U.S. PRESIDENT'S MALARIA INITIATIVE

# PMI VECTORLINK CÔTE D'IVOIRE 2019 ANNUAL ENTOMOLOGICAL REPORT
# APRIL 2019–DECEMBER 2019

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# ACRONYMS

| | |
|---|---|
| ***ace-1*** | Acetylcholinesterase |
| **bph** | bites per person per hour |
| **bpn** | bites per person per night |
| **CDC** | Centers for Disease Control and Prevention |
| **EIR** | entomological inoculation rate |
| **frd** | females per room per day |
| **DDT** | dichlorodiphenyltrichloroethane |
| **HBR** | human biting rate |
| **HLC** | human landing catch |
| **IRS** | indoor residual spraying |
| **ITN** | insecticide-treated net |
| ***kdr*** | **k**nockdown resistance |
| **NMCP** | National Malaria Control Program |
| **PBO** | Piperonyl butoxide |
| **PMI** | President's Malaria Initiative |
| **PSC** | pyrethrum spray catch |
| **WHO** | World Health Organization |

# EXECUTIVE SUMMARY

From April through December 2019, the U.S. President's Malaria Initiative (PMI) Project in Côte d'Ivoire, through its subcontractor Centre Suisse de Recherche Scientifiques, conducted malaria vector surveillance in four sites and insecticide resistance monitoring in 15 sites in Côte d'Ivoire.

Monthly longitudinal vector monitoring was conducted from April through December 2019 in four districts (i.e., sites) identified by the National Malaria Control Program (NMCP) in collaboration with PMI VectorLink. This included two sites scheduled to receive indoor residual spraying (IRS) in 2020 (Nassian and Sakassou), and two control sites (Béoumi and Dabakala). Three different methods were used to collect adult mosquitoes, including human landing catch (HLC), pyrethrum spray catch (PSC), and Centers for Disease Control and Prevention (CDC) light traps. The entomological parameters assessed were vector composition, seasonality, distribution, biting and resting behaviour, sporozoite infection, parity, and entomological inoculation rate (EIR).

In addition, insecticide susceptibility tests, resistance intensity tests, and synergist assays were conducted in 15 sites, including the four vector surveillance sites and an additional 11 sites (Abengourou, Aboisso, Adzopé, Bettié, Bouaké, Bouna, Daloa, Gagnoa, Odienné, San Pedro, and Yamoussoukro). World Health Organization (WHO) susceptibility tube tests and CDC bottle assays were used to test the local malaria vectors against different insecticides used in public health efforts.

Species composition across all longitudinal monitoring sites showed that *An. gambiae* s.l. was the predominant malaria vector, representing 84.5% (n=79,246) of the total *Anopheles* collected. The overall *An. gambiae* s.l. female biting activities were highest between 10:00 p.m. and 3:00 a.m. during the nine months of collection, both indoors and outdoors, in all four sites.

The highest human biting rate (HBR) was recorded in Sakassou, with an overall rate ranging from 97.5 to 230 bites per person per night (bpn) during the collection period. Parity rates were high in all sites, ranging from 65.3% in Dabakala to 92.5% in Nassian. Indoor resting densities of *An gambiae* s.l. recorded during PSC collections were highest in Dabakala—up to 35 females per room per day (frd)— and lowest in Nassian (0 to 6 frd).

Resistance was observed in *An. gambiae* s.l. to the diagnostic dose of all pyrethroids and bendiocarb in all sites surveyed for insecticide resistance monitoring. Susceptibility to the diagnostic dose of pirimiphos-methyl was observed in 10 sites, while the 5 other sites recorded low resistance. Pre-exposure to piperonyl butoxide did not yield full susceptibility to pyrethroids, but induced a substantial increase in mortality in nine of the 15 sites when combined with deltamethrin only (43.2% on average), and less so with alpha-cypermethrin (30%) and permethrin (22.8%). That suggests that deltamethrin + piperonyl butoxide insecticide-treated nets (ITNs) may be the most appropriate type of ITN in these areas. *An. gambiae* s.l. was susceptible to chlorfenapyr in one of the 15 sites at the dose of 100 µg/bottle, and in nine of the sites at the dose of 200 µg/bottle. For clothianidin, susceptibility was observed in seven out of 15 sites seven days post-exposure.

Molecular characterization of mosquitoes collected for insecticide susceptibility testing indicated that *An. coluzzii* represented the predominant species in all sites except in Nassian, Bouna, Odienné, Bouaké, and Yamoussoukro. One hundred percent of the population of Nassian, Bouna, and Odienné were *An. gambiae* s.s., while the population of Daloa Gagnoa and Sakassou were entirely *An. coluzzii.* A few hybrids of the two species were found in Yamoussoukro (2.9%) and San Pedro (1.9%).

Among adult mosquitoes collected in the four longitudinal monitoring sites, *An. coluzzii* were predominant in Béoumi and Dabakala, regardless of the collection method. *An. coluzzii* was the only species identified in Sakassou, while *An. gambiae* s.s. was the only vector identified in Nassian. A few hybrid *An. coluzzii*/*An. gambiae* s.s. were identified in Béoumi.

Both knockdown-resistant- (*kdr*-) West and East mutations were present in all sites except Nassian and Odienné. That is consistent with the high resistance to pyrethroids previously observed in Côte d'Ivoire. The acetylcholinesterase (*ace-1*) target site mutation observed in all sites was also consistent with phenotypic resistance to bendiocarb in all sites and to pirimiphos-methyl in some sites. These findings suggest that clothianidin-based products may be an appropriate insecticide for IRS in the targeted sites. Based on the susceptibility status of vector populations, pirimiphos-methyl-based products could be considered for IRS in other districts or for insecticide rotation in Nassian. Furthermore, piperonyl butoxide synergist ITNs and dual-active-ingredient ITNs (e.g., Interceptor G2) should be considered for insecticide resistance management in the country.

# I. INTRODUCTION

Malaria is a leading cause of morbidity and mortality in Côte d'Ivoire. It accounts for about 33% of outpatient visits in health facilities, with an incidence of 134 per 1,000 in the general population and 247 per 1,000 among children under 5, according to the 2017 NMCP report. To reduce the malaria burden, the main malaria vector control method used in Côte d'Ivoire is the distribution and use of ITNs through mass campaigns and routine distribution. Since the country started ITN mass and routine distributions, only pyrethroid-based ITNs have been distributed there. The National Malaria Strategic Plan 2016–2020 prioritized IRS as an additional vector control method to reduce malaria morbidity and mortality.

In 2019, VectorLink Côte d'Ivoire, in collaboration with the Centre Suisse de Recherche Scientifiques (the subcontractor) and all local entomological research institutes, conducted its second consecutive year of insecticide susceptibility testing of *An. gambiae* s.l. in 15 sites across the country, and longitudinal entomological surveillance in four sites selected by the NMCP. The data collected aimed to support the NMCP and the malaria vector control stakeholders (including PMI VectorLink) in determining the optimal timing and insecticides for IRS programming and to inform the selection of ITNs for future net distribution campaigns.

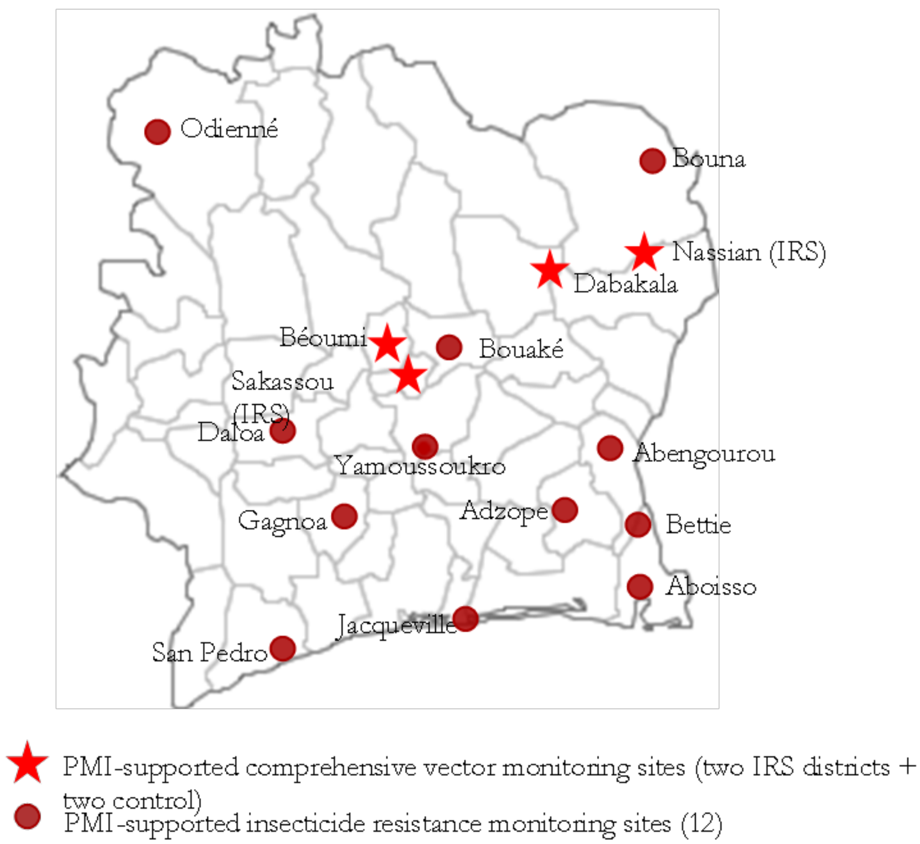
# 2. METHODOLOGY

From April through December 2019, VectorLink Côte d'Ivoire conducted longitudinal entomological surveillance in four sites selected by the National Malaria Control Program (NMCP,) and insecticide resistance monitoring in 15 sites including the four longitudinal monitoring sites.

## 2.1 ENTOMOLOGICAL MONITORING SITES

VectorLink Côte d'Ivoire conducted comprehensive monitoring (vector surveillance and insecticide resistance monitoring) in two sites targeted to receive IRS in 2020 (Nassian and Sakassou) and two control sites (Béoumi and Dabakala) and insecticide resistance monitoring only) in 11 additional sites including Abengourou, Aboisso, Adzope, Bettie, Bouaké, Bouna, Daloa, Gagnoa, Odienné , San Pedro, and Yamoussoukro (Figure 1).

**FIGURE 1: MAP OF CÔTE D'IVOIRE SHOWING THE 2019 PMI VECTORLINK ENTOMOLOGICAL MONITORING SITES**



## 2.2 VECTOR BIONOMICS MONITORING

Adult mosquitoes were collected via HLC, PSC, and CDC light traps. HLCs were conducted during two consecutive nights in four houses per site per month: two urban, two rural, at a minimum of five km from the town. The PSCs were conducted in 30 houses (15 urban and 15 rural) per site per month. The CDC light trap collections were performed in four different houses from HLCs (two urban and two rural) during two consecutive nights per site per month. The same houses were maintained for HLC and CDC light trap collections throughout the longitudinal monitoring, while randomly selected houses were used each month for PSC collections depending on the availability of households. Collections were conducted every month from April through December 2019, representing nine months of collection. The collection times and sampling methods are shown in Table 1.

### TABLE 1: LONGITUDINAL MONITORING COLLECTION METHODS

| Collection Method | Time | Frequency | Sample |
|---|---|---|---|
| HLC | 6:00 p.m. to 6:00 a.m. | Two nights per site per month | Four houses per site (two urban and two rural); same houses each month |
| PSC | 6:00 am to 8:00 a.m. | Two days per site per month | 30 houses per site (15 urban and 15 rural) |
| CDC light trap | 6:00 p.m. to 6:00 a.m. | Two nights per site per month | Four houses per site (two urban and two rural; same houses each month) |

HLCs were performed indoors and outdoors to collect adult mosquitoes landing on human baits. With legs exposed to attract host-seeking mosquitoes, one human bait (mosquito collector) was seated indoors and another one outdoors. The collectors switched between indoors and outdoors each hour, basis to control for potential differences in attractiveness. The doors of the houses were kept closed when collections were under way. The collectors used flashlights and hemolysis tubes to collect mosquitoes that landed on their legs, before they could be bitten. The tubes were covered with cotton after individual collection of mosquitoes. The teams transferred the mosquitoes hourly to custom-made labelled bags, over 12 hours.

The PSCs were carried out between 6:00 a.m. and 8:00 a.m. White sheets were placed on the floor from wall to wall in sampled rooms. The rooms were sprayed with the commercial pyrethroid and piperonyl butoxide (PBO) insecticide, after closing the house's windows and doors and removing drinking water and food items from the room. For houses with open eaves, collectors sprayed from the outside through the eaves before entering and spraying indoors. Ten minutes after spraying, all mosquitoes knocked down by the chemical were collected from the white sheets. The mosquitoes were kept in Petri dishes and then sorted by species using an identification key (Gillies and Coetzee 1987). The abdominal status of all female anophelines was determined and sorted into four categories: unfed, blood-fed, half-gravid, and gravid.

CDC light traps were installed indoors in selected houses where people slept under an ITN. The CDC light traps were suspended in a bedroom 1.5 meters above the floor, above the sleeper's legs. Traps were set from 6:00 p.m. to 6:00 a.m. to ensure that surveillance was conducted during the suspected peak host-seeking periods.

All mosquitoes collected through each method were morphologically identified to genus. *Anopheles* mosquitoes were identified to species or species complex by microscope, using identification keys (Gillies and Coetzee 1987). The identification was done by a team of well-trained technicians from research institutes and VectorLink staff. A subsample of *An. gambiae* s.l. from each site was dissected to estimate parity rate. All mosquitoes were preserved on silica gel in Eppendorf tubes for further laboratory processing to identify sibling species, resistance mechanisms, infection status, and source of blood meal, using polymerase chain reaction and enzyme-linked immunosorbent assay.

The indicators listed in Table 2 were calculated based on the number of mosquitoes collected through each collection method.

**TABLE 2: VECTOR SURVEILLANCE INDICATORS PER COLLECTION METHOD**

| Collection Method | Indicator | Definition |
|---|---|---|
| HLC | HBR | Mean number of bpn |
| | Peak biting time | Hour of highest HBR |
| | Parity rate | Percentage of parous mosquitoes/total dissected |
| | Exophagic rate | Percentage of mosquitoes biting outside |
| | Endophagic rate | Percentage of mosquitoes biting inside |
| PSC | Indoor resting density | Mean number of mosquitoes/house/day |
| | % of fed females | Number of fed mosquitoes/total collected by PSC |
| CDC light trap | Indoor density | Mean number of mosquitoes collected indoors/trap/night |

## 2.3 INSECTICIDE RESISTANCE MONITORING

From June through August 2019, VectorLink Côte d'Ivoire completed insecticide resistance monitoring in 15 sites across the country including the four longitudinal monitoring sites (Figure 1). Larvae and pupae of *An. gambiae* s.l. were collected in each site from several larval habitats, pooled, and reared to adulthood in the field laboratory. Insecticide susceptibility tests were conducted on two- to five-day-old adult females using WHO tube tests and CDC bottle assays.

For each tube test, about 100 female *An. gambiae* s.l. were tested against the insecticide (in 4 batches of 25) and an additional 40–50 were tested in two control tubes (20–25 each) in parallel.

The diagnostic concentrations of permethrin (0.75%), deltamethrin (0.05%), alpha-cypermethrin (0.05%), bendiocarb (0.1%), and pirimiphos-methyl (0.25%) were tested in all sites. Resistance was defined following the WHO criteria (WHO, 2016):

98% or greater mortality indicates susceptibility

- *between* 90 and 98% mortality indicates possible resistance
- *less than* 90% mortality indicates confirmed resistance

When insecticide resistance was confirmed, resistance intensity (high, moderate, and low) was also tested at 5 and 10 times the diagnostic concentration of permethrin, deltamethrin, alpha-cypermethrin, and pirimiphos-methyl.

Clothianidin papers (13.2 mg/paper) were treated locally using a protocol designed by VectorLink. The susceptibility testing was conducted as described above, and the mortality was recorded up to seven days post-exposure.

Synergist assays with PBO were conducted for deltamethrin, permethrin, and alpha-cypermethrin according to the WHO tube test protocol to determine the involvement of cytochrome P450s in pyrethroid resistance. A high percent mortality and/or reversal of susceptibility when pre-exposed to PBO indicates probable involvement of enzymes such as P450s in the resistance mechanism.

CDC bottle assays were conducted using chlorfenapyr at the doses of 100µg/bottle and 200µg/bottle. Testing was done following the protocol of Brogdon and Chan (2010), but the exposure time was one hour, and mortality was recorded every 24 hours up to 3 days (72 hours).

## 2.4 MOLECULAR CHARACTERIZATION

Insecticide resistance in mosquitoes can be related to target-site mutations. Among these, resistance to pyrethroids and dichlorodiphenyltrichloroethane [i.e., DDT] is described as a substitution of the amino acid leucine with either phenylalanine (L1014F, referred as knockdown rate (*kdr)* -West) or serine (L1014S, referred to as *kdr*-East) at the position 1014 in the sodium channel gate. For organophosphate and carbamate insecticide, the target site mechanism, known as *ace-1,* is a substitution of an amino acid glycine to serine at position 119.

About 50 *An. gambiae* s.l. mosquitoes were randomly selected from each of the 15 sites among the dead and surviving mosquitoes from the WHO susceptibility tests, and further analyzed to identify species and assess molecular markers of insecticide resistance. In the four longitudinal monitoring sites, a subsample of about 400, 100, and 100 females per site was preserved from the HLC, PSC, and CDC-light trap collections, respectively, to determine subspecies of *An. gambiae* s.l. The DNA of each individual mosquito was extracted using the protocol designed by Collins et al., 1987. *An. gambiae* complex species were identified as *An. gambiae*, *An. Coluzzii,* or hybrids of the two species, following the Short Interspersed Element protocol described by Santolamazza et al. (2008). The presence of *kdr*-West and East was characterized using the Taqman protocol described by Bass et al. (2007).

The sporozoite infection status of a subsample of mosquitoes collected by HLC from each site was determined using the enzyme-linked immunosorbent assay protocol for identification of *Plasmodium falciparum* circumsporozoite infection. The blood sources of blood fed female *An. gambiae* s.l. collected using PSC were examined using ELISA protocol to determine the human blood index.

# 3. RESULTS

## 3.1 VECTOR BIONOMICS MONITORING

Vector bionomics activities were conducted in the two IRS targeted and two control sites during nine consecutive months. Vector species composition, density and behavior were collected per sites.

### 3.1.1 SPECIES COMPOSITION

#### *OVERALL SPECIES COMPOSITION*

A total of 93,748 mosquitoes, 10% (n=9,404) of which were culicines, were collected over nine months (April through December 2019) using the three collection methods described above. *An. gambiae* s.l. (n=79,246; 84.5%) was the most predominant malaria vector species, representing 94.0% of the total *Anopheles* mosquitoes collected across all sites and methods (n=84,344; Table 3). *An. funestus* s.l. represented 2% (n=1,655) and *An. nili* represented 0.7% (n=574) of all mosquitoes collected. The other *Anopheles* species represented 3.4% (n=2,869) and were composed primarily of *An. pharoensis.*

A total of 75,026 mosquitoes were collected by HLC. *An. gambiae* s.l. was the most collected malaria vector species (94.8%; n=66,635), followed by *An. funestus* s.l. (1%; n=717) and *An. nili* (0.6%; n=440) across all sites during the nine months. The other *Anopheles* species found was *An. pharoensis* (3.5%, n= 2.477) (Figure 2; Annex Tables 8–11). Culicine mosquitoes represented 6.3% (n=4,757) of the total mosquitoes caught using HLCs.

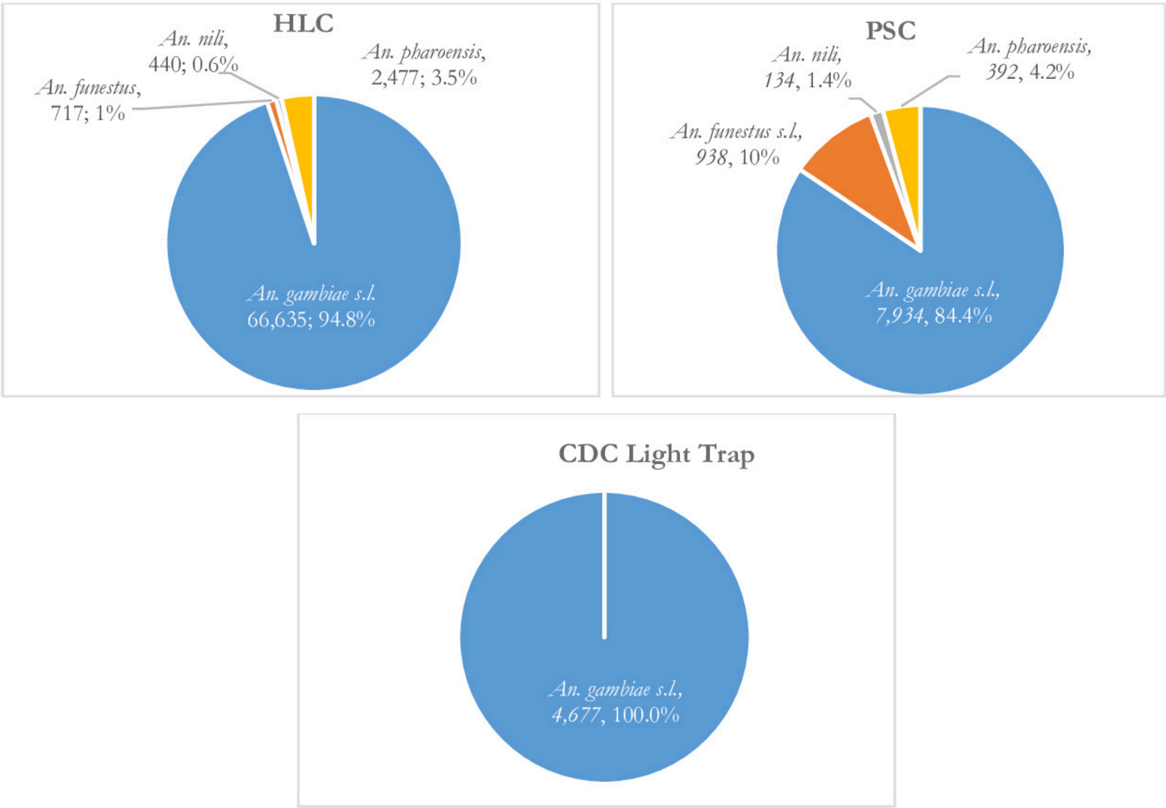
A total of 12,274 mosquitoes were collected by PSC. An. gambiae s.l. represented 84.4% (n=7,934) of the total Anopheles collected (n=9,398), all sites included. An. funestus s.l. and An. nili represented the other malaria vector species recorded, at 10% (n=938) and 1.4% (134). The other Anopheles species found was An. pharoensis (Figure 2; Annex Tables 8–11). Culicine mosquitoes represented 23.4% (n=2,876) of the total mosquitoes caught using PSC (Annex: Tables 8–11).

A total of 6,448 mosquitoes were collected by CDC light trap. *An. gambiae* s.l. was the only *Anopleles* species collected (100% of *Anopheles;* n=4,677) (Figure 2; Annex Tables 8–11). Culicine mosquitoes represented 27.5% (n=1,771) out of all mosquitoes caught using the CDC light trap collection method (Annex: Tables 8–-11).

**TABLE 3: NUMBER OF MOSQUITOES COLLECTED IN ALL SITES USING THE THREE COLLECTION METHODS**

| | Béoumi | Dabakala | Nassian | Sakassou | Total |
|---|---|---|---|---|---|
| *An. gambiae* s.l. | 7,930 | 14,864 | 3,968 | 52,484 | **79,246** |
| *An. funestus* s.l. | 7 | 998 | 368 | 282 | **1,655** |
| *An. nili* | 1 | 560 | 0 | 13 | **574** |
| Other *Anopheles* | 819 | 310 | 7 | 1733 | **2,869** |
| Culicine | 1,486 | 2408 | 629 | 4881 | **9,404** |
| **Total** | **10,243** | **19,140** | **4,972** | **59,393** | **93,748** |

**FIGURE 2: SPECIES COMPOSITION OF THE *ANOPHELES* MOSQUITOES COLLECTED IN THE FOUR SITES USING HLC, PSC, AND CDC LIGHT TRAPS FROM APRIL THROUGH DECEMBER 2019**




### *SPECIES COMPOSITION IN BÉOUMI*

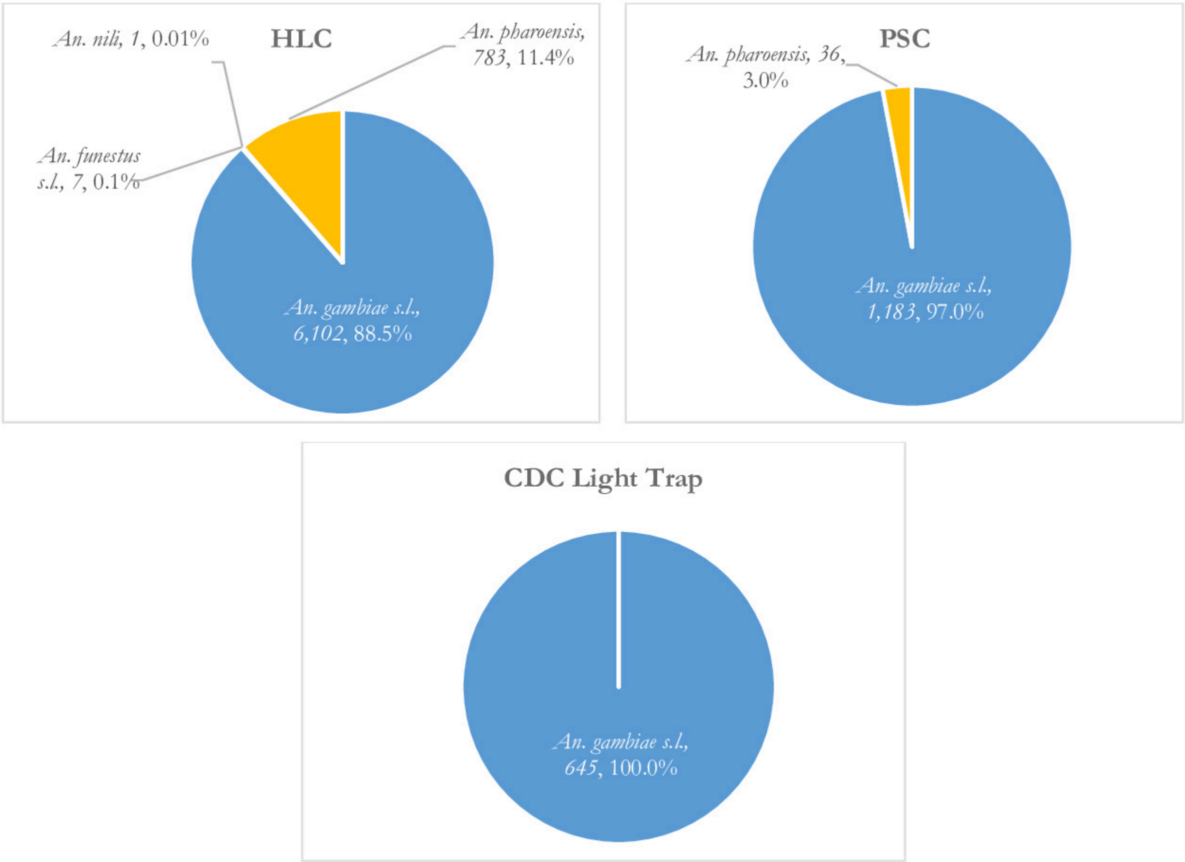
In Béoumi, a total of 10,243 mosquitoes were collected from May through December 2019 using the three collection methods. *An. gambiae* s.l. was the most collected malaria vector species (90.6%; n=7,930). Only a few *An. funestus* s.l. (0.1%; n=7) were collected. The other *Anopheles* species represented 9.4% (n=819) of all *Anopheles* species. Culicines (14.5%, n=1,486) were also collected during the same period and using all methods.

Using HLC, a total of 7,611 mosquitoes were collected. *An. gambiae* s.l. was the most collected (88.5%; n=6,102) vector species. Only a few *An. funestus* s.l. (0.1%; n=7) were recorded. The other *Anopheles* species found was *An. pharoensis* (11.4%; n=783) (Figure 3; Annex Table 8).

With PSC, a total of 1,557 mosquitoes were collected. *An. gambiae* s.l. was the only vector species, representing 97% (n=1,183) of the total *Anopheles* collected. The other *Anopheles* species found was *An. pharoensis* (Figure 3; Annex Table 8).

With the CDC light trap, 1,075 mosquitoes were collected. All *Anopheles* mosquitoes collected were *An. gambiae* s.l. (100%; n=645) (Figure 3; Annex Table 8).

**FIGURE 3: SPECIES COMPOSITION OF THE *ANOPHELES* MOSQUITOES COLLECTED IN BÉOUMI USING HLC, PSC, AND CDC LIGHT TRAP FROM MAY THROUGH DECEMBER 2019**




### *SPECIES COMPOSITION IN DABAKALA*

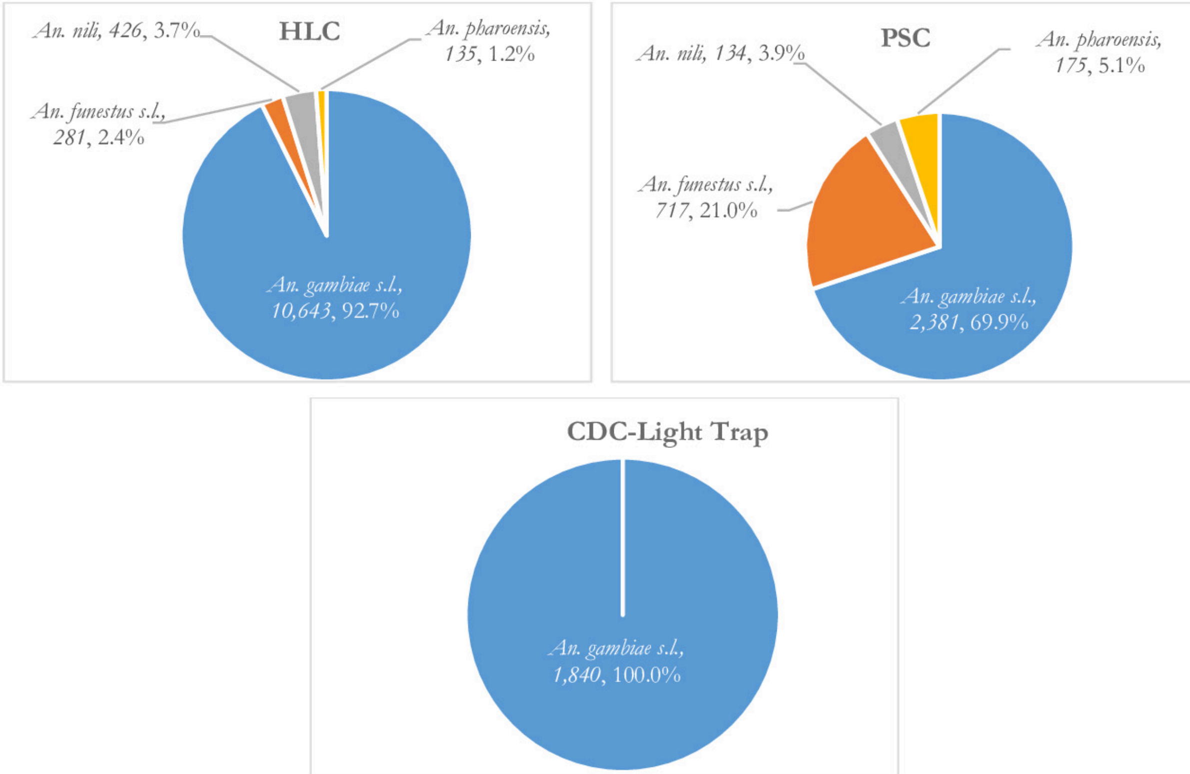
In Dabakala, a total of 19,140 mosquitoes were collected from May through December 2019 using the three collection methods. *An. gambiae* was the predominant malaria vector species collected (88.8%; n=14,864). *An. funestus* s.l. and *An. nili* represented the other malaria vectors found, at 6% (n=560) and 3.4% (n=310) respectively. The other *Anopheles* species represented 1.9% (n=310) of all *Anopheles* collected all collection methods included. Two thousand four hundred and eight (2,408) culicines were collected during the same period and using all collection methods.

Using HLC, a total of 12,984 mosquitoes were collected during the eight months. *An. gambiae* s.l. was the main malaria vector species collected (92.7%; n=10,643). *An. funestus* s.l. and *An. nili* were the other vector species collected, at 2.4% (n=281) and 3.7% (n=426) respectively. *Anopheles pharoensis* represented the other *Anopheles* species found (1.2%; n=135) (Figure 4; Annex Table 9).

With PSC, a total of 3,557 mosquitoes were collected. *An. gambiae* s.l. was the most collected (69.9%; n=2,381). *An. funestus* s.l. and *An. nili* were the other vector species collected: 21% (n=717) and 3.9% (n=134) respectively. The other *Anopheles* species found was *An. pharoensis* (5.1%; n=175; Figure 4; Annex Table 9).

With the CDC light trap, 2,399 mosquitoes were collected with *An. gambiae* s.l. (100%; n=1,840) representing the only *Anopheles* and vector species collected (Figure 4; Annex Table 9).

**FIGURE 4: SPECIES COMPOSITION OF THE *ANOPHELES* MOSQUITOES COLLECTED IN DABAKALA USING HLC, PSC, AND CDC LIGHT TRAPS FROM MAY THROUGH DECEMBER 2019**




## *SPECIES COMPOSITION IN NASSIAN*

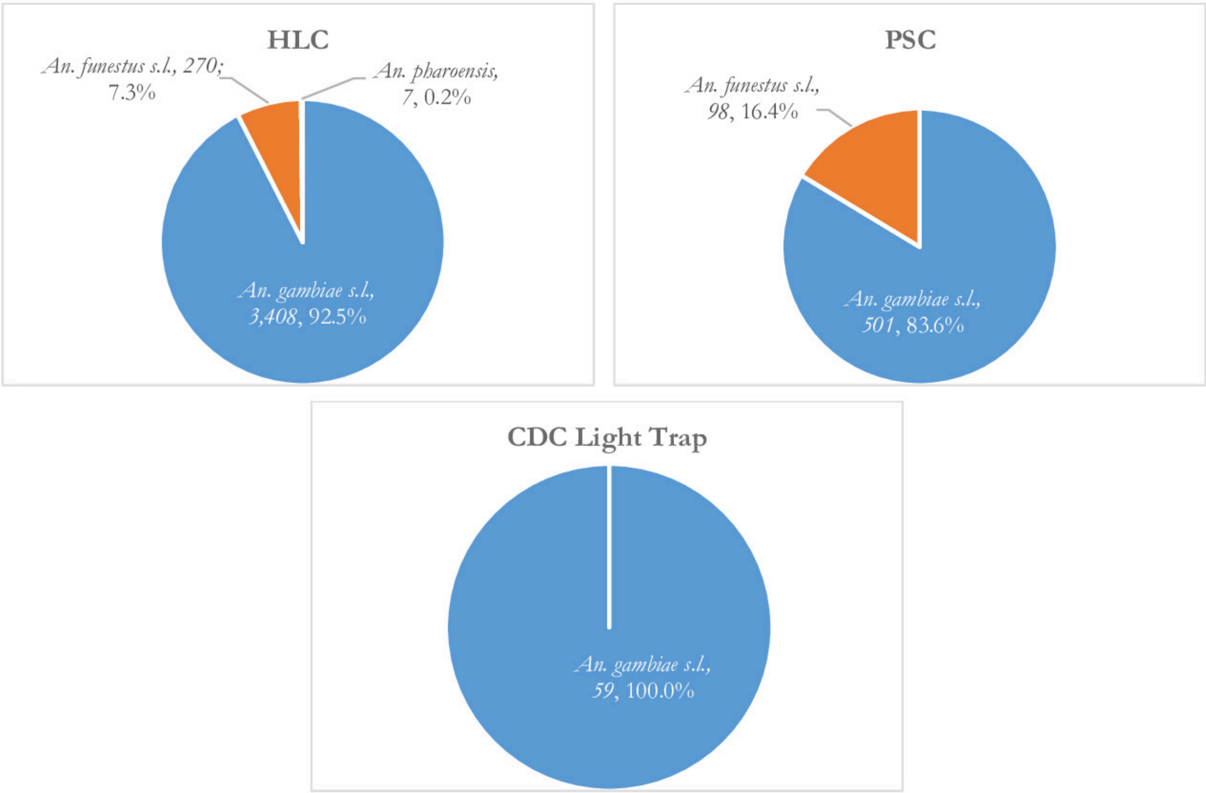
In Nassian, a total of 4,972 mosquitoes including culicines were collected from May through December 2019 using the three collection methods. *An. gambiae* s.l. was the main vector species collected (91.4%; n=14,864). *An. funestus* s.l. represented 8.5% (n=368). The other *Anopheles* species represented 0.2% (n=7) of all *Anopheles* collected. More than twelve percent of culicines were also collected during the period and using all collection methods (12.7%, n=629).

Using HLC, a total of 4,075 mosquitoes were collected in Nassian during the eight months. *An. gambiae s.l.* was the predominant vector species collected (92.5%; n=3,408). *An. funestus* s.l. was the other vector species collected, at 7.3% (n=270; Figure 5; Annex Table 10).

With PSC, a total of 701 mosquitoes were collected. *An. gambiae* s.l. was the most collected vector species (83.6%; n=501). *An. funestus* s.l. was the second malaria vector collected, at 16.4% (n=98), followed by *An. pharoensis* (0.2%, n=7; Figure 5; Annex Table 10).

With the CDC light trap, 196 mosquitoes were collected. *An. gambiae* s.l. (100%; n=59) was the only *Anopheles* species collected (Figure 5; Annex Table 10).

**FIGURE 5 : SPECIES COMPOSITION OF THE *ANOPHELES* MOSQUITOES COLLECTED IN NASSIAN USING HLC, PSC, AND CDC LIGHT TRAPS FROM MAY THROUGH DECEMBER 2019**




### SPECIES COMPOSITION IN SAKASSOU

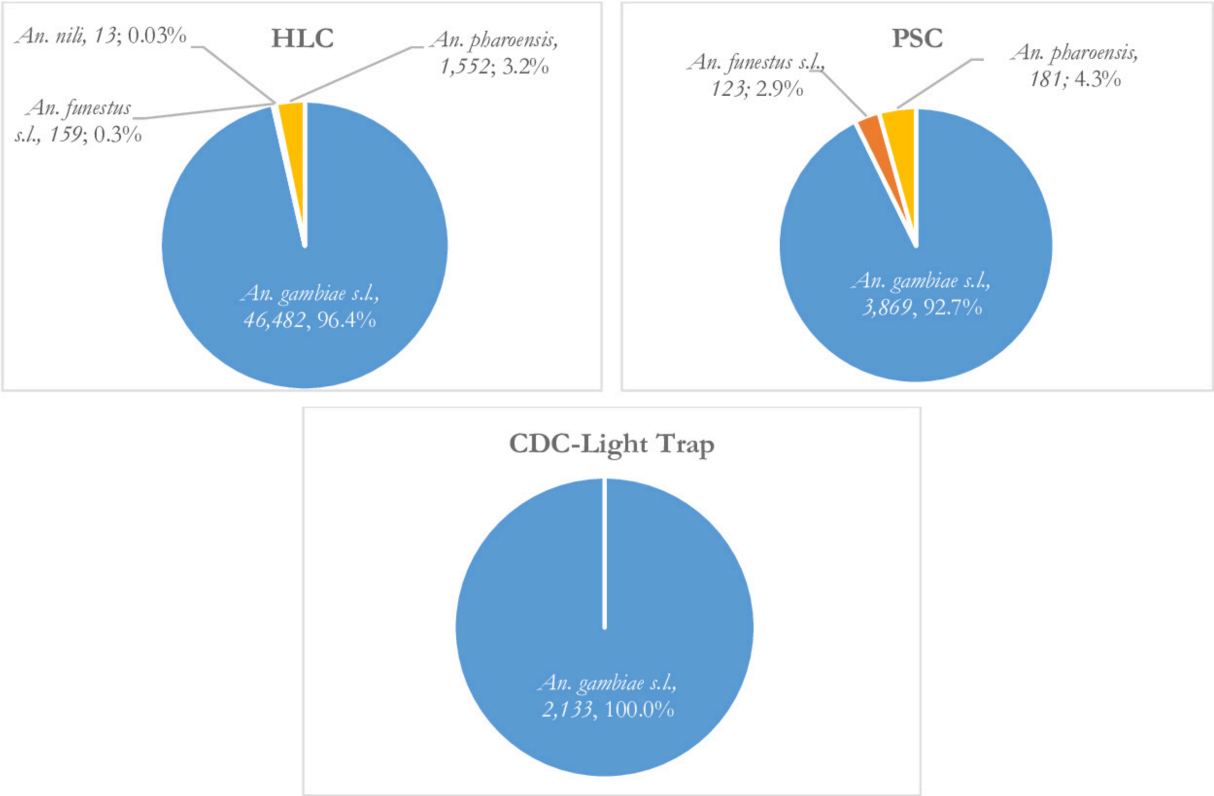
Sakassou was the most productive site among the four where vector surveillance was conducted. A total of 59,393 mosquitoes were collected from April through December 2019 using the three collection methods. *An. gambiae* s.l. was the main vector species collected (96.3%; n=52,484). Few *An. funestus* s.l. and *An. nili* were collected (0.5%; n=282 and 0.1%). The other *Anopheles* species represented 8.2% (n=4,881) of all *Anopheles* collected. More than eight percent of culicines were also collected in Sakassou (8.2%, n=4,881) during the same period, all methods included.

Using HLC, a total of 50,356 mosquitoes including 4.3% (n=2,150) culicines were collected in Sakassou during the nine months. *An. gambiae* s.l. was the most collected (96.4%; n=46,482). *Anopheles funestus* s.l. and *An. nili* were also collected (0.3%, n=159 and 0.1%, n=13). The other *Anopheles* species found was *An. pharoensis* (1.2%; n=135; Figure 6; Annex Table 11.

With PSC, a total of 6,259 mosquitoes including 33.3% (n=2,086) culicines were collected. *An. gambiae* s.l. was the predominant vector species collected (92.7%; n=3,869). Few *An. funestus* s.l. were collected: 2.9% (n=123). The other *Anopheles* species found was *An. pharoensis* (4.3%; n=181) (Figure 6; Annex Table 11).

With the CDC light trap, 2,778 mosquitoes including 23.2% (n=645) culicines were collected, and all *Anopheles* mosquitoes collected were *An. gambiae* s.l. (100%; n=2,133; Figure 6; Annex Table 11).

**FIGURE 6: SPECIES COMPOSITION OF THE *ANOPHELES* MOSQUITOES COLLECTED IN SAKASSOU USING HLC, PSC, AND CDC-LIGHT TRAP FROM APRIL THROUGH DECEMBER 2019**



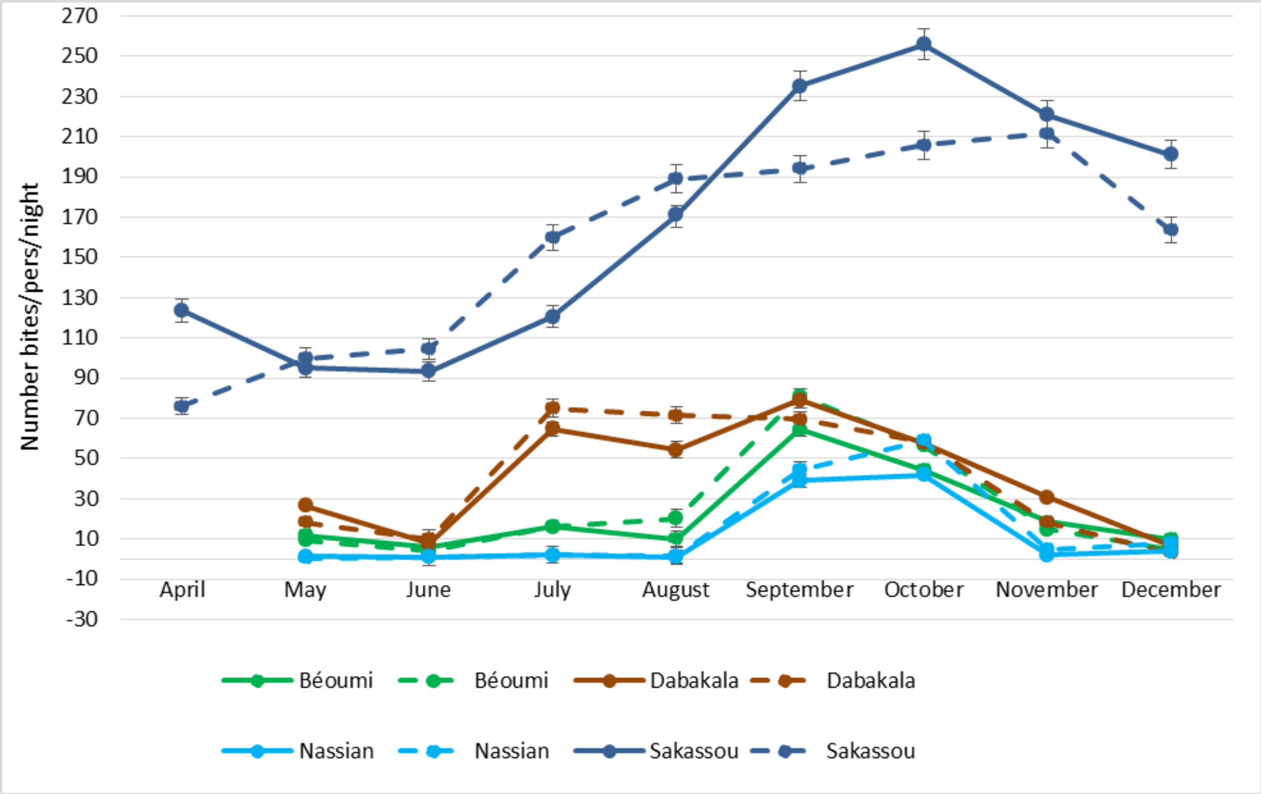

### 3.1.2 BITING CYCLE OF *AN. GAMBIAE* S.L.

The hourly and monthly collections using HLC methods permitted the determination of the biting cycle of the main malaria vector, *An. gambiae* s.l. within the four longitudinal monitoring sites.

#### *OVERALL TRENDS IN AN. GAMBIAE S.L. HBR*

The HBRs were very high in Sakassou, with an overall rate ranging from 97.5 in May to 231 bpn in October 2019 (Figure 3; Annex Table 11). The indoor rate was between 76 and 212 bpn, and the outdoor rate ranged from 93 to 255 bpn from April 2019 through December 2019 (Figure 3). The biting rate ranged from 5 to 74 bpn in Dabakala and from 5 to 73 bpn in Béoumi. The biting rates were lowest in Nassian (from 1 to 50 bpn) regardless of the months and the collection place (Figure 7; Annex Table 12).

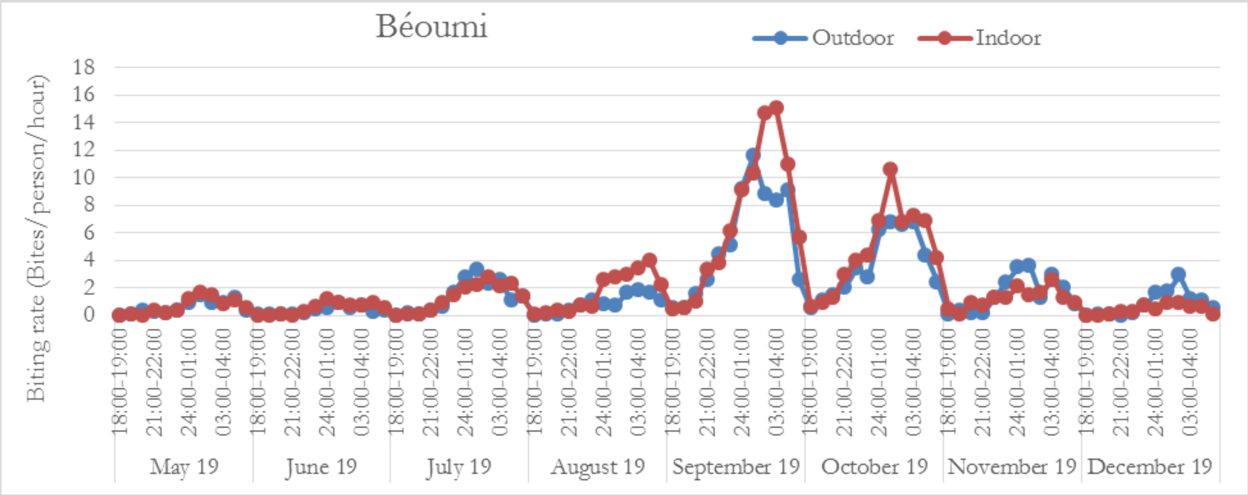
**FIGURE 7: *ANOPHELES GAMBIAE* S.L. BITING RATE USING HLC AT ALL SITES**




### *BITING CYCLE OF AN. GAMBIAE S.L. FROM BÉOUMI*

*An. gambiae* s.l. showed variable biting behavior across the four districts (Annex Table 12). In Béoumi *An. gambiae* s.l. was endophilic (53.5%). The hourly biting rates peaked between 10:00 p.m. and 4:00 a.m. outdoors and indoors, and the highest average rate was recorded in September 2019, with about 15 bites per person per hour (bph) indoors and 11.6 bph outdoors (Figure 8; Annex Table 13).

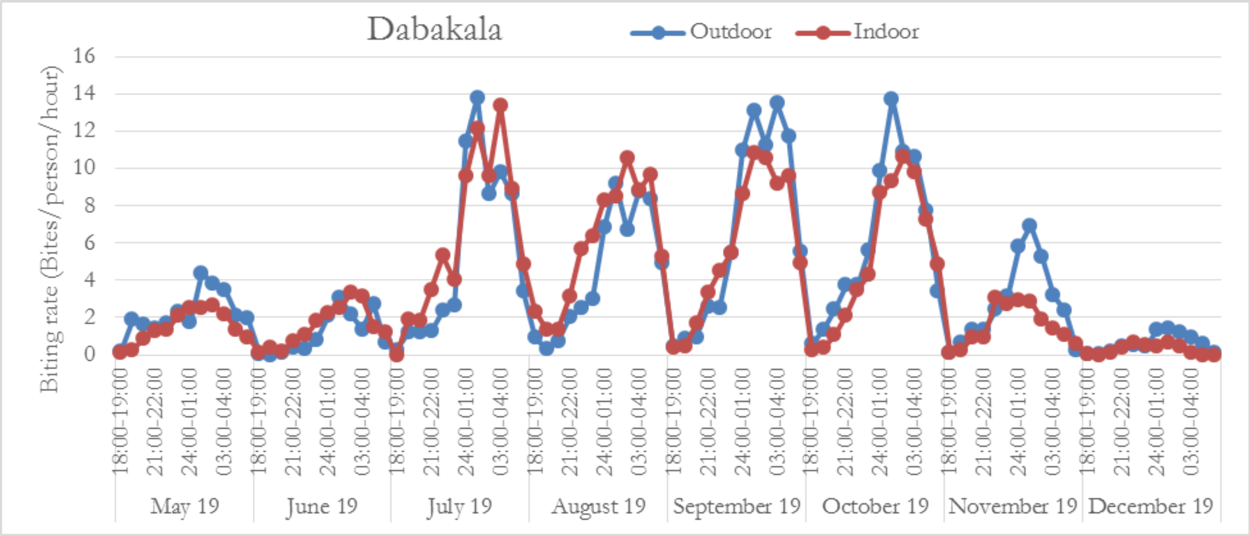
**FIGURE 8: BITING RATE AND CYCLE OF *AN. GAMBIAE* S.L. COLLECTED USING HLC IN BÉOUMI**



### *BITING CYCLE OF AN. GAMBIAE S.L. FROM DABAKALA*

The densities of *An. gambiae* s.l. were overall higher outdoors in Dabakala (51.4%). The average biting activities were higher between 10:00 p.m. and 03:00 a.m. during the eight-month collection period, both indoors and outdoors. The highest biting peak was recorded in July (13.4 bph indoors and 13.8 bph outdoors) with additional similar peaks in September and October (Figure 9; Annex Table 13).

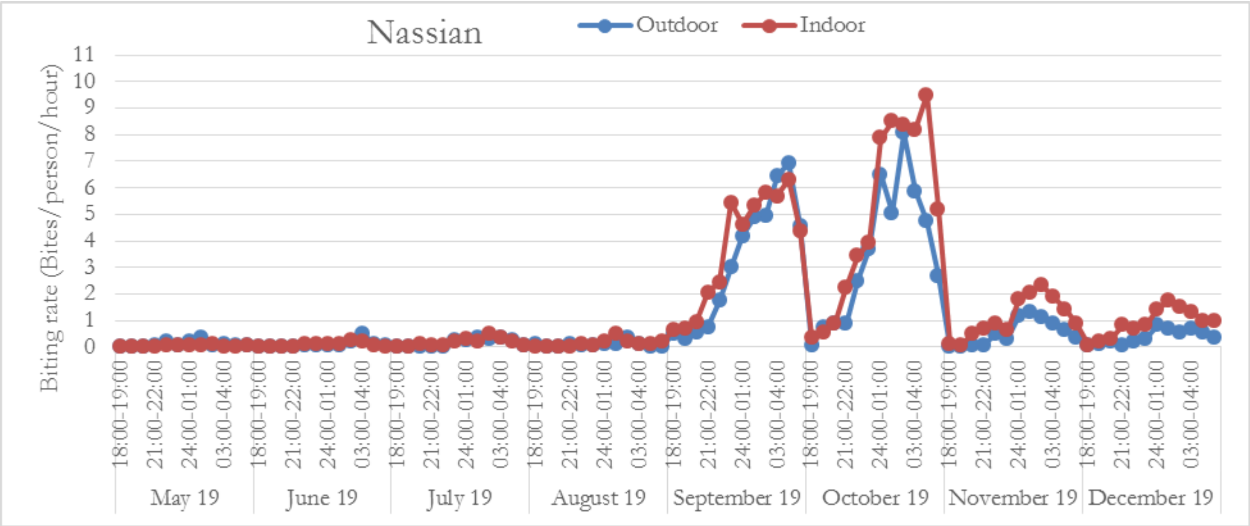
**FIGURE 9: BITING RATE AND CYCLE OF *AN. GAMBIAE* S.L. COLLECTED USING HLC IN DABAKALA**




### *BITING CYCLE OF AN. GAMBIAE S.L. FROM NASSIAN*

*An. gambiae* s.l. showed variable biting behavior in Nassian (Annex Table 12). The densities of *An. gambiae* s.l. were higher indoors in Nassian (57.8%). *An. gambiae* females biting activities were averagely higher between 01:00 a.m. and 03:00 a.m. during the eight months, both indoors and outdoors. The highest biting rates were recorded in October 2019 with 9.5 bph indoors and 8.4 bph outdoors (Figure 10; Annex Table 13).

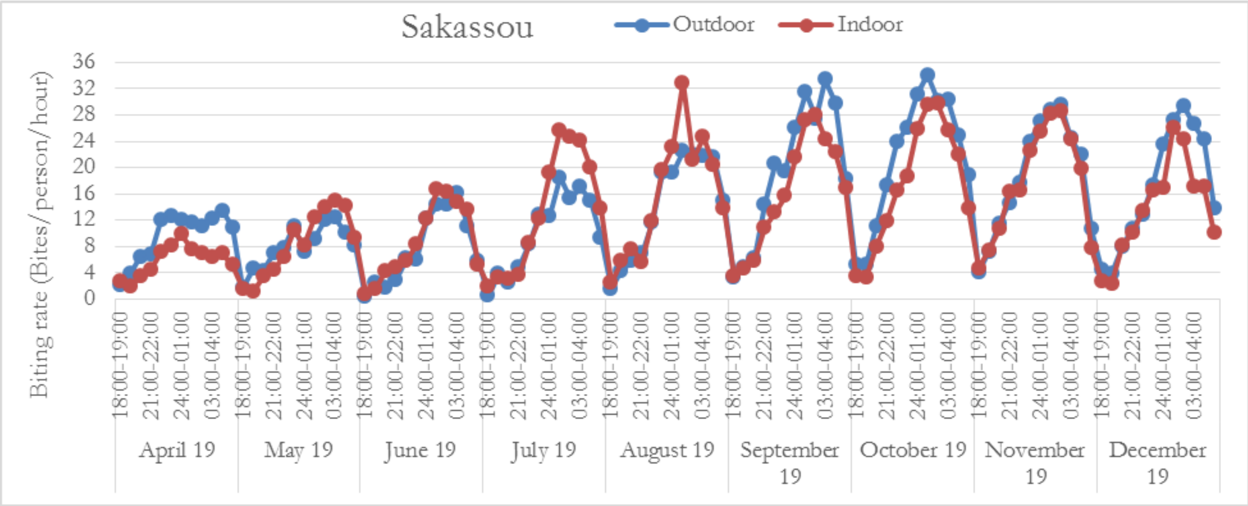
**FIGURE 10: BITING RATE AND CYCLE OF *AN. GAMBIAE* S.L. COLLECTED USING HLC IN NASSIAN**



### *Biting cycle of An. gambiae s.l. from Sakassou*

The HBRs were very high in Sakassou, with an overall rate ranging from 97.5 to 231 bpn in May 2019 and October 2019 respectively (Figure 4; Annex Table 12). The indoor rate was between 76 and 212 bpn, and outdoor rate range was 93 to 255 bpn from April 2019 to December 2019. In Sakassou the biting rates peaked between 11:00 p.m. and 04:00 a.m. outdoors and indoors with the highest hourly indoor biting rate observed in August 2019 (32.9 bph) and outdoor in September and October 2019 (34 bph) (Figure 11; Annex Table 13).

**Figure 11: Biting rate and cycle of *An. gambiae s.l.* collected using HLC in Sakassou**

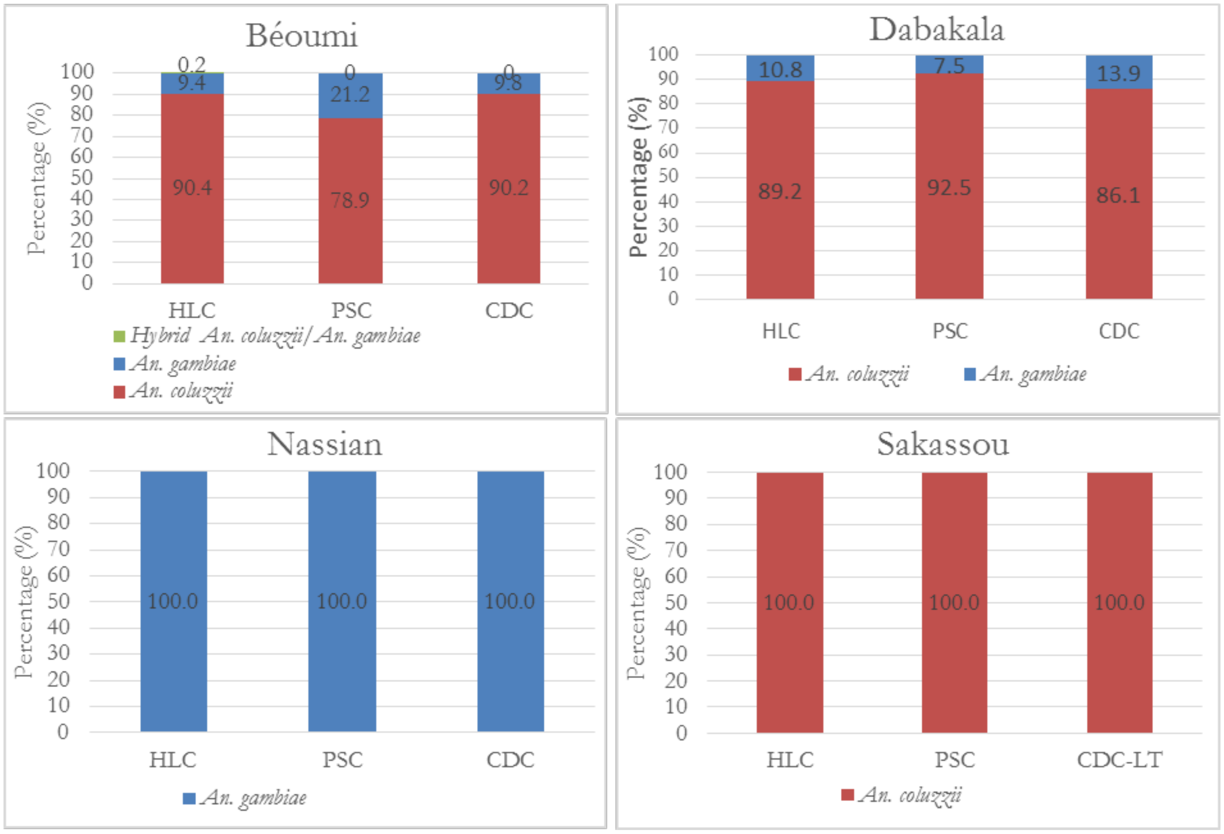



## 3.1.3 Molecular Species Identification of adults *An. gambiae* s.l. Collected through Bionomics monitoring

Subsamples of *An. gambiae* s.l. were randomly analyzed for species identification using PCR. A total of 694 *An. gambiae* s.l. from Béoumi were molecularly identified for the identification of the species of the *An. gambiae* complex (488 from HLC, 104 from PSC, and 102 from CDC-light trap). The numbers were 698 from Dabakala (490 from HLC, 107 from PSC and 101 from CDC-light trap), 608 from Nassian (483 from HLC, 101 from PSC and 24 from CDC light trap), and 731 from Sakassou (521 from HLC, 106 from PSC, and 104 from CDC-light trap).

*An. coluzzii* represented the predominant species in Béoumi and Dabakala (all collection methods included) and was the only species found in Sakassou. In Nassian, *An. gambiae* was the only vector species found using the three methods (Figure 12).

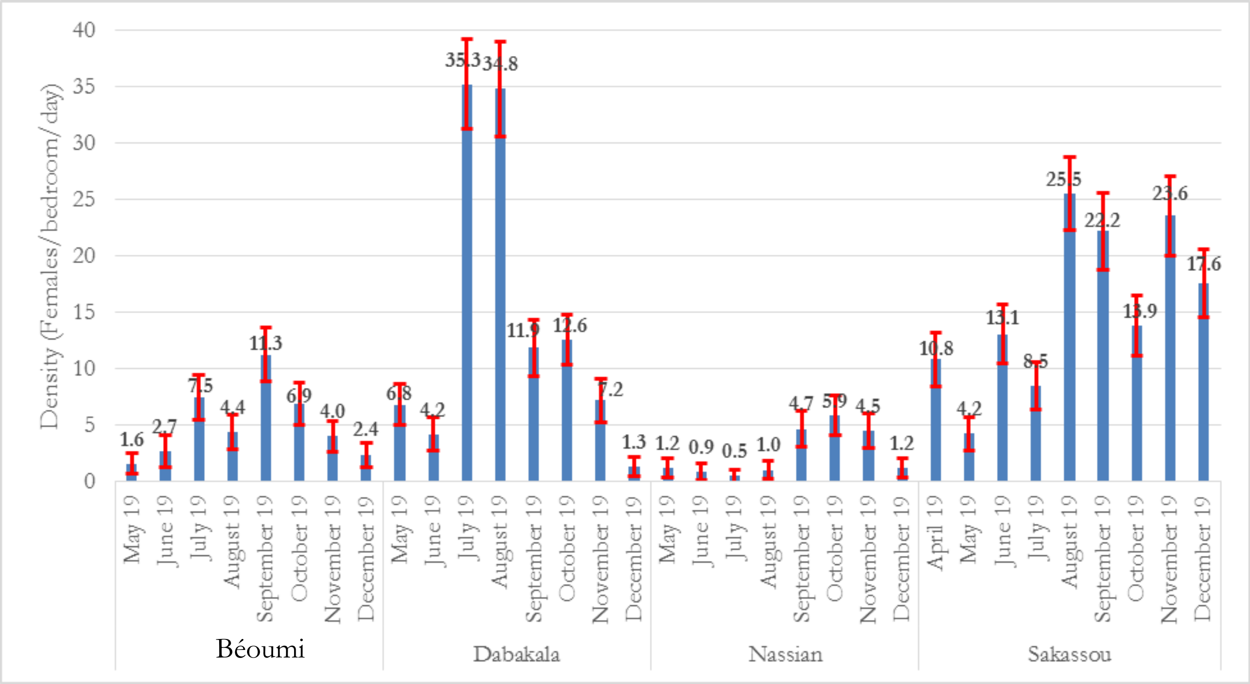
**FIGURE 12: PROPORTION OF *AN. GAMBIAE* S.L. PER COLLECTION METHODS IN THE BIONOMIC MONITORING SITES**




### 3.1.4 INDOOR RESTING DENSITY

*An gambiae* s.l. indoor resting densities, as measured by PSCs, were highest in Dabakala (1 to 35 frd), followed by Sakassou (4 to 5 frd). Indoor resting densities were between 2 and 11 frd in Béoumi and lowest in Nassian (0 to 6 frd) (Figure 13). The percentage of blood-fed *An. gambiae* s.l. collected ranged from 7 to 99% during the nine months of monitoring (Annex Table 14).

**FIGURE 13: MEAN INDOOR RESTING DENSITY OF *AN. GAMBIAE* S.L. BY MONTH AND BY SITE**

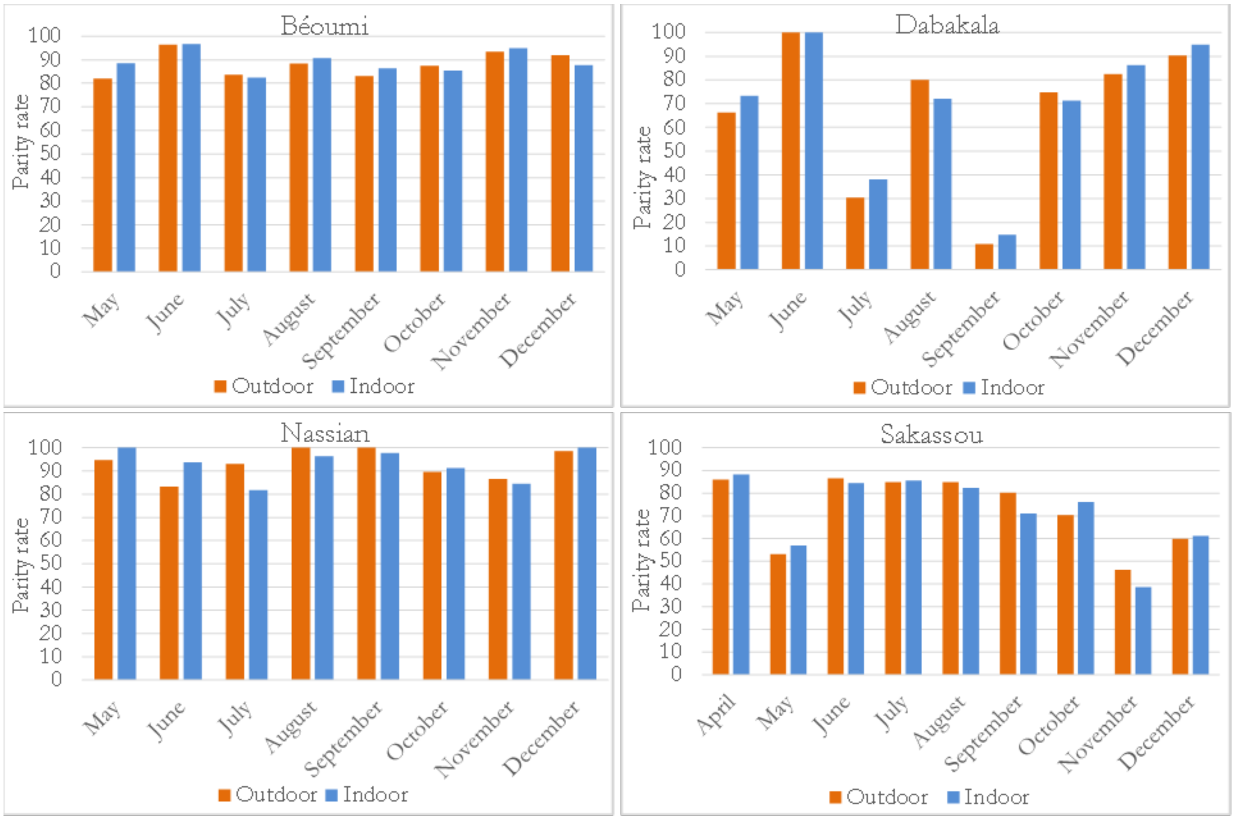



Note: Error bars represent the standard errors.

### 3.1.5 PARITY RATE

For a better control of the ovary dissection and parity rate recording, the field teams were rotated between sites every month. Additionally, subsamples of 100 mosquitoes dissected per site and per month were 100% confirmed in the laboratory at CSRS. Thus, a total of 11,275 mosquitoes collected using HLCs over the collection period were dissected to determine parity across all four sites: Béoumi (3,042), Dabakala (2,726), Nassian (1,035), and Sakassou (4,472). Out of the dissected mosquitoes, 2,679 (88.1%) were found to be parous in Béoumi, 1,781 (65.3%) in Dabakala, 957 (92.5%) in Nassian, and 3,257 (72.8%) in Sakassou (Annex Table 15). Overall, the parity rate was similar for mosquitoes collected indoors and outdoors (Figure 14 ; Annex Table 16).

**FIGURE 14: PARITY RATE OF *AN. GAMBIAE* S.L. COLLECTED USING HLC**




### 3.1.6 PLASMODIUM FALCIPARUM SPOROZOITE RATES

The proportions of *An. gambiae* s.l. mosquitoes infected by *Plasmodium falciparum* ranged from 1.7 in Béoumi to 5.48% in Nassian (Table 4). May through July were the months with the highest number of *An. gambiae* s.l. infected bites in Béoumi, Dabakala, and Sakassou, while Nassian recorded high infection throughout the semester (June–December) except in August (Annex Table 17). With 37 *An. gambiae* s.l. infected, Nassian recorded the largest number of infectious bites among the four sites. Additionally, 16 *An. funestus* s.l. mosquitoes were found with sporozoite infections among the samples collected in November and December 2019, representing 32.6% of infected mosquitoes in Nassian.

### TABLE 4: VECTOR SPOROZOITE INFECTION RATE IN THE FOUR VECTOR BIONOMICS SITES

| | *An. gambiae* s.l. | | | | *An. funestus* s.l. | | | |
|---|---|---|---|---|---|---|---|---|
| **District** | **TC** | **TA** | **P** | **SR** | **TC** | **TA** | **P** | **SR** |
| Béoumi | 7,930 | 900 | 9 | **0.010** | 7 | 0 | | |
| Dabakala | 14,864 | 868 | 16 | **0.018** | 998 | 0 | | |
| Nassian | 3,968 | 675 | 37 | **0.055** | 368 | 215 | 16 | **0.074** |
| Sakassou | 52,484 | 900 | 19 | **0.021** | 282 | 0 | | |

TC=total collected; TA=total analyzed; P=positive; SR=sporozoite rate.

## 3.1.7 ENTOMOLOGICAL INOCULATION RATE

Table 5 shows the overall EIRs for *An. gambiae* s.l. in the four districts recorded between April and December 2019 in Sakassou and from May through December 2019 in Béoumi, Dabakala, and Nassian. Specific average EIR of 0.61 infective bpn for *An. funestus* s.l. was reported for the collection of November and December in Nassian. Sakassou was still recording the highest EIR (3.59 infective bpn) of *An. gambiae* s.l., while Nassian and Dabakala had similar EIRs of 0.73 and 0.75 infective bpn. Béoumi recorded the lowest EIR among the four sites, with 0.24 infective bpn. This will be an important comparison factor for Dabakala and Nassian, as Dabakala represents the control site for IRS in Nassian (Annex Table 17).

### TABLE 5: EIR OF MALARIA VECTORS COLLECTED USING HLC IN THE FOUR VECTOR BIONOMICS SITES

| **District** | **Sporozoite Rate** | **HBR (bpn)** | **EIR (infective bpn)** |
|---|---|---|---|
| *An. gambiae* s.l. | | | |
| Béoumi | 0.010 | 24.26 | 0.24 |
| Dabakala | 0.018 | 40.71 | 0.75 |
| Nassian | 0.055 | 13.33 | 0.73 |
| Sakassou | 0.021 | 170.04 | 3.59 |
| *An. funestus* s.l. | | | |
| Nassian | 0.074 | 8.19 | 0.61 |

## 3.1.8 HUMAN BLOOD INDEX

Table 6 describes the human blood index of *An. gambiae* s.l. from the four sites. A total of 801 blood fed female mosquitoes collected throughout the period using PSC method were analyzed from all four sites, and 72.7% had evidence of a human blood source. In Béoumi, 60.2% (n=121) of *An. gambiae* s.l. were blood fed from humans between May and December 2019. In Dabakala and Nassian, 73% and 72.5% were fed from a human source during the same time period. The highest blood index was observed in Sakassou, with 85% (n=170) of tested mosquitoes having fed on human blood. The results also showed that there the mosquitoes were feeding from other sources of blood (cattle: 2.1%; sheep: 1.4%, goats: 1.4%; and chickens: 0.6%; Annex Table 18)

**TABLE 6: HUMAN BLOOD INDEX OF *AN. GAMBIAE* S.L. BY SITE**

| District | Human Blood | | |
|---|---|---|---|
| | Total Tested | Human Blood Positive | Human Blood Index (%) |
| Béoumi | 201 | 121 | **60.2** |
| Dabakala | 200 | 146 | **73.0** |
| Nassian | 200 | 145 | **72.5** |
| Sakassou | 200 | 170 | **85.0** |
| Total | 801 | 582 | **72.7** |

## 3.2 INSECTICIDE RESISTANCE MONITORING

Sixteen sites were planned to be tested for insecticide susceptibility within the 2019 PMI VectorLink work plan. Jacqueville could not be tested because not enough mosquito larvae could be collected and attempts to collect adult mosquitoes for an F1 generation have not been successful.

Table 7 shows the resistance status to the different insecticides tested against *An. gambiae* s.l. collected from the 15 different sites. All insecticides were tested in all sites.

Resistance was observed to the diagnostic dose of all pyrethroids and bendiocarb in all sites surveyed

Susceptibility to pirimiphos-methyl was recorded at the diagnostic dose in 10 sites, while resistance was observed in the 5 others, but at a low intensity.

The intensity of resistance was high in all sites for all the three pyrethroids (deltamethrin, permethrin, and alpha-cypermethrin), except in Nassian and Odienné, where permethrin resistance was moderate.

The pre-exposure of mosquitoes to PBO before deltamethrin, permethrin, and alphacypermethrin did not reverse the resistance status of the *An. gambiae* s.l. populations, but yielded partial increment of the mortality in all of the sites surveyed. Deltamethrin showed the highest increase in mortality among the pyrethroids when combined with PBO in all the sites (Table 7).

## TABLE 7: INSECTICIDE SUSCEPTIBILITY TEST RESULTS IN 15 SITES

| Insecticide Tested | Abengourou | Aboisso | Adzopé | Béoumi | Bettié | Bouaké | Bouna | Daloa |
|---|---|---|---|---|---|---|---|---|
| | **Number Tested (% mortality)** | **Number Tested (% mortality)** | **Number Tested (% mortality)** | **Number Tested (% mortality)** | **Number Tested (% mortality)** | **Number Tested (% mortality)** | **Number Tested (% mortality)** | **Number Tested (% mortality)** |
| Deltamethrin (0.05%) 1x | 90 (0) | 84 (6) | 91 (0) | 76 (6.6) | 97 (10.3) | 100 (0) | 86 (9.3) | 95 (1.1) |
| PBO + deltamethrin (0.05) | 89 (14.6) | 84 (56) | 91 (49.5) | 73 (80.8) | 94 (44.7) | 95 (57.9) | 95 (71.6) | 85 (10.6) |
| Deltamethrin (0.25%) 5x | 104 (27.9) | 84 (11.9) | 91 (53.1) | 89 (75.3) | 99 (31.3) | 62 (38.7) | 85 (18.8) | 93 (22.6) |
| Deltamethrin (0.5%) 10x | 99 (45.5) | 80 (57.5) | 91 (68.1) | 73 (79.5) | 107 (61.3) | 97 (49.5) | 85 (51.8) | 89 (33.7) |
| Permethrin (0.75%) 1x | 94 (1.1) | 84 (0) | 96 (2.1) | 83 (0) | 98 (1) | 95 (2.1) | 96 (7.3) | 91 (1.1) |
| PBO + permethrin (0.75%) | 89 (4.5) | 84 (10.7) | 95 (14.7) | 77 (6.5) | 100 (8) | 93 (19.4) | 101 (53.5) | 93 (11.8) |
| Permethrin (3.75%) 5x | 109 (42.2) | 95 (17.9) | 92 (27.2) | 84 (63.1) | 98 (67.3) | 87 (37.9) | 90 (60) | 95 (11.6) |
| Permethrin (7.5%) 10x | 97 (62.9) | 97 (64.9) | 95 (60) | 74 (95.9) | 100 (80)) | 101 (57.4) | 90 (88.9) | 86 (40.7) |
| Alpha-cypermethrin (0.05) 1x | 90 (0) | 81 (0) | 91 (4.4) | 88 (4.5) | 105 (1.9) | 106 (4.7) | 93 (2.2) | 93 (4.3) |
| PBO + alpha-cypermethrin (0.05) | 77 (3.9) | 84 (83.3) | 97 (26.8) | 83 (53) | 105 (73.5) | 103 (44.7) | 96 (21.9) | 93 (9.7) |
| Alpha-cypermethrin (0.25) 5x | 93 (18.3) | 80 (16.3) | 86 (10.5) | 80 (46.3) | 100 (15) | 88 (14.8) | 93 (11.8) | 95 (9.5) |
| Alpha-cypermethrin (0.5) 10x | 100 (43) | 80 (21.3) | 97 (21.6) | 75 (77.3) | 95 (48.4) | 101 (42.6) | 96 (16.7) | 91 (23.1) |
| Lambdacyhalothrin (0.05%) 1x | 103 (0) | x | x | 84 (54.8) | 100 (99) | 78 (0) | 89 (2.2) | 91 (38.5) |
| Bendiocarb (0.1%) 1x | 95 (50.5) | 98 (1) | 89 (53.9) | x | 88 (60.2) | 78 (11.5) | 98 (50) | 96 (1) |
| Bendiocarb (1%) 10x | 94 (77.7) | x | x | x | x | 91 (37.4) | 100 (64) | x |
| Pirimiphos-methyl (0.25%) 1x | 105 (100) | 97 (73.2) | 98 (90.8) | 86 (100) | 101 (25.7) | 84 (100) | 98 (100) | 97 (34) |
| Pirimiphos-methyl (1.25%) 5x | x | 95 (100) | 99 (100) | x | 97 (100) | x | x | 92 (100) |

X *represents the tests that were not completed, either because not enough mosquitoes were collected or because these mosquitoes were not needed for the intensity assay.*

*Resistance confirmed* *Suspected resistance* *Susceptible*

| Insecticide tested | Dabakala | Gagnoa | Nassian | Odienné | Sakassou | San Pedro | Yamous soukro |
|---|---|---|---|---|---|---|---|
| | **Number Tested (% mortality)** | **Number Tested (% mortality)** | **Number Tested (% mortality)** | **Number Tested (% mortality)** | **Number Tested (% mortality)** | **Number Tested (% mortality)** | **Number Tested (% mortality)** |
| Deltamethrin (0.05%) 1x | 97 (5.2) | 87 (4.6) | 81 (2.5) | 96 (7.3) | 101 (5) | 91 (4.4) | 183 (3.8) |
| PBO + deltamethrin (0.05) | 94 (45.7) | 99 (47.5) | 65 (40) | 96 (70.8) | 90 (30) | 89 (21.3) | 93 (73.1) |
| Deltamethrin (0.25%) 5x | 107 (10.3) | 92 (30.4) | 79 (73.4) | 104 (38.5) | 98 (17.3) | 100 (14) | 187 (26.7) |
| Deltamethrin (0.5%) 10x | 97 (27.8) | 102 (50) | 58 (91.4) | 107 (61.7) | 102 (62.7) | 101 (46.5) | 209 (61.2) |
| Permethrin (0.75%) 1x | 100 (4) | 95 (6.3) | 101 (5) | 98 (2) | 99 (5.1) | 94 (4.3) | 179 (4.5) |
| PBO + permethrin (0.75%) | 100 (11) | 94 (46.8) | 92 (47.8) | 100 (20) | 84 (54.8) | 91 (26.4) | 97 (51.5) |
| Permethrin (3.75%) 5x | 47 (42.6) | 98 (36.7) | 76 (94.7) | 96 (70.8) | 101 (87.1) | 92 (45.7) | 151 (35.1) |
| Permethrin (7.5%) 10x | 43 (76.7) | 100 (85) | 87 (98.9) | 101 (100) | 97 (87.) | 91 (76.9) | 179 (78.2) |
| Alpha-cypermethrin (0.05) 1x | 98 (4.1) | 95 (8.4) | 86 (11.6) | 91 (1.1) | 693 (0) | 100 (3) | 100 (0) |
| PBO + alpha-cypermethrin (0.05) | 95 (9.5) | 91 (19.8) | 63 (31.7) | 101 (49.5) | 88 (30.7) | 99 (17.2) | 92 (25) |
| Alpha-cypermethrin (0.25) 5x | 44 (25) | 96 (15.6) | 71 (42.3) | 103 (11.7) | 99 (23.2) | 83 (8.4) | 93 (5.4) |
| Alpha-cypermethrin (0.5) 10x | 48 (45.8) | 98 (39.8) | 107 (56.1) | 101 (80.2) | 93 (28) | 99 (35.4) | 96 (13.5) |
| Lambdacyhalothrin (0.05%) 1x | 95 (2.1) | 96 (5.2) | 93 (2.2) | 104 (1.9) | 97 (9.3) | x | x |
| Bendiocarb (0.1%) 1x | 95 (31.6) | 93 (15.1) | 95 (35.8) | 106 (51.9) | 23 (0) | 82 (32.9) | 175 (7.4) |
| Bendiocarb (1%) 10x | x | 97 (78.4) | 90 (57.8) | 97 (78.4) | x | 98 (90.8) | x |
| Pirimiphos-methyl (0.25%) 1x | 98 (100) | 89 (100) | 110 (98.2) | 98 (100) | 85 (51.8) | 101 (100) | 85 (97.6) |
| Pirimiphos-methyl (1.25%) 5x | x | x | x | x | 88 (100) | x | x |

X *represents the tests that were not completed, either because not enough mosquitoes were collected or because these mosquitoes were not needed for the intensity assay.*

*Resistance confirmed*   *Suspected resistance*   *Susceptible*

The results of the CDC bottle assays using chlorfenapyr and WHO susceptibility test using clothianidin are shown in Figures 15–17.

Resistance to chlorfenapyr was observed at the dose of 100 µg/bottle after observing mortality for 72 hours in all sites except Bettie. The lowest mortality rate was recorded in Sakassou using the 100 µg/bottle. Susceptibility to chlorfenapyr at 200µg/bottle was recorded in 9 of the 15 sites surveyed. Béoumi, Bouaké, Gagnoa, Nassian, Sakassou, and San Pedro showed resistance at the dose of 200µg/bottle.

For clothianidin, full susceptibility was observed in seven out of 15 sites. Resistance was observed in Abengourou, Aboisso, Bettié, Bouaké, Bouna, Daloa, Odienné, and Yamoussoukro.

In Figures 15–17, the horizontal dashed red line represents the 90% threshold for resistance, and the green line represents the 98% threshold for susceptibility

**FIGURE 15: SUSCEPTIBILITY OF *AN. GAMBIAE* S.L. TO CHLORFENAPYR 100µG/BOTTLE BY SITE**

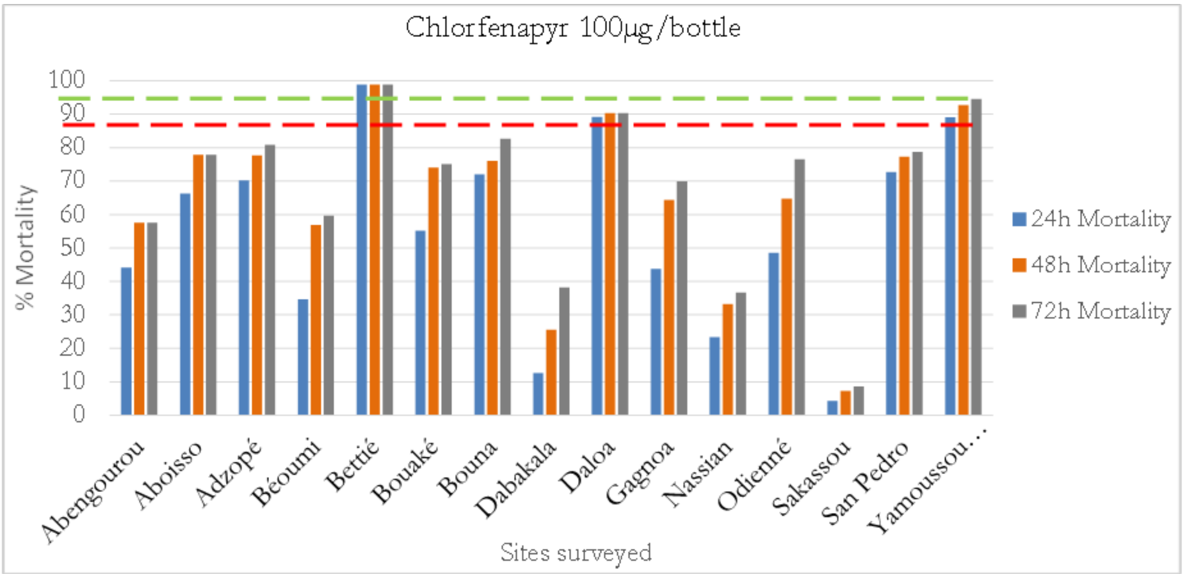


**FIGURE 16: SUSCEPTIBILITY OF *AN. GAMBIAE* S.L. TO CHLORFENAPYR 200µG/BOTTLE BY SITE**

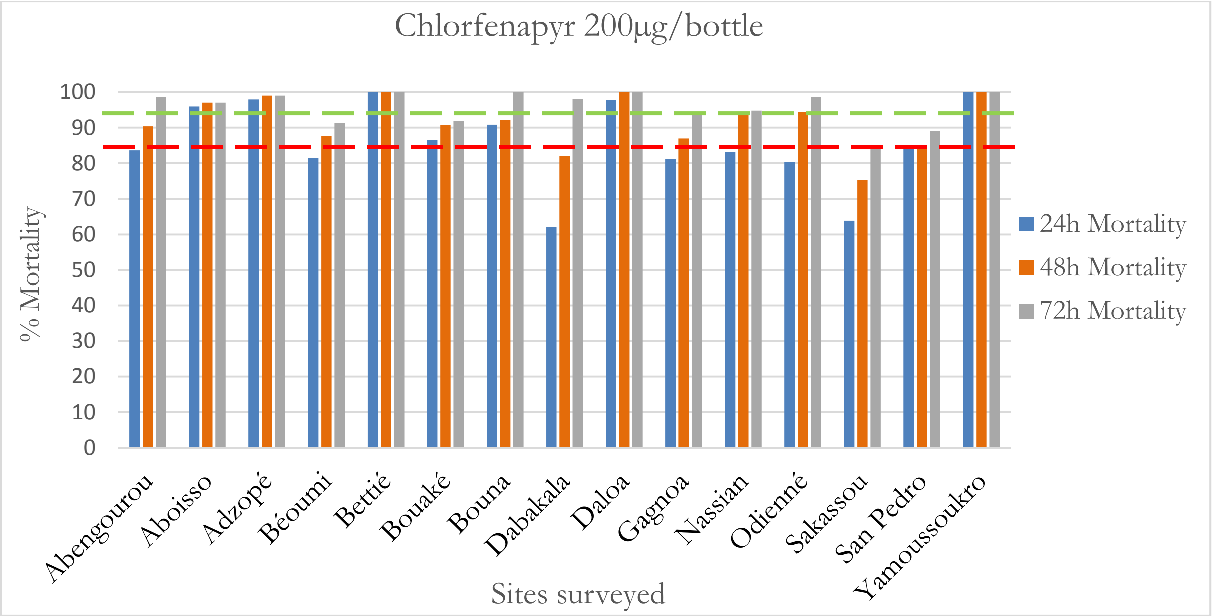
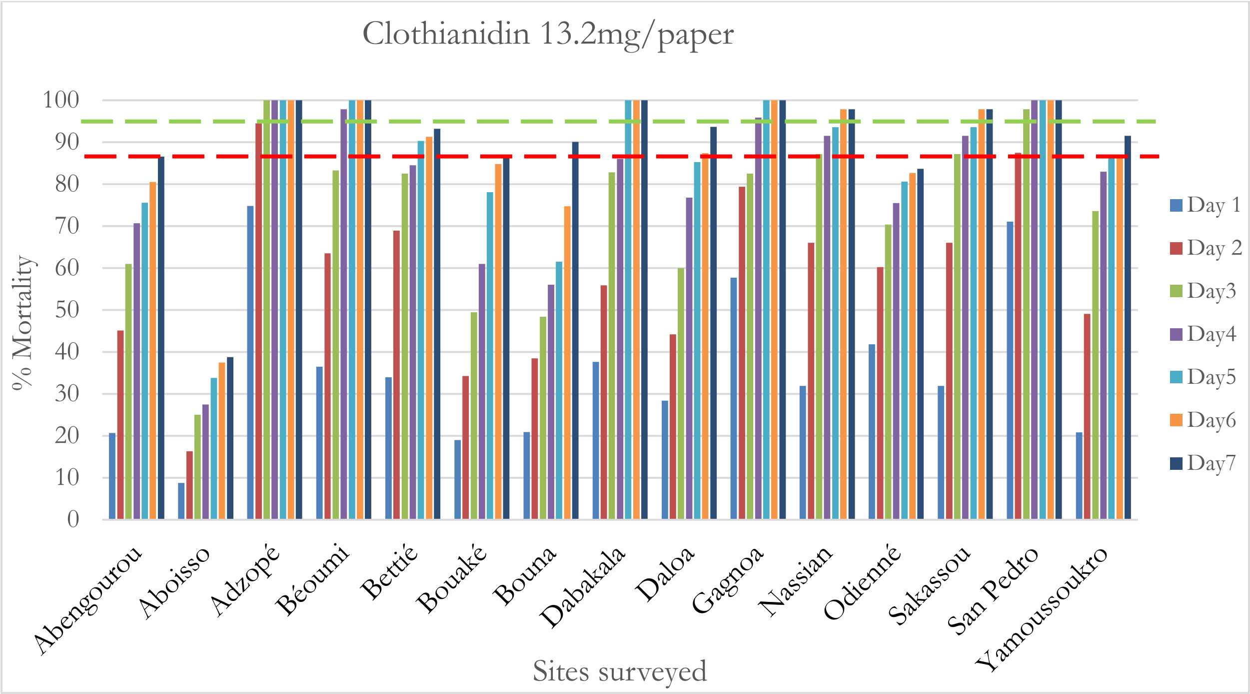



**FIGURE 17: SUSCEPTIBILITY OF *AN. GAMBIAE* S.L. TO CLOTHIANIDIN BY SITE**

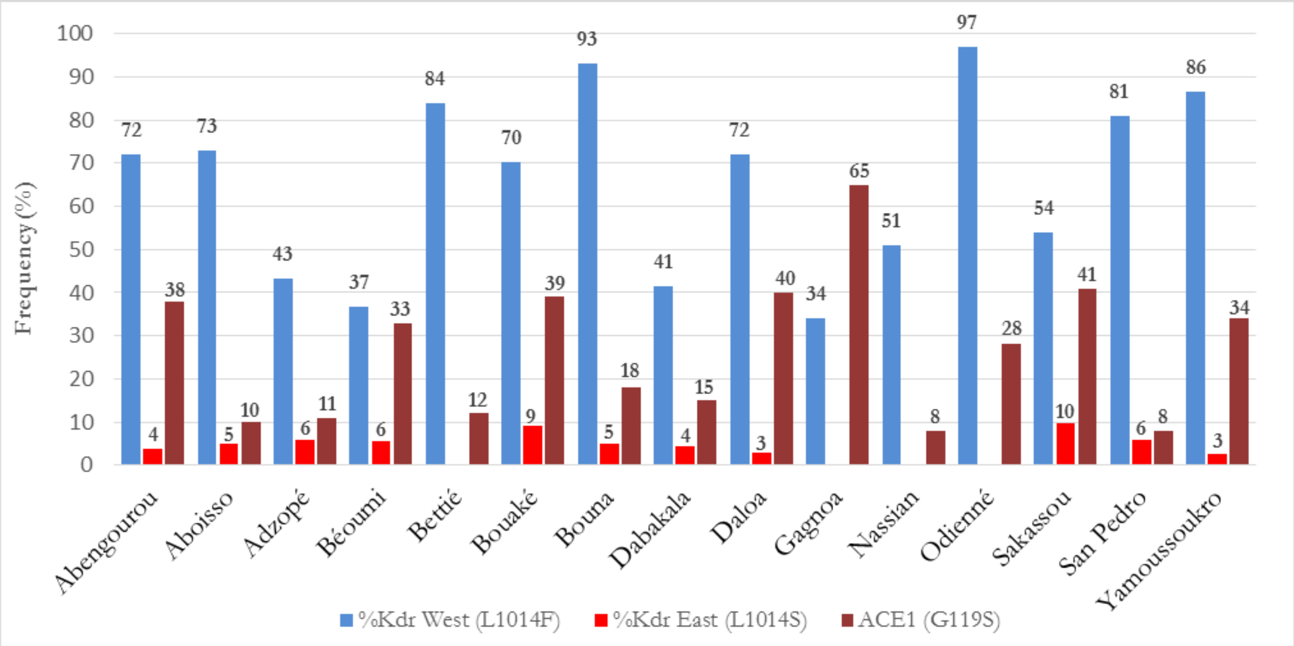


## 3.3 MOLECULAR MARKERS OF RESISTANCE

Figure 18 shows the distribution of *kdr* (West and East) and *ace-1* for *An. gambiae sl.* from the 15 sites. Overall, *kdr* -West mutations were highly present (ranged from 36.8 to 97%) in all study sites while the *kdr*-East mutation was found at low frequency in 11 sites but not in Bettié, Gagnoa, Nassian and Odienné. The highest *kdr*-East frequency was recorded in Sakassou and Bouaké (9.8% and 9.3%). In other locations, *kdr* -East was present at frequencies that ranged between 2.5 and 5.9%. The presence of both *kdr* mutations in the country is consistent with the high phenotypic resistance observed to pyrethroid insecticides, and represents a real threat to pyrethroid-based vector control tools. About 7.6% (59/778) of the mosquitoes tested across all sites were carrying both mutations.

As with *kdr*, the *ace-1* mutation was found in all sites, and the frequencies ranged from 8% in San Pedro to 65% in Gagnoa, confirming the resistance observed in all sites to bendiocarb and in some sites to pirimiphos-methyl.

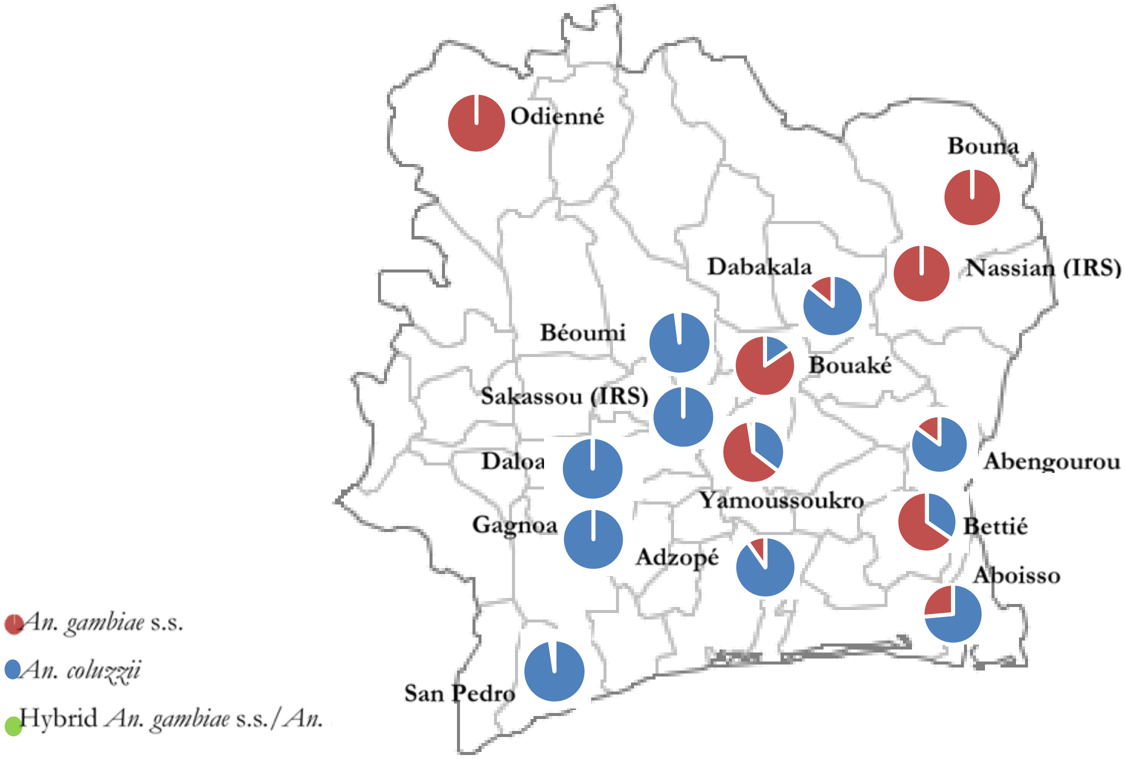
**FIGURE 18: DISTRIBUTION OF TARGET SITE MUTATIONS IN *AN. GAMBIAE* S.L IN ALL SITES TESTED FOR INSECTICIDE RESISTANCE IN CÔTE D'IVOIRE**




### 3.3.3 MOLECULAR SPECIES IDENTIFICATION OF ADULT *AN. GAMBIAE* S.L. TESTED FOR SUSCEPTIBILITY

Figure 19 shows the species composition of *An. gambiae* s.l. used for susceptibility testing per site. *An. coluzzii* represented the predominant species in Abengourou, Abidjan, Aboisso, Adzopé, Béoumi, Daloa, Dabakala, Sakassou, and San Pedro. One hundred percent of the population in Daloa, Gagnoa, and Sakassou were *An. Coluzzi,* while the population of Bouna, Nassian, and Odienné were entirely *An. gambiae* s.s. The populations from Bettié, Bouaké, and Yamoussoukro were dominated by *An. gambiae* s.s. A few hybrids of the two species were characterized in San Pedro (1.2%) and Yamoussoukro (2.5%).

**FIGURE 19: PROPORTION OF *AN. GAMBIAE* S.L. USED FOR SUSCEPTIBILITY TEST ACROSS THE 15 DISTRICTS**



# 4. CONCLUSION

*An. gambiae* s.l. represented the predominant malaria vector collected in all the four longitudinal monitoring sites during the nine months of collection. *Anopheles coluzzii* and *An. gambiae* s.s. were the two species of the complex recorded in all sites. *An. coluzzii* was predominant in Béoumi, Dabakala, and Sakassou. *An. gambiae* s.s. represented the main vector only in Nassian

The molecular species identification of the mosquitoes used for susceptibility testing showed the predominance of *An. coluzzii* in 9 of the 15 sites. *An. gambiae* s.s. was the predomineant species in five sites, mainly among the samples collected in the north of the country.

The presence of different species in Nassian (*An. gambiae* s.s.) and Sakassou (*An. coluzzii*) is important, because IRS will be implemented in both districts in 2020. That will help understand any behavior changes that could occur within each species population in the future after IRS. The different site's vector species will also support the assessment of the impact of the IRS on entomological indicators.

Sakassou recorded the highest density of malaria vectors collected (82.7%). The average HBR of *An. gambiae* s.l. in Sakassou ranged from 97.5 to 231 bpn; the next highest HBR was in Dabakala (5 to 74 bpn). This trend, combined with it having the highest reported malaria incidence in the country, makes Sakassou the top candidate for IRS in Côte d'Ivoire. April through August represents the long rainy season in the Southern and Central part of the country where Sakassou is located. Therefore, the higher number of mosquito vectors collected could support conducting IRS in April. Furthermore, Nassian recorded the highest number of infected vectors, validating the selection of Nassian as the second IRS targeted site. Similar EIRs were observed in Dabakala and Nassian, and only Nassian will receive IRS in 2020, so Dabakala will serve as a good control against which to measure the entomological impact of IRS.

The overall parity rate was high across all four sites, and ranged from 65.3% to 92.5%, increasing the probability of mosquitoes carrying *Plasmodium falciparum* and transmitting malaria. The longevity of the mosquitoes before and after IRS is an important indicator of IRS impact as well.

*An. gambiae* s.l. was endophilic in Dabakala (indoor densities 1–35 frd) and Sakassou (4-–5 frd), and mainly exophilic in Béoumi and Nassian. Continued longitudinal monitoring in all the sites after IRS will help detect any biting behavior changes.

High pyrethroid and carbamate resistance was observed in all 15 sites monitored for susceptibility of *An. gambiae* s.l., emphasizing the potentially limited efficacy of pyrethroid-only treated tools for vector control that need to be reoriented appropriately for more efficiency in controling malaria.

Pre-exposure to PBO before exposure to deltamethrin, permethrin, and alpha-cypermethrin yielded partial but substantial increases in the mortality of the *An. gambiae* s.l. population in all sites, particularly with deltamethrin. This shows that enzymes such as P450s might be involved in the insecticide resistance, but further molecular analysis may be required to confirm the extent of their involvement.. These additional investigations could also support the NMCP in selecting areas that could be considered for PBO ITN distribution.

Susceptibility to the diagnostic dose of pirimiphos-methyl was observed in 10 sites, while the intensity of the resistance was low in the 5 remaining sites. If IRS is extended to new districts, the current data could guide insecticide selection, with a possible rotation of pirimiphos-methyl- and clothianidin-based products to mitigate further development of insecticide resistance.

Susceptibility to clothianidin was observed in seven sites seven days after exposure, including the two sites targeted for IRS in 2020.

Chlorfenapyr at the dose of 200µg/bottle yielded full susceptibility in 10 of the sites, while only Bettié yielded full susceptibility at the lower dose of 100µg/bottle. As the insecticide is still under assessment for diagnostic concentration, the "resistance" observed already in few sites in the country should calls for continuous monitoring to delimitate its extent. Furthermore, the data gathered on chlorfenapyr across several will help the WHO prequalification team determine the appropriate diagnostic dose for this insecticide. Morever, vector control tools incorporating chlorfenapyr, such as Interceptor G2 nets, could be recommended in a stratified distribution of ITNs for malaria vector control and resistance management in Côte d'Ivoire.

# Annex

#### Table 8: Species Composition Of Mosquitoes Collected in Béoumi using the Three Collection Methods

| BÉOUMI | Species | May 2019 | June 2019 | July 2019 | August 2019 | September 2019 | October 2019 | November 2019 | December 2019 | Total |
|---|---|---|---|---|---|---|---|---|---|---|
| HLC | *An. gambiae* s.l. | 234 (69) | 162 (98.2) | 517 (77.39) | 481 (92) | 2,326 (90.4) | 1,612 (78.7) | 535 (66.9) | 235 (47.6) | 6,102 (80.17) |
| | *An. funestus* s.l. | | 1 (0.6) | | | | | | 6 (1.2) | 7 (1.1) |
| | *An. nili* | | | 1 (0.15) | | | | | | 1 (0.01) |
| | Other *Anopheles* | 10 (2.95) | 2 (1.2) | 43 (6.44) | 9 (1.7) | 150 (5.8) | 323 (15.8) | 167 (20.9) | 79 (16.0) | 783 (10.29) |
| | Culicine | 95 (28.02) | | 107 (16.02) | 33 (6.3) | 97 (3.8) | 114 (5.6) | 98 (12.3) | 174 (35.2) | 718 (9.43) |
| | **Total** | **339** | **165** | **668** | **523** | **2573** | **2049** | **800** | **494** | **7611** |
| PSC | *An. gambiae* s.l. | 43 (75.4) | 81 (41.5) | 225 (75.0) | 130 (72.6) | 338 (79.0) | 201 (97.6) | 101 (84.2) | 64 (88.9) | 1,183 (76) |
| | Other *Anopheles* | 4 (7) | | | | | 5 (2.4) | 19 (15.8) | 8 (11.1) | 36 (2.3) |
| | Culicine | 10 (17.5) | 114 (58.5) | 75 (25.0) | 49 (27.4) | 90 (21.0) | | | | 338 (21.7) |
| | **Total** | **57** | **195** | **300** | **179** | **428** | **206** | **120** | **72** | **1557** |
| CDC light trap | *An. gambiae* s.l. | 15 (12.8) | 3 (11.5) | 89 (60.1) | 27 (36.5) | 360 (90.2) | 72 (50.3) | 38 (76.0) | 41 (34.7) | 645 (60) |
| | Culicine | 102 (87.2) | 23 (88.5) | 59 (39.9) | 47 (63.5) | 39 (9.8) | 71 (49.7) | 12 (24.0) | 77 (65.3) | 430 (40) |
| | **Total** | **117 (100)** | **26 (100)** | **148 (100)** | **74 (100)** | **399 (100)** | **143 (100)** | **50 (100)** | **118 (100)** | **1,075 (100)** |

## Table 9: Species Composition of Mosquitoes Collected in Dabakala using the Three Collection Methods

| DABAKALA | Species | May 2019 | June 2019 | July 2019 | August 2019 | September 2019 | October 2019 | November 2019 | December 2019 | Total |
|---|---|---|---|---|---|---|---|---|---|---|
| HLC | *An. gambiae* s.l. | 716 (61.8) | 512 (64.7) | 2,233 (86.7) | 2,010 (87.3) | 2,374 (87.0) | 1,859 (90.7) | 781 (97.6) | 158 (32.0) | 10,643 (82) |
| | *An. funestus* s.l. | 3 (0.3) | 3 (0.4) | 24 (0.9) | 72 (3.1) | 140 (5.1) | 12 (0.6) | 18 (2.3) | 9 (1.8) | 281 (2.2) |
| | *An. nili* | 12 (1.0) | 50 (6.3) | 28 (1.1) | 1 (0.0) | | 304 (14.8) | 27 (3.4) | 4 (0.8) | 426 (3.3) |
| | Other *Anopheles* | 4 (0.3) | 3 (0.4) | 23 (0.9) | 36 (1.6) | 38 (1.4) | 21 (1.0) | 9 (1.1) | 1 (0.2) | 135 (1) |
| | Culicine | 423 (36.5) | 223 (28.2) | 268 (10.4) | 183 (7.9) | 177 (6.5) | 102 (5.0) | 38 (4.8) | 85 (17.2) | 1499 (11.5) |
| | **Total** | **1,158** | **479** | **2,576** | **2,302** | **2,729** | **2,298** | **873** | **257** | **12,984** |
| PSC | *An. gambiae* s.l. | 191 (62.8) | 125 (57.6) | 543 (47.9) | 679 (61.6) | 356 (96.7) | 286 (75.9) | 175 (81.0) | 26 (66.7) | 2381 (63.4) |
| | *An. funestus s.l.* | | | 310 (27.3) | 360 (32.7) | | | 34 (15.7) | 13 (33.3) | 717 (19.1) |
| | *An. nili* | | | 36 (3.2) | | | 91 (24.1) | 7 (3.2) | | 134 (3.6) |
| | Other *Anopheles* | | | 169 (14.9) | 6 (0.5) | | | | | 175 (4.7) |
| | Culicine | 113 (37.2) | 92 (42.4) | 76 (6.7) | 57 (5.2) | 12 (3.3) | | | | 350 (9.3) |
| | **Total** | **304** | **217** | **1,134** | **1,102** | **368** | **377** | **216** | **39** | **3,757** |
| CDC light trap | *An. gambiae* s.l. | 27 (46.6) | 12 (27.3) | 599 (82.8) | 829 (74.9) | 107 (74.4) | 213 (89.1) | 40 (87.0) | 13 (43.3) | 1840 (76.7) |
| | Culicine | 31 (53.4) | 32 (72.7) | 124 (17.2) | 278 (25.1) | 45 (29.6) | 26 (10.9) | 6 (13.0) | 17 (56.7) | 559 (23.3) |
| | **Total** | **58 (100)** | **44 (100)** | **723 (100)** | **1,107 (100)** | **152 (100)** | **239 (100)** | **46 (100)** | **30 (100)** | **2,399 (100)** |

## TABLE 10: SPECIES COMPOSITION OF MOSQUITOES COLLECTED IN NASSIAN USING THE THREE COLLECTION METHODS

| NASSIAN | Species | May 2019 | June 2019 | July 2019 | August 2019 | September 2019 | October 2019 | November 2019 | December 2019 | Total |
|---|---|---|---|---|---|---|---|---|---|---|
| HLC | *An. gambiae* s.l. | 26 (41.3) | 34 (36.6) | 63 (67.7) | 42 (43.3) | 1,330 (95.9) | 1,612 (78.7) | 112 (14.0) | 189 (38.3) | 3,408 (83.6) |
| | *An. funestus* s.l. | | | | 5 (5.2) | 3 (0.2) | | 203 (25.4) | 59 (11.9) | 270 (6.6) |
| | Other *Anopheles* | | | | 5 (5.2) | | 2 (0.1) | | | 7 (0.2) |
| | Culicine | 37 (58.7) | 59 (63.4) | 30 (32.3) | 45 (46.4) | 54 (3.9) | 86 (4.2) | 34 (4.3) | 45 (9.1) | 390 (9.6) |
| | **Total** | **63** | **93** | **93** | **97** | **1,387** | **1,700** | **349** | **293** | **4,075** |
| PSC | *An. gambiae* s.l. | 37 (44.6) | 27 (67.5) | 15 (36.6) | 31 (73.8) | 140 (73.8) | 176 (100) | 58 (42.6) | 17 (45.9) | 501 (71.5) |
| | *An. funestus* s.l. | | | | | | | 78 (57.4) | 20 (54.1) | 98 (14.0) |
| | Culicine | 46 (55.4) | 13 (32.5) | 26 (63.4) | 11 (26.2) | 6 (4.1) | | | | 102 (14.6) |
| | **Total** | **83** | **40** | **41** | **42** | **146** | **176** | **136** | **37** | **701** |
| CDC light trap | *An. gambiae* s.l. | 0 | 3 (3.7) | 0 (0) | 5 (19.2) | 3 (42.9) | 22 (81.5) | 25 (89.3) | 1 (20.0) | 59 (30.1) |
| | Culicine | 3 (100) | 78 (96.3) | 19 (100) | 21 (80.8) | 4 (57.1) | 5 (18.5) | 3 (10.7) | 4 (80.0) | 137 (69.9) |
| | **Total** | **3 (100)** | **81 (100)** | **19 (100)** | **26 (100)** | **7 (100)** | **27 (100)** | **28 (100)** | **5 (100)** | **196 (100)** |

## TABLE11: SPECIES COMPOSITION OF MOSQUITOES COLLECTED IN SAKASSOU USING THE THREE COLLECTION METHODS

| SAKASSOU | Species | Apr 2019 | May 2019 | June 2019 | July 2019 | August 2019 | September 2019 | October 2019 | November 2019 | December 2019 | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|
| HLC | *An. gambiae* s.l. | 2959 (97.7) | 3,121 (96.5) | 3,162 (87.8) | 4,486 (84.6) | 5,761 (90.1) | 6,863 (92.2) | 7,384 (94.6) | 6,916 (96.2) | 5,830 (94.1) | 46,482 (92.3) |
| | *An. funestus* s.l. | | | 24 (0.7) | 8 (0.2) | 6 (0.1) | 1 (0.0) | 74 (0.9) | 15 (0.2) | 31 (0.5) | 159 (0.3) |
| | *An. nili* | | | 5 (0.1) | 4 (0.1) | 3 (0.1) | 1 (0.0) | | | | 13 (0.03) |
| | Other *Anopheles* | 100 (3.1) | 114 (3.5) | 153 (4.2) | 403 (7.6) | 192 (3) | 236 (3.2) | 180 (2.3) | 71 (1.0) | 103 (1.7) | 1,552 (3.1) |
| | Culicine | 133 (4.2) | | 259 (7.2) | 400 (7.5) | 430 (6.7) | 344 (4.6) | 166 (2.1) | 184 (2.6) | 234 (3.8) | 2,150 (4.3) |
| | **Total** | **3,192** | **3,235** | **3,603** | **5,301** | **6392** | **7,445** | **7,804** | **7,186** | **61,98** | **50,356** |
| PSC | *An. gambiae* s.l. | 325 (94.5) | 121 (94.5) | 380 (79.5) | 243 (76.2) | 534 (57.9) | 666 (86.4) | 414 (50) | 707 (50) | 479 (45.4) | 3,869 (61.8) |
| | *An. funestus* s.l. | | | 1 (0.2) | | 74 (8.0) | | | | 48 (4.6) | 123 (2) |
| | Other *Anopheles* | | | 11 (2.3) | 12 (3.8) | 158 (17.1) | | | | | 181 (2.9) |
| | Culicine | 20 (5.5) | 7 (5.5) | 86 (18.0) | 64 (20.1) | 156 (16.9) | 105 (13.6) | 414 (50) | 707 (50) | 527 (50) | 2,086 (33.3) |
| | **Total** | **345** | **128** | **478** | **319** | **922** | **771** | **828** | **1,414** | **1,054** | **6,259** |
| CDC-light trap | *An. gambiae* s.l. | 159 (64.3) | 54 (64.5) | 206 (43.2) | 69 (45.1) | 67 (57.8) | 220 (73.8) | 796 (96.1) | 337 (92.8) | 225 (93.4) | 2133 (76.8) |
| | Culicine | 59 (35.7) | 30 (35.7) | 271 (56.8) | 84 (54.9) | 49 (42.2) | 78 (26.2) | 32 (3.9) | 26 (7.2) | 16 (6.6) | 645 (23.2) |
| | **Total** | **218 (100)** | **84 (100)** | **477 (100)** | **153 (100)** | **116 (100)** | **298 (100)** | **828 (100)** | **363 (100)** | **241 (100)** | **2778 (100)** |

### TABLE 12 : MONTHLY BITING RATE RESULTS USING HLC

| Sites | Month | Outdoor Biting Rate (bpn) | Indoor Biting Rate (bpn) | Total Biting Rate (bpn) |
|---|---|---|---|---|
| **Béoumi** | May 19 | 11.9 | 9.2 | 10.5 |
| | June 19 | 5.8 | 4.3 | 5.1 |
| | July 19 | 16.4 | 15.9 | 16.2 |
| | August 19 | 9.9 | 20.1 | 15.0 |
| | September 19 | 64.4 | 80.9 | 72.7 |
| | October 19 | 44.4 | 56.4 | 50.4 |
| | November 19 | 18.7 | 14.8 | 16.7 |
| | December 19 | 10.1 | 4.6 | 7.3 |
| **Dabakala** | May 19 | 26.6 | 18.1 | 22.4 |
| | June 19 | 7.8 | 9.9 | 8.8 |
| | July 19 | 64.6 | 75.0 | 69.8 |
| | August 19 | 54.3 | 71.4 | 62.8 |
| | September 19 | 78.9 | 69.4 | 74.2 |
| | October 19 | 57.8 | 58.4 | 58.1 |
| | November 19 | 30.6 | 18.2 | 24.4 |
| | December 19 | 6.9 | 3.0 | 4.9 |
| **Nassian** | May 19 | 1.2 | 0.4 | 0.8 |
| | June 19 | 1.1 | 1.0 | 1.1 |
| | July 19 | 1.9 | 2.1 | 2.0 |
| | August 19 | 1.1 | 1.5 | 1.3 |
| | September 19 | 38.8 | 44.3 | 41.6 |
| | October 19 | 41.7 | 59.1 | 50.4 |
| | November 19 | 2.0 | 5.0 | 3.5 |
| | December 19 | 3.9 | 7.9 | 5.9 |
| **Sakassou** | April 19 | 123.7 | 75.8 | 99.8 |
| | May 19 | 95.1 | 99.9 | 97.5 |
| | June 19 | 93.3 | 104.4 | 98.8 |
| | July 19 | 120.6 | 159.8 | 140.2 |
| | August 19 | 171.1 | 188.9 | 180.0 |
| | September 19 | 235.1 | 193.9 | 214.5 |
| | October 19 | 255.8 | 205.8 | 230.8 |
| | November 19 | 220.8 | 211.5 | 216.1 |
| | December 19 | 201.1 | 163.3 | 182.2 |

### TABLE 13: BITING BEHAVIOR OF *AN. GAMBIAE* S.L. PER SITE

| Sites | | April 19 | May 19 | June 19 | July 19 | August 19 | September 19 | October 19 | November 19 | December 19 | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Béoumi** | Out N (%) | | 121 (51.7) | 94 (57.7) | 263 (50.8) | 159 (33.1) | 1,031 (44.3) | 710 (44.0) | 299 (55.9) | 163 (67.6) | 2,840 (46.5) |
| | In N (%) | | 113 (48.7) | 69 (42.3) | 255 (49.2) | 322 (66.9) | 1,295 (55.7) | 902 (56.0) | 236 (44.1) | 78 (32.4) | 3,272 (53.5) |
| | **Total** | | **236** | **163** | **518** | **481** | **2,326** | **1,612** | **535** | **241** | **6,112** |
| **Dabakala** | Out N (%) | | 434 (59.4) | 261 (46.2) | 1,050 (46.0) | 913 (43.8) | 1,358 (54) | 1,180 (54.3) | 525 (63.6) | 117 (68.4) | 5,838 (51.4) |
| | In N (%) | | 297 (40.6) | 304 (53.8) | 1,235 (54.0) | 1,170 (56.2) | 1,156 (46) | 995 (45.7) | 301 (36.4) | 54 (31.6) | 5,512 (48.6) |
| | **Total** | | **731** | **565** | **2,285** | **2,083** | **2,514** | **2,175** | **826** | **171** | **11,350** |
| **Nassian** | Out N (%) | | 19 (73.1) | 18 (52.9) | 30 (47.6) | 20 (42.6) | 621 (46.6) | 667 (41.4) | 103 (32.7) | 74 (29.8) | 1,552 (42.2) |
| | In N (%) | | 7 (26.9) | 16 (47.1) | 33 (52.4) | 27 (57.4) | 712 (53.4) | 945 (58.6) | 212 (67.3) | 174 (70.2) | 2,126 (57.8) |
| | **Total** | | **26** | **34** | **63** | **47** | **1,333** | **1,612** | **315** | **248** | **3,678** |
| **Sakassou** | Out N (%) | 1,835 (62) | 15,22 (48.8) | 1,513 (47.4) | 1,933 (43.0) | 2,743 (47.5) | 3,763 (54.8) | 4,128 (51.1) | 3,540 (51.1) | 3,233 (55.2) | 24,210 (51.9) |
| | In N (%) | 1130 (38) | 1,599 (51.2) | 1,678 (52.6) | 2,565 (57.0) | 3,027 (52.5) | 3,102 (45.2) | 3,330 (48.9) | 3,391 (48.9) | 2,628 (44.8) | 22,450 (48.1) |
| | **Total** | **2,965** | **3,121** | **3,191** | **4,498** | **5,770** | **6,865** | **7,458** | **6,931** | **5,861** | **46,660** |

In: indoor; Out: outdoor

## TABLE 14: PERCENTAGE OF BLOOD-FED *AN. GAMBIAE* S.L. COLLECTED USING PSC

| Sites | Months | Blood-fed | Half Gravid | Gravid | Unfed | Total | % Blood-Fed |
|---|---|---|---|---|---|---|---|
| **Béoumi** | May 19 | 23 | 18 | 0 | 2 | 43 | 53 |
| | June 19 | 50 | 15 | 6 | 3 | 74 | 68 |
| | July 19 | 134 | 28 | 31 | 29 | 222 | 60 |
| | August 19 | 52 | 22 | 50 | 6 | 130 | 40 |
| | September 19 | 183 | 67 | 68 | 19 | 337 | 54 |
| | October 19 | 110 | 44 | 37 | 13 | 204 | 54 |
| | November 19 | 100 | 22 | 5 | 1 | 128 | 78 |
| | December 19 | 43 | 13 | 7 | 3 | 66 | 65 |
| | **Total** | **695** | **229** | **204** | **76** | **1,204** | **58** |
| **Dabakala** | May 19 | 77 | 52 | 62 | 14 | 205 | 38 |
| | June 19 | 21 | 48 | 53 | 3 | 125 | 17 |
| | July 19 | 402 | 13 | 38 | 95 | 548 | 73 |
| | August 19 | 347 | 6 | 149 | 167 | 669 | 52 |
| | September 19 | 167 | 58 | 43 | 77 | 345 | 48 |
| | October 19 | 182 | 68 | 11 | 26 | 287 | 63 |
| | November 19 | 58 | 61 | 49 | 7 | 175 | 33 |
| | December 19 | 5 | 6 | 13 | 3 | 27 | 19 |
| | **Total** | **1,259** | **312** | **418** | **392** | **2,381** | **53** |
| **Nassian** | May 19 | 32 | 0 | 3 | 2 | 37 | 86 |
| | June 19 | 2 | 15 | 9 | 1 | 27 | 7 |
| | July 19 | 14 | 0 | 1 | 0 | 15 | 93 |
| | August 19 | 15 | 8 | 12 | 0 | 35 | 43 |
| | September 19 | 101 | 26 | 1 | 12 | 140 | 72 |
| | October 19 | 174 | 0 | 0 | 2 | 176 | 99 |
| | November 19 | 23 | 30 | 25 | 1 | 79 | 29 |
| | December 19 | 13 | 6 | 9 | 1 | 29 | 45 |
| | **Total** | **374** | **85** | **60** | **19** | **538** | **70** |
| **Sakassou** | April 19 | 254 | 0 | 0 | 71 | 325 | 78 |
| | May 19 | 98 | 0 | 8 | 28 | 134 | 73 |
| | June 19 | 270 | 36 | 55 | 20 | 381 | 71 |
| | July 19 | 113 | 13 | 41 | 73 | 240 | 47 |
| | August 19 | 307 | 30 | 48 | 137 | 522 | 59 |
| | September 19 | 292 | 78 | 119 | 177 | 666 | 44 |
| | October 19 | 233 | 5 | 35 | 144 | 417 | 56 |
| | November 19 | 453 | 19 | 58 | 165 | 695 | 65 |
| | December 19 | 244 | 29 | 94 | 114 | 481 | 51 |
| | **Total** | **2,264** | **210** | **458** | **929** | **3,861** | **59** |

### TABLE 15: MONTHLY PARITY RATES OF DISSECTED MOSQUITOES PER SITE

| District | Month | Dissected | Parous | Parity Rate |
|---|---|---|---|---|
| **Béoumi** | May 19 | 232 | 196 | 84.5 |
| | June 19 | 144 | 139 | 96.5 |
| | July 19 | 384 | 319 | 83.1 |
| | August 19 | 422 | 380 | 90.0 |
| | September 19 | 272 | 231 | 84.9 |
| | October 19 | 944 | 816 | 86.4 |
| | November 19 | 407 | 383 | 94.1 |
| | December 19 | 237 | 215 | 90.7 |
| | **Total** | **3,042** | **2,679** | **88.1** |
| **Dabakala** | May 19 | 419 | 289 | 69.0 |
| | June 19 | 268 | 268 | 100.0 |
| | July 19 | 422 | 144 | 34.1 |
| | August 19 | 470 | 360 | 76.6 |
| | September 19 | 308 | 39 | 12.7 |
| | October 19 | 298 | 218 | 73.2 |
| | November 19 | 409 | 342 | 83.6 |
| | December 19 | 132 | 121 | 91.7 |
| | **Total** | **2,726** | **1,781** | **65.3** |
| **Nassian** | May 19 | 26 | 25 | 96.2 |
| | June 19 | 34 | 30 | 88.2 |
| | July 19 | 62 | 54 | 87.1 |
| | August 19 | 49 | 48 | 98.0 |
| | September 19 | 167 | 165 | 98.8 |
| | October 19 | 181 | 164 | 90.6 |
| | November 19 | 297 | 253 | 85.2 |
| | December 19 | 219 | 218 | 99.5 |
| | **Total** | **1,035** | **957** | **92.5** |
| **Sakassou** | April 19 | 366 | 318 | 86.9 |
| | May 19 | 471 | 259 | 55.0 |
| | June 19 | 490 | 419 | 85.5 |
| | July 19 | 579 | 494 | 85.3 |
| | August 19 | 594 | 497 | 83.7 |
| | September 19 | 616 | 469 | 76.1 |
| | October 19 | 458 | 335 | 73.1 |
| | November 19 | 428 | 182 | 42.5 |
| | December 19 | 470 | 284 | 60.4 |
| | **Total** | **4,472** | **3,257** | **72.8** |
| **Grand total** | | **11,275** | **8,674** | **76.9** |

## TABLE 16: MONTHLY PARITY RATES OF DISSECTED MOSQUITOES FORM INDOOR AND OUTDOOR COLLECTIONS

| District | Month | Outdoor | | | Indoor | | |
|---|---|---|---|---|---|---|---|
| | | Dissected | Parous | Parity Rate | Dissected | Parous | Parity Rate |
| **Béoumi** | May 19 | 144 | 118 | 81.9 | 88 | 78 | 88.6 |
| | June 19 | 84 | 81 | 96.4 | 60 | 58 | 96.7 |
| | July 19 | 178 | 149 | 83.7 | 206 | 170 | 82.5 |
| | August 19 | 138 | 122 | 88.4 | 284 | 258 | 90.8 |
| | September 19 | 125 | 104 | 83.2 | 147 | 127 | 86.4 |
| | October 19 | 464 | 406 | 87.5 | 480 | 410 | 85.4 |
| | November 19 | 231 | 216 | 93.5 | 176 | 167 | 94.9 |
| | December 19 | 163 | 150 | 92.0 | 74 | 65 | 87.8 |
| **Dabakala** | May 19 | 255 | 169 | 66.3 | 164 | 120 | 73.2 |
| | June 19 | 118 | 118 | 100.0 | 150 | 150 | 100.0 |
| | July 19 | 220 | 67 | 30.5 | 202 | 77 | 38.1 |
| | August 19 | 270 | 216 | 80.0 | 200 | 144 | 72.0 |
| | September 19 | 158 | 17 | 10.8 | 150 | 22 | 14.7 |
| | October 19 | 166 | 124 | 74.7 | 132 | 94 | 71.2 |
| | November 19 | 285 | 235 | 82.5 | 124 | 107 | 86.3 |
| | December 19 | 93 | 84 | 90.3 | 39 | 37 | 94.9 |
| **Nassian** | May 19 | 19 | 18 | 94.7 | 7 | 7 | 100.0 |
| | June 19 | 18 | 15 | 83.3 | 16 | 15 | 93.8 |
| | July 19 | 29 | 27 | 93.1 | 33 | 27 | 81.8 |
| | August 19 | 22 | 22 | 100.0 | 27 | 26 | 96.3 |
| | September 19 | 80 | 80 | 100.0 | 87 | 85 | 97.7 |
| | October 19 | 77 | 69 | 89.6 | 104 | 95 | 91.3 |
| | November 19 | 97 | 84 | 86.6 | 200 | 169 | 84.5 |
| | December 19 | 65 | 64 | 98.5 | 154 | 154 | 100.0 |
| **Sakassou** | April 19 | 214 | 184 | 86.0 | 152 | 134 | 88.2 |
| | May 19 | 239 | 127 | 53.1 | 232 | 132 | 56.9 |
| | June 19 | 247 | 214 | 86.6 | 243 | 205 | 84.4 |
| | July 19 | 238 | 202 | 84.9 | 341 | 292 | 85.6 |
| | August 19 | 299 | 254 | 84.9 | 295 | 243 | 82.4 |
| | September 19 | 344 | 276 | 80.2 | 272 | 193 | 71.0 |
| | October 19 | 240 | 169 | 70.4 | 218 | 166 | 76.1 |
| | November 19 | 221 | 102 | 46.2 | 207 | 80 | 38.6 |
| | December 19 | 244 | 146 | 59.8 | 226 | 138 | 61.1 |

## Table 17: Monthly Sporozoite and EIRs of the Four Districts of Bionomic Survey

| District | Month | Total Tested | Number of Circumsporozoite Positive | Sporozoite Rate (%) | HBR (bpn) | EIR (infective bpn) |
|---|---|---|---|---|---|---|
| | | | *An. gambiae* s.l. | | | |
| Béoumi | May | 100 | 3 | 3.0 | 10.6 | 0.318 |
| | June | 100 | 4 | 4.0 | 5.1 | 0.204 |
| | July | 74 | 0 | 0.0 | 16.2 | 0.000 |
| | August | 120 | 0 | 0.0 | 15 | 0.000 |
| | September | 114 | 0 | 0.0 | 72.7 | 0.000 |
| | October | 120 | 2 | 1.7 | 50.4 | 0.840 |
| | November | 134 | 0 | 0.0 | 16.7 | 0.000 |
| | December | 138 | 0 | 0.0 | 7.4 | 0.000 |
| | **Total** | **900** | **9** | **1.0** | **24.26** | **0.243** |
| Dabakala | May | 103 | 5 | 4.9 | 22.4 | 1.087 |
| | June | 100 | 3 | 3.0 | 8.9 | 0.267 |
| | July | 124 | 4 | 3.2 | 69.8 | 2.252 |
| | August | 125 | 2 | 1.6 | 62.9 | 1.006 |
| | Sept | 100 | 0 | 0.0 | 74.2 | 0.000 |
| | October | 100 | 0 | 0.0 | 58.1 | 0.000 |
| | November | 100 | 0 | 0.0 | 24.4 | 0.000 |
| | December | 116 | 2 | 1.7 | 5 | 0.086 |
| | **Total** | **868** | **16** | **1.8** | **40.71** | **0.750** |
| Nassian | May | 22 | 0 | 0.0 | 0.8 | 0.000 |
| | June | 29 | 1 | 3.4 | 1.1 | 0.038 |
| | July | 59 | 4 | 6.8 | 2 | 0.136 |
| | August | 42 | 0 | 0.0 | 1.3 | 0.000 |
| | September | 116 | 4 | 3.4 | 41.6 | 1.434 |
| | October | 157 | 5 | 3.2 | 50.4 | 1.605 |
| | November | 89 | 10 | 11.2 | 3.5 | 0.393 |
| | December | 161 | 13 | 8.1 | 5.9 | 0.476 |
| | **Total** | **675** | **37** | **5.5** | **13.33** | **0.730** |
| Sakassou | April | 99 | 0 | 0.0 | 99.8 | 0.000 |
| | May | 100 | 8 | 8.0 | 97.5 | 7.800 |
| | June | 100 | 4 | 4.0 | 98.9 | 3.956 |
| | July | 100 | 1 | 1.0 | 140.2 | 1.402 |
| | August | 100 | 1 | 1.0 | 180 | 1.800 |
| | September | 100 | 1 | 1.0 | 214.5 | 2.145 |
| | October | 100 | 0 | 0.0 | 230.8 | 0.000 |

| District | Month | Total Tested | Number of Circumsporozoite Positive | Sporozoite Rate (%) | HBR (bpn) | EIR (infective bpn) |
|---|---|---|---|---|---|---|
| | November | 102 | 1 | 1.0 | 216.2 | 2.120 |
| | December | 99 | 3 | 3.0 | 182.2 | 5.521 |
| | **Total** | **900** | **19** | **2.1** | **170.04** | **3.59** |
| | | | *An. funestus* s.l. | | | |
| **Nassian** | November | 161 | 13 | 8.1 | 12.69 | 1.024 |
| | December | 54 | 3 | 5.5 | 3.68 | 0.202 |
| | **Total** | **215** | **16** | **7.4** | **8.18** | **0.609** |

## TABLE 18: ELISA BLOOD FEEDING (BLOOD ORIGIN) IN FOUR DISTRICTS OF VECTOR BIONOMIC SURVEY

| District | Total tested N | Status | Human | | Cattle | | Sheep | | Goat | | Chicken | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Total (n) | % | Total (n) | % | Total (n) | % | Total (n) | % | Total (n) | % |
| **Beoumi** | 201 | Positive | 121 | 60.20 | 4 | 1.99 | 2 | 1.00 | 2 | 1.00 | 0 | 0.00 |
| | | Negative | 80 | 39.80 | 197 | 98.01 | 199 | 99.00 | 199 | 99.00 | 201 | 100.00 |
| **Dabakala** | 200 | Positive | 146 | 73.00 | 11 | 5.50 | 3 | 1.50 | 3 | 1.50 | 5 | 2.50 |
| | | Negative | 54 | 27.00 | 189 | 94.50 | 197 | 98.50 | 197 | 98.50 | 195 | 97.50 |
| **Nassian** | 200 | Positive | 145 | 72.50 | 1 | 0.50 | 2 | 1.00 | 2 | 1.00 | 0 | 0.00 |
| | | Negative | 55 | 27.50 | 199 | 99.50 | 198 | 99.00 | 198 | 99.00 | 200 | 100.00 |
| **Sakassou** | 200 | Positive | 170 | 85.00 | 1 | 0.50 | 4 | 2.00 | 4 | 2.00 | 0 | 0.00 |
| | | Negative | 30 | 15.00 | 199 | 99.50 | 196 | 98.00 | 196 | 98.00 | 200 | 100.00 |

## TABLE 19: SPECIES IDENTIFICATION IN THE FOUR DISTRICTS OF BIONOMIC SURVEY

| District | Methods | Total Tested | *An. coluzzii* | | *An gambiae* s.s. | | Hybrid *An. coluzzii/gambiae* | |
|---|---|---|---|---|---|---|---|---|
| | | N | Total (n) | % | Total (n) | % | Total (n) | % |
| **Béoumi** | HLC | 488 | 441 | 90.37 | 46 | 9.43 | 1 | 0.20 |
| | PSC | 104 | 82 | 78.85 | 22 | 21.15 | 0 | 0 |
| | CDC | 102 | 92 | 90.20 | 10 | 9.80 | 0 | 0 |
| **Dabakala** | HLC | 490 | 437 | 89.18 | 53 | 10.82 | 0 | 0 |
| | PSC | 107 | 99 | 92.52 | 8 | 7.48 | 0 | 0 |
| | CDC | 101 | 87 | 86.14 | 14 | 13.86 | 0 | 0 |
| **Nassian** | HLC | 483 | 0 | 0 | 483 | 100 | 0 | 0 |
| | PSC | 101 | 0 | 0 | 101 | 100 | 0 | 0 |
| | CDC | 24 | 0 | 0 | 24 | 100 | 0 | 0 |
| **Sakassou** | HLC | 521 | 521 | 100 | 0 | 0 | 0 | 0 |
| | PSC | 106 | 106 | 100 | 0 | 0 | 0 | 0 |
| | CDC | 104 | 104 | 100 | 0 | 0 | 0 | 0 |

## TABLE 20: FREQUENCIES OF KDR-WEST IN MOSQUITOES

| District | *Kdr* -West | | | | |
|---|---|---|---|---|---|
| | **Total (n)** | **RR** | **RS** | **SS** | **% Kdr West** |
| Abengourou | 52 | 29 | 17 | 6 | 72.12 |
| Aboisso | 50 | 27 | 19 | 4 | 73.00 |
| Adzopé | 51 | 10 | 24 | 17 | 43.14 |
| Béoumi | 53 | 16 | 7 | 30 | 36.79 |
| Bettié | 50 | 38 | 8 | 4 | 84.00 |
| Bouaké | 54 | 30 | 16 | 8 | 70.37 |
| Bouna | 50 | 46 | 1 | 3 | 93.00 |
| Dabakala | 58 | 18 | 12 | 28 | 41.38 |
| Daloa | 50 | 26 | 20 | 4 | 72.00 |
| Gagnoa | 50 | 0 | 34 | 16 | 34.00 |
| Nassian | 50 | 15 | 21 | 14 | 51.00 |
| Odienné | 50 | 47 | 3 | 0 | 97.00 |
| Sakassou | 51 | 9 | 37 | 5 | 53.92 |
| San Pedro | 50 | 31 | 19 | 0 | 81.00 |
| Yamoussoukro | 59 | 45 | 12 | 2 | 86.44 |

*RR: Homozygous with two resistant Kdr West alleles;*

*RS : Heterozygous with one resistant Kdr West allele and one susceptible allele;*

*SS: Homozygous with two susceptible alleles.*

## TABLE 21: FREQUENCIES OF KDR-EAST IN MOSQUITOES

| District | *Kdr*-East | | | | |
|---|---|---|---|---|---|
| | **Total (n)** | **RR** | **RS** | **SS** | **%*Kdr*-East** |
| Abengourou | 52 | 0 | 4 | 48 | 3.85 |
| Aboisso | 50 | 0 | 5 | 45 | 5 |
| Adzopé | 51 | 0 | 6 | 45 | 5.88 |
| Béoumi | 53 | 0 | 6 | 47 | 5.66 |
| Bettié | 50 | 0 | 0 | 50 | 0 |
| Bouaké | 54 | 0 | 10 | 44 | 9.26 |
| Bouna | 50 | 1 | 3 | 46 | 5 |
| Dabakala | 58 | 0 | 5 | 53 | 4.31 |
| Daloa | 50 | 1 | 1 | 48 | 3 |
| Gagnoa | 50 | 0 | 0 | 50 | 0 |
| Nassian | 50 | 0 | 0 | 50 | 0 |
| Odienné | 50 | 0 | 0 | 50 | 0 |
| Sakassou | 51 | 1 | 8 | 42 | 9.8 |
| San Pedro | 50 | 1 | 4 | 45 | 6 |
| Yamoussoukro | 59 | 0 | 3 | 56 | 2.54 |

*RR: Homozygous with two resistant Kdr East alleles;*

*RS : Heterozygous with one resistant Kdr East allele and one susceptible allele;*

*SS: Homozygous with two susceptible alleles.*

## TABLE 22: FREQUENCIES OF ACE-1 IN MOSQUITOES

| District | ACE-1 G119S | | | | |
|---|---|---|---|---|---|
| | **Total (n)** | **RR** | **RS** | **SS** | **%ACE-1 (G119S)** |
| Abengourou | 50 | 8 | 22 | 20 | 38.00 |
| Aboisso | 50 | 0 | 10 | 40 | 10.00 |
| Adzopé | 50 | 0 | 11 | 39 | 11.00 |
| Béoumi | 50 | 0 | 33 | 17 | 33.00 |
| Bettie | 50 | 0 | 12 | 38 | 12.00 |
| Bouaké | 50 | 6 | 27 | 17 | 39.00 |
| Bouna | 50 | 3 | 12 | 35 | 18.00 |
| Dabakala | 50 | 2 | 11 | 37 | 15.00 |
| Daloa | 50 | 4 | 32 | 14 | 40.00 |
| Gagnoa | 50 | 15 | 35 | 0 | 65.00 |
| Nassian | 50 | 0 | 8 | 42 | 8.00 |
| Odienné | 50 | 6 | 16 | 28 | 28.00 |
| Sakassou | 50 | 9 | 23 | 18 | 41.00 |
| San Pedro | 50 | 1 | 6 | 43 | 8.00 |
| Yamoussoukro | 50 | 4 | 26 | 20 | 34.00 |

*RR: Homozygous with two resistant Ace-1G119St alleles;*

*RS : Heterozygous with one resistant Ace-1G119Sallele and one susceptible allele;*

*SS: Homozygous with two susceptible alleles.*

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