RESEARCH PAPER

Origin of the blood meal of the *Aedes aegypti* mosquito in five localities in Benin

Tchibozo Carine[1], Yadouleton Anges[*1,2,3], Dramane Gado[1,2,3], Hounkanrin Gildas[1], Adewumi Praise[1], Joest Hanna[4]

[1] *Laboratoire des Fièvres Hémorragiques Virales et des Arbovirus du Bénin*

[2] *Ecole Normale Supérieure de Natitingou, Université Nationale des Sciences, Technologies, Ingénierie et Mathématiques (UNSTIM), Bénin*

[3] *Centre de Recherche Entomologique de Cotonou, Bénin*

[4] *Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany*

Abstract

With the aim of learning about the multiple origins of blood meal sources in *Aedes aegypti* in Benin, a study was conducted in five localities from the south to the north of the country (Cotonou, Porto-Novo, Calavi, Dassa and kandi) from June 2020 to October 2021 to capture adult populations of *A. aegypti*. To achieve this objective, BG-Sentinel and Aedes Gravid traps were set daily inside and outside four randomly selected concessions in each of the above-mentioned sites, three times a week for the duration of the study. Populations of blood-feeding *A. aegypti* mosquitoes were identified using the Polymerase Chain Reaction (PCR) technique. PCR results were confirmed by sequencing to identify the origin of the blood meal. Out of a total of 3,749 mosquitoes collected, *Aedes aegypti* (79.22%) and *Culex quinquefaciatus* (20.08%) were the two main species caught. With a total of 2,970 *A. aegypti* populations, 2,684 (71.7%) were non-blood-fed, compared with 286 (7.6%) blood-fed. For *Culex quinquefasciatus*, out of 753 populations caught, 733 (19.5%) were non-gorged versus 20 (0.5%) blood-fed. Research into the origin of the blood meal using the PCR technique showed that out of 1019 mosquitoes analyzed, 987 (96.8%) had taken their blood meal from humans. This result was confirmed by sequencing analysis of PCR-positive pools. The anthropophagous nature of *A. aegypti* confirmed by the sequencing results during this study remains an important clue in the implementation of arbovirus control strategies, particularly against *A. aegypti* mosquitoes in Benin.

*Corresponding Author: Yadouleton Anges ✉ anges33@yahoo.fr

Introduction

According to the World Health Organization in 2016, several vector-borne diseases are caused by arthropods and the most important are found in sub-Saharan Africa. The main mosquito general responsible for these diseases are: *Anopheles*, *Aedes* and *Culex*.

Aedes aegypti is the main vector of arboviruses such as dengue, zika, yellow fever and chikungunya. This highly hematophagous domestic mosquito is found in human dwellings and, is present in jars, abandoned cans, from northern to southern Benin throughout the year with risks of spreading dengue fever (Yadouleton *et al.,* 2018). The recent and rapid spread of this mosquito to new geographical areas, including rural environments, means that it is considered a public health problem (WHO, 2016), Moreover, these blood-requiring mosquitoes most often develop a preference for the most available and stable source of blood (Roiberg and Gordon *et al.,* 2005; O'Meara *et al.,* 2020). The preference of mosquito vectors for a specific host for their blood meal undoubtedly affects the mosquitoes' ability to transmit pathogens.

Over the past decade, several cases of arboviruses, notably dengue, have been reported in sub-Saharan Africa, notably in Gabon (Abe *et al.,* 2020), Côte d'Ivoire (Moi *et al.,* 2010), Senegal (Faye *et al.,* 2014) and Burkina-Faso (Ouédraogo *et al.,* 2019), with over 1,500 cases recorded. The preference of mosquito vectors for a specific host for their blood meal surely affects the mosquitoes' ability to transmit pathogens. Consequently, understanding mosquito blood feeding patterns in different environments can help determine which host species can influence the maintenance and epidemic transmission of viruses. With this in mind, this study was carried out in 5 cities in Benin, in order to determine the origin of the blood meal in *Aedes aegypti* populations.

Materials and methods

Study sites

This study was carried out in five localities in Benin from June 2020 to October 2021. Three urban sites were selected in southern Benin: Abomey-Calavi (6.418736ºN, 2.3425287ºE), Cotonou (6.364528ºN, 2.441564ºE) and Porto Novo (6.510439ºN, 2.604147ºE). In the center of the country, a semi-urban site in the locality of Dassa-Zoumè (7.783625ºN, 2.185264ºE) was chosen, and in the north-east of Benin, the W National Park (12.040653°, 3.034178°); the choice of each of these sites is justified by previous work carried out by Yadouléton *et al.* which showed ecological niches and artificial gites that favour the development of *Aedes aegypti*.
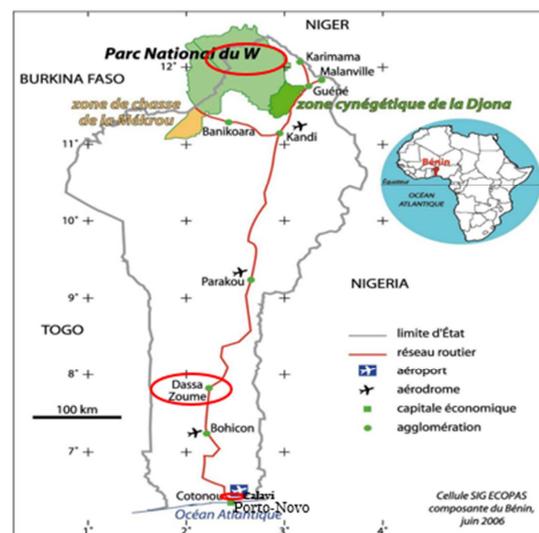

Fig. 1. Map of study sites.

Mosquito collection

Adult mosquitoes were collected using BG-Sentinel (BGS) mosquito traps (Biogents, Regensburg, Germany), Gravid Aedes Trap (GAT) passive traps, and human bait catches (HLC).

The traps were set from 2pm to 6pm, and all catchers were vaccinated against yellow fever and protected against malaria with sulfadoxine-pyrimethamine chemoprophylaxis. Five BGS and GAT traps were set up at each site from 2pm to 7pm. Trapped mosquitoes are immediately placed in a portable refrigerated box and transported to the laboratory, where they were stored at -20°C.

Identification of blood meal hosts

Morphological identification of individuals of the genus *Aedes* was carried out using a stereomicroscope

following the taxonomic key proposed by Forattini (1965) to identify insects at genus level, and the taxonomic examination of Cova-Garcia *et al.* (1966) was used to identify species. Molecular identification of individual *Aedes* species was achieved by amplifying a fragment of the COI gene (Folmer *et al.*, 1994) from insect RNA.

After identification, mosquitoes with fresh or visible blood remains are used for blood meal identification. They are classified as partially fed, freshly fed, late fed and placed in sterile 2ml cryotubes, then stored at -20°C until the blood meal host is identified.

For blood-meal host identification, mosquitoes are homogenized with 500ml of high-glucose Dulbecco's modified Eagle's medium (DMEM; Sigma-Aldrich, St. Louis, USA) and two stainless steel beads. After centrifugation at 8,000 rpm for 2 min, the supernatant is used directly for PCR analysis. The homogenate is then used in a PCR reaction targeting Cytochrome B from avian and mammalian species (Pautasso *et al.*, 2013). PCR reactions are performed using the Phusion Blood Direct PCR kit (Thermo Fischer Scientific, Waltham, USA). DNA amplifications are visualized after electrophoresis on 2% agarose gels stained with Midori Green Advance (Biozym Biotech, Hessisch Oldendorf, Germany). The aim of each reaction is to amplify a mitochondrial gene fragment for the cytochrome b protein, by means of which vertebrate species can be genetically differentiated (Burkett-Cadena *et al.*, 2008; Kitano *et al.*, 2007).

All positive PCR samples are sequenced using the Sanger method (LGC Genomics, Berlin, Germany).

Sequence results are evaluated using Geneious version 9.0.5 (http://www.geneious.com, Kearse *et al* 2012) and the Basic Local Alignment Search Tool (BLAST) from the National Center for Biotechnology Information (NCBI) (https://blast.ncbi.nlm.nih.gov/ Blast.cgi, Altschul *et al* 1990). An identification value of at least 95% is required as an internal threshold value, which we set ourselves, to comply with a quality standard.

Results and discussion

A total of 3749 mosquitoes, comprising two genera and four species, were captured during the sampling period. The dominant species was *Ae. aegypti* with 2,970 specimens, 9.6% of which were bloodstained, followed by 753 specimens of *Cx. quinquefasciatus* with 2.7% bloodstained.

Table 1. Number and species of gorged and non-gorged mosquitoes collected.

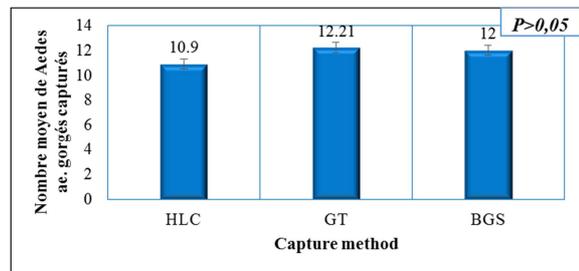|	*Aedes aegypti*	*Aedes albopictus*	*Aedes vittatus*	*Culex quinque fasciatus*	Unspecified
Gorged,% (CI at 95%)	90.4 ([89.6 ; 96.3])	100 ([98.78 ; 100])	100 ([98.76 ; 100])	97.3 ([94.7 ; 99.3])	100 ([92.4 ; 100])
No-gorged (CI at 95%))	9.6 ([6.2 ; 11,8])	0 (0 ; 3])	0 ([0 ; 3.1])	2.7 ([0.2 ; 3.5])	0 ([0 ; 4])
P value	< 0,05				

Fig. 2. Average number of gorged *Aedes aegypti* collected according to capture method.

The number of *Aedes aegypti* captured ranged from 0 to 18, from 1 to 20 and from 3 to 18 respectively according to the HLC, GT and BGS capture methods. With respective averages of 10.9±4.15 (CI: [9.39; 12.41]), 12.21±4.65 (CI: [10.51; 13.9]) and 12±3.69 (CI: [10.65; 13.34]), there was no significant difference between the average numbers of gorged mosquitoes from the different capture methods (P>0.05). Consequently, all the methods used in this study can be recommended for capturing mosquitoes of the *Aedes* genus.
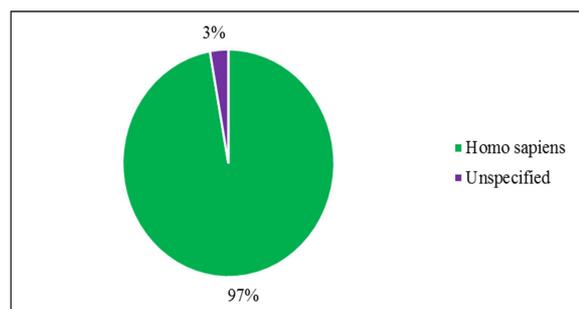

Fig. 2. Origin of *Aedes aegypti* blood meal.

Investigation of the origin of the blood meal using the PCR technique showed that the majority of gorged mosquitoes had taken their blood meal from humans. This result was confirmed by the sequencing test. Of the 286 mosquitoes analysed, 278 had taken their blood meal from humans. The origin of the blood meal in the remaining mosquitoes (8) was undetermined.

Discussion

Our results showed four different mosquito species, with *Aedes aegypti* being the predominant species in all locations studied. The presence of both *A. aegypti* and *A. albopictus* across Benin suggests that the environmental factors prevailing in the country are favourable to the development of both species. However, the low representation of *Aedes albopictus* across the entire country could be explained by natural selection within this population. This study also reveals that almost all gorged *Aedes* mosquitoes have taken their blood meal from humans, giving them an antropohilic character. This undoubtedly influences the ability of these mosquitoes to transmit arboviruses such as dengue, which are present in these vectors in Benin (Tchibozo *et al.*, 2022).

These results concur with the hypothesis of Camison *et al.* (2020) who reported that humans were the preferred source of blood supply for *Aedes aegypti*. According to Raji and De Gennaro (2017) and Gonzales and Hansen (2016), the host's natural odour could play a stimulating or motivating role in activating mosquitoes towards a specific host for its blood supply. Similarly, Carolyn S *et al.* (2015) showed that the evolution of human odour preference in domesticated mosquitoes is linked to the odorant receptor AaegOr4. Indeed, the increased expression and ligand sensitivity of this receptor recognises a compound present at high levels in human odour. The preference for humans is therefore correlated with increased expression and sensitivity to ligands of the Or4 odorant receptor. These changes due to the domestication of *Aedes aegypti* may help these mosquitoes to distinguish humans from animals by increasing their behavioural sensitivity to human odorant sulcatone stimulation.

It should be noted that sulcatone has been described as a mosquito repellent when added to human odour at certain concentrations (McBride *et al.*, Logan *et al.*, 2010; Menger *et al.*, 2014) and sometimes as an attractant when added at low concentrations or when administered alone (Menger *et al.*, 2014; Bernier *et al.*, 2000)

Conclusion

Our results indicate that, *A. aegypti* has a high rate of human bites in 5 ecological zones with low-medium and high antropogenic activity in Benin. This confirms the role of humans as hosts in local epidemic transmission of the dengue virus. The anthropophagous nature of this mosquito, confirmed by the results of this study, is a highly favourable indicator for strategies to control *Aedes aegypti* populations in Benin.

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