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Evaluation of Plasmodial Species in Humans and in Malaria Vectors in the Central Arrondissement of Abomey-Calavi and in the Health Zone of Cotonou I and IV in Benin, West Africa

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ABSTRACT

Background: Malaria remains a recurrent public health disease. Several plasmodial species are vigorously implicated in malaria infection. The aim of this study is to assess the risk of exposure to malaria in the populations of southern Benin, more specifically in the central arrondissement of Abomey-calavi and in the Cotonou I and IV health zones, by identifying the plasmodial species responsible for malaria. Method: Mosquito collections were carried out in July 2021 following nocturnal captures of mosquitoes from volunteer subjects and intradomiciliary spraying at dawn on the days following the capture nights. At the same time, thick drop-positive blood samples were collected from tributary healthcare facilities per work zone. The species of mosquitoes collected were identified on the basis of their morphological characteristics, and *P. falciparum* (Pf) CSP antigens were screened for in *Anopheles* using the enzyme-linked immunosorbent assay (ELISA) technique. PCRs were performed to detect *Plasmodium* infection in anopheles, with characterization of the anopheles species involved and identification of the plasmodial species involved. The XN 31 automated system was used to identify plasmodial species in blood samples. Results: A total of 112 female anopheles were collected in Abomey-calavi and 264 in Cotonou. The *An. gambiae sl* complex was the predominant anopheles species, accounting for 20.1% in Abomey-calavi and 15.5% in Cotonou. *An. pharoensis* is

found with a low percentage in Abomey-calavi (0.2%). Following molecular characterization of the *An. gambiae sl* complex, *An. coluzzii* was the predominant species in Cotonou (82.2%), in contrast to Abomey-calavi where *An. gambiae* was in the majority (82.1%). The sporozoites infection rate and entomological inoculation rate were 4.5% and 0.3 bi/h/night or 125 bi/h/year respectively in Abomey-calavi, and 0.75% and 0.12 bi/h/night or 43.8 bi/h/year in Cotonou. Of these anopheles obtained in Abomey-calavi, 17 were carriers of *P. falciparum* (*Pf*), 02 of *Plasmodium vivax* (*Pv*) and 01 of *P. malariae* (*Pm*). In Cotonou, 07 were carriers of *P. falciparum* and 03 of *Plasmodium vivax*. In the same period, 118 blood samples were collected in Abomey-calavi and 183 in Cotonou. After analysis, *Pf*, *Po* and *P. malariae* (*Pm*) were found singly or mixed in the blood samples collected and we have the next plasmodial formula: *Pf* = 97.54%; *Po* = 1.64%; *Pm* = 0.82% was obtained in Abomey-calavi, followed by *Pf* = 96.08%; *Po* = 3.48%; *Pm* = 0.43%. Conclusion: The present study mentions the presence of *Pv* alongside *Pf*, *Pm* and *Po* in *Anopheles*. This finding was made in vectors and not in humans, who carried the three usually encountered, *Pv*, *Pm* and *Po*. It is therefore important to extend research work to other regions and health centers in the country, in order to obtain a broader estimate of the plasmodia profile.

1. INTRODUCTION

Malaria still remains a health problem in Africa and particularly in Benin. In 2018, sub-Saharan Africa carried 93% of the total burden of disease due to malaria worldwide that is 228 million malaria cases with 405,000 deaths [1]. From 228 million cases recorded in 2018, 229 million cases are recorded in 2019 with 409,000 deaths [2]. Most recently, in 2021, the disease affected some 247 million people worldwide [3]. 95% of the total burden announced for 2021 is in Africa alone, which is a cause for concern [3]. Also, for the year 2021, the Yearbook of Health Statistics (SAS in French) generated by the National Health Information and Management System (SNIGS in French) of the Ministry of Health for the year 2022 presents malaria as the cause of 44.2% and 49.5% of the reasons for consultation in the general population and in children under five respectively.

Mosquitoes of the genus Anopheles are the exclusive vectors of malaria, and certain species such as *Anopheles gambiae* and *Anopheles funestus* are particularly effective in transmitting *P. falciparum*, the most widespread and deadly species in Benin. Malaria is transmitted by the bite of a female *Anopheles* infected with the *Plasmodium* parasite. Many *Plasmodium* species are known to cause malaria in humans. Previously, five *Plasmodium* species were known to cause malaria: *P. falciparum* (*Pf*), *Plasmodium vivax* (*Pv*), *P. ovale* (*Po*), *P. malariae* (*Pm*) and *Plasmodium knowlesi* [4]. Of these species, *Pf* and *Pv* are the most frequent, with *Pf* the most virulent, accounting for 99.7% of malaria cases in sub-Saharan Africa [1]. In common practice, malaria has long been attributed to *Pf*, in the full knowledge that there are other parasites that cause malaria. Studies have demonstrated the potential risk of *Pv* malaria through its ability to form dormant clones in the liver "hypnozoites", which are insensitive to most antimalarial drugs (Wells *et al.*, 2010) [5]. It important to emphasize that although not to be overlooked, since the changing patterns of this species, which used to be found exclusively in animals, are rapidly being detected in humans [6]. In Malaysian Borneo, *P. knoxlesi* has been highlighted as a cause of death due to malaria.

In 2021, six additional plasmodial species have been identified, increasing the risk of malaria transmission: *P. cynomolgi*, *P. inui*, *P. coatneyi*, *P. inui-like*, *P. simiovale*, and *P. simium* [7]. As a result, it is necessary to reinforce both preventative and curative measures to reduce the morbidity and mortality burden resulting from malaria. The rapid and accurate diagnosis of malaria cases is necessary, requiring prior knowledge of the types of species involved, as well as those circulating in each region.

In Benin, the *Anopheles gambiae sl* (sensu lato) complex and the *Anopheles funestus* and *Anopheles nili* groups are the major vectors involved in *Plasmodium* transmission [8]. In 2010, three of the causal

species of malaria were identified in the southern region of Benin, the species *Pf*, *Pm* and *Po*, following the work of 2010 of Damien *et al*. [9]. A new species of the Plasmodium parasite causing malaria, known as *Pv*, which was previously absent in Benin, has been found in blood pockets of asymptomatic Beninese donors who lack the Duffy antigen on the surface of their red blood cells [10]. Recently, in 2023, it has also been identified in vectors [11].

Benin's malaria endemicity, *Plasmodium* species circulating, and vector transmission dynamics were considered. Therefore, this study aims to establish the current incidence profile of plasmodia in both humans and anopheles' mosquitoes, malaria vectors, in the central Abomey-calavi district and Cotonou I-IV health zone in the southern region of Benin.

2. MATERIAL AND METHODS

2.1. Areas of Study

The rainy season provides a favorable environment for mosquito breeding, which is conducive to larva production. The study was conducted during the major rainy season in Benin (April to July) with two study areas: the central district of Abomey-calavi and the health zone Cotonou I and IV. The selection of the central district of Abomey-calavi and the health zones of Cotonou I and IV for the collection of mosquitoes was justified by the proximity of these areas to our collection hospitals (research objectives) on the one hand, and by the fact that the central district of Abomey-calavi and the health zones of Cotonou I and IV offer a variety of environments with breeding sites for mosquitoes, such as temporary pools of water in swamps. These conditions favors the development of mosquito larvae and the emergence of adult mosquitoes. The capture points were chosen for their easy access for the capture teams. Hospitals located in the study area were selected for the purpose of blood sample collection, as well as study sites for mosquito collection. Therefore, in the central district of Abomey-calavi, the Lab polyclinic on campus and the Calavi zone hospital were the two hospitals that were used for blood sample collection, followed by CM and HOMEL in the Cotonou I and IV health district for mosquito collection.

Figure 1 illustrates the study sites in each respective area: Agori Finanfa/Tankpè for Calavi and Djidjè II in Cotonou. Additionally, their proximity to blood sample collection hospitals facilitated the correlation between plasmodial species in the transmitting vector and the species in the sick human hosts. The selection of these sites was based on the high vector nuisance, allowing for the collection of a significant number of mosquitoes.

2.2. Collection of Adult Mosquitoes

Two techniques were used to collect adult mosquitoes in the different study areas. These were the catching technique on human volunteers (9 am to 5 am) and the morning pyrethrum spraying technique (6 am to 7 am). The technique of capturing mosquitoes on human volunteers helped us to assess the nocturnal aggressiveness of mosquitoes and their human preference (**Figure 2**), while the morning spray enabled us to collect mosquitoes at rest after their nocturnal cycle [12].

The catching technique on human volunteers (**Figure 3**) took place inside and outside four houses. Eight catchers were trained using two catchers per house (one outside and one inside) per night in each neighborhood, including Agori Finanfa in Abomey-calavi and Djidjè II in Cotonou. The catchers sit in the dark with their legs uncovered and wait for the mosquito to land [13].

As soon as they feel the mosquito land on their legs, they turn on the electric lamp on the mosquito to see it and at the same time to immobilize it so that it is skillfully collected in a tube and then plugged with absorbent cotton (**Figure 3**) (Service 1977; World Health Organization 2013) [14]. Every quarter of an hour during the night, a team of two people supervised the catch to ensure the smooth running of the collection.

Morning sprays (**Figure 4**) are designed to trap mosquitoes that entered the chamber during the night. It was conducted between 6 and 7 a.m. at dawn. The traps were sprayed with insecticide. A white sheet was then placed on the floor to collect the mosquitoes after spraying. Spraying starts from the bottom of the chamber towards the exit. Windows and other vents are closed.

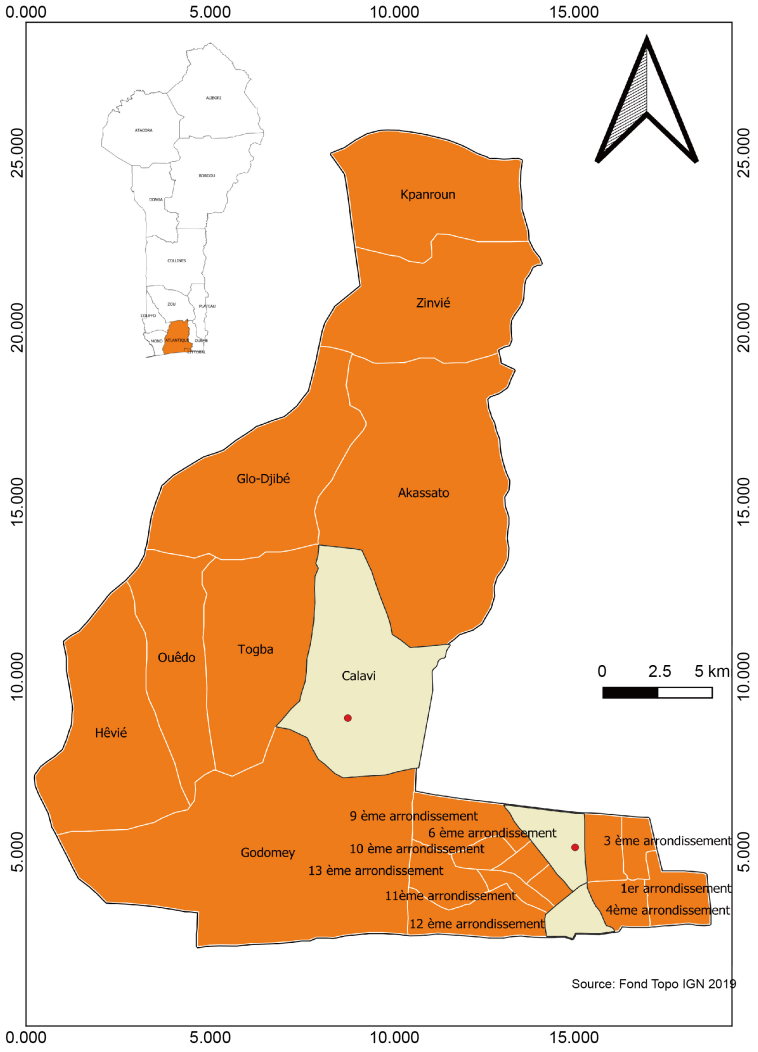

Figure 1. Carte of Benin illustrating the study areas.

Figure 2. Images of the capture technique for aggressive mosquitoes on humans By Gounou Yerima July 2021.

Figure 3. Images of a technique for collecting mosquitoes by house spraying By Gounou Yerima July 2021.

After 15 minutes, the fallen mosquitoes were collected by lifting the sheet from the ends to the outside according to the standard WHO protocol [15, 16].

Mosquitoes from the catches were identified by their morphological characteristics using the identification keys of (Coetzee, 2020) and the binocular magnifying glass (Coetzee *et al.*, 2000) [17, 18].

2.3. Entomological Parameters of Malaria Transmission

Entomological parameters are used to assess vector transmission.

2.3.1. Anopheline Parturition Rate

The ovaries of a number of female anopheline mosquitoes were dissected and the results were used to assess the rate at which anopheline mosquitoes mature.

The maturity rate = (number of female anophelines tested/total number of female anophelines tested) × 100 [16, 19].

2.3.2. Anopheles Aggressiveness Rate

The anopheles aggressiveness rate (**Ma**) corresponds to the number of female anopheles bites received by a person per hour or night of capture.

Ma = number of Anopheles bites man/(number of catchers × time unit) in b/h/h or b/h/n (bites per person per hour or per night or per year) [20].

2.3.3. Circumsporozoite Index

The circumsporozoite index (**Is**) refers to the percentage of Anopheles carrying the CSP antigen.

Is = (number of CSP Ag positive mosquitoes/total number of mosquitoes examined) × 100 expressed as a percentage (%) [20, 21].

2.3.4. Entomological Inoculation Rate

The entomological inoculation rate (EIR) represents the number of infective bites received by a person in a given time interval.

EIR = aggressiveness rate × circumsporozoite index in bi/h/year; expressed as infectious bites per person per year [21].

2.4. Determination of the Circumsporozoït Index in the Complex *Anopheles gambiae*

Anopheles gambiae infectivity was assessed by Elisa on the head and thorax of individual vector

samples. Mosquito carcasses are stored for future use. This method uses specific monoclonal antibodies directed against the circumsporozoite protein of *P. falciparum* [22].

2.5. Molecular Identification of Species of *Anopheles gambiae* Complex

Anopheles gambiae, *Anopheles coluzzii* and *Anopheles arabiensis* species of the *Anopheles gambiae sl* complex were identified by PCR sine described in the protocol of Santolamazza [23].

Table 1 shows the different species to be characterized, their respective molecular weights and the primers used for their detection.

Table 1. Some species of the *An. gambiae sl* complex, their specific primers and molecular weights.

Complex *An. gambiae sl* species	Molecular weight	Sequence of Primers (5'-3')
An. Gambiae	249 pb	TCG CCT TAG ACC TTG CGT TA
An. Coluzzii	479 pb	CGC TTC AAG AAT TCG AGA TAC
An. Arabiensis	223 pb	CGC TTC AAG AAT TCG AGA TAC

2.6. Molecular Identification of Plasmodial Species in *Anopheles* of the *An. gambiae* Complex

DNA extracted from the carcasses of *An. gambiae* species was used to identify the different *plasmodium* species involved in the infectivity of these mosquitoes, following the well-described protocol of 2003 of Padley [24].

Table 2 shows the different *plasmodium* species identified in *An. gambiae sl*, together with the primers used for their detection and their molecular weight.

Table 2. Some plasmodial species, their molecular weights and specific primers according to the Padley *et al.* protocol [24].

Plasmodium species		Sequence of Primers (5'-3')
P. falciparum	276 pb	5'AAC AGA CGG GTA GTC ATG ATT GAG3'
P. vivax	300 pb	5'CGG CTT GGA AGT CCT TGT3'
P. ovale	375 pb	5'CTG TTC TTT GCA TTC CTT ATGC3'
P. malariae	412 pb	5'CGT TAA GAA TAA ACG CCA AGCG3'

2.7. Collection of Blood Samples

With the agreement of wealthy health care institutions registered in our working area, blood samples with positive thick samples were collected, followed by anonymized data from each of these samples. A venous blood sample in an EDTA tube is taken before the microscopic analysis for a thick sample. The thick drop and the slides of the blood smear are read by the technician of the day at each hospital according to the standard procedure of the WHO [25].

After this reading, the samples declared positive by the hospital are regularly collected and sent to the laboratory of the Centre de recherche entomologique de cotonou (CREC). They are then placed in a refrigerator at 4˚ to reach a relatively large number before being processed and, for most of them, 30 days after being placed in the refrigerator.

Transfer to the XN 31 system was the next step. This system directly detects and quantifies red blood

cells parasitized by *Plasmodium* parasites [26] using fluorescence flow cytometry.

For each sample passed, it generates the parasite species involved: "*Pf*" or "others" if it's not *Pf,* the parasite density, a complete blood count with a short delay in transmitting the results. It absorbs 60 ul of blood and has a detection limit of 20 parasites per microliter of blood. In the case of parasite species other than *Pf,* marked "other", we compared the scattergram image provided by the machine for the sample with the reference image for each species contained in the abacus of the device to identify the species in question.

2.8. Statistical Analysis

The identifier, the blood count, the parasite density and the completion of the causal species not mentioned by the automaton are the data recorded to generate a file by Excel 2013 software, from which was established the plasmodial formula that determines the representativeness rate for each species.

3. RESULTS

1) Diversity of mosquito species collected

The nights spent collecting mosquitoes in the two study areas allowed us to collect a total of 2,265 mosquitoes: 1,708 mosquitoes in the Cotonou I and IV health zones and 557 in the Abomey-calavi central district. Of these 2265 mosquito species collected, *Culex quinquefasciatus* was the predominant species (77.8%), followed by *Anopheles gambiae sl* (16.6%), *Aedes aegypti* (4.1%), *Culex anulioris* (1.45%) and *Anopheles pharoensis* (0.04%). *Culex quinquefasciatus* was the most abundant species in both study areas. Among the 380 *Anopheles* collected, *Anopheles gambiae sl* (99.73%) remains the most common vector for malaria transmission in our study areas (Table 3). In Abomey-calavi (20.1%) and Cotonou (15.5%), *Anopheles gambiae sl* remains the predominant anopheline species.

Table 3. Distribution of anopheles collected in Abomey-calavi central district and Cotonou I and IV sanitary zone.

Species /Study site	Abomey-calavi district Number (%)		Cotonou I and IV sanitary zone Number (%)		Total Number (%)	
Culex quinquefasciatus	382 (68.6)	[64.55; 72.42]	1380 (80.8)	[78.85; 82.64]	1762 (77.8)	[76.02; 79.49]
Anopheles gambiae s.l	**112 (20.1)**	[16.86; 23.68]	**264 (15.5)**	[13.77; 17.26]	**376 (16.6)**	[15.09; 18.20]
Aedes aegypti	29 (5.2)	[3.51; 7.39]	64 (3.7)	[2.90; 4.76]	93 (4.1)	[3.33; 5.01]
Culex anulioris	33 (5.9)	[4.11; 8.22]	0 (0)		33 (1.45)	[1.01; 204].
***Anopheles* pharoensis**	**1 (0.2)**	[0.005; 0.996]	**0 (0)**		1 (0.04)	[0.001; 0.25]
Total Number (%)	**557 (100)**		**1708 (100)**		**2265 (100)**	

2) Number of bites and estimate aggressivity per person per night in July 2021 in health zones Cotonou I and IV

Table 4 shows a breakdown by species of the 1708 mosquitoes collected in the Cotonou I and IV health zones, based on indoor and outdoor captures, and allows us to estimate the rate of bites per person per night and per species. In health zones Cotonou I and IV, a person receives a higher rate of bites from *Culex*

quinquefasciatus (43.1), followed by *Anopheles gambiae sl* (8.2) and a very low rate of bites from *Aedes aegypti* [2].

Table 4. Diversity, culicid abundance and estimated number of bites per person per night in July 2021 in the Cotonou I and IV health zones.

Mosquitoe species	Outdoor/ Indoor	Capture Night	Number of mosquito	HBR	95%CI	rate ratio [95%CI]	p-value
CX. Quinquefasciatus	Indoor	16	755	47.2	[43.9; 50.7]	1.208 [1.09; 1.35]	0.0005111
	Outdoor	16	625	39.1	[36.1; 42.3]	1	
An. gambiae sl	Indoor	16	116	7.3	[6.0; 8.7]	1	0.0562
	Outdoor	16	148	9.3	[7.8; 10.9]	1.276 [0.994; 1.641]	
Ae. aegypti	Indoor	16	35	2.2	[1.5; 3.0]	1.207 [0.717; 2.047]	0.5323
	Outdoor	16	29	1.8	[1.2; 2.6]	1	
All species	Indoor	16	906	56.6	[53.0; 60.4]	1.130 [1.026; 1.244]	0.01267
	Outdoor	16	802	50.1	[46.7; 53.7]	1	

The number of catchers per night being 08, the anopheles aggressivity rate amounts to: ma = 16.5 p/h/n

3) Number of bites and estimated aggressivity per person per night in July 2021 in the central arrondissement of Abomey-calavi.

In the central arrondissement of Abomey-calavi, more precisely in Agori Finanfa, an individual receives a rate of 18.43 mosquito bites per night. The vector species *Culex qinquefasciatus* is responsible for the highest rate of bites per person per night, at 12.12. The anopheline species *Anopheles gambiae sl* and *Anopheles pharoensis* had rates of 2.87 and 0.06 respectively.

The number of catchers per night being 08, the anopheles aggressivity rate amounts to:

ma = 7.06 p/h/n

4) Characterization of *An. gambiae* and *An.coluzzii* species of the *An.gambae sl* complex obtained per study area

Of a total of 376 mosquitoes of the *Anopheles gambiae* complex collected, 264 in health zones Cotonou I and IV and 112 in the central arrondissement of Abomey Calavi (Table 5), the majority species of the *An. gambiae s l* complex obtained by sine PCR was *An. coluzzii* (58.2%).

In the Abomey-calavi district, Anopheles gambiae represented 82.1% of the mosquitoes collected, whereas in the Cotonou I and IV health zones, its proportion (or frequency) was 4.5%. This 77.6% difference highlights a marked difference in species composition between the two zones.

Conversely, *Anopheles coluzzii* is much more present in Cotonou I and IV with 82.2%, whereas it

represents only 1.8% in Abomey-calavi. This shows that the ecology favours this species in the urban area of Cotonou.

Table 5. Diversity, culicid abundance and estimated number of bites per person per night in July 2021 in the central arrondissement of Abomey-calavi.

Mosquitoe species	Outdoor/ Indoor	Capture Night	Number of mosquito	HBR	95%CI	Rate Ratio [95%CI]	p-value
Cx. quinquefasciasus	**Indoor**	16	188	11.8	[10.1; 13.6]	1	0.7981
	Outdoor	16	194	12.1	[10.5; 14.0]	1.03 [0.84; 1.27]	
An. gambiae sl	**Indoor**	16	66	4.1	[3.2; 5.3]	1.44 [0.97; 2.14]	0.07213
	Outdoor	16	46	2.9	[2.1; 3.8]	1	
Cx. anuluoris	**Indoor**	16	2	0.1	[0.02; 0.5]	1	<0.0001
	Outdoor	16	31	1.9	[1.3; 2.8]	15.5 [3.94; 133.67]	
Ae. aegypti	**Indoor**	16	6	0.38	[0.14; 0.82]	1	0.002316
	Outdoor	16	23	1.44	[0.9; 2.16]	3.83 [1.52; 11.51]	
An. pharoensis	**Indoor**	16	0	0	[0.00; 0.23]		1
	Outdoor	16	1	0.06	[0.00; 0.35]	Inf [0.026; Inf]	
All species	**Indoor**	16	**262**	16.38	[14.45; 18.48]	1	0.1751
	Outdoor	16	**295**	18.44	[16.39; 20.67]	1.13 [0.95; 1.34]	

The presence of hybrids is very low in both zones, with only 0.9% in Abomey-Calavi and 0.4% in Cotonou. The 95% confidence intervals (CI) do not overlap for the two zones:

- *An. gambiae*: [73.78; 88.74] (Abomey-calavi) vs [2.37; 7.81] (Cotonou).
- *An. coluzzii*: [0.22; 6.30] (Abomey-calavi) vs [77.04; 86.62] (Cotonou).

This indicates a significant difference ($p < 0.05$) in species distribution. (Table 6).

5) Parturition rates of the anopheline population from pyrethrum spraying

The anopheles resulting from pyrethrum spraying were dissected to assess the parturition rate. A total of 12 anopheles were dissected in the central arrondissement of Abomey-calavi, with a maturity rate of

85.7%, and 98 anopheles were dissected in the Cotonou I and IV health zones, with a maturity rate of 81.6%. High share of anopheles in the 2 study areas.

Table 6. Species of the *Anopheles gambiae s.l* complex obtained with sine PCR.

Species/Study area	Abomey-calavi district		Cotonou I and IV sanitary zone		Total	
	Number of mosquitoe (%)	[IC 95%]	Number of mosquitoe (%)	[IC 95%]	Number of mosquitoe (%)	[IC 95%]
An. gambiae	92 (82.1)	[73.78; 88.74]	12 (4.5)	[2.37; 7.81]	104 (27.7)	[23.20; 32.48]
An. coluzzii	2 (1.8)	[0.22; 6.30]	217 (82.2)	[77.04; 86.62]	219 (58.2)	[53.08; 63.28]
An. gambiae/ An. coluzzi	1 (0.9)	[0.02; 4.87]	1 (0.4)	[0.00; 2.09]	2 (0.5)	[0.06; 1.91]
Total Number (%)	112 (100)		264 (100)		376(100)	

6) The entomological inoculation rate is 0.3 pi/h/year to 0.3 bi/h/night or 125 bi/h/year

One hundred twelve and two hundred sixty-four head-thoraxes of *An. gambiae s.l.* complex from the traps in the central arrondissement of Abomey-calavi and Cotonou health zones I and IV, respectively, were tested by ELISA. In this way, 5 Anopheles from the Abomey-calavi captures were found positive, allowing the sporozoite index to be calculated at 4.5%. The inoculation rate can be evaluated as a function of the aggressivity rate thanks to the sporozoite index obtained. The entomological inoculation rate is 0.3 pi/h/year.

On the other hand, in the health zones of Cotonou I and IV, 2 Anopheles were found positive, giving a circumsporozoite index of 0.75% and an entomological inoculation rate of 0.12 (EIR = 0.12 bi/h/night or 43.8 bi/h/year).

7) Molecular identification of *P. falciparum*, *P. ovale*, *P. malariae* and *P. vivax* in the *Anopheles gambiae* complex

Of 112 samples tested, 17 *Anopheles* species were found to carry *Pf,* 2 *Pv* and 1 *Pm* in the central arrondissement of Abomey-calavi (Figure 1).

Out of 265 samples tested, 7 *Anopheles* carried *Pf,* then 4 *Pv* in Cotonou I and IV health zones (Figure 4).

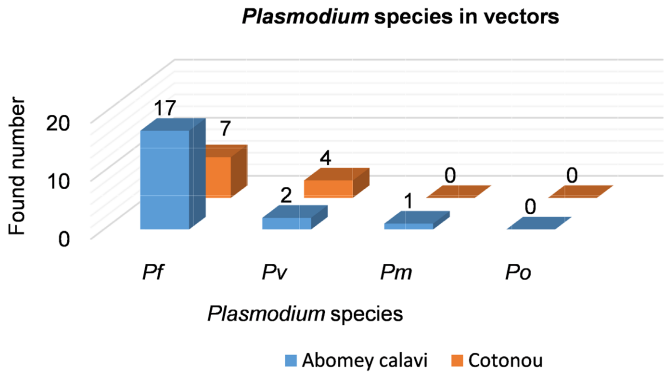

Figure 4. Summary of the different plasmodial species found in vectors in the two study areas. *Pf*: *P. falciparum*, *Pv*: *Plasmodium vivax*, *Pm*: *Plasmodium malariae*, *Po*: *Plasmodium ovalae*.

7) Plasmodium species obtained with the XN31 automated system

For each sample passed to the XN31 automated system, the latter generated an image (Figure 5 and Figure 6) providing information on various parameters, presented as illustrated below:

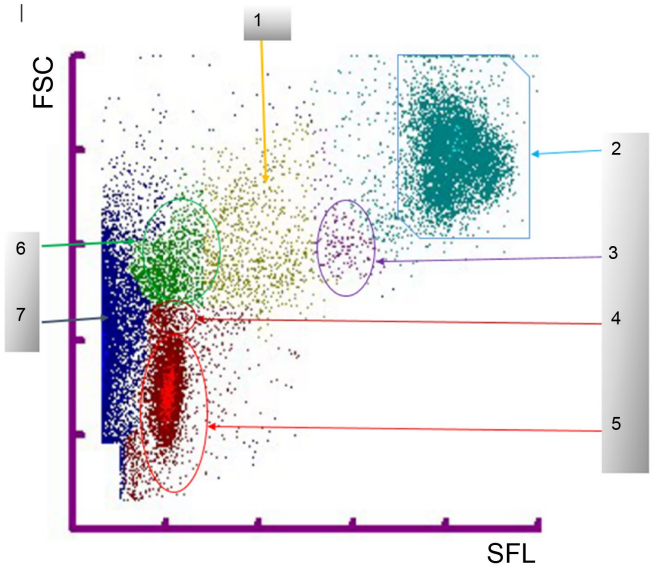

Figure 5. XN31 blood sample results: presence of *Pf* and other species to be identified. Legend: 1-) Free circulating Trophozoites; 2-) Lysed red cell debris; 3-) Schizonts; 4-) Other species to be identified; 5-) Pf trophozoites; 6-) Gametocytes; 7-) Débris des leucocytes.

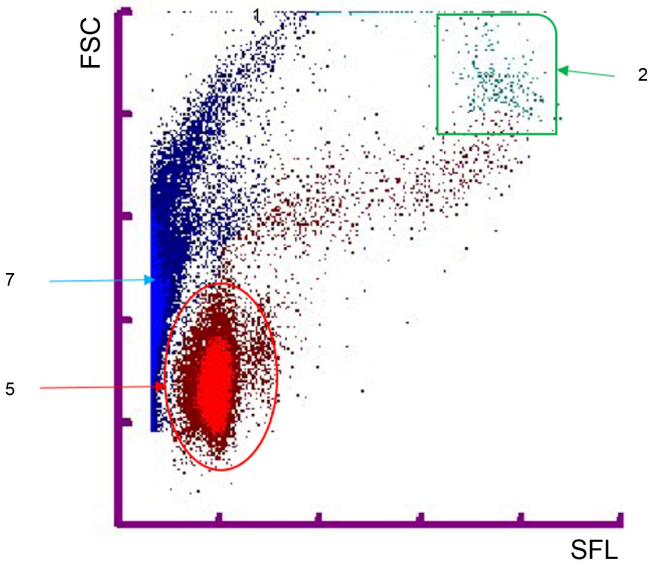

Figure 6. Result of a blood sample run through XN31: Presence of *Pf*. Legend: 1-) Free circulating Trophozoites; 2-) Lysed red cell debris; 3-) Schizonts; 4-) Other species to be identified; 5-) Pf trophozoites; 6-) Gametocytes; 7-) Débris des leucocytes.

Of 118 blood samples collected and tested in the central arrondissement of Abomey-calavi, 108 were positive for *Pf*, 02 for *Po*, 03 for *Pm*, 04 for *Pf* and *Po*, 1 for *Pf* and *Pm* (Figure 7).

In addition, 176 *Pf*, 3 *Po*, 01 *Pm* and one combination (*Pf, Pm*) were identified in 183 blood samples collected in health zones Cotonou I and IV (Figure 7).

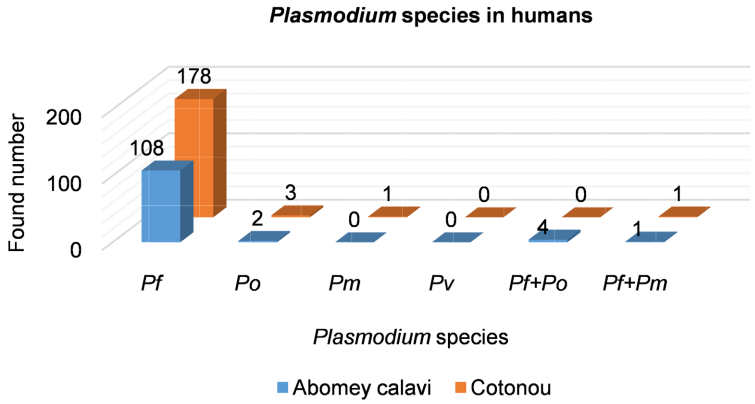

Figure 7. Summary of the different plasmodial species found in blood samples collected in the two study areas. *Pf*: *P. falciparum*, *Pv*: *Plasmodium vivax*, *Pm*: *Plasmodium malariae*, *Po*: *Plasmodium ovalae*; The plasmodial formula for a total of 118 samples tested for calavi is *Pf* = 97.54%; *Po* = 1.64%; *Pm* = 0.82%; For 183 samples in the Cotonou I and IV health zones, the plasmodial formula is *Pf* = 96.08%; *Po* = 3.48%; Pm = 0.43%.

8) Plasmodium species in Anopheles gambiae complex and humans in the two study areas

In the central arrondissement of Abomey-calavi and in the Cotonou I and IV health zones, *Pv* was detected alone or in combination with *Pf*, *Pm* and *Po* in anopheles and *Pf*, *Pm* and *Po* in humans (Figure 8 and Figure 9).

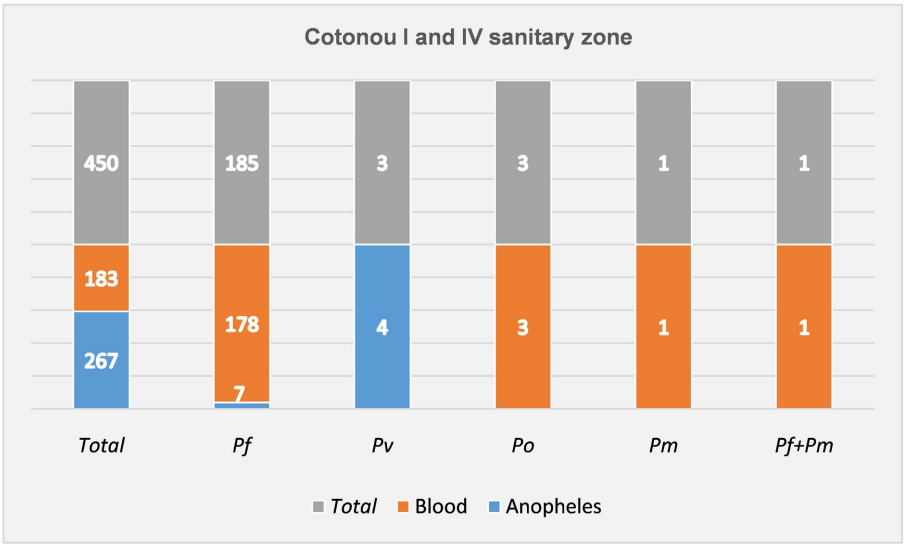

Figure 8. Summary of plasmodial species in vectors and humans in Cotonou health zone I and IV.

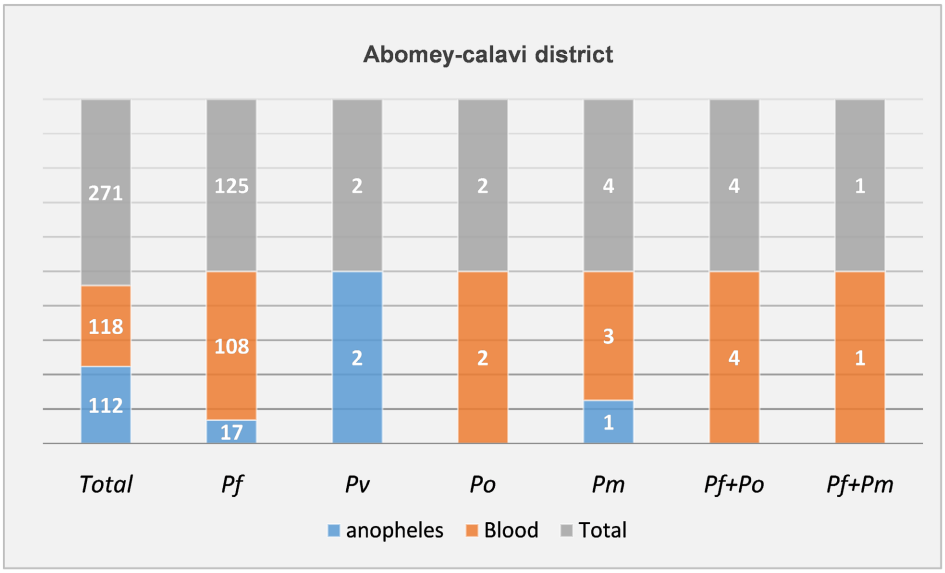

Figure 9. Summary of plasmodial species in vectors and humans in Abomey-calavi central district.

4. DISCUSSION

In the current context of malaria elimination, the identification of *Plasmodium* species in humans and vectors is a prerequisite not only for understanding the epidemiology of the disease, but also for implementing an effective control strategy against these parasites, not to mention adequate diagnosis, especially when work has already mentioned the presence of *Plasmodium vivax* in the subregion. The present study covered the central arrondissement of Abomey-calavi and the Cotonou I and IV health zones. A total of 2265 mosquitoes were collected, 557 in Abomey-calavi and 1708 in Cotonou, divided into three species: *Anopheles*, *Culex* and *Aedes*. The species *Anopheles*, the vector of malaria, was more aggressive in the health zones of Cotonou I and IV (16.5 b/h/n) than in the central arrondissement of Abomey-calavi (7.06 b/h/n). This difference in aggressivity observed in the two study zones is probably due to the difference in anopheline abundance observed (112 in Abomey-calavi and 264 in Cotonou). *Anopheles* were dissected and tested by ELISA. A total of 12 *Anopheles* were dissected in the central arrondissement of Abomey-calavi, with a percentage of 85.7%, and 98 *Anopheles* were dissected in health zones Cotonou I and IV, with a percentage of 81.6%. The high maturity rate reflects the high reproductive and epidemiological risk of *Anopheles* in the study areas. Out of 112 *anopheles* tested by ELISA, 5 were positive and out of 264 tested, 2 were positive in the central arrondissement of Abomey-calavi and in the Cotonou I and IV health zones, respectively. These ELISA results allow us to assess the circumsporozoite index and the entomological inoculation rate. The circumsporozoite index is estimated at 0.045 in Abomey-calavi and 0.075 in Cotonou. The circumsporozoite index in Abomey-calavi is higher than that obtained in Zogbadjè in 2022, also in the central arrondissement of Abomey-calavi (Mémoire Boko 2022) [27]. The indices obtained are also comparable to those of neighboring communes, based on the work of Tokponnon *et al.* [28] in a study providing information on the entomological dynamics and transmission profile of malaria in several departments of Benin. Thus, 0.08 is the sporozoite index obtained in Zè (a commune close to Calavi), which twice that is obtained in our study in Abomey-calavi. At the same time, 0.042 is the sporozoite index obtained in Littoral in the 9th arrondissement, which is half of that obtained in our study.

In this study, among the mosquito species identified, the majority is anopheline species: *Anopheles gambiae sl.* These results are in line with those obtained in the work of [8]. A study that highlighted the involvement of *Anopheles nili* in malaria transmission in northern Benin (**Table 1**). According to the characterization of species of the *An. gambiae sl* complex by PCR sine, we obtained more *Anopheles coluzzii*

than Anopheles gambiae this in the rainy season in the central arrondissement of Abomey-calavi. This observation is consistent with the results obtained in Toffo in the Atlantic department [28], but contradicts the work of [29] following a study of the genetic profile of *Anopheles gambiae s.l.* in relation to its resistance to insecticides in the cotton growing zone of Benin. This study concluded that "Irrespective of the season, *An. gambiae ss* remains the predominant species". This would probably be due to the nature (permanent or temporary) of the breeding sites favoring the emergence of these mosquitoes and the period of capture.

On the other hand, it should be noted that in health zones Cotonou I and IV, more *An. gambiae* were obtained than *An. coluzzii*, confirming the results of the work of [11, 29] and contradicting the recent results in the 3rd Littoral district from the work of Tokponnon *et al.* [28].

With regard to the plasmodial species that these anopheles are capable of transmitting, the study showed the presence in the two study areas of *Pf, Pm* and *Pv* in anopheles of the *An. gambiae* complex, the main malaria vector in Benin. The presence of *Pf, Pm* and *Pv* in Anopheles gambiae highlights the diversity of malaria parasites circulating in Benin. Microscopic tests and RDTs (rapid diagnostic tests) mainly target *P. falciparum*, leaving *P. vivax* infections undetected. The detection of *P. vivax* requires the integration of molecular (PCR) or serological tools to identify submicroscopic *P. vivax* infections. In addition, *P. vivax* infections are often asymptomatic but contribute to the silent transmission of this species. This means that screening campaigns need to be rolled out, including tests that are sensitive to all plasmodial species [11]. Moreover, the health zone of cotonou I-IV where *P. vivax* has been detected requires targeted interventions. Screening for *P. vivax* should be systematically included in national health surveys.

This study allowed the characterization of plasmodia by real-time PCR in southern Benin. As shown by the results of our study, it should be noted that recent work already indicates the presence of *P. vivax* in malaria vectors in Benin [11].

In terms of malaria transmission and spread, this involves not only plasmodial species in vectors, but also those found in humans. With regard to the plasmodial species found in the blood samples collected, the results of our study reveal the presence of *Pf*, *Pm* and *Po*, which were present alone or in mixtures. The plasmodial formula was *Pf* = 97.54%; *Po* = 1.64%; *Pm* = 0.82% in Abomey-calavi and *Pf* = 96.08%; *Po* = 3.48%; *Pm* = 0.43% in Cotonou. In both study areas, *Pf* is predominant. Obtaining Pv in the vector opens up the possibility of the emergence of severe forms following possible mutation or the emergence of new virulent Pv strains capable of infecting duffy-negative phenotypes. These results show the importance of implementing genomic monitoring of parasites to detect the emergence of new virulent strains. In addition, the detection of *Pv* poses a new diagnostic challenge, since rapid tests and microscopy may underestimate *P. vivax* in the case of co-infection with *P. falciparum* in humans. These results are similar to those of Damien *et al.*, [9] on the evaluation of new vector control strategies in an area of vector resistance to pyrethroids in southern Benin, where the plasmodial formula was *Pf* = 0.95; *Pm* = 0.05 and *Po* = 0.05. Our results also revealed an absence of *P. vivax*, in contrast to the work of [10], which revealed the presence of this plasmodial species in asymptomatic donors in Benin.

Furthermore, the quantity of mosquitoes captured depends on the exposure of the catchers to the mosquitoes, which may under-represent the density and variation of mosquitoes captured. In addition, rainy season collections do not consider seasonal variations in dry season densities or transmission of mosquito parasite species in the dry season. Similarly, the health centers selected cover specific areas and do not consider all the health care points where we could find more positivity to the different plasmodial species. The study was carried out in the Cotonou I and IV health zones and the results may not reflect the profile or prevalence of parasite occurrence in the vector as in humans in other health zones where environmental conditions, mosquito behavior and malaria transmission efficiency may differ. In view of these limitations, studies should be conducted over several months or years to capture mosquitoes according to seasonal variations in the mosquito populations studied. It would also be advisable to extend the collection to other geographical areas with varying characteristics in order to assess the generalizability of the results. Finally, it will be necessary to provide capacity building for technicians in the diagnosis of *P.v,* and to set up a *P.v* surveillance system.

The results of our study showed heterogeneity in the distribution of *Plasmodium* species in vectors and humans. *P. vivax* is found in vectors but absent in blood samples. This variation in *Plasmodium* species obtained in these localities can be explained by sample size, and particular attention should be paid to *P. vivax* retro.

5. CONCLUSION

The presence of *P. vivax* in parts of Africa is confirmed day by day, as in the present article. This study mentions its existence in the central arrondissement of Abomey-calavi and in the Cotonou I-IV health zone in Benin, in human-captured anopheles, vectors of malaria. In the human blood samples collected, clinical and XN31 confirmation data indicated the presence of *P. falciparum*, *P. ovale* and *Plasmodium malariae*, but no *P. vivax*.

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AUTHORS' CONTRIBUTIONS

TFT, RO, IJGY, FH and MA designed the research; TFT, IJGY, and FH conducted data collection; all authors conducted data analysis. TFT, IJGY, AS, GGP and FH coded the data; TFT, FH, RO and IJGY led the drafting with substantive input from MA, IJGY and FH in the results section; all authors revised the manuscript.

All authors read and approved the final manuscript.

ETHICS APPROVAL

The study was conducted in accordance with the Declaration of Helsinki and approved by the institutional ethics committee of the Cotonou Entomological Research Center (N06/IECC of January 7, 2021).

CONFLICTS OF INTEREST

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

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