Scientific Research Publishing

Advances in Entomology, 2023, 11, 156-171
https://www.scirp.org/journal/ae
ISSN Online: 2331-2017
ISSN Print: 2331-1991

Evaluation of Malaria Transmission and Vector Control Strategies in the Dry Season in the Cotonou V Health Zone, Benin, West Africa

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How to cite this paper: Filémon, T., Razaki, O., Franck, Z.A.R., Marlène, A., Zoulkifilou, S.D., Festus, H., Idayath, G.Y., André, S. and Martin, A. (2023) Evaluation of Malaria Transmission and Vector Control Strategies in the Dry Season in the Cotonou V Health Zone, Benin, West Africa. *Advances in Entomology*, **11**, 156-171.
https://doi.org/10.4236/ae.2023.113012

Received: June 9, 2023
Accepted: July 22, 2023
Published: July 25, 2023

Abstract

The anarchic urbanization of certain African cities favors the multiplication of the malaria parasite. Thus, the urgent mobilization of African cities is essential to combat this health risk. It is, therefore, with the objective of contributing to the investigation of problem areas that the present study evaluates malaria transmission and vector control strategies in the Cotonou V health zone in particular. This is a cross-sectional study taking into account four neighborhoods, including Wologuèdè, Sainte Rita, Gbèdjromèdé and the area around Etoile Rouge. Two nocturnal captures on voluntary humans and the method of spray were carried out in the dry season from December 2021 to February 2022. On the captured *Anopheles*, the ELISA Circum-Sporozoite Protein test was performed to determine the infectivity and calculate some transmission parameters. Finally, we conducted a survey using the second stage sampling method with one step to ask selected households about their knowledge of vector control methods, their use and the physical integrity of LLINs. We collected 2386 culicidae of which the majority was *Culex quinquefasciatus*. After the ELISA test, the 29 *Anopheles* tested, showed no infectivity, *i.e.* an EIR of 0 pi/h/n. In addition, 99% of the populations in the Cotonou V area use LLINs to protect themselves. However, coils, door and window screens, aerosol sprays, skin and household repellents, and periodic indoor spraying were used. Finally, the majority of nets observed had T1 tears, but there were also T2, T3 and T4 nets (P-value = 0.0). This study confirms that malaria transmission during the dry season in the Cotonou V health zone is almost negligible but not non-existent. Also, populations are exposed to the nuisance of *Culex quinquefasciatus* mosquitoes continuously throughout the year.

Keywords

Anopheles, Transmission, Urban Malaria, Cotonou V Health Zone, Vector Control

1. Introduction

Malaria tops the list of priority communicable diseases in Benin. It remains one of the leading causes of mortality in the world with a percentage of 80% of morbidity cases in 2018 [1]. Transmitted by the infecting bite of mosquitoes of the genus *Anopheles* inducing in the blood the presence of hematozoa of the genus *Plasmodium*, malaria constitutes a public health threat with an incidence rate of 21.9% in 2019 [2]. It is a public health problem for more than 3.5 billion people living in 106 countries and territories worldwide.

In 2014, approximately 198 million cases and 584,000 deaths were recorded worldwide with more than 80% occurring in Sub-Saharan Africa [3].

Despite a 60% reduction in morbidity in 15 years and more than 6 million lives saved by malaria control programs, the number of cases is stagnating, particularly in African countries. Due to the anarchic urbanization of certain African cities and global warming, the mosquito's breeding sites favor the dangerous multiplication of the malaria parasite. We are thus witnessing the emergence of urban malaria, which is more difficult to localize, leads to greater health complications and can turn into an epidemic at any time [4]. In 2000, an estimated 40.1% of Benin's population lived in urban areas, and the country could be considered urbanized by Sub-Saharan African standards. Also, urbanization has a significant impact on the composition of the vector system and the dynamics of malaria transmission: the variability of malaria transmission and vector density in Cotonou [5]. Assessment, understanding, and control should not simply be initiatives taken in rural communities [6]. Therefore, the urgent mobilization of large African cities is paramount to combat this health risk [4].

Several studies have been conducted in Benin to gain further information on malaria in certain regions [7]. A study conducted in Copargo in northeastern Benin again shows that *Anopheles gambiae* remains the main vector of malaria transmission with high aggressive densities in this commune [8].

Similarly, a study conducted during the dry season in Benin in the coastal area of Gbégamey, Sainte Rita and Ladji shows that malaria transmission in urban areas is lower than in rural areas, but the intensity of transmission in Cotonou is remarkably high [9].

However, these studies did not take into account the health zone of Cotonou V, the different seasonal periods, the transmission of malaria and the means of vector control.

Knowing that some population groups are at higher risk of contracting malaria and being severely affected than others [4], this study assesses malaria transmission and vector control strategies in the dry season in the Cotonou V health zone.

2. Material and Method

2.1. Area and Type of Study

The study was conducted in Benin in the coastal department, specifically in the city of Cotonou from December 2021 to February 2022. This city is located in southern Benin. It is characterized by a tropical climate with a hot and dry season from December to February and a rainy season from mid-March to October. The work was carried out specifically in four clusters of Cotonou, namely Sainte Rita, Gbèdjromèdé, Wologuèdè and the vicinity of the Etoile Rouge.

This is an analytical cross-sectional study that allows highlighting a relationship between the exposure of the resident populations in the Cotonou V health zone to culicidae, the transmission of malaria in this zone and the vector control strategies of the study population.

2.2. Sampling of Mosquito Populations

The collection of information on the Culicidal diversity in our study area was based on night captures. These captures were carried out twice during the study period in January and February respectively. The methods used were capture on human volunteers and capture by morning spray.

The collection of mosquitoes was done between 21:00 and 05:00 for each of the two different captures. In the study area, 8 houses were selected, *i.e.* 2 houses per neighborhood and 4 captors per neighborhood, for a total of 16 captors in the Cotonou V health zone, specifically in Wologuèdè, Sainte Rita, Gbèdjromèdé and the area around Etoile Rouge.

The most direct method of measuring host-vector contact is the human subject capture technique (HSC). It consists of an individual ("captor"), sitting on a chair or stool, capturing, with the help of tubes (glass or plastic), mosquitoes that come to rest on a part of the body left bare (often the lower part of the legs, below the knees). Ideally, the mosquitoes are captured before they have time to bite. In order to estimate the aggressiveness of both endophagous (biting inside the house) and exophagous (biting outside the house) anopheles, captures were made simultaneously inside and outside the houses. Entomological methods of sampling adult anopheles that land on or near humans allow us to estimate the number of bites a person receives per unit of time.

The second method that was used for the collection is the capture by spray, which consists of an intra-domestic spray of insecticide inside the houses for the collection of the residual fauna in the morning. Thus, 2 rooms were selected per night of capture in different neighborhoods to collect in the morning at 06:00, the mosquitoes that entered during the night. This morning spraying was done using aerosol cans and a white sheet for the collection of residual fauna.

The adult mosquitoes thus collected were identified on the basis of their morphological characteristics using the identification keys of Gillies and De Meillon (1968) [10] with a binocular magnifying glass and the vector anopheles were transported to the laboratory. These *Anopheles* species are stored individually in Eppendorf tubes containing silica gel and cotton and kept at −20˚C until the time of treatment.

2.3. Entomological Parameters of Malaria Transmission

From a quantitative point of view, several mathematical indices are calculated from the entomological data.

2.4. Aggressive Density

The aggressive density or aggressiveness rate ma, is the product of the anophelian density in relation with humans (m) and the anthropophilic rate (a).

It is expressed as the number of anopheles bites per human per unit time. It is obtained by dividing the total number of anopheles captured by the number of subjects used, per unit of time.

2.5. Sporozoite Index

The sporozoite index (SI) is the percentage of anopheles carrying circumsporozoite antigens (Ag CSP). This value is expressed as a percentage.

2.6. Entomological Inoculation Rate

Malaria transmission is expressed as the entomological inoculation rate (EIR) which is the product of the human bite rate during a specific period and the sporozoite index out of 100.

2.7. Treatment in the Laboratory

Following identification, the identified female Anopheles mosquitoes were individually incubated in eppendorf tubes under silica gel. The head and thorax together and the residue separately.

On the head and thorax was carried out the evidence of the infestation of female anopheles to Plasmodium by the ELISA technique (Enzyme Linked Immuno Sorbent Assay) which allows detecting the presence of the circumsporozoite protein of plasmodium (CSP) in *anopheles*. The technique used is that of Burkot *et al.* (1984) [11] improved by Wirtz *et al.* (1987) [12].

It consists in coupling the CSP protein to a monoclonal capture antibody (ACm) against circumsporozoite (anti-CSP) previously fixed on the wall of the wells of a plate.

The antigen-antibody complex formed is then revealed by a peroxidase-coupled (labeled) anti-CSP monoclonal antibody. The addition of a substrate which will be degraded by the enzyme induces a visible colored reaction whose optical density will be measured by spectrophotometry.

2.8. Survey on Vector Control Strategies Used by the Population of Cotonou V

Data were collected on the basis of a questionnaire that was designed with questions assessing knowledge, physical integrity of nets and malaria prevention measures in the Cotonou V health zone and integrated into the ODK Collect platform in order to digitize the information and facilitate its processing. Strategically, we calculated the size of the representative sample using Schwart's formula [13]:

$$N = \frac{Z^2 \times P(1-P)}{i^2}$$

P: Prevalence of malaria in the general population with $P = 17\%$;

Z: Target confidence level with $Z = 1.96$;

I: Acceptable margin of error or accuracy $I = 5\%$;

N: Representative sample size;

$N = 216$ households.

This allowed us to interview 1216 households selected by the second-stage sampling method with a step in the areas of Wologuèdè, Sainte Rita, Gbèdjromèdé and the vicinity of Etoile Rouge to be able to cover a certain distance throughout the selected area and especially taking into account the places where the captures will be made.

The questionnaire allowed us to collect information on socio-demographic characteristics (age, sex, marital status, level of education, occupation, religion, area of residence, number of people in the household, presence or absence of swamps in the area), people's knowledge of malaria vectors and vector control measures, possession of LLINs and their use. We also checked the integrity of the nets and counted the holes in torn nets.

2.9. Data Analysis

Word processing was done using Word 2016 software. The processing of the capture data and the net surveys was done by Excel 2016 software and finally the statistical analysis of the tables and figures at the statistician.

3. Results

3.1. Diversity of Culicid Fauna in the Study Area

A total of 2386 mosquitoes were collected by both capture methods during the two nights of capture with *Culex quinquefasciatus* being the majority species for 98.11% followed by *Anopheles gambiaes.l* which makes 0.67%, *Anopheles ziemanni* 0.54%, *Mansonia africana* 0.33%, *Aedes aegypti* 0.29% and finally *Mansonia uniformis* 0.04%. Thus, *Culex quinquefasciatus* is the most predominant culicid in the Cotonou V health zone that causes nuisance to the population (**Figure**

1).

3.2. Exophagous and Endophagous Characteristics of the Different Species of Mosquitoes Captured

We identified *Culex quinquefasciatus*, *Anopheles gambiaes* s.l, *Anopheles ziemanni*, *Aedes aegypti, Mansonia africana* and *Mansonia uniformis*. In addition, *Anopheles gambiaes* s.l, from capture are much more endophagous (Table 1).

3.3. Relationship between Aggression Rate, Sporozoite Index and Entomological Inoculation Rate in the Study Area

Among the study areas, the vicinity of Etoile Rouge73% and Gbèdjromèdé 87% are the places with a high density of mosquitoes followed by Sainte Rita 69% and finally Wologuèdè 63% (Table 2). With the results of the CSP ELISA test, out of the 29 anopheles tested, there are none with the CSP antigen, which means that the sporozoite index is zero. Since the sporozoite index is zero, the EIR is also zero for all the study areas in the dry season.

3.4. Socio-Demographic Characteristics of the Populations Interviewed for the Vector Control Strategies and the Source of the Nets in the Households Interviewed and the Brand of LLINs Observed

The majority of people interviewed for this study were female (75.46%), married (82.40%), educated (77.29%) and Christian (81.48%) (Table 3).

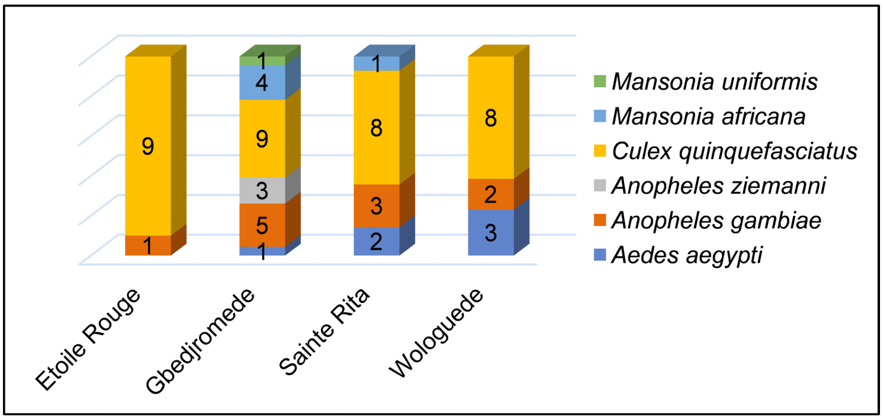

Figure 1. Diversity of culicid fauna in the study area.

Table 1. Exophagous and endophagous characteristics of the different mosquito species captured.

	Aedes aegypti	*Anopheles gambiae*	*Anopheles ziemanni*	*Culex quinquefasciatus*	*Mansonia africana*	*Mansonia uniformis*	Total
Outdoor	3	4	1	16	3		27
Indoor	3	7	2	18	2	1	33
Total	6	11	3	34	5	1	60

Table 2. Relationship between aggressiveness rate, sporozoite index and entomological inoculation rate in the study area.

	Etoile Rouge	Gbedjromèdé	Sainte Rita	Wologuèdè
Aggression Rate: Ma (p/h/n)	73	87	69	63
Sporozoite Index (%)	0	0	0	0
Entomological Inoculation Rate (p/h/n)	0	0	0	0

Table 3. Socio-demographic characteristics of households surveyed on vector control strategies.

Variables	Modalities	Number	Frequencies	Total
Gender	Female	163	75.46%	216
	Male	53	24.53%	
Marital Status	Single	38	17.59%	216
	Married	178	82.40%	
Level of Education	Primary	20	9.25%	216
	Secondary	67	31.01%	
	Superior	80	37.03%	
	Not Instructed	49	22.68%	
Religion	Animist	4	1.85%	216
	Christian	176	81.48%	
	Muslim Woman	36	16.66%	

The nets used by most of the population came from the successive distribution campaigns of 2017, 2019 and 2020 (73%), some took their nets from street vendors (13%), others got it from the pharmacy (8%) and the rest received it from the hospital (6%).There are several brands of LLINs (**Figure 2**). However, the majority of people in Cotonou V health zone use PermaNet 2 nets (42%), followed by Yorkool (17%), Dawa (11%) and Olyset (8%). It should also be noted that 22% of nets do not have brand identification paper (**Figure 3**).

This was due to the extended time of use, which was more than a year, and also to the multiple washing, which caused the identifying paper to disappear from the nets, making it impossible to identify the brand of the nets.

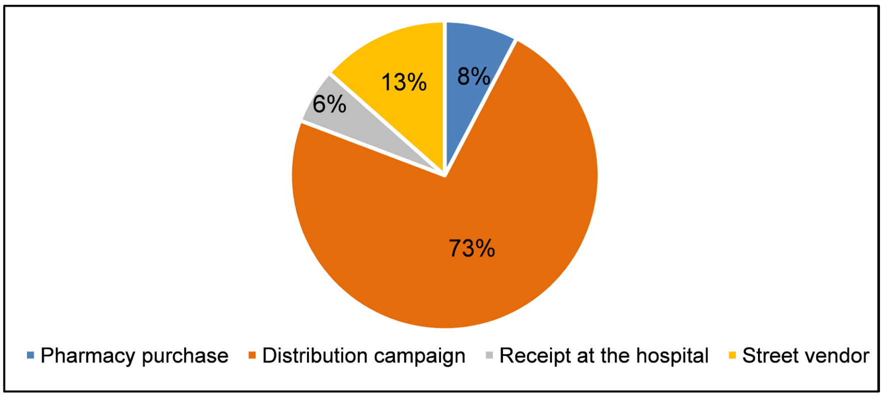

Figure 2. Source of nets for surveyed households.

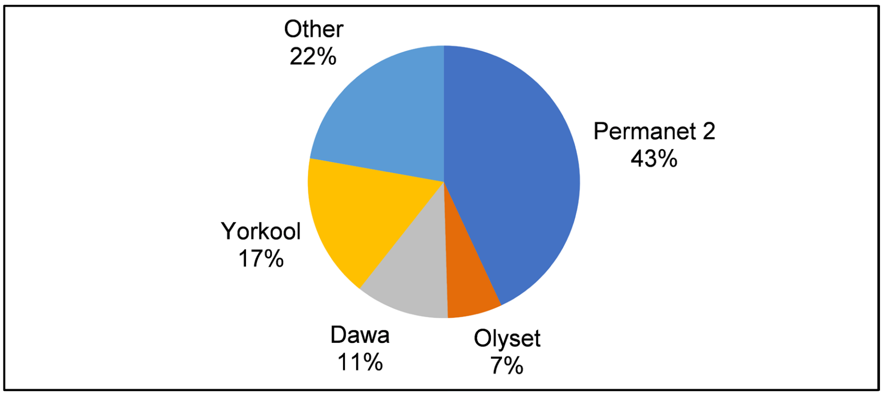

Figure 3. Markings of observed LLINs.

3.5. Physical Integrity of LLINs Observed in Households and Hole Size of Torn LLINs

According to the household surveys, 99% of the population owns LLINs, 95% of whom sleep under them regularly. Of the 80% of LLINs observed, approximately 126 nets were found to be torn (62.70%) and sewn (53.24%) (**Table 4**). Moreover, among the torn nets, the tears are much more T1 size (62.70%) followed by T2 size holes (14.28%). Few nets have T3 (5.55%) and T4 (2.38%) holes (**Table 5**).

3.6. Other Means of Vector Control Used by the Populations in the Study Area

The major finding is based on the use of LLINs by the majority of the population as a primary means of protection to reduce human-vector contact, we were also able to identify other complementary means that also reduce human-vector contact. These include coils, the smell of which repels mosquitoes, followed by aerosol cans, then screens in front of doors and windows that serve as a physical barrier to prevent mosquitoes from entering rooms, followed by indoor spraying, which has almost the same process as skin repellents, the smell of which repels mosquitoes (**Figure 4**). Note that there is another category of people who do not use any other means besides LLINs because of their body's intolerance.

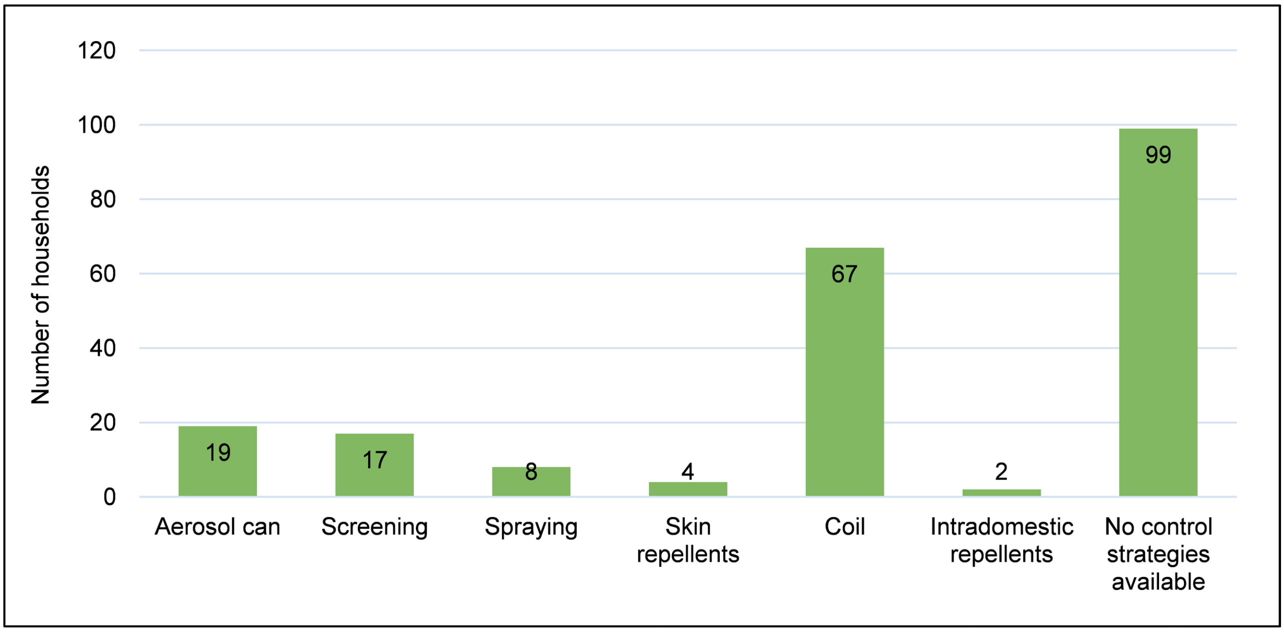

Figure 4. Distribution of other control methods used by people in the study area.

Table 4. Physical integrity of LLINs observed in households.

Physical Integrity of LLINs Observed in Households				
	Number	Frequencies	Total	
Untorn LLINs	47	37.30%	126	P-value < 0.001
Torn LLINs	79	62.70%		
Unsewn LLINs	101	46.76%	216	
Sewn LLINs	115	53.24%		

Table 5. Hole size of torn LLINs.

Size of the Holes of the Torn LLINs				
	Number	Frequencies	Total	
Size 1	79	62.70%	79	P-value < 0.001
Size 2	18	14.28%		
Size 3	7	5.55%		
Size 4	3	2.38%		

4. Discussion

In the Cotonou V health zone, malaria transmission is almost negligible in the dry season. The study of mosquito vectors and the dynamics of malaria transmission is an essential first step not only for understanding the epidemiology of the disease, but also for implementing effective and targeted control of these vectors [7]. The aim of the study that we conducted in four different neighborhoods, namely Wologuèdè, Sainte Rita, Gbèdjromèdé and the area around Etoile Rouge in the Littoral Department in the heart of Cotonou, was to evaluate malaria transmission and vector control strategies in the Cotonou V health zone during the dry season. This work was carried out over a period of 3 months allowing us to capture 2386 mosquitoes in two nights with six species, including *Aedes aegypti*, *Culex quinquefasciatus*, *Mansonia africana* and *uniformis*, *Anopheles ziemanni* and *Anopheles gambiaes* s.l present in the neighborhoods of Wologuèdè, Sainte Rita, Gbèdjromèdé and the area around of the Etoile Rouge. Among the anopheline species we found *Anopheles gambiaes* s.l already incriminated in malaria transmission in Benin, these results are in line with those of Ossè *et al.* (2023) [14] and Tokponnon *et al.* (2023) [7]. This diversity can be explained by the different ecological factors that favored the presence of the different sites for the development of each species. In addition, the anopheles density was low (29 *Anopheles* captured despite the number of captors, 16 collectors for the 4 quarters with 2 nights of captures) compared to the mosquitoes density. The nuisance of *Culex* is the major characteristic. During the dry season, the *Anopheles* density is low in the intermediate and peripheral zones and nil zero in the center of the city; this result from the work of Akogbeto *et al.* (1992) [9] is consistent with our results. This decrease is influenced by the drought that is present during the months of December to February; these results align with the work of Klinkenberg *et al.* (2008) [15] in Ghana and Akono *et al.* (2015) [16]. Each increase or decrease in *Anopheles gambiaes* s.l density is a function of increasing or decreasing rainfall [8]. But we found that mosquito aggression is higher indoors than outdoors which is justified by the endophagic behavior of *Anopheles gambiae* [8]. Through the study of *Plasmodium falciparum* infectivity by CSP-ELISA of the 29 *Anopheles*, it was proven that no *Anopheles* was infected. This would probably be due to the small number of mosquitoes collected and tested.

Malaria transmission was almost negligible, but not non-existent during the dry season from December to February with an EIR of 0 f/h/n; this result is not in line with those obtained by Djènontin *et al.* (2010) [17] in southern Benin. In addition, we collected statistics from thick drop examinations that confirm that transmission occurs in the dry season, but with a rather reduced frequency. These results are consistent with those of Gnanguenon *et al.* (2014) [18] in Benin. In assessing people's knowledge of malaria vectors and insecticide-treated nets, as well as means of control, we found that the majority, nearly 99% of the population surveyed, had knowledge of malaria vectors and means of control. This proportion is higher than that observed by Yandai *et al.* in Tchad (2017) [19].

The household survey in the study area showed that 99% of the population surveyed owned LLINs, 95% of which always slept under a net. This result is higher than those conducted by PNLP 2010 [20] in Benin and by Yandai *et al.* (2017) [19] in Tchad. Of the LLINs observed more than half have at least one hole; the majority have T1 size holes, the others T2, T3 and a few T4 size nets. We also found that most of the nets are from the successive distribution campaigns of 2017, 2019 and 2020, which justifies that the majority are torn since they have been used for nearly 2 years or more, this result is in line with the results of Ahogni *et al.* (2020) [21] in Benin which show that the longer LLINs last, the more formidable the integrity is. Also, the majority of respondents were female housewives or shopkeepers because the survey was conducted during the day on working days and Tokponnon *et al.* (2014) [22]. In addition to mosquito nets, people also use other means of control, mainly coils during the night, others use aerosol cans, then doors and windows with screens, then periodic spraying of houses with insecticide, not forgetting skin repellents. The latter are ointments or products that are applied to the skin and whose smell is supposed to repel mosquitoes. This concept is the same as that of in-home repellents. Nevertheless, these vector control methods do not really reduce the nuisance of the vectors. Our work also revealed that the brands of mosquito nets used by the population are PermaNet 2, followed by Yorkool, Dawa and Olyset. The quality of the nets could also be the origin of the durability of LLINs and the integrity of the nets.

It would be wise to evaluate the malaria transmission during the rainy season in the Cotonou V health zone to better assess the result. This work could also be done in other areas where the vector density is considerably high in order to better characterize the situation.

5. Conclusions

The study of entomological indices in the urban health zone in Cotonou V confirms that malaria transmission is diversified in the dry season and that permanent culicidal density does not influence urban malaria transmission in the populations of Wologuèdè, Sainte Rita, Gbèdjromèdé and the area around Etoile Rouge.

Because of the permanent nuisance provided by *Culex* in the houses and the living environment of the populations, the latter has taken into account the measures of vector control, especially the reduction of the contact between man and vector through the use of impregnated mosquito nets, skin repellents and aerosol cans and fences.

In order to better participate in the well-being of the inhabitants and their satisfaction, it would be wise to find a way to reduce the nuisance caused by *Culex* to the populations of this area.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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Appendix

Questionnaire for assessing the knowledge of the physical integrity of mosquito nets and preventive measures against malaria in Cotonou

1) Age: Sex:

2) Marital Status: Married Single

3) Level of Study: Primary Secondary Superior Uneducated

4) Occupation……………………………………………………………………….

5) Religion…………………………………………………………………………

6) Area of Residence……………………………………………………………

7) How many people live in your household?

8) Is it a marshy area? Yes No

9) Take the geographical coordinates of the household…………………………

10) What is the frequency of mosquito bites in the locality?

Quite Frequent Frequent Very Common

11) From what time do mosquitoes attack you the most?

……………………………………………………………………………………..

12) Do you know the vectors of malaria? Yes No

13) What promotes the proliferation of Anopheles mosquitoes?

Vegetation Channels

Puddle of Water

Used Tires

Others

To Be Specified:

14) To your knowledge, where do Anopheles mosquitoes breed?

Vegetation Channels

Puddle of Water

Used Tires

Others

To Be Specified

15) How can the proliferation of mosquitoes be prevented?

Cleaning the Surroundings Dwellings

Covering Water Tanks

Eliminate Stagnant Water

Weeding

16) In your opinion, can we prevent the proliferation of roosts next to your house? Yes No

If yes, explain your answer?

If no, comment

17) Do you have a mosquito net? Yes No

18) How did you get your mosquito net? Purchase at the pharmacy Received in hospital Mass sharing (campaign) Street vendor

19) How is the mosquito net? Tender Hard

20) How many mosquito nets do you have in the household?…………………

21) How many of these nets are LLINs?……………………………………………

22) Is the mosquito net enough to fit your mattress or bed?

Yes No

23) How long have you been using the mosquito net?

………………………………………………………………………………………..

24) Do you sleep under an LLIN?

No Yes Rarely Often Enough

Very Often Always

25) How many people sleep with you in the mosquito net?

………………………………………………………………………………………..

26) Is your mosquito net that you use torn? No Yes

27) If yes, observe and count the number of holes that cannot pass a finger

28) Count the number of holes that can pass:

The thumb of your hand

The number of holes that can let a fist of the closed hand

The number of holes that a person's head can pass through

29) Is the mosquito net attached in places?

Very Little Several Places Not Attached

30) At what time do you put under the mosquito net?…………………………

31) From what time are you inside your room?…………………………………

32) Do you wash the mosquito net (maintenance)?

No Yes How many times in 3 months

33) Where do you dry the mosquito net? Under the Sun In the Shade

34) Do you frequently get malaria? No

Yes How many times in 3 months

35) How long have you lived in your house? Less than 3 months More than 3 months More than 6 months

36) Do all household members have mosquito nets for sleeping? Yes or No

37) All the people sleep under mosquito nets continuously during the year in your household?

Yes Or No

38) What other means of mosquito control do you use?

Serpentine

Untreated Mosquito Net

Spraying of Insecticide

Cleaning of the Oh Surroundings of Dwellings

Covering of Water Tanks

Eliminating Stagnant Water

Weeding around Houses

Mesh on Doors Oh and Windows

Other Materials Oh Impregnated with Insecticides

Other

39) How many mosquito nets are there in the household in good condition in

use?

40) Do you ever swap mosquito nets in the household?

No Yes How many times per year

41) What is the brand of your mosquito net?

Permanet 2 or 3 OlysetDAWA net DuranetYorkool Interceptor

Other

Thank you for taking the time to respond to us.