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PMI VECTORLINK COTE D'IVOIRE ANNUAL ENTOMOLOGICAL REPORT APRIL 2018–MARCH 2019

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PMI VECTORLINK COTE D'IVOIRE ANNUAL ENTOMOLOGICAL REPORT APRIL 2018-MARCH 2019

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Acronyms

CDC	Centers for Disease Control and Prevention
CSP	Circumsporozoite
ELISA	Enzyme-Linked Immuno-Sorbent Assay
HBR	Human Biting Rate
HLC	Human Landing Catch
IRD	Indoor Resting Densities
IRM	Insecticide Resistance Monitoring
IRS	Indoor Residual Spraying
KDR	Knock Down Resistance
LLIN	Long Lasting Insecticidal Net
LT	Light traps
NMCP	National Malaria Control Program
MOP	Malaria Operational Plan
PBO	Piperonyl butoxide
PCR	Polymerase Chain Reaction
PMI	President's Malaria Initiative
PSC	Pyrethrum Spray Catch
USAID	United States Agency for International Development
WHO	World Health Organization

EXECUTIVE SUMMARY

Entomological monitoring of malaria vectors was conducted from April 2018 to March 2019 to inform site selection and planning for indoor residual spraying (IRS) in Cote d'Ivoire.

Longitudinal vector monitoring was conducted every month from November 2018 to March 2019at sites in four districts (Gagnoa, Sakassou, Bocanda, and Jacqueville) identified by the National Malaria Control Program (NMCP) for potential spray campaigns. Three different methods were used to collect adult mosquitoes: human landing catch (HLC), pyrethrum spray catch (PSC) and the Centers for Disease Control and Prevention (CDC) light traps (LT). 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, intensity of resistance, and synergist assays with piperonyl butoxide (PBO) were completed in these four sites (Bocanda, Gagnoa, Jacqueville and Sakassou) and an additional six (Aboisso, Adzope, Bouake, Bouna, Daloa, Odienne) using WHO tube tests to monitor the resistance status of *Anopheles gambiae* s.l. to insecticides used for IRS (clothianidin and pirimiphos methyl) long-lasting insecticidal net (LLIN) impregnation (pyrethroids). Chlorfenapyr susceptibility was assessed using CDC bottle assays.

Monthly collections showed that *An. gambiae* s.l. was the predominant malaria vector in all four vector surveillance sites (68%; n=17,726), with subsequent molecular species identification analyses confirming that these specimens were almost entirely *An. coluzzii*, collected using the three collection methods (HLC, PSC and CDC-LT) The peak biting of *An. gambiae* s.l. ranged between 10:00 pm and 12:00 pm outdoors, and 00:00 am and 02:00 am indoors in Sakassou and between 01:00 am and 03:00 am in Gagnoa both indoors and outdoors with the highest peak recorded in November 2018 for both outdoor and indoor in Sakassou and Gagnoa. Indoor biting peaked in both November 2018 and March 2019 for Sakassou while Gagnoa recorded the highest in November only. A single peak was recorded in Bocanda and Jacqueville in November and December 2018 for outdoor and indoor respectively in Bocanda and in February and March 2019 for indoor and outdoor respectively in Jacqueville.

Overall, female *An. gambiae* s.l. biting activity was consistently highest between 10:00 PM and 03:00 AM both indoors and outdoors in all sites, with more biting mosquitoes collected outdoors compared to indoors in Bocanda (53.3%), Gagnoa (72.2%) and Sakassou (53.9%), but not in Jacqueville (47.1%). The highest human biting rate (HBR) was recorded in Sakassou, with an overall rate ranging from 107 to 200 bites per person per night (b/p/n) during the five month collection period. Parity rates were high in all sites with 84.4% (76/90), 67% (1,070/ 1,598), 80% (668/835) and 61.7% (1,349/ 2,186) in Bocanda, Gagnoa, Jacqueville, and Sakassou, respectively. Indoor resting densities of *An gambiae s.l.* were highest in Sakassou (0.4-18 female/room/day (f/r/d)), followed by Gagnoa (0.5-4.5 f/r/d). A total of 1870 *An. gambiae* s.l. mosquitoes were analysed for sporozoite detection across all four sites with mean sporozoite rates of 0.061 in Bocanda (N = 82), 0.022 in Gagnoa (N = 462), 0.025 in Jacqueville (N = 200) and 0.011 in Sakassou (N = 1126). The highest seasonal entomological inoculation rate (EIR) was recorded in Sakassou (7.41 ib/p/n) followed by Gagnoa (3.17 ib/p/n). Bocanda and Jacqueville recorded the least with less than 1 ib/p/n.

Molecular characterization of mosquitoes collected for insecticide susceptibility testing in the other six sites indicated that *Anopheles gambiae s.s* was the predominant species of *An. gambiae* s.l. in the North (Odienné and Bouna), while *An. coluzzii* were predominant in Aboisso, Adzopé, and Daloa. Both species were equally present in Bouaké (50% respectively). Hybrids (11%) of the two species (*An. gambiae*/*An. coluzzii*) were recorded only in Aboisso. Both species were sympatric in five districts.

Resistance was observed in *An. gambiae* s.l. to the diagnostic dose of all pyrethroids, pirimiphos-methyl and bendiocarb in all sites surveyed except Gagnoa for pirimiphos methyl. Moderate resistance intensity to pirimiphos-methyl was observed while resistance to pyrethroids was very high in all the sites tested. Pre-exposure to PBO did not yield full susceptibility but induced a significant increase in mortality in nine sites for deltamethrin as compared to alpha-cypermethrin and permethrin, suggesting that deltamethrin + PBO insecticide treated bed nets (ITNs) may be appropriate for deployment in all districts tested except Jacqueville. The average percentage increment was about 45% with deltamethrin compared to permethrin (6.5%) and alpha-cypermethrin (34.3%). *An. gambiae* s.l. was susceptible to chlorfenapyr in five of the ten sites (98–100% mortality) at the dose of 100 μg/bottle, and in seven of ten sites (99–100% mortality) at the dose of 200 μg/bottle. For clothianidin, susceptibility (98-100% mortality) was observed after 4-6 days post exposure for the majority of the sites including Adzopé, Bouake, Gagnoa, Jacqueville, Odienné and Sakassou. Bocanda recorded 100% mortality after 7 days while Aboisso and Daloa showed possible resistance with 78% and 94% mortality 7 days post-exposure, respectively.

Knock-down resistance-West (*kdr*-West) and *kdr*-East represent the main pyrethroid and DDT target site resistance mechanisms, while *ace-1* is the target site resistance mechanism for carbamates (bendiocarb) and organophosphates (pirimiphos-methyl). *Kdr*-West was detected in *An. gambiae* s.l. in all study sites (frequency ranged from 71 to 97%) while *kdr*-East was found in six sites except Bouake, Bouna, Gagnoa, Jacqueville, with the frequency ranging from 5 to 10%. The*ace-1* mutation was found in all sites except Bouna and the frequencies ranged from 16% (lowest in Bouaké) to 83% (highest in Daloa).

Overall, the results confirmed that there is high resistance to pyrethroids in Cote d'Ivoire, with the presence of both *kdr*-West and East mutations, in addition to a high *ace-1* mutation frequency in all the sites except Bouna. These findings suggest that clothianidin based products are the only insecticide option for IRS in the four districts identified for potential deployment of IRS, with the exception of Gagnoa where the vector was also susceptible to pirimiphos-methyl., Furthermore, PBO-synergist ITNs and dual active ingredient long-lasting insecticide treated nets (LLINs) (e.g., Interceptor G2) should be considered for insecticide resistance management in the country. Based on the insecticide resistance profiles and high human biting rates, Sakassou and Gagnoa would be the most suitable of the four potential districts targeted for IRS.

I. INTRODUCTION

Malaria is a leading public health challenge in Côte d'Ivoire. It accounts for about 33 percent of outpatient visits in health facilities with an incidence of 134 per 1000 in general population and 247 per 1000 among children under five years 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 utilization of long-lasting insecticidal nets (LLINs) through mass campaigns and routine distribution. The National Malaria Strategic Plan 2016-2020 prioritized Indoor Residual Spraying (IRS) as an additional vector control method to reduce malaria morbidity and mortality.

In September 2017, Abt Associates was awarded a five-year Task Order, the PMI VectorLink Project, to support PMI, as well as USAID Missions, Country Offices, and Bureaus with malaria programs, in planning and implementing IRS programs with the overall goal of reducing the burden of malaria in Africa. This task order will enhance USAID's ability to implement IRS and integrated malaria vector control programs.

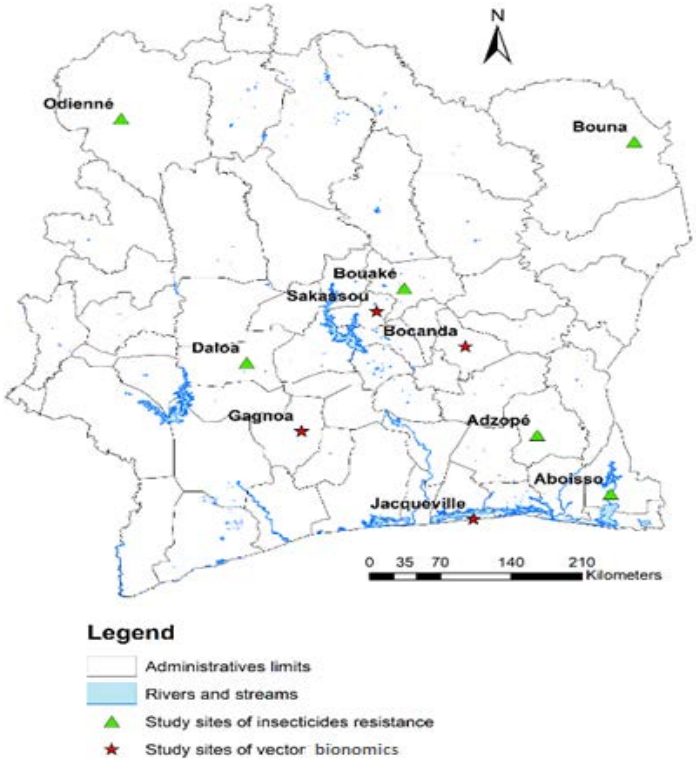
In order to inform and guide the implementation of IRS in Côte d'Ivoire, VectorLink conducted entomological monitoring including insecticide susceptibility testing of *Anopheles gambiae* s.l. in ten sites across the country and longitudinal entomological surveillance to assess the variation of vector density, composition, and behavior in four sites selected by NMCP as potential IRS sites (Gagnoa, Bocanda, Jacqueville, Sakassou). The data collected aimed to support the NMCP and the malaria vector control stakeholders (including PMI VectorLink) in the selection of the districts, insecticide, and timing for IRS programming and to inform the selection of LLINs for future mass campaigns.

2. METHODOLOGY

2.1 ENTOMOLOGICAL MONITORING SITES

Both vector bionomics and insecticide susceptibility monitoring were conducted in four potential IRS sites (Bocanda, Gagnoa, Jacqueville and Sakassou) and additional six sites were tested for insecticide susceptibility (Aboisso, Adzope, Bouake, Bouna, Daloa and Odienne) (Figure 1).

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



2.2 VECTOR BIONOMICS MONITORING

Adult mosquitoes were collected using human landing catches (HLCs), pyrethrum spray catches (PSCs) and CDC Light Traps (CDC LTs). HLCs were conducted during two consecutive nights in four houses (2 urban 2 rural at a maximum of 5 km from the town) per site per month. The PSCs were conducted in 30 houses (15 urban and 15 rural) per site per month. The CDC LT collections were performed in four different houses from HLC's (two urban and two rural) during two consecutive nights per site per month. Collections were conducted every month from November 2018 to March 2019, representing five 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 pm to 6:00 am	Two nights per site per month	4 houses per site (2 urban and 2 rural)
PSC	6:00 am to 8:00 am	Two days per site per month	30 houses per site (15 urban and 15 rural)
CDC LT	6:00 pm to 6:00 am	Two nights per site per month	4 houses per site (2 urban and 2 rural)

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 was seated indoors and another one outdoors and served as mosquito collectors. The collectors switched between indoors and outdoors on an hourly basis. The doors of the houses were kept closed when collections were underway. The collectors used flashlights and hemolysis tubes to collect mosquitoes that landed on their legs before the mosquitoes could bite. The tubes were covered with cotton after individual collection of mosquitoes. The teams transferred the mosquitoes hourly to custom-made bags for a total of 12 hours.

The PSCs were carried out during morning hours, between 6:00 a.m. and 8:00 a.m. White cloth/sheets were placed on the floor from wall to wall in sampled rooms. The rooms were sprayed with the commercial pyrethroid+ PBO insecticide, in the house after closing windows and doors of the house and covering or removing drinking water and food items from the room before spraying. For houses with open eaves, collectors sprayed from 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. The abdominal status of all female anophelines was determined, and individuals were sorted into four categories: unfed, blood-fed, half-gravid, and gravid.

CDC light traps were installed indoors in selected houses where people slept under a LLIN that they received during the distribution campaign. 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. until the morning shift (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, M.T. & Coetzee, M. 1987). The identification was done by a team of well-trained technicians from research institutes and VectorLink staff members. A subsample of *An. gambiae* s.l. from each site was dissected for parity rate estimation. 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 (PCR) and Enzyme-Linked Immunosorbent Assay (ELISA).

The following indicators were calculated based on the number of mosquitoes collected through each collection method (Table 2):

TABLE 2: VECTOR SURVEILLANCE INDICATORS PER COLLECTION METHODS

Collection Method	Indicator	Definition
HLC	Human Biting Rate	# bites / person / night
	Peak biting time	Hour of highest human biting rate
	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	# mosquitoes / house / day
	% of fed females	# fed mosquitoes / total collected by PSC
CDC LT	Indoor Density	# mosquitoes collected indoors / trap / night

2.3 INSECTICIDE RESISTANCE MONITORING

The field teams visited each site for larval collections and insecticide susceptibility tests. *Anopheles gambiae* s.l. larvae and pupae were collected from different larval habitats across each site using the dipping method. Collected larvae and pupae were pooled and reared to adults in the field laboratory. The adult mosquitoes were kept under controlled conditions (25 ºC ± 2 ºC and 70% ± 10% humidity) and fed with 10% sugar solution soaked in cotton. The GPS coordinates of the larval collection sites were recorded for the geo mapping of the larval breeding sites and distribution in each locality.

Insecticide susceptibility testing was conducted using WHO tube tests on 2-5-day old adult female *An. gambiae* s.l. emerged from larvae collections. The mosquitoes were exposed to the diagnostic doses of the insecticides for one hour and the mortality recorded after 24-hours post-exposure.

- The diagnostic concentrations of deltamethrin (0.05%), permethrin (0.75%), alpha-cypermethrin (0.05%), lambda-cyhalothrin (0.05%), bendiocarb (0.1%), etofenprox (0.5%) and pirimiphos-methyl (0.25%) were tested on site. The resistance status was determined following WHO criteria with < 90% as confirmed resistance, 90% - 97% as possible resistance, and ≥98% as susceptible (WHO, 2016).
- Synergist assays with piperonyl butoxide (PBO) were conducted for deltamethrin, permethrin and alpha-cypermethrin according to the WHO susceptibility test protocol to determine the involvement of P450s in any pyrethroid resistance detected in a site, and to assess whether PBO synergist ITNs would be an effective vector control option in Cote d'Ivoire. One-hour pre-exposure of the mosquitoes to PBO 4% was done before exposure to each pyrethroid insecticide and mortality was recorded 24 hours post-exposure. A high increment of the mortality after pre exposure to PBO represents an involvement of enzyme activities such as P450s in the insecticide resistance of the population tested.
- Resistance intensity at 5x and 10x the diagnostic concentration of deltamethrin, permethrin, alpha-cypermethrin and pirimiphos methyl were also tested and the intensity of the resistance was defined following the WHO criteria of high, moderate or low intensity (WHO, 2016).
- CDC bottle assays were conducted using chlorfenapyr at the doses of 100µg/bottle and 200µg/bottle. Testing was done following the protocol of Brogdon et al. (2010) but the exposure time was one hour and mortality was recorded every 24 hours for 3 days (72 hours).

- Wild mosquitoes were also tested against clothianidin following a protocol designed and shared by VectorLink. The papers were impregnated using a solution of SumiShield (commercial formulation of clothianidin) and distilled water at the dose of 13.2 mg/m^2. The testing was conducted following WHO tube assay procedures and the mortality was recorded up to 7 days post-exposure. The susceptibility of the mosquitoes was determined following WHO criteria (WHO, 2016).

2.4 MOLECULAR CHARACTERIZATION

Insecticide resistance in mosquitoes can be related to target site mutations. Among them, resistance to pyrethroids and DDT is described as a substitution of amino acid leucine to either phenylalanine (L1014F, referred as *kdr* -West) or serine (L1014S, referred as *kdr*-East) at the position 1014 in the sodium channel gate. For organophosphate and carbamate insecticide, 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 per site among the dead and surviving mosquitoes from the WHO susceptibility tests and were further analysed to determine species identification and assess molecular markers of insecticide resistance. The DNA of each individual mosquito was extracted using the protocol designed by Collins et al, 1987. The presence of *kdr*-West and East was characterized using the Taqman protocol described by Bass et al, 2007.

A subsample of about 150 *An. gambiae* s.l. from Gagnoa and Jacqueville and 189 from Sakassou collected using HLC were further identified to the species level by polymerase chain reaction (PCR). Furthermore, 100 *An. gambiae* s.l. from Gagnoa and Sakassou, 15 from Bocanda, and 7 from Jacqueville collected using CDC-LT were also identified. Those specimens identified as *An. gambiae* s.s., were further identified as *An. gambiae*, *An. coluzzii* or hybrids of the two species following the Short Interspersed Element (SINE) protocol described by Santolamazza et al, 2008.

The sporozoite infection status of a subsamples of mosquitoes collected from each site by HLC was determined using the Enzyme Linked Immunosorbent Assay (ELISA) protocol for identification of *Plasmodium falciparum* circumsporozoite infection. This included 82 *An. gambiae* s.l. form Bocanda, 462 from Gagnoa, 200 from Jacqueville and 1126 from Sakassou. Blood source of the mosquitoes collected through PSCs was also determined by ELISA.

3. RESULTS

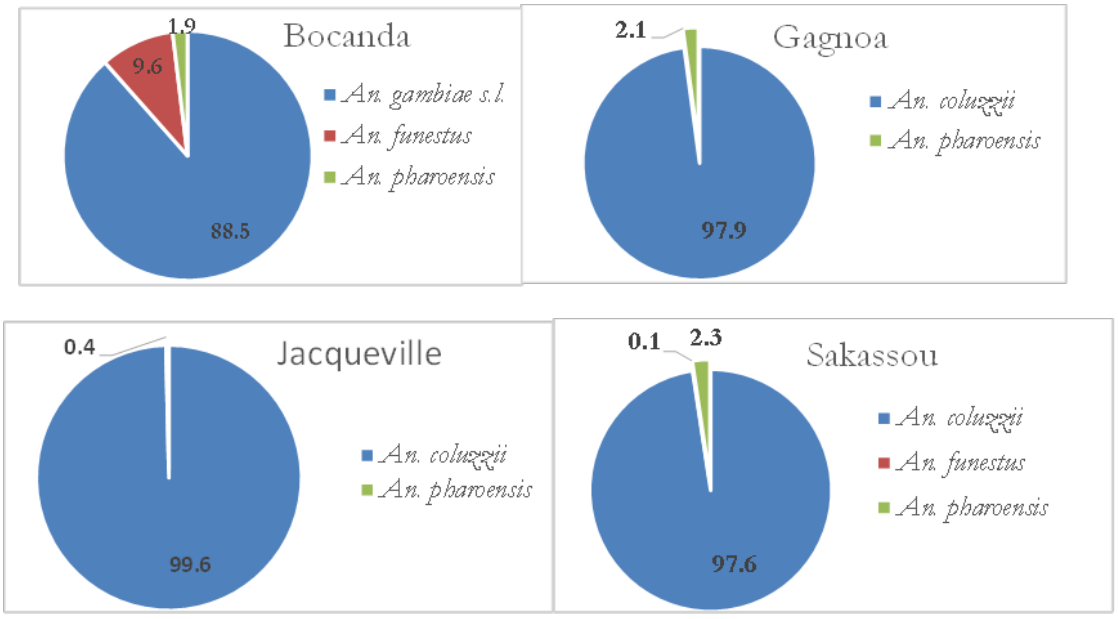
3.1 VECTOR BIONOMICS MONITORING

3.1.1 SPECIES COMPOSITION

A total of 26,110 mosquitoes, including 30.6% culicines (n=7986), were collected over five months (November 2018 to March 2019) using the three collection methods described above. *An. gambiae s.l.* was the predominant malaria vector species in all sites, representing 68% of the total mosquitoes collected (n= 17,726). Furthermore, culicines represented the majority of mosquitoes collected in Bocanda and Jacqueville using all collection methods during the five month-collection with respectively 86% (n= 1119) and 75% (n=2595) of the total mosquitoes of both localities. (Annex Table 7)

For HLC, *An. gambiae s.l.* was the most collected *Anopheles* species (n = 14,324, 97.7 %) across all sites during the five months. The overall abundance of *An. gambiae s.l.* was 88.5% (n= 92) in Bocanda, 97.9% (n= 2212) in Gagnoa 99.6% (n=853) in Jacqueville and 97.6% (n=11167) in Sakassou. *An. coluzzii* represented the only species of the *An. gambiae* s.l. complex in Gagnoa, Jacqueville and Sakassou *An. funestus s.l.* was collected at a lower rate and only in Bocanda (9.6%; n=10) and Sakassou (0.1%; n=6). The other *Anopheles* species found was *An. pharoensis*. (Figure 2; Annex: Table 8)

FIGURE 2: SPECIES COMPOSITION OF THE *ANOPHELES* MOSQUITOES COLLECTED USING HLC IN THE FOUR SITES



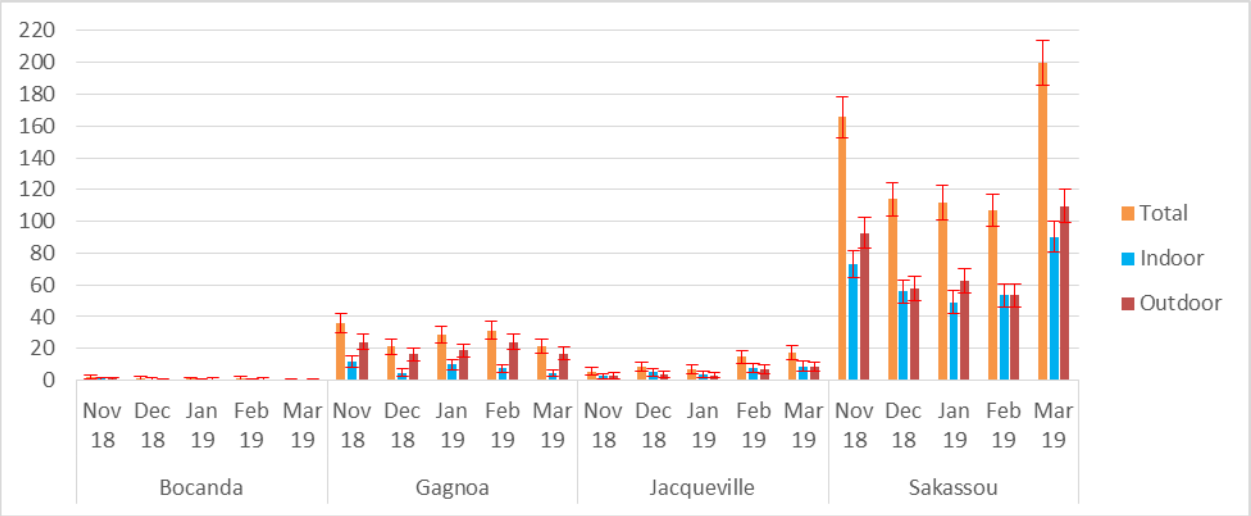
A total of 1769) *An. gambiae s.l.* were collected by PSC in all the sites including 200 in Gagnoa, 1502 in Sakassou, 62 in Bocanda, and and 5 Jacqueville. *An. gambiae* complex was composed of only *An. coluzzii* (100%) in all the sites except Bocanda where a single *An. gambiae* s.s was recorded among those molecularly identified. Indoor resting densities of *An. coluzzii* were highest in Sakassou and Gagnoa compared to the two other sites where *An. gambiae s.l.* of Bocanda and *An. coluzzii* of Jacqueville represented less than 4% of all mosquitoes collected. Culicine mosquitoes represented the predominant mosquitoes collected in Bocanda (96.3%), Jacqueville (96.2%) and Sakassou (60.3%). The percentage of blood fed *An. Gambiae* s.l. collected ranged from 44 to 100% during the five months of monitoring (Annex: Table 9).

CDC-LT results were similar to the PSC with 100% of the anopheles mosquitoes being An. coluzzii and in higher density in Sakassou (1,366) and Gagnoa (306) respectively. In Bocanda and Jacqueville, only 15 An. gambiae and 7 A. coluzzii were collected respectively. In both sites, other culicine mosquitoes represented the predominant mosquito collected using CDC-LT collection method (Annex: Table 10).

3.1.2 HUMAN BITING RATE OF *AN. GAMBIAE S.L.*

The human biting rates were very high in Sakassou, with an overall rate of 166, 114, 112, 107 and 200 bites per person per night (b/p/n) in November 2018 , December 2018, January 2019, February 2019 and March 2019, respectively (Figure 3; Annex: Table 11). The indoor rate was between 49 and 90 b/p/n, and the outdoor rate ranged from 46 to 99 b/p/n from November 2018 to March 2019. The indoor biting rate in Gagnoa ranged from 4 to 12 b/p/n however it was two times higher outdoors during the same period. The biting rates were lower in Jacqueville (from 2.8 to 8.4 b/p/n) and Bocanda (from 0.1 to 1 b/p/n) regardless of the months and the collection place (Figure 3 Annex: Table 11).

FIGURE 3: *AN GAMBIAE S.L.* BITING RATE USING HUMAN LANDING CATCHES (HLC) AT ALL SITES



3.1.3 BITING BEHAVIOUR OF *AN. GAMBIAE S.L.*

Anopheles gambiae s.l. showed variable biting behavior across the four districts (Annex: Table 10). The densities of *An. gambiae* s.l. were overall higher outdoor in Bocanda (53.3%), Gagnoa (72.2%) and Sakassou (53.9%) compared to indoor. On the other hand, *An. gambiae* s.l. was generally endophilic in Jacqueville (52.9%).

Overall, *An. gambiae* females biting activities were highest between 10:00 pm and 03:00 am across the five months, indoors and outdoors in all sites. The biting rates peaked between 10:00 pm and 12:00 pm outdoors, and 00:00 am and 02:00 am indoors for Sakassou and between 01:00 am and 03:00 am for Gagnoa both indoors and outdoors. Overall, Bocanda recorded a biting peak time of around 3:00 am outdoors and 01:00 am indoors. Jacqueville showed several peak biting times during the five months of collection both indoors and outdoors (Figures 4-7; Annex: Table 12).

FIGURE 4: BITING RATE AND CYCLE OF *AN. GAMBIAE S.L.* COLLECTED USING HLC AT BOCANDA

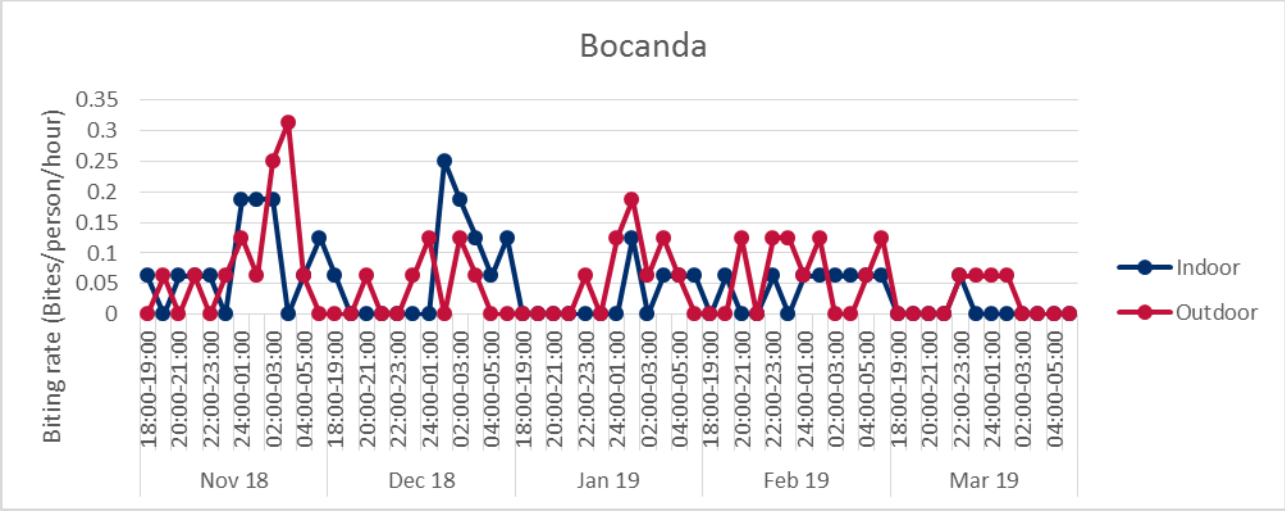


FIGURE 5: BITING RATE AND CYCLE OF *AN. GAMBIAE S.L.* COLLECTED USING HLC AT GAGNOA

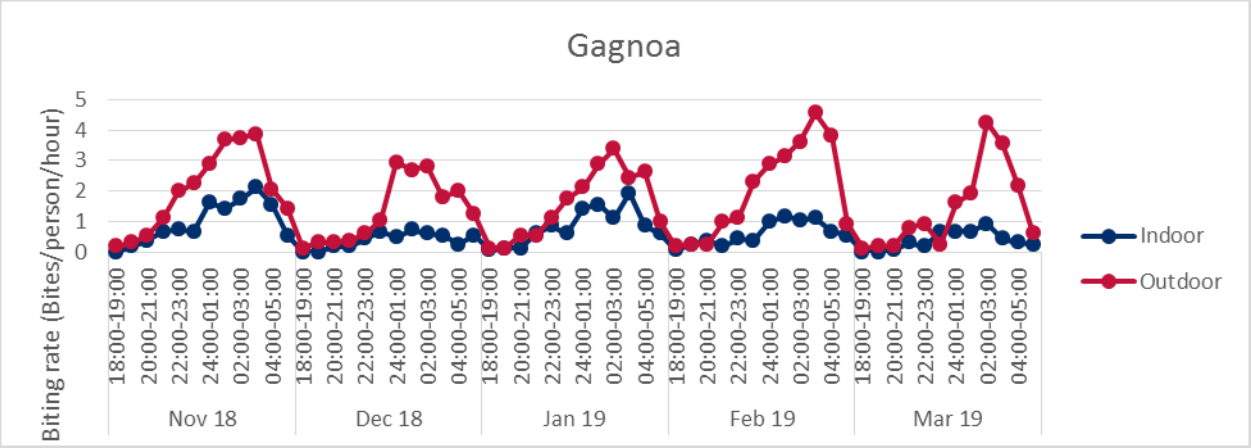


FIGURE 6: BITING RATE AND CYCLE OF *AN. GAMBIAE S.L.* COLLECTED USING HLC AT JACQUEVILLE

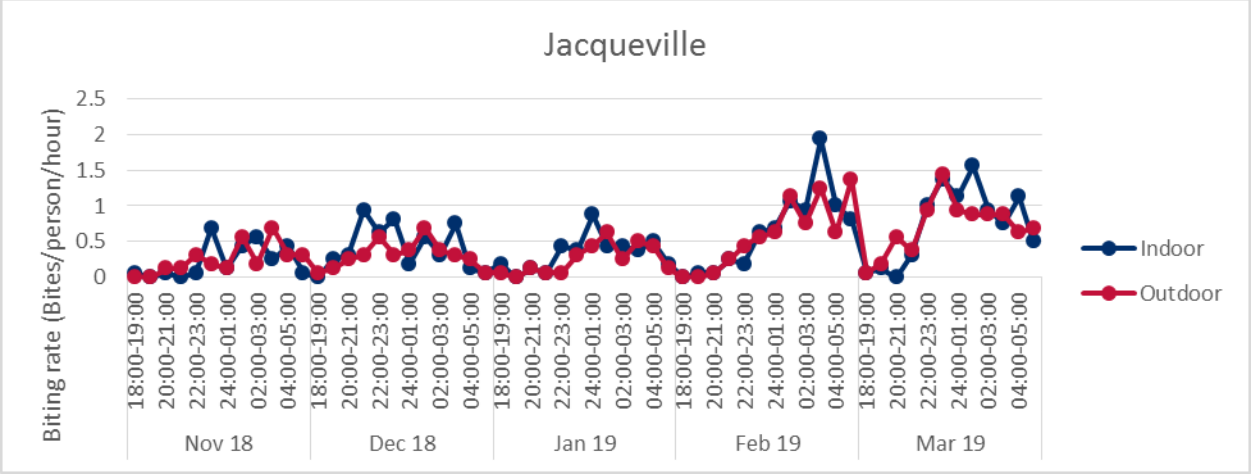
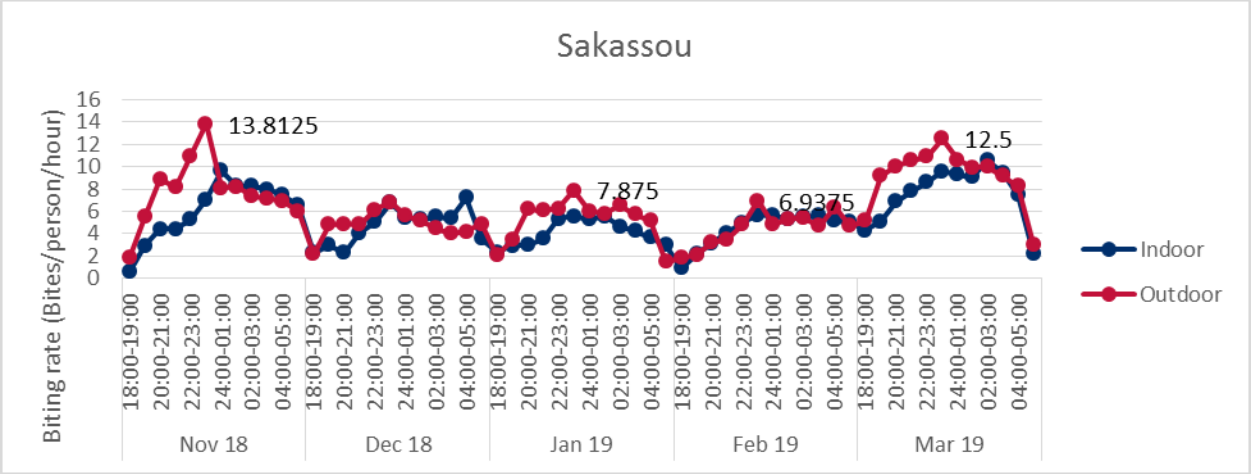


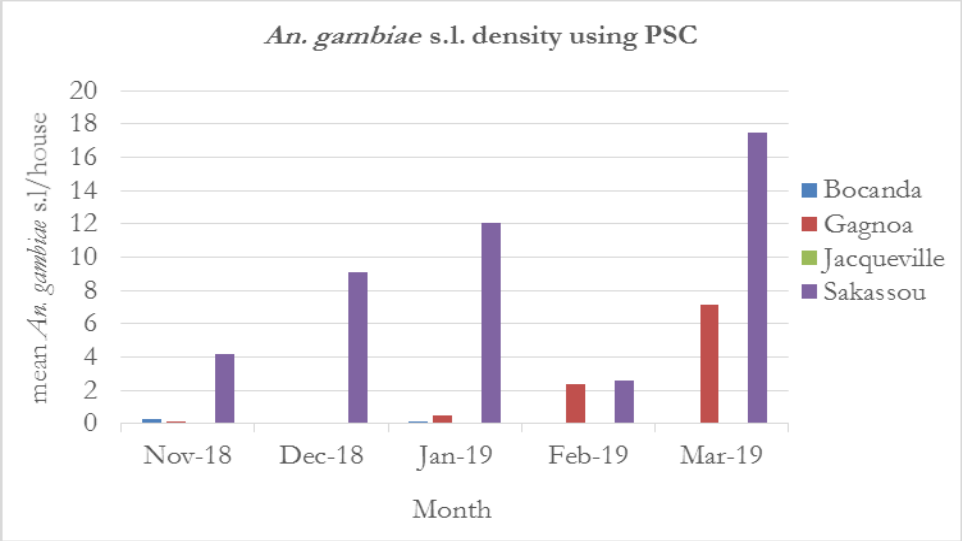
FIGURE 7: BITING RATE AND CYCLE OF *AN. GAMBIAE S.L.* COLLECTED USING HLC AT SAKASSOU



3.1.4 INDOOR RESTING DENSITY

The overall mean vector indoor resting density using PSC was calculated using the number of *An. gambiae* s.l. collected per 30 houses per day during the five months of collection. The mean monthly density was higher in Sakassou in all months throughout the collection period with an average of (10 *An. gambiae* s.l./house /day) compared to all the three other sites. Gagnoa recorded an overall mean of 1.33 *An. gambiae* s.l./house /day while Jacqueville and Bocanda recorded less than 1 vector per house per day (0.007 and 0.03 for Bocanda and Jacqueville respectively) over the collection period (Figure 8).

FIGURE 8: MEAN DENSITY OF *AN. GAMBIAE S.L.* PER HOUSE USING PSC, BY SITE

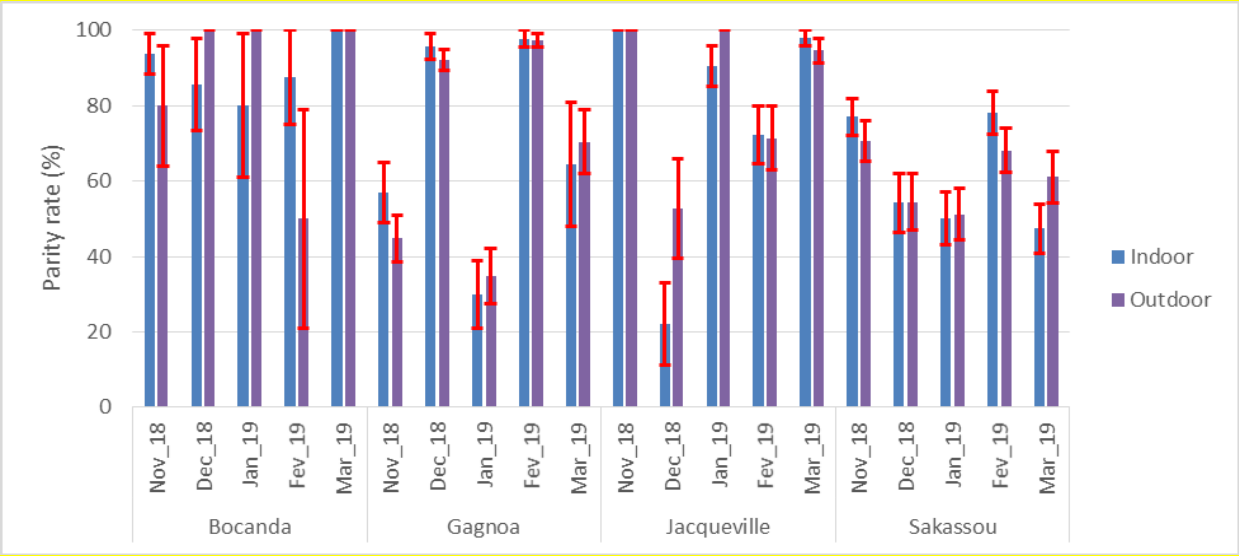


3.1.5 PARITY RATE

A subsample of 4,709 mosquitoes collected in human landing collections over the five months, was dissected for parity across all four sites including 2,186 in Sakassou, 1,598 in Gagnoa, 90 in Bocanda and 835 in Jacqueville. Out of the numbers dissected, 1,349 (61.7%), 1,070 (67%), 76 (84.4%), 668 (80%) were parous in Sakassou, Gagnoa, Bocanda and Jacqueville, respectively. Overall, the parity rate was similar for mosquitoes collected indoors and outdoors (Figure 9; Annex: Tables 13 & 14).

The parity rates were also higher in the rural areas than the urban areas in Bocanda (89.3% vs 56.4%) and Gagnoa (73.1% vs 62.6%), but similar in both settings in Sakassou (61.5% and 62.1% respectively). Only Jacqueville recorded a higher parity rate in urban area (86.3%) compared to in rural area (74.9%) (Annex Table 15).

FIGURE 9: PARITY RATE OF *AN. GAMBIAE* COLLECTED USING HLC



3.1.6 PLASMODIUM FALCIPARUM SPOROZOITE RATES

A total of 1870 *An. gambiae* s.l. mosquitoes were analysed for sporozoite infection from all four sites including 82 from Bocanda, 462 from Gagnoa, 200 from Jacqueville, and 1126 from Sakassou (Table 3).. Five *An. gambiae* s.l. and *An. coluzzii* were recorded positive for circumsporozoite per sites in Bocanda and Jacqueville respectively, while 10 positive were found within the *An. coluzzii* from Gagnoa and 12 in Sakassou. Overall, the sporozoite rate (SR) ranged from 0.011 in Sakassou to 0.061 in Bocanda. However, sporozoite infections were recorded at every month in Sakassou compared to the other sites. The highest SR recorded in a given month was 0.125 in Bocanda in November 2018. No infection was detected in *An. funestus* s.l. and *An. nili* tested in Bocanda, though the numbers were very low.

TABLE 3: SPOROZOITE RATE OF *AN. GAMBIAE* S.L. AND *AN. FUNESTUS* S.L.

Districts	Month	An. gambiae s.l.				An. funestus				An. nili			
		C	T	P	SR	C	T	P	SR	C	T	P	SR
Bocanda	November	32	32	4	0.125	7	-	-	-	-	-	-	-
	December	20	18	1	0.056	3	2	0	0	-	-	-	-
	January	15	13	0	0	0	0	0	0	-	7	0	0
	February	20	15	0	0	-	-	-	-	-	-	-	-
	March	5	4	0	0	-	-	-	-	-	1	0	0
	Total	**92**	**82**	**5**	**0.061**	**-**	**3**	**0**	**0**	**-**	**8**	**0**	**0**
Gagnoa	November	574	92	5	0.054	-	-	-	-	-	-	-	-
	December	337	105	4	0.038	-	-	-	-	-	-	-	-
	January	459	69	1	0.014	-	-	-	-	-	-	-	-
	February	502	113	0	0	-	-	-	-	-	-	-	-
	March	340	83	0	0	-	-	-	-	-	-	-	-
	Total	**2212**	**462**	**10**	**0.022**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**

Districts	Month	An. gambiae s.l.				An. funestus				An. nili			
		C	T	P	SR	C	T	P	SR	C	T	P	SR
Jacqueville	November	91	0	0	0	-	-	-	-	-	-	-	-
	December	138	42	1	0.024	-	-	-	-	-	-	-	-
	January	112	0	0	0	-	-	-	-	-	-	-	-
	February	235	78	3	0.038	-	-	-	-	-	-	-	-
	March	277	80	1	0.013	-	-	-	-	-	-	-	-
	Total	**853**	**200**	**5**	**0.025**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
Sakassou	November	2648	276	5	0.018	-	-	-	-	-	-	-	-
	December	1822	209	1	0.005	-	-	-	-	-	-	-	-
	January	1789	198	1	0.005	-	-	-	-	-	-	-	-
	February	1711	213	3	0.014	-	-	-	-	-	-	-	-
	March	3197	230	2	0.009	-	-	-	-	-	-	-	-
	Total	**11167**	**1126**	**12**	**0.011**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**

C= # Collected; T= # Tested; P = Positives; SR = Sporozoite rate

3.1.7 ENTOMOLOGICAL INOCULATION RATE

Table 4 shows the monthly and seasonal entomological inoculation rates (EIRs) for *An. gambiae* s.l. in the four districts. The Overall EIRs recorded for the period of November 2018 to March 2019 were 0.064 for Bocanda, 0.634 for Gagnoa, 0.198 for Jacqueville and 1.482 for Sakassou. The highest EIRs were recorded in November for Bocanda, Gangoa and Sakassou with respectively 0.25, 1.95 and 3 ib/person/month, while Jacqueville recorded the highest EIR in February (0.56 ib/person/month). Sakassou recorded the highest seasonal EIR (7.45 ib/person/season) followed by Gagnoa (3.17 ib/person/season) (Table 4).

TABLE 4: EIR OF MALARIA VECTORS COLLECTED USING HLC

District	Month	An. gambiae s.l.		
		HBR	SR	EIR
Bocanda	November	2.0	0.125	0.25
	December	1.3	0.056	0.07
	January	0.9	0	0
	February	1.3	0	0
	March	0.3	0	0
	Seasonal	**6.8**	**0.181**	**0.32**
Gagnoa	November	35.9	0.054	1.95
	December	21.1	0.038	0.80
	January	28.7	0.014	0.42
	February	31.4	0	0
	March	21.3	0	0
	Seasonal	**138.4**	**0.106**	**3.17**

District	Month	An. gambiae s.l.		
		HBR	SR	EIR
Jacqueville	November	5.7	0	0
	December	8.6	0.024	0.21
	January	7.0	0	0
	February	14.7	0.038	0.56
	March	17.3	0.013	0.22
	Seasonal	**53.3**	**0.075**	**0.99**
Sakassou	November	166	0.018	3.00
	December	114	0.005	0.54
	January	112	0.005	0.56
	February	107	0.014	1.51
	March	200	0.009	1.8
	Seasonal	**699**	**0.051**	**7.41**

3.1.8 HUMAN BLOOD INDEX

The human blood index (HBI) representing the percentage of human blood meal taken by the population of the mosquitoes analyzed is described in the table 5 below. More than 60% of the *An. gambiae* s.l. analysed in Bocanda, Gagnoa and Jacqueville were fed on human blood. Additionally, 78% of the *An. funestus* s.l. from Bocanda have also had human blood meal. Sakassou recorded the lowest human blood index with only 39% of the blood meal of *An. gambiae* s.l. showing availability of other blood meal sources such as cow (15%), goat (3%) and sheep (3%) (Table 5).

TABLE 5: HUMAN BLOOD INDEX OF *AN. GAMBIAE* S.L. BY SITE

Site	Species	Total analyzed	Total human blood	Human blood index
Bocanda	*An. gambiae* s.l	44	28	63.6
	An. funestus s.l.	9	7	77.8
Gagnoa	*An. gambiae* s.l	124	78	62.9
jacqueville	*An. gambiae* s.l	8	5	62.5
Sakassou	*An. gambiae* s.l	200	78	39.0

3.2 INSECTICIDE RESISTANCE MONITORING

Table 6 describes the resistance status to the different insecticides tested against *An. gambiae* s.l. collected from the ten different sites. All insecticides were tested in all sites except in Bouna where the intensity of the resistance and the clothianidin susceptibility testing were not fully completed due to the limited number of mosquitoes.

- Resistance was observed to the diagnostic dose of all pyrethroids, pirimiphos methyl and bendiocarb in all sites surveyed.
- The intensity of resistance tested indicated moderate resistance of *An. gambiae* s.l. to pirimiphos

methyl (100% at 5x diagnostic dose) while the resistance to the pyrethroids was very high in all the sites where the tests were completed.

- Daloa and Jacqueville recorded the highest pyrethroid resistance intensity with less than 40% mortality at 10x the diagnostic doses of the three pyrethroids
- The pre-exposure of mosquitoes to PBO before deltamethrin, permethrin and alpha-cypermethrin yielded partial increase of the mortality in most of the sites surveyed. PBO + deltamethrin showed the highest increase in mortality among the three pyrethroids in all the sites, followed by PBO + alpha-cypermethrin. Although full susceptibility was not recorded after the pre exposure to PBO, the significant increase observed indicates that P450s may be involved in the insecticide resistance of the *An. gambiae* s.l. from most of the sites except Daloa, Gagnoa and Jacqueville.

TABLE 6: SUSCEPTIBILITY TEST RESULTS OF THE TEN SITES SURVEYED

Insecticide tested	Bocanda	Gagnoa	Jacqueville	Sakassou	Aboisso	Adzopé	Bouaké	Bouna	Daloa	Odienné
	Number tested (% mortality)	Number tested (% mortality)	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	75 (2.7)	79 (5.1)	93 (5.4)	91 (4.4)	100 (4)	84 (9.5)	81 (0)	89 (85.4)	100 (5)	96 (3.1)
PBO + Deltamethrin (0.05)	101 (61.4)	82 (31.7)	82 (9.8)	103 (58.3)	100 (96)	100 (77)	97 (50.5)	89 (100)	100 (20)	94 (69.1)
Deltamethrin (0.25%) 5x	92 (21.7)	72 (8.3)	90 (2.2)	101 (18.8)	100 (28)	96 (25)	95 (24.2)	72 (83.3)	100 (9)	97 (13.4)
Deltamethrin (0.5%) 10x	99 (93.9)	74 (27)	86 (33.7)	107 (63.6)	100 (67)	95 (68.4)	87 (74.7)	x	100 (22)	90 (30)
Permethrin (0.75%) 1x	75 (4)	78 (10.3)	94 (2.1)	91 (1.1)	98 (5.1)	99 (0)	83 (1.2)	80 (72.5)	100 (1)	97 (1)
PBO + Permethrin (0.75%)	101 (7.9)	76 (5.3)	69 (1.4)	101 (5)	100 (47)	91 (0)	89 (3.4)	80 (76.3)	100 (7)	91 (3.3)
Permethrin (3.75%) 5x	97 (51.5)	76 (81.6)	92 (28.3)	98 (74.5)	99 (22.2)	97 (32)	92 (81.5)	75 (94.7)	100 (59)	94 (43.6)
Permethrin (7.5%) 10x	102 (93.1)	x	87 (42.5)	102 (91.4)	100 (21)	100 (60)	91 (86.8)	x	100 (35)	100 (69)
Alpha-cypermethrin (0.05) 1x	104 (15.4)	79 (3.3)	90 (0)	87 (1.1)	94 (0)	94 (2.1)	94 (2.1)	97 (59.8)	100 (5)	93 (0)
PBO + Alpha-cypermethrin (0.05)	97 (63.9)	97 (7.2)	90 (2.2)	95 (22.1)	100 (90)	100 (47)	90 (52.2)	97 (97.9)	100 (25)	91 (24.2)
Alpha-cypermethrin (0.25) 5x	96 (14.6)	x	x	89 (11.2)	73 (5.5)	93 (0)	95 (9.5)	75 (76)	100 (7)	98 (7.1)
Alpha-cypermethrin (0.5) 10x	103 (66)	76 (50)	90 (26.7)	96 (43.8)	76 (2.1)	96 (37.5)	94 (38.3)	x	100 (12)	94 (29.8)
Lambdacyhalothrin (0.05%) 1x	95 (0)	94 (2.1)	90 (0)	99 (7.1)	100 (1)	82 (7.3)	74 (0)	x	97 (8.2)	95 (3.2)
Etofenprox (0.5%) 10x	98 (0)	x	78 (0)	x	100 (1)	89 (0)	85 (0)	x	96 (5.2)	100 (7)
Bendiocarb (0.1%) 1x	98 (55.1)	78 (15.4)	95 (0)	86 (0)	100 (84)	92 (32.6)	93 (19.4)	x	100 (40)	98 (43.9)
Bendiocarb (0.5%) 5x	103 (50.5)	x	x	92 (17.4)	x	x	x	x	x	x
Bendiocarb (1%) 10x	102 (68.6)	68 (82.4)	76 (10.5)	97 (12.4)	x	x	x	x	x	x
Pirimiphos-methyl (0.25%) 1x	92 (56.5)	144 (100)	177 (3.3)	193 (6.2)	100 (87)	90 (100)	82 (23.2)	75 (94.7)	100 (27)	100 (4)
Pirimiphos-methyl (1.25%) 5x	96 (100)	x	91 (83.7)	97 (95.9)	100 (100)	x	95 (100)	x	95 (100)	100 (100)
Pirimiphos methyl (2.5%) 10x	x	x	62 (100)	100 (1000	x	x	x	x	x	x

[x] *represents the uncompleted tests due to limited number of mosquitoes or not needed for intensity assay*

Resistant confirmed *Suspected resistance* *Susceptible*

The results of the CDC bottle assays using chlorfenapyr and WHO susceptibility test using clothianidin are shown in Figures 10-11.

- For all the ten sites where chlorfenapyr was tested, susceptibility was recorded in five sites (Adzopé, Bocanda, Bouna, Daloa and Gagnoa) at the dose of 100 µg/bottles after 72 hours post exposure and seven sites at the dose of 200 µg/bottles. Three sites (Odienne, Jacqueville and Sakassou) remained with 72-hour mortality below 98%.
- For clothianidin, susceptibility was observed after seven days post exposure in seven of the nine sites where tests were completed and including particularly in the four potential IRS sites. However, resistance was observed in Aboisso with 78% mortality and possible resistance in Daloa with 94% mortality after 7 days post-exposure (Figure 12).

For all the figures, the horizontal dashed red line represents the 90% threshold for resistance and the green line represents the 98% threshold for susceptibility.

FIGURE 10: SUSCEPTIBILITY OF *AN. GAMBIAE* S.L. TO CHLORFENAPYR 100µG/BOTTLE PER SITE

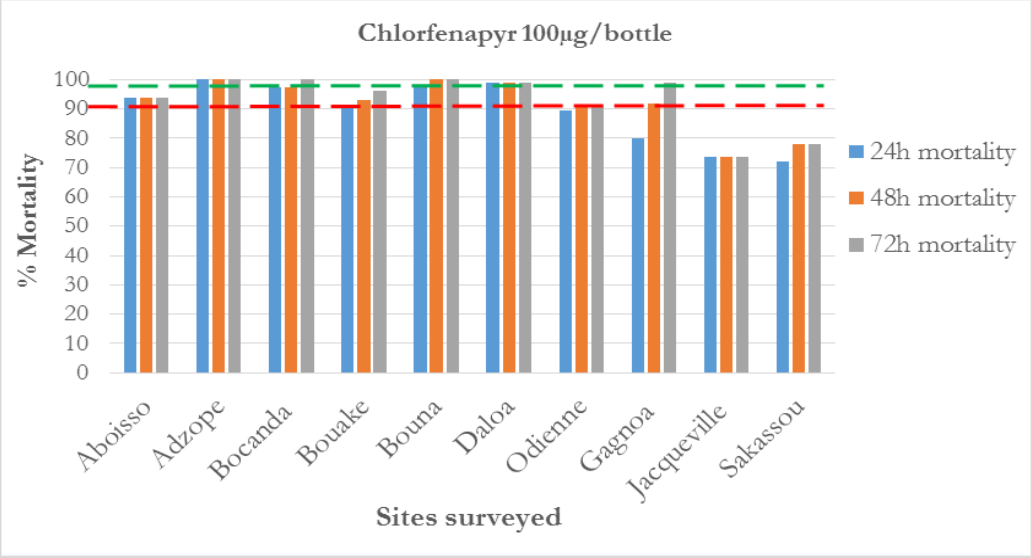


FIGURE 11: SUSCEPTIBILITY OF *AN. GAMBIAE* S.L. TO CHLORFENAPYR 200µG/BOTTLE PER

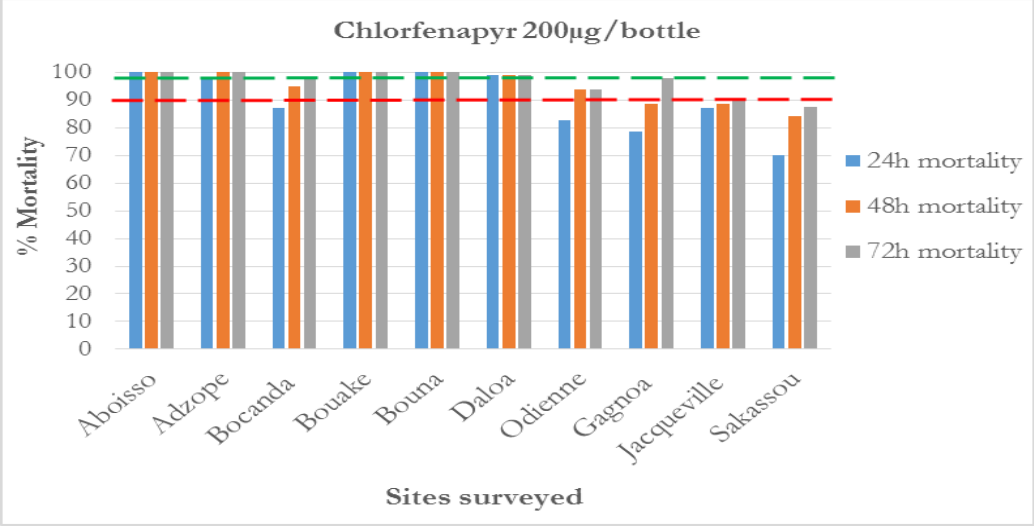
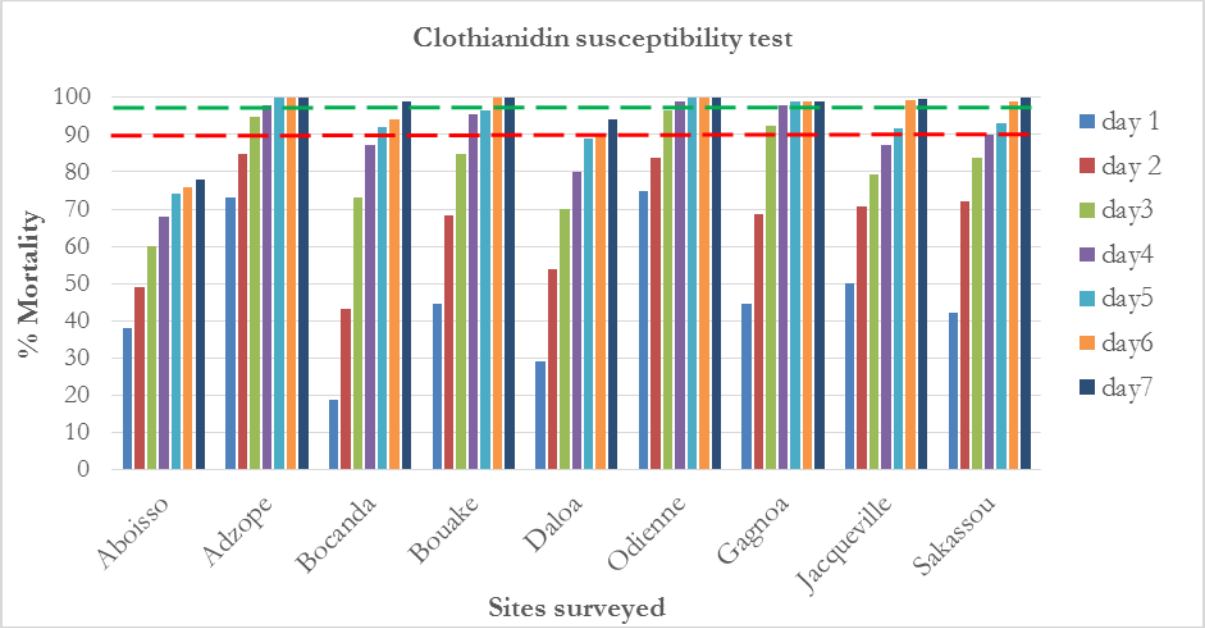


FIGURE 12: SUSCEPTIBILITY OF *AN. GAMBIAE* S.L. TO CLOTHIANIDIN PER SITE



3.3 MOLECULAR MARKERS OF RESISTANCE

Figure 13 (A,B) shows the distribution of Knock-down West, East and *Ace-1* mutations in *An. gambiae sl.* from study sites. Overall, *kdr* -West mutations were highly present (ranged from 71 to 97%) in all study sites. The *kdr*-East mutation was found in all the sites except Bouna, with the highest frequency (52%) in Jacqueville. In other locations, *kdr* -East was present at frequencies that ranged between 6 and 10%. The presence of both *kdr* mutations in the country confirmed the high resistance of the vectors to pyrethroid insecticides and represents a significant threat to pyrethroid-based vector control tools.

Similar to *kdr*, the *ace-1* mutation was found in all sites except Bouna and the frequencies ranged from 16% (in Bouaké) to 83% (in Daloa) confirming the resistance to pirimiphos-methyl and bendiocarb observed in the majority of the sites tested for susceptibility. Apart from Daloa, where the highest *ace-1* frequency was recorded (83%), Aboisso and Sakassou yielded 50% while Jacqueville 52%.

FIGURE 13: DISTRIBUTION OF TARGET SITE MUTATIONS IN *AN. GAMBIAE* S.L USED FOR SUSCEPTIBILITY TESTING IN A) FOUR COMPREHENSIVE MONITORING SITES AND B) SIX INSECTICIDE RESISTANCE SITES IN COTE D'IVOIRE

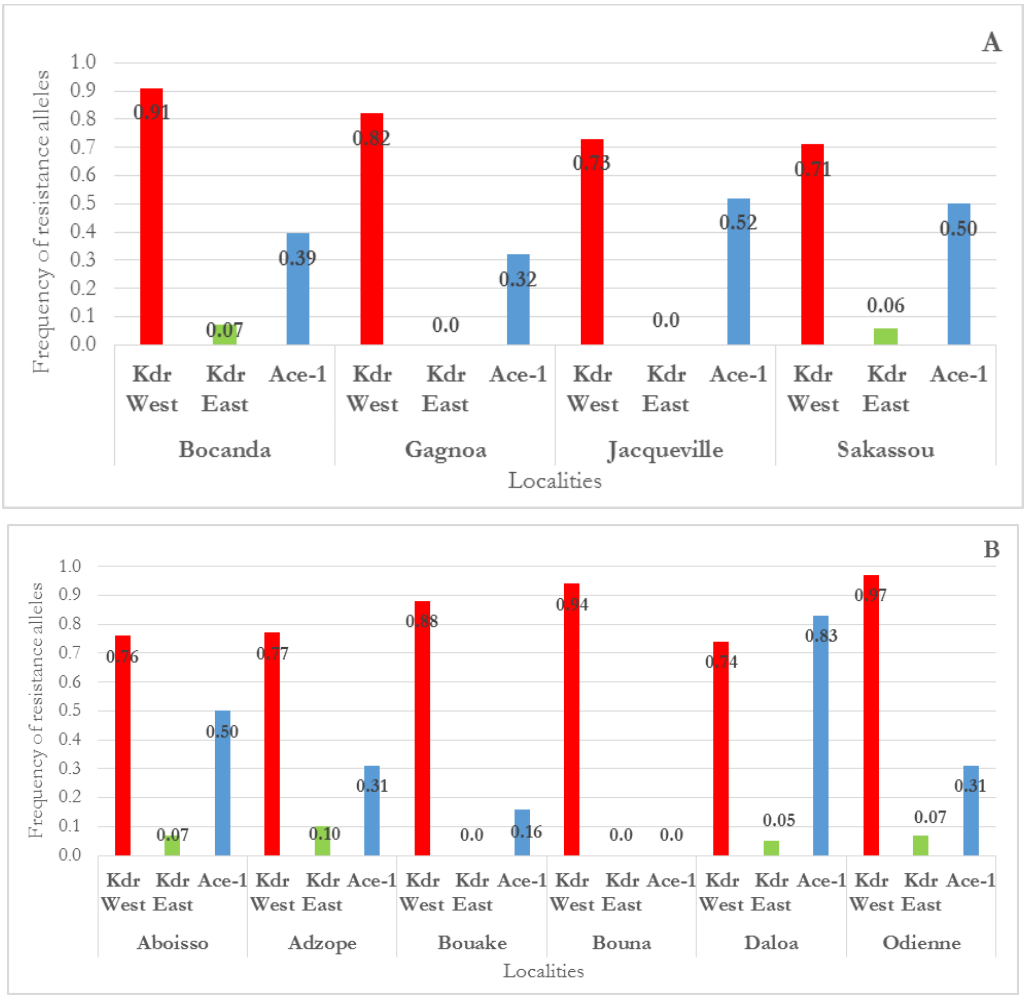
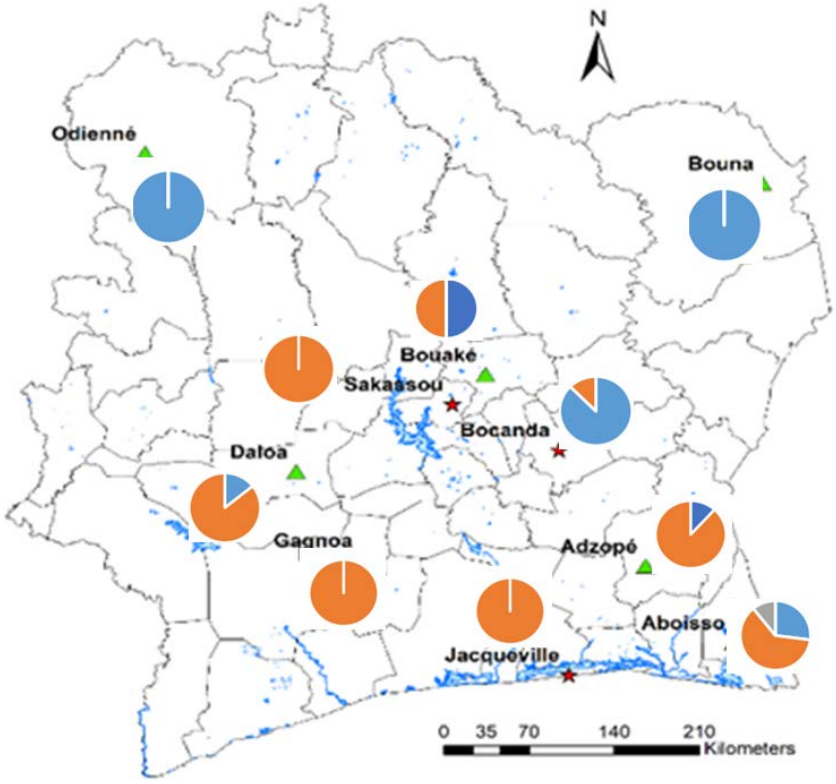


Figure 14 shows species composition of *An. gambiae* s.l. per site. *An. gambiae s.s* represents the predominant species in the North (Odienné and Bouna), while *An. coluzzii* is mainly found in the South West (Gagnoa) and Centre West (Sakassou). Overall, *An. coluzzii* were predominant in six out of 10 districts, including Aboisso, Adzopé, Daloa, Gagnoa, Jacqueville and Sakassou. *An. gambiae s.s* were predominant in Bocanda, Bouna and Odienné. Co-occurrence of both species is detected in Bouaké (50% respectively). Few hybrids (11%) of both species (*An. gambiae*/*An. coluzzii*) were recorded only in Aboisso. In general, both species were sympatric in five districts (Figure 14).

FIGURE 14: PROPORTION OF *AN. GAMBIAE* S.L. ACROSS 10 DISTRICTS



● *An. gambiae* s.s. ● *An. coluzzii* ● Hybrid *An.gambiae* s.s./*An. coluzzii*
▲ Insecticide resistance monitoring sites ★ Vector surveillance sites

4. CONCLUSION

Bionomics data collected during five months in the four potential IRS sites showed that *An. gambiae s.l.,* was the predominant malaria vector in all the sites using human landing catch (HLC), pyrethrum spray catch (PSC) and CDC light traps (CDC-LT) methods. Out of the 26,049 mosquitoes collected, HLCs produced the highest numbers and particularly, Sakassou recorded the highest density among the four sites followed by Gagnoa. Both sites are irrigated rice field areas and the availability of favorable and permanent larvae habitat conditions has contributed to the large number of vectors collected

All vector surveillance data collections were completed in two settings (rural and urban) within each district to better understand the impact of the neighbourhood's structures on the densities and populations of malaria vectors. Thus, the results of HLCs showed that *An. gambiae* s.l. biting rate was higher in urban area in Bocanda and Gagnoa both outdoors and indoors while the trends were opposite in Jacqueville and Sakassou recording the highest densities in rural area than in urban, both outdoors and indoors. Sakassou recorded particularly the highest vector population density with 11,167 *An. gambiae* s.l. and 6 *An. funestus* collected over the five months with and average human biting rate (HBR) of 140 bites/person/night. This proportion of vectors collected in Sakassou represents about 93% of the total malaria vectors collected in the four sites using HLCs. This, combined with having the highest reported malaria incidence in Cote d'Ivoire, makes Sakassou a top candidate for IRS.

The longevity of the mosquitoes represents an important parameter to consider for IRS implementation in a district. For the four sites surveyed, the overall parity rate was high and above 60% in all the sites. Furthermore, the parity rate was similar for mosquitoes collected indoors and outdoors in each district. The similarity observed within indoor and outdoor parity rates will be a good parameter of comparison after IRS is conducted.

High sporozoite rates were recorded in all the four vector surveillance sites with Sakassou yielding the highest monthly infection rate in November 2018. Furthermore, the average entomological inoculation rate (EIR) varied from 0.064 in Bocanda to 1.482 in Sakassou. This entomological finding supports epidemiological reports indicating higher malaria prevalence within the population of Sakassou compared to all the districts of the country. Per NMCP report, Sakassou represents the district of highest malaria incidence within the country since 2015. Therefore, malaria control strategies need to be carefully managed in Sakassou, as elsewhere, in order to reduce malaria cases.

For the CDC light trap and PSC collections, higher densities were recorded in the rural settings than urban. Similar to the HLC collection method, Sakassou provided the majority of the mosquitoes collected. The PSC method is particularly important while aiming for IRS. The densities collected in the different sites could support any decision making for IRS implementation in addition to the HLC data. This will be a good parameter of comparison for any change on vector biting behavior such as outdoor biting after IRS is conducted.

The resistance of *An. gambiae s.l.* mosquitoes to pyrethroids was observed in all ten sites surveyed. Moreover, high resistance was recorded in all sites where resistance intensity assays were

completed. Furthermore, the pre-exposure of the mosquitoes to the synergist PBO before deltamethrin, permethrin and alpha-cypermethrin did not yield full susceptibility but induced significant increment of mortality in all sites for deltamethrin than alpha-cypermethrin and permethrin respectively except in Daloa, Gagona and Jacqueville. This shows that enzymes such as P450s may be involved in the insecticide resistance of the vectors in some sites and that need to be taken into consideration before the distribution of PBO based LLINs in the country.

Resistance was also observed to pirimiphos methyl and bendiocarb in all sites surveyed, except in Gagnoa. For clothianidin, the tests completed in 9 sites showed susceptibility in 7 sites and particularly all the IRS potential sites recorded full susceptibility to the insecticide. This is important as far as the country is embarking in the implementation of IRS as an additional vector control strategy. In a prospect of extending IRS to other district where malaria prevalence would be increasing, the current data would guide insecticide selection with a passible rotation of pirimiphos methyl and clothianidin in Gagnoa.

The results of the CDC bottle assays using chlorfenapyr at 100 µg/bottle and 200 µg/bottle doses showed susceptibility of *An. gambiae* s.l. mosquitoes from seven out of the ten sites surveyed (Adzopé, Bocanda, Gagnoa, Daloa, Bouna, Aboisso and Bouaké). Odienné, Jacqueville and Sakassou were still recording survivals at 200ug/bottle after 72-hour post exposure. Based on these results, chlorfenapyr-based vector control tool like the Interceptor G2, could be recommended in a stratified distribution for malaria vector control and resistance management in Cote d'Ivoire while the probable resistance observed in the three localities needs to be confirmed.

The *An. gambiae* s.l. population of the country observed from mosquitoes reared from larval collections, includes mainly *An. coluzzii* in the large majority around the southern part of the country and *An. gambiae* s.s. predominant in the North, but both species living in sympatry. Cote d'Ivoire is a country with economic growth relying mostly on agricultural activities and the intense use of insecticides and herbicides in a threat to vector control and insecticide resistance management. As a result, *kdr*-West mutation has been found in all the sites, and at a very high frequency (ranged from 71 to 97%) and *kdr*-East in six of the ten sites. Additionally, the *ace-1* mutation is a marker for carbamate and organophosphate resistance, occurs concomitantly with all *kdr* mutations in some study districts. All of these findings impact greatly the vector control strategies and insecticide resistance management plans that the country could undertake.

ANNEX

TABLE 7: DISTRIBUTION OF TOTAL *ANOPHELES GAMBIAE* COLLECTED IN THE DIFFERENT SITE SETTINGS

Collection methods / Sites		18-Nov				18-Dec				19-Jan				19-Feb				19-Mar				Total			
		Rural		Urban		Rural		Urban		Rural		Urban		Rural		Urban		Rural		Urban		Rural		Urban	
		In	Out	In	Out	In	Out	In	Out	In	Out	In	Out	In	Out	In	Out	In	Out	In	Out	In	Out	In	Out
HLC	Bocanda	3	2	13	14	0	0	13	7	1	2	4	8	7	5	1	7	1	3	0	1	12	12	31	37
	Gagnoa	107	135	81	251	24	92	52	169	46	104	114	195	50	255	67	130	50	166	23	101	277	752	337	846
	Jacqueville	44	47	0	0	77	57	2	2	64	48	0	0	88	100	34	13	132	118	10	17	405	370	46	32
	Sakassou	725	954	440	529	636	643	258	285	607	778	179	225	715	670	138	188	1024	1145	422	606	3707	4190	1437	1833
PSC	Bocanda	0		0		0		1		0		0		0		0		0		0		0		1	
	Gagnoa	6		10		27		23		0		0		0		0		106		28		139		61	
	Jacqueville	0		0		4		1		0		0		0		0		0		0		4		1	
	Sakassou	297		68		260		21		399		149		259		37		9		3		1224		278	
CDC-LT	Bocanda	1		9		0		0		1		3		0		0		1		0		3		12	
	Gagnoa	1		4		0		1		9		5		68		3		211		4		289		17	
	Jacqueville	1		0		0		1		0		2		0		1		2		0		3		4	
	Sakassou	67		59		260		14		318		44		77		2		509		16		1231		135	

TABLE 8: SPECIES COMPOSITION OF MOSQUITOES COLLECTED USING HLC

District	Species	November 2018	December 2018	January 2019	February 2019	March 2019	Total
		N (%)	N (%)	N (%)	N (%)	N (%)	N (%)
Bocanda	*An. gambiae* s.l.	32 (28.3)	20 (16.1)	15 (16.7)	20 (9.2)	5 (4.2)	92 (13.9)
	An. funestus	7 (6.2)	3 (2.4)	0 (0)	0 (0)	0 (0)	10 (1.5)
	An. pharoensis	2 (1.8)	0 (0)	0 (0)	0 (0)	0 (0)	2 (0.3)
	Culicine	72 (63.7)	101 (81.5)	75 (83.3)	198 (90.8)	113 (95.8)	559 (84.3)
Total		113 (100)	124 (100)	90 (100)	218 (100)	118 (100)	663 (100)
Gagnoa	*An. gambiae* s.l.	574 (84.2)	337 (72.6)	459 (69.3)	502 (67.3)	340 (55.5)	2212 (69.8)
	An. pharoensis	8 (1.2)	19 (4.1)	16 (2.4)	5 (0.7)	0 (0)	48 (1.5)
	Culicine	100 (14.7)	108 (23.3)	187 (28.2)	239 (32)	273 (44.5)	907 (28.6)
Total		682 (100)	464 (100)	662 (100)	746 (100)	613 (100)	3167 (100)
Jacqueville	*An. gambiae* s.l.	91 (22.2)	138 (17.9)	112 (16.9)	235 (31.5)	277 (45.2)	853 (26.9)
	An. pharoensis	0 (0)	0 (0)	1 (0.2)	0 (0)	2 (0.3)	3 (0.1)
	Culicine	319 (77.8)	631 (82.1)	586 (88.5)	606 (81.2)	0 (0)	2142 (67.6)
Total		410 (100)	769 (100)	699 (100)	841 (100)	279 (100)	2998 (100)
Sakassou	*An. gambiae* s.l.	2648 (92.2)	1822 (96.2)	1789 (89.2)	1711 (96.3)	3197 (91.3)	11167 (92.7)
	An. funestus	3 (0.1)	3 (0.2)	0 (0)	0 (0)	0 (0)	6 (0.05)
	An. pharoensis	83 (2.9)	29 (1.5)	57 (2.8)	32 (1.8)	67 (1.9)	268 (2.2)
	Culicine	137 (4.8)	39 (2.1)	160 (8.0)	34 (1.9)	236 (6.7)	606 (5.0)
Total		2871 (100)	1893 (100)	2006 (100)	1777 (100)	3500 (100)	12047 (100)

TABLE 9: SPECIES COMPOSITION OF MOSQUITOES COLLECTED USING PSC

District	Species	November 2018	December 2018	January 2019	February 2019	March 2019	Total
		N (%)	N (%)	N (%)	N (%)	N (%)	N (%)
Bocanda	*An. gambiae* s.l.	0 (0)	1 (3.7)	24 (10.5)	25 (11.2)	12 (3.0)	62 (7.1)
	Culicine	0 (0)	26 (96.3)	0	0	0	26 (96.3)
	Total	0 (0)	27 (100)	0	0	0	27 (100)
Gagnoa	*An. gambiae* s.l.	16 (57.1)	50 (100)	0	0	134 (44.7)	200 (52.9)
	Culicine	12 (42.9)	0 (0.0)	0	0	166 (55.3)	178 (47.1)
	Total	28 (100)	50 (100)	0	0	300 (100)	378 (100)
Jacqueville	*An. gambiae* s.l.	0 (0)	5 (6.5)	0	0	0	5 (3.8)
	Culicine	54 (100)	72 (93.5)	0	0	0	126 (96.2)
	Total	54 (100)	77 (100)	0	0	0	131 (100)
Sakassou	*An. gambiae* s.l.	365 (99.5)	281 (99.3)	548 (48.4)	296 (44.9)	12 (0.9)	1502 (39.7)
	Culicine	2 (0.5)	2 (0.7)	585 (51.6)	363 (55.1)	1330 (99.1)	2282 (60.3)
	Total	367 (100)	283 (100)	1133 (100)	659 (100)	1342 (100)	3784 (100)

TABLE 10: SPECIES COMPOSITION OF MOSQUITOES COLLECTED USING CDC-LT

District	Species	November 2018	December 2018	January 2019	February 2019	March 2019	Total
		N (%)	N (%)	N (%)	N (%)	N (%)	N (%)
Bocanda	*An. gambiae* s.l.	10 (34.5)	0 (0)	4 (1.8)	0 (0)	1 (1.1)	15 (2.7)
	Culicine	19 (65.5)	159 (100)	241 (98.4)	23 (100)	92 (98.9)	534 (97.3)
Total		29 (100)	159 (100)	245 (100)	23 (100)	93 (100)	549 (100)
Gagnoa	*An. gambiae* s.l.	5 (14.7)	1 (4.5)	14 (21.5)	71 (65.7)	215 (96.0)	306 (67.5)
	Culicine	29 (85.3)	21 (95.5)	51 (78.5)	37 (34.3)	9 (4.0)	147 (32.5)
Total		34 (100)	22 (100)	65 (100)	108 (100)	224 (100)	453 (100)
Jacqueville	*An. gambiae* s.l.	1 (1.1)	1 (0.6)	2 (50)	1 (6.3)	2 (4.2)	7 (2.1)
	Culicine	94 (98.9)	170 (99.4)	2 (50)	15 (93.8)	46 (95.8)	327 (97.9)
Total		95 (100)	171 (100)	4 (100)	16 (100)	48 (100)	334 (100)
Sakassou	*An. gambiae* s.l.	126 (85.1)	274 (88.4)	362 (88.7)	79 (83.2)	525 (94.3)	1366 (90.0)
	Culicine	22 (14.9)	36 (11.6)	46 (11.3)	16 (16.8)	32 (5.7)	152 (10)
Total		148 (100)	310 (100)	408 (100)	95 (100)	557 (100)	1518 (100)

TABLE 11: MONTHLY BITING RATE RESULTS USING HLC

District	Month	Position	Bite Rate	Lower 95% limit Confidence intervals	Upper 95% limit Confidence intervals
Bocanda	November 2018	Indoor	1.0	0.0	2.0
Gagnoa	November 2018	Indoor	11.8	8.3	15.2
Jacqueville	November 2018	Indoor	2.8	1.1	4.4
Sakassou	November 2018	Indoor	72.8	64.3	81.3
Bocanda	December 2018	Indoor	0.8	-0.1	1.7
Gagnoa	December 2018	Indoor	4.8	2.6	6.9
Jacqueville	December 2018	Indoor	4.9	2.7	7.2
Sakassou	December 2018	Indoor	55.9	48.4	63.3
Bocanda	January 2019	Indoor	0.3	-0.2	0.9
Gagnoa	January 2019	Indoor	10.0	6.8	13.2
Jacqueville	January 2019	Indoor	4.0	2.0	6.0
Sakassou	January 2019	Indoor	49.1	42.1	56.1
Bocanda	February 2019	Indoor	0.5	-0.2	1.2
Gagnoa	February 2019	Indoor	7.3	4.6	10.0
Jacqueville	February 2019	Indoor	7.6	4.9	10.4
Sakassou	February 2019	Indoor	53.3	46.0	60.6
Bocanda	March 2019	Indoor	0.1	-0.2	0.3
Gagnoa	March 2019	Indoor	4.6	2.4	6.7
Jacqueville	March 2019	Indoor	8.9	5.9	11.9
Sakassou	March 2019	Indoor	90.4	80.9	99.9
Bocanda	November 2018	Outdoor	1.0	0.0	2.0
Gagnoa	November 2018	Outdoor	24.1	19.2	29.0
Jacqueville	November 2018	Outdoor	2.9	1.2	4.7
Sakassou	November 2018	Outdoor	92.7	83.1	102.3
Bocanda	December 2018	Outdoor	0.4	-0.2	1.1
Gagnoa	December 2018	Outdoor	16.3	12.3	20.4
Jacqueville	December 2018	Outdoor	3.7	1.8	5.6
Sakassou	December 2018	Outdoor	58.0	50.4	65.6
Bocanda	January 2019	Outdoor	0.6	-0.2	1.4
Gagnoa	January 2019	Outdoor	18.7	14.4	23.0
Jacqueville	January 2019	Outdoor	3.0	1.3	4.7
Sakassou	January 2019	Outdoor	62.7	54.8	70.6
Bocanda	February 2019	Outdoor	0.8	-0.1	1.6
Gagnoa	February 2019	Outdoor	24.1	19.2	29.0
Jacqueville	February 2019	Outdoor	7.1	4.4	9.7
Sakassou	February 2019	Outdoor	53.6	46.3	60.9
Bocanda	March 2019	Outdoor	0.3	-0.3	0.8
Gagnoa	March 2019	Outdoor	16.7	12.6	20.8
Jacqueville	March 2019	Outdoor	8.4	5.5	11.3
Sakassou	March 2019	Outdoor	109.4	99.0	119.9

TABLE 12: BITING BEHAVIOR OF *AN. GAMBIAE* S.L. PER SITE

District		November 2018	December 2018	January 2019	February 2019	March 2019	Total
Bocanda	In N (%Endo)	16 (50)	13 (65)	5 (33.3)	8 (40)	1 (20)	43 (46.7)
	Out N (%Exo)	16 (50)	7 (35)	10 (66.7)	12 (60)	4 (80)	49 (53.3)
	Total	32	20	15	20	5	92
Gagnoa	In N (%Endo)	188 (32.8)	76 (22.6)	160 (34.9)	117 (23.3)	73 (21.5)	614 (27.8)
	Out N (%Exo)	386 (67.2)	261 (77.4)	299 (65.1)	385 (76.7)	267 (78.5)	1598 (72.2)
	Total	574	337	459	502	340	2212
Jacqueville	In N (%Endo)	44 (48.4)	79 (57.2)	64 (57.1)	122 (51.9)	142 (51.3)	451 (52.9)
	Out N (%Exo)	575 (51.6)	59 (42.8)	48 (42.9)	113 (48.1)	135 (48.7)	402 (47.1)
	Total	91	138	112	235	277	853
Sakassou	In N (%Endo)	1165 (44)	894 (49.1)	786 (43.9)	853 (49.9)	1446 (45.2)	5144 (46.1)
	Out N (%Exo)	47 (56.0)	928 (50.9)	1003 (56.1)	858 (50.1)	1751 (54.8)	6023 (53.9)
	Total	2648	1822	1789	1711	3197	11167
TOTAL	In N (%Endo)	1413 (42.2)	1062 (45.8)	1015 (42.7)	1100 (44.6)	1662 (43.5)	6252 (43.6)
	Out N (%Exo)	1932 (57.8)	1255 (54.2)	1360 (57.3)	1368 (55.4)	2157 (56.5)	8072 (56.4)
	Total N	3345	2317	2375	2468	3819	14324

Endo: endophagy rate; exo: exophagy rate; In: Indoor; Out: Outdoor

TABLE 13: MONTHLY PARITY RATES OF DISSECTED MOSQUITOES PER SITE

District	Month	Dissected	Number of parous	Parity rate (%)
Bocanda	November 2018	31	27	87.1
	December 2018	20	18	90.0
	January 2019	14	13	92.9
	February 2019	20	13	65.0
	March 2019	5	5	100.0
	Total	90	76	84.4
Gagnoa	November 2018	462	227	49.1
	December 2018	323	300	92.9
	January 2019	326	107	32.8
	February 2019	351	342	97.4
	March 2019	136	94	69.1
	Total	1598	1070	67.0
Jacqueville	November 2018	88	88	100.0
	December 2018	132	46	34.8
	January 2019	110	104	94.5
	February 2019	231	166	71.9
	March 2019	274	264	96.4
	Total	835	668	80.0
Sakassou	November 2018	530	390	73.6
	December 2018	390	212	54.4
	January 2019	431	218	50.6
	February 2019	407	297	73.0
	March 2019	428	232	54.2
	Total	2186	1349	61.7
Overall		4709	3163	67.2

TABLE 14: MONTHLY PARITY RATE OF MOSQUITOES DISSECTED FROM INDOOR AND OUTDOOR COLLECTIONS

District	Month	Position	Dissected	Number parous (Parous rate %)	Lower 95% limit Confidence intervals	Upper 5% limit Confidence intervals
Bocanda	November 2018	Indoor	16	15 (93.8)	70	99
Gagnoa	November 2018	Indoor	165	94 (57)	49	65
Jacqueville	November 2018	Indoor	42	42 (100)	92	100
Sakassou	November 2018	Indoor	240	185 (77.1)	71	82
Bocanda	December 2018	Indoor	14	12 (85.7)	57	98
Gagnoa	December 2018	Indoor	69	66 (95.6)	88	99
Jacqueville	December 2018	Indoor	77	17 (22.1)	13	33
Sakassou	December 2018	Indoor	188	102 (54.3)	47	62
Bocanda	January 2019	Indoor	5	4 (80)	28	99
Gagnoa	January 2019	Indoor	127	38 (29.9)	22	39
Jacqueville	January 2019	Indoor	64	58 (90.6)	81	96
Sakassou	January 2019	Indoor	216	108 (50)	43	57
Bocanda	February 2019	Indoor	8	7 (87.5)	47	100
Gagnoa	February 2019	Indoor	90	88 (97.8)	92	100
Jacqueville	February 2019	Indoor	119	86 (72.3)	63	80
Sakassou	February 2019	Indoor	197	154 (78.2)	72	84
Bocanda	March 2019	Indoor	1	1 (100)	2.5	100
Gagnoa	March 2019	Indoor	31	20 (64.5)	45	81
Jacqueville	March 2019	Indoor	144	141 (97.9)	94	100
Sakassou	March 2019	Indoor	217	103 (47.5)	41	54
Bocanda	November 2018	Outdoor	15	12 (80)	52	96
Gagnoa	November 2018	Outdoor	297	133 (44.8)	39	51
Jacqueville	November 2018	Outdoor	46	46 (100)	92	100
Sakassou	November 2018	Outdoor	290	205 (70.7)	65	76
Bocanda	December 2018	Outdoor	6	6 (100)	54	100
Gagnoa	December 2018	Outdoor	254	234 (92.1)	88	95
Jacqueville	December 2018	Outdoor	55	29 (52.7)	39	66
Sakassou	December 2018	Outdoor	202	110 (54.5)	47	62
Bocanda	January 2019	Outdoor	9	9 (100)	66	100
Gagnoa	January 2019	Outdoor	199	69 (34.7)	28	42
Jacqueville	January 2019	Outdoor	46	46 (100)	92	100
Sakassou	January 2019	Outdoor	215	110 (51.2)	44	58
Bocanda	February 2019	Outdoor	12	6 (50)	21	79
Gagnoa	February 2019	Outdoor	261	254 (97.3)	95	99
Jacqueville	February 2019	Outdoor	112	80 (71.4)	62	80
Sakassou	February 2019	Outdoor	210	143 (68.1)	61	74
Bocanda	March 2019	Outdoor	4	4 (100)	40	100
Gagnoa	March 2019	Outdoor	105	74 (70.5)	61	79
Jacqueville	March 2019	Outdoor	130	123 (94.6)	89	98
Sakassou	March 2019	Outdoor	211	129 (61.1)	54	68

TABLE 15: RURAL AND URBAN PARITY RATES OF *AN. GAMBIAE* S.L. USING HLC

	Rural			Urban				
		# Parous	% Parous	# Dissected	# Parous	% Parous	Total Parous	% Parous
Bocanda	56	50	89.3	55	31	56.4	81	73.0
Gagnoa	668	488	73.1	930	582	62.6	1070	67.0
Jacqueville	762	605	79.4	73	63	86.3	668	80.0
Sakassou	1170	719	61.5	1018	632	62.1	1351	61.7

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