



BLOOD MEAL ANALYSIS OF FILARIAL VECTOR *CULEX QUINQUEFASCIATUS* IN SOUTHERN BENIN

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ABSTRACT

To evaluate the host-feeding preference of *Cx. quinquefasciatus* in southern Benin, a cross-sectional study was carried out in five neighborhoods, two at Cotonou and three at Porto-Novo, from December 2017 to March 2018. In each neighborhood, 4 inhabited houses were selected as mosquito catching sites. The collection of mosquitoes was conducted by pyrethrum spray captures (PSC) from 07:00am to 9:00am every morning and twice a week. Fifteen minutes after the spray, knocked down mosquitoes falling on white bed sheets were preserved in Eppendorf tubes labeled with an indication of the place, date of collection, and room number for further identification. Each collected mosquito was identified and classified as *Culicinae* or *Anophelinae* after an examination on a binocular microscope and identification keys. Finally, host-feeding preference of *Cx. quinquefasciatus* was determined based on serological characteristics of the plasma using immunoenzymatic ELISA (Enzyme Linked Immunosorbent Assay) technique on fed *Cx. quinquefasciatus* collected. From our investigation, a total of 978 mosquitoes were collected during the study period in the different areas with *Culex quinquefasciatus* as the most predominant specie. The majority of mosquitoes collected from neighborhoods of Cotonou and Porto Novo tested for blood meal revealed that 85.71% of the collected mosquitoes fed on human and 5.72% on various animals (beef, sheep and pork). The results of this study confirm the anthropophagic behavior of *Cx. quinquefasciatus* which tend to feed more on human than animal. This feeding preference is an important result to the implementation of effective strategies to control *Cx. quinquefasciatus*.

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INTRODUCTION

Culex quinquefasciatus an anthropophilic mosquito is the principal vector of *Bancroftian filariasis* and a potential vector of *Dirofilaria immitis*. This mosquito species is also a potential vector of several arboviruses like West Nile virus (WNV), Rift Valley fever virus, avian pox and protozoa like *Plasmodium relictum* that causes bird malaria (Aziz et al., 2012, Yadouleton et al., 2015). In 2016, WHO reported 120 million people worldwide infected with *Bancroftian filariasis* the main parasite of *Cx. quinquefasciatus* where 34% of infected people

were in Africa (WHO, 2016). Indeed, the rapid urbanization in many African countries creates collections of stagnant water which represents good breeding sites of *Cx. quinquefasciatus*. This mosquito is a major biting nuisance, particularly in urban areas where it thrives in wet pit latrines, blocked open drains, and polluted puddles (Yadouleton et al., 2015). In Benin, *Cx. quinquefasciatus* is a common mosquito that lives close to people due to the presence of large number of *Cx. quinquefasciatus* breeding sites (Yadouleton et al., 2015). To control this mosquito, the use of Insecticide-Treated Nets (ITN) and the implementation of Indoor Residual Spraying

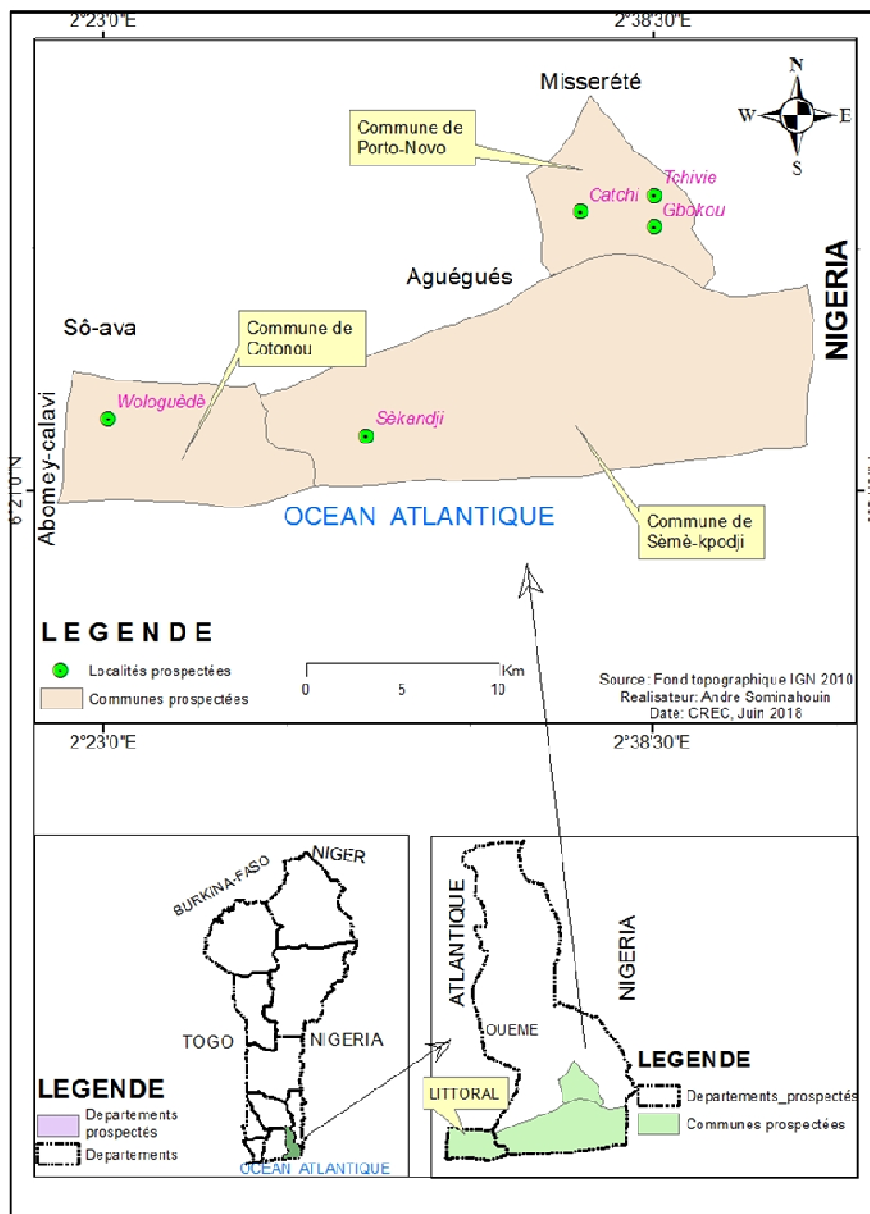


Figure 1. Map of Benin showing the study area

(IRS) as vector control strategies were adopted in many African countries where lymphatic filariasis (LF) is present (Jones *et al.*, 2012). However, despite these strategies to control this mosquito and to reduce the incidence of LF, *Cx. quinquefasciatus* is still able to feed on its hosts and to collect parasite-infected blood during its blood meal (Simonsen *et al.*, 2008; Hamon *et al.*, 1963; Akogbeto *et al.*, 2010). Therefore, for better control of this mosquito, the Knowledge of the blood-feeding preferences of *Cx. quinquefasciatus* needs to be understood. In Benin, little work has been done on the blood-feeding preferences of mosquitoes. The few studies carried out on this topic were done on *Anopheles gambiae*, the main vector of malaria (Anagonou *et al.*, 2015, Padonou *et al.*, 2012) despite the presence of *Cx. quinquefasciatus* throughout the year with several cases of lymphatic filariasis (Yadouleton *et al.*, 2015). It is therefore crucial to study the blood-feeding preferences of *Cx. quinquefasciatus* in southern Benin for a better control of this mosquito.

MATERIAL AND METHODS

Data collection areas: The study was conducted in southern Benin in the cities of Cotonou (6°45'N and 2°31'E) and Porto Novo (6° 30'N and 2°47'E).

At Cotonou the neighborhoods of Wologuèdè and Sèkandji whereas at Porto-novo, neighborhoods of Catchi, Tchivié and Agbokou were chosen for mosquito collection. The choice of these neighborhoods is based on the weak level of urbanization (Figure 1). The southern Benin is characterized by a tropical coastal Guinean climate with two rainy seasons (April–July and September–November). The main annual rainfall is more than 1300 mm.

Mosquito collection: In each study site, 4 inhabited houses have served as capture points. The collection of mosquitoes was conducted by pyrethrum spray captures (PSC) from 07:00am to 9:00am every morning and twice a week from December 2017 to March 2018. Fifteen minutes after the spray, knocked down mosquitoes falling on white bed sheets were preserved in Eppendorf tubes labeled with an indication of the place, date of collection, and room number for further identification.

Morphological identification of captured mosquitoes: Mosquitoes collected were identified and classified as *Culicinae* or *Anophelinae* according to the identification keys of Gillies and Meillon (1968), Gillies and Coetzee (1987), Doby (1955) and Edwards (1941).

Table 1. Mosquito fauna recorded at Cotonou and Porto-Novo in the different neighborhoods.

Mosquito species	Neighborhoods						Total
	Wologuèdè	Sèkandji	Catchi	Tchivié	Agbokou		
<i>Aedesluteocephalus</i>	0	1	0	1	0		2
<i>Ae. Aegypti</i>	54	47	10	23	16		150
<i>Ae. vittatus</i>	0	0	2	0	1		3
<i>Anopheles gambiae</i>	17	21	11	07	13		69
<i>An. pharoensis</i>	3	6	2	4	7		22
<i>An. Ziemanni</i>	2	6	0	2	1		11
<i>Culex tigripes</i>	0	0	3	1	0		4
<i>Cx.gr decens</i>	2	1	3	1	5		12
<i>Cx. quinquefasciatus</i>	105	97	111	203	148		664
<i>Mansonia africana</i>	10	7	3	9	12		41
Total	193	186	145	251	203		978

Table 2. Host-feeding preference of *Cx. Quinquefasciatus*

Neighborhoods	Nb Tested	Human		Beef		Sheep		Pork		Negative	
		N	%	N	%	N	%	N	%	N	%
Wologuèdè	70	60	85,71 ^a	2	2,86 ^b	1	1,43 ^b	1	1,43 ^b	6	8,57
Sèkandji	70	54	77,14 ^a	5	7,14 ^b	3	4,3 ^b	3	4,3 ^b	5	7,14
Catchi	70	25	35,71 ^a	6	8,57 ^b	6	8,57 ^b	9	12,86 ^c	24	34,29
Tchivié	70	63	90 ^a	0	0 ^b	0	0 ^b	0	0 ^b	7	10
Agbokou	70	65	92,86 ^a	0	0 ^b	1	1,43 ^b	0	0 ^b	4	5,71
Total	350	267	76,29	13	3,71	11	3,14	13	3,71	46	13,14

The percentages assigned the same letter are not significantly different ($p > 0.05$). Those assigned different letters are significantly different ($p < 0.05$).

Host-feeding preference of *Cx. Quinquefasciatus*: 350 blood-fed *Culex quinquefasciatus* were individually prepared for the test by grinding with a plastic pestle in 750µl of 0.01M phosphate-buffered saline (PBS), pH 7.4, with 0.1% gelatin and centrifuged at 10,000Xg for 10 min, and the supernatant was used for blood meal analyses. To standardize the test, positive control samples consisted of a 10µl aliquot of each supernatant from the 14 females, which had fed on the blood sample of each host, and the negative control sample was constituted of a 10µl aliquot of each supernatant from the 14 males. ELISA was conducted using ninety-six well microplates (Nunc®, Maxisorp, Denmark) covered with 50µl/well of each anti-IgG (H+L) host-specific [anti-human IgG 6284-00 Zymed, USA; anti-rat IgG 6295-00, Zymed, USA], diluted in PBS at concentrations of 20µg/ml, 10µg/ml, 5µg/ml, 2.5µg/ml, 1.25µg/ml and 0.625µg/ml and incubated overnight at 4°C. Plates were blocked with PBS/1% gelatin and kept covered at room temperature for 3h. Each plate was washed five times with PBS-0.05%Tween20 (P-1379, Sigma, USA); then, the competitive reaction was performed in two consecutive wells by addition of 50µl/well of the PBS/0.1% gelatin and 50µl/well of each positive control in each plate. Those samples were diluted from 1:50 to 1:3,200. After 18h at 4°C, biotin conjugated anti-human IgG, anti-beef IgG, anti-sheep IgG, anti-pork IgG anti-were added. After 1h at room temperature, 50µl/well of avidin-alkaline phosphatase conjugate (A7294, Sigma,USA) in PBS/1% gelatin was distributed. After 1h, the enzymatic reaction was obtained by addition of p-nitrophenyl phosphate (Sigma Chemical) in diethanolamine buffer. Absorbance was measured by spectrophotometry (Multiskan ® EX) at 405nm.

RESULTS

Mosquito fauna

A total number of 978 mosquitoes were collected from pyrethrum spray captures (PSC) at the five neighborhoods of the study site (Table 1). The majority was *Culex* spp (67.89%). Of the remaining 32%, 15.34% were *Aedes aegypti* and 7.1% were *Anopheles gambiae* and the rest were *Mansonia Africana* and *Anopheles* (Table 1).

Host-feeding preference of *Cx. Quinquefasciatus*: From the 350 females blood-fed *Culex quinquefasciatus* tested by enzyme-linked immunosorbent assay (ELISA) blood meal, 85.71% of *Culex quinquefasciatus* from the five neighborhoods took their blood meal on human whereas 5.72% on various animals (beef, sheep, pork) (Table 2).

DISCUSSION

10 species of adult mosquitoes were collected by pyrethrum spray captures (PSC) method where *Cx. quinquefasciatus* is the most important specie. The diversity of mosquitoes species in the five neighborhoods of the study site was previously found by many scientists (Lingenfelter *et al.* (2010; Djènonatin *et al.*, 2010; Padonou *et al.*, 2012; Agbanrin *et al.* 2015) however, they found more species than what was found in this study. This can be explained by the various methods of adult collections which were used, the time duration of the study, the dynamic of mosquito population subjected to the urbanization pressure. In fact, the rapid urbanization in many African countries particularly in southern Benin contributes to the change of biotope which offers good breeding sites for the preimaginal populations of each species: permanent aquatic vegetation, stagnant water, tires (Agbanrin *et al.*, 2015). The predominant of *Cx. quinquefasciatus* as the most abundant species collected during our study could be explained by the global urbanization of the different neighborhoods chosen for our study which offering a good breeding sites for mosquitoes development particularly for *Cx. quinquefasciatus*. This finding confirmed previous reports in Benin (Yadouleton *et al.*, 2014) and in Burkina Faso (Robert *et al.*, 2001) on the predominant of *Cx. quinquefasciatus* in urban and peri-urban areas. Moreover, the evaluation of host-feeding preference of *Cx. quinquefasciatus* showed that the majority of *Cx. quinquefasciatus* population took its blood on human regardless the neighborhoods where the mosquitoes were collected. This is probably due to the fact that domestic animals are rare in the neighborhoods chosen at Cotonou and Porto-novo for the study. This finding confirmed results found by Yadouleton *et al.* (2015) in a neighborhood of Cotonou (Agbalilame) where 93% of *Cx. quinquefasciatus* fed on human. This result confirmed the anthropophagic behavior of

Cx. quinquefasciatus (Tirados et al., 2006). However, the presence of animals caused a significant zoophagic deviation and explained the percentage of *Cx. quinquefasciatus* fed in pork (12.86%), beef (8.57%) and sheep (8.57%) found in this study.

Conclusion

The results of this study confirm the anthropophagic behavior of *Cx. quinquefasciatus* which tend to feed more on human than animal. This feeding preference is an important result to the implementation of effective strategies to control *Cx. quinquefasciatus*.

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