume using 1 unit Taq 2× Master mix 25 μl (Cat. # K0701, Thermoscientific, Pittsburgh, PA, USA), 6 μl of genomic DNA template, 2 μl of each of following primers: ACE pip for *Cx. pipiens*, ACE quin for *Cx. quinquefasciatus*, and B1246s and 13 μl nuclease free water. The PCR reaction was performed with an Initial denaturation at 95^0C for 5 min, followed by 35 cycles at 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 1 min with a final 5 min extension at 72 °C (Nchoutpouen et al., 2019).

2.3.3. Gel electrophoresis

The amplified DNA were loaded onto an agarose gel (2%) with the 1000-bp ladder loading marker (Bio-Rad, Richmond, Calif., USA), stained with ethidium bromide (Amresco Inc., Solon, Ohio, USA), run for 45 min at 120 V, and visualized on a UV transilluminator (Sigma-Aldrich Chemie GmbH, Germany). The gels showing only primer dimers indicating that DNAs were extracted but are not specific for the primers added were conducted in triplicates individually (Supplementary materials).

2.4. Data analysis

The data obtained from the studies were expressed as percentages and presented as tables. Statistical Package for Social Sciences (SPSS Version 20.0) was used for the analysis and Chi- square test association was used and values of $P \leq 0.05$ were considered statistically significant.

3. Result

3.1. Entomological data

A total of ($n = 413$) adult mosquitoes, belonging to 3 genera, *Culex*, *Anopheles* and *Aedes* were caught and collected, of which *Culex* spp. had the highest occurrences; 201 species. Female were 120 (59.70%), while male were 81(40.29%). This was followed by *Anopheles* spp.; 117 species collected with 73 (36.32%) being female and 44 (21.89%) male. *Aedes* spp. were the lowest occurrence 95; female 62 (30.85%) and male were 33(16.41%) (Table 1).

3.2. Distribution of mosquitoes in the study area

Monthly distribution of *Culex* spp. caught in the study area. Chi-square analysis of variance was used to analyze the data. In August, 65 *Culex* spp. were caught comprising of home with livestock and home without livestock. This indicates that the calculated chi-square of 6.00 and the P.value of 0.199 which is less than the calculated chi-square. The implication here is that August had highest calculated chi-square and P. value compared to the months of September and October (Fig. 1).

To the best of our knowledge in Kano, northwestern Nigeria, *Culicoides* spp. were caught (Supplementary materials). This is significant because of its zoonotic and public health importance.

Table 1
Relative occurrence and sex distribution of mosquitoes caught in the study area.

Genus	Total number of collected species	Sex			
		Female (%)		Male	(%)
Culex	201	120	59.70	81	40.29
Anopheles	117	73	36.32	44	21.89
Aedes	95	62	30.85	33	16.41
Total	413	255		158	

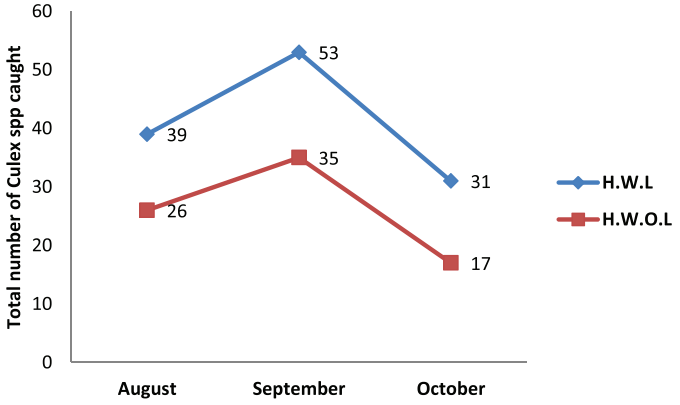

Fig. 1. Monthly distribution of *Culex* spp. in homes with livestock (H.W.L) and homes without livestock (H.W.O.L) in the study area.

4. Discussions

The mosquitoes belonging to the genera *Culex* (Table 1) and Culicoides (Table 3) collected and identified in Kura Local Government Area, North-western Nigeria are capable of transmitting pathogens causing many different arbo-viral diseases (Linthicum et al., 2016; Opayele et al., 2018; Nyakarahuka et al., 2018; Lagare et al., 2019). Little is known about the occurrence of *Cx. pipiens* complex mosquitoes in Kura Local Government Area. This information is quintessential for assessing the epidemic potential of RVF in Kano State. Vector control in the study area is majorly through the use of insecticides. Among the species collected, *Culex* spp. had the highest occurrences; 201 species. Females had the highest occurrences 120 (59.70%), while male had 81 (40.29%). Similar high occurrences were recorded for *Culex* spp. (52.5%) in Makurdi, Nigeria (Msugh-Ter et al., 2017) but lower than the results recorded in Minna, Nigeria (24%) (Rabi'u and Ahmed, 2019).

These studies displayed for the first time ever the relative abundance and molecular characterisation of *Cx. pipiens* complex in Kura Local Government Area, North-western Nigeria, this information is necessary for establishing effective surveillance and targeted control programs to prevent or control RVF outbreaks. The abundance of hybrid mosquitoes increased in the area is between August and October associated with a decrease in temperature (Fig. 1).

Even though the multiplex PCR aimed at the molecular identification of the *Culex pipiens* complex and therefore the specific siblings and perhaps hybrid spp. are still not very clear (Supplementary materials), although this may not represent the true picture as the major limitations to this study were a smaller sample size of the field mosquitoes collected and our inability to consistently test each sampled field mosquito using PCR due to high cost. Hence, only a small proportion of identified mosquito species were tested using PCR. The presence of *Culex* mosquitoes encountered in the present study should be a source of concern to residents and the Kano State Government. It was noticeable that greater proportion of *Culex* mosquitoes than *Aedes* were collected (Table 1). A probable reason for this is that the former are usually more active from dusk to dawn (night-biting species) unlike *Aedes* that are mostly day-biting species (World Health Organization (WHO), 2013) (Table 3). Baba et al., 2006 in Nigeria, (Özer et al., 2007) in Turkey, LaBeaud et al. (LaBeaud et al., 2011) in Kenya, and Vaux et al. (Vaux et al., 2015) in the United Kingdom also collected greater proportion of *Culex* mosquitoes in their studies. This study revealed the abundance of Culicines for the first time in the study area and was found to be higher than other mosquito genera in houses with livestock. This is primarily because presence of livestock attracts Culicines into a compound as previously shown (Kirby et al., 2008; Forattini et al., 1993). More significantly however, RVF was previously isolated from *Culicoides* spp. and *Culex antennatus* in Ibadan, Nigeria (Fagbami and Ojeh, 1983; Oluwayelu et al., 2018).

5. Conclusions

This study provides an insight for the first time into the relative abundance of *Culex* mosquito species in Kura Local Government Area, Northwestern Nigeria. And is suggestive of the prevalence of vector-borne diseases such as yellow fever, dengue fever and filariasis.

in the study area. Even though the multiplex PCR aimed at the molecular identification of the *Culex* spp. and therefore the specific species perhaps hybrid spp. are still not very clear, this work shows the importance of the occurrence of these potential arboviral vectors in the study area. Of significance also was the identification of *Culicoides* spp. for the first time in the study area. Therefore, intensive vector control programmes and public enlightenment especially on human activities that encourage mosquito breeding are recommended. Further studies through designing and validating primers for *Culex antennatus*; an important species previously associated with transmission of RVF in Nigeria to reveal if these species are still in existence is recommended. It will also be interesting if the harvested *Culex* spp. mosquitoes are investigated using Real Time – Polymerase Chain Reaction (RT-PCR), to determine or otherwise the presence of the Rift Valley Virus in the study area.

Declaration of competing interest

The authors declare that there are no competing interests regarding the publication of this paper.

Acknowledgements

The authors thank Dr. C.S. Wilding, of Liverpool John Moores University, Liverpool L2 2QP, United Kingdom, for his helpful comments on the manuscript. We also thank Staff of the Center for Infectious Diseases, Bayero University, Kano and Molecular Biology unit of the National Arbovirus and Vector Research Center (NAVRC), Enugu State, Nigeria for their contribution in the conduct of this research.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.parepi.2021.e00213.

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