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SENEGAL 2018 FINAL ENTOMOLOGICAL MONITORING REPORT

JANUARY 1, 2018 – FEBRUARY 28, 2019

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Acronyms

AIRS	Africa Indoor Residual Spraying Project
CDC	Centers for Disease Control and Prevention
CSP	Circumsporozoite
EIR	Entomological Inoculation Rate
ELISA	Enzyme-Linked Immuno-Sorbent Assay
HBR	Human Biting Rate
HLC	Human Landing Catch
IRD	Indoor Resting Density
IRM	Insecticide Resistance Monitoring
IRS	Indoor Residual Spraying
kdr	Knock Down Resistance
LEVP	Laboratory of Vector and Parasite Ecology
LLIN	Long-lasting Insecticidal Nets
NMCP	National Malaria Control Program
PBO	Piperonyl Butoxide
PCR	Polymerase Chain Reaction
PMI	President's Malaria Initiative
PSC	Pyrethrum Spray Catch
SI	Sporozoite Index
WHO	World Health Organization

EXECUTIVE SUMMARY

This report presents entomological surveillance data that the PMI VectorLink project in Senegal, in collaboration with the country's National Malaria Control Program (NMCP), conducted from January 2018 through February 2019. The NMCP will use the findings to inform its malaria vector control decisions.

An. gambiae s.l. was the main vector collected, followed by *An. funestus* – specifically *An. funestus* s.s. – which was found in a limited area. *An. gambiae* s.s. was the main vector in the southeast, whereas *An. arabiensis* was predominant in the north and center regions. *An. coluzzii* was recorded at the lowest density but was sympatric with the two other species of the complex.

Collection of *An. gambiae* s.l. peaked from August to October. Overall, females were found more endophagic than exophagic; however, resting densities inside the houses were higher in the Sudanese area and lower in the Sudano-Guinean area. No difference was observed in the exophagic and endophagic behaviors of *An. funestus* except in the Sudano-Guinean area, where the anthropophilic rate was low – vectors were mainly blood feeding on horses and cows. Infective females were found in all areas except in the north (Sahelian), and the highest entomological inoculation rate was recorded in Sudano-Guinean area.

Vector density and behavior was similar in former IRS districts and control sites. In former IRS districts, the parity rate and sporozoite rate have increased and become as high as in the control districts.

An. gambiae s.l. was resistant to all three pyrethroid insecticides tested in the majority of the districts and to bendiocarb in the Dakar suburbs and Diourbel District. The Dakar suburbs were the only sites where *An. gambiae* s.l. showed resistance to pirimiphos-methyl. In Kédougou District, it showed high resistance to the three pyrethroid insecticides tested (deltamethrin, permethrin, and alpha-cypermethrin). Pre-exposure to the synergist piperonyl butoxide significantly increased its susceptibility to deltamethrin and permethrin. Susceptibility to clothianidin was observed in all the sites surveyed. Susceptibility also was recorded for chlorfenapyr in all sites except in the Dakar suburbs, especially at the dose of 200 µg/bottle.

Both mutations *L1014S* (*kdr-east*) and *L1014F* (*kdr-west*) were present in all the sentinel districts. The co-occurrence of the two mutations in the same individual was noted in all areas except in the south, with the highest frequencies in urban areas. *Ace-1R* (*G119S*) mutation was found in all study sites, and mostly within the heterozygous allele (RS). Allelic frequencies were relatively low, except in urban areas and in the southeast. The presence of both *kdr* and *Ace-1R* mutations was found in *An. arabiensis*, *An. gambiae*, and *An. coluzzii*. In Diourbel, the potential breeding sites monitored from July 2018 to January 2019 showed that the ravine with permanent water in the Keur Serigne Mbaye Sarr area was responsible for the presence of *An. gambiae* s.l. during the dry season.

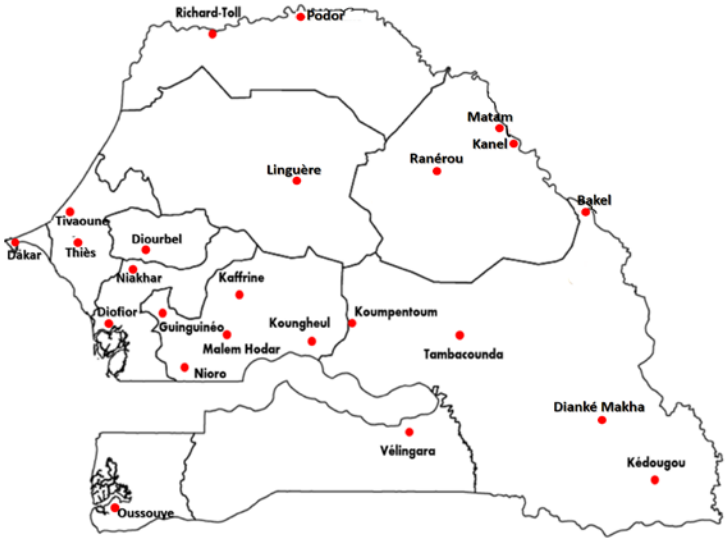
1. Introduction

In 2007, the U.S. President's Malaria Initiative (PMI) /Senegal, in collaboration with the National Malaria Control Program (NMCP), contracted the Laboratory of Vector and Parasite Ecology (LEVP) in the Faculty of Science and Technology at the University Cheikh Anta Diop in Dakar to implement entomological surveillance activities in Senegal. In 2015, PMI made the PMI African Indoor Residual Spraying (AIRS) project responsible for entomological monitoring, and the project executed a contract directly with LEVP to continue the surveillance activities. In 2017, the PMI AIRS project transitioned to the five-year PMI VectorLink project, which is currently implementing vector control activities in up to 23 African countries, including Senegal.

Over the same period, PMI supported the NMCP's Indoor Residual Spraying (IRS) program. IRS implementation began as a pilot in three health districts (Velingara, Nioro, and Richard Toll) in 2007. With the transition to the PMI AIRS project in 2015, IRS was targeted to districts where health posts reported high malaria incidence (> 15 cases/ 1,000 inhabitants). In 2018, as PMI VectorLink began, the NMCP decided to discontinue PMI-funded IRS, and instead focus PMI support on entomological surveillance, including the monitoring of malaria vectors' resistance to various insecticides, and evaluation of vector density and of sporozoite infection rates in the areas after withdrawal of IRS.

In collaboration with the NMCP, LEVP was selected to lead the implementation of the activities, in collaboration with the Research Institute for Development, Institute Pasteur, and Anti Parasite Control Service. In 2018 and early 2019, PMI VectorLink Senegal conducted routine entomological surveillance in 24 sites located throughout the country (Figure 1) and insecticide resistance monitoring (IRM) in 15 of the sites to provide data to inform malaria vector control decisions for the country. Entomological surveillance was done monthly at routine surveillance sites and periodically at others, using human landing catches (HLCs) and pyrethrum spray catches (PSCs) to collect mosquitoes to identify vector composition, density, and behavior. In the 15 sites selected for IRM activities, VectorLink assessed *An. gambiae* s.l. susceptibility to selected insecticides at diagnostic doses, intensity of any confirmed resistance, and susceptibility/resistance to insecticide when the synergist piperonyl butoxide (PBO) was used.

Figure 1. Geographical Locations of Districts with Entomological Monitoring



The main objectives of VectorLink Senegal entomological surveillance activities were to:

- Assess *anopheline* vector bionomics, including species composition, density, and behavior in 24 sites including former IRS sites and control sites;
- Determine insecticide susceptibility of *An. gambiae* s.l., the primary local malaria vector, to selected insecticides used for the impregnation long-lasting insecticidal nets (LLINs) and IRS in 15 sites across the country;
- Determine the sporozoite infection rates of vector mosquitoes collected during routine surveillance; and
- Identify the blood meal source of the *Anopheles* vectors.

This report presents the results of entomological surveillance conducted from January 2018 through February 2019.

2. METHODOLOGY

2.1 DISTRICTS AND SENTINEL VILLAGES

Sentinel sites for entomological surveillance were in former IRS intervention and control areas, and in other selected sites (Table 1). Two sentinel sites in areas with high malaria burden were selected in each of the four IRS districts (Nioro, Koumpentoum, Koungheul, and Malem Hodar). One sentinel site was selected in the control districts of Kaffrine and Tambacounda). Ndoffane, the other control district, received two sentinel sites; the NMCP recommended the additional site be located in the Keur Socé health post area, where the NMCP and the University Cheikh Anta Diop have monitored parasitological and clinical indicators for over 10 years. Geographical coordinates available for IRS and control sites in Annex A. Data on species composition, vector abundance, vector behavior, and vector susceptibility were collected at all sentinel sites.

TABLE 1: SENTINEL SITES SELECTED FOR ENTOMOLOGY SURVEILLANCE, 2018

District	Sentinel Site	Entomological Activity	Frequency	Former IRS and Control Districts
Sahalian				
Podor	Ndiayène Pendao and Niandane	HLC, PSC	Once or twice in rainy season	
Richard Toll*	Mbagame, Rosso Béthio, Ndiandiou, Maka Diama, Taba Darou Salam, Mallé, Gnith, Ronkh, Khor, and Reynabé	HLC, PSC		
Sudano-Sahelian				
Thies**	Thiaye and Beer	HLC, PSC, and IRM	Every two months	
Tivaouane**	Ngadiaga, Ndiambalo, and Touba Taw Fekh	HLC, PSC, and IRM		
Linguere	Barkedji and Ouarkhokh	HLC, PSC	Once or twice in rainy season	
Ranerou	Oudalaye and Fourdou	HLC, PSC		
Matam	Sadel and Nabadji Ciwol	HLC, PSC		
Kanel	Haouré and Dembankané	HLC, PSC		
Bakel	Gabou and Moudéry	HLC, PSC		
Diourbel	Keur Serigne Mbaye Sarr and Keur Cheikh Anta	HLC, PSC, and IRM	Monthly	
Dakar Suburbs	Pikine, Guédiawaye, Mbao, and Rufisque	IRM	Monthly	
Sudanese				
Nioro	Bamba Diakhatou and Ndramé Ndimb	HLC, PSC, and IRM	Monthly	X
Ndoffane	Tawa Mboudaye and Sagnanème	HLC, PSC, and IRM	Monthly	X (Control)
Koumpentoum	Village 1 and Kouthiaba	HLC, PSC, and IRM	Monthly	X
Tambacounda	Koussanar	HLC, PSC, and IRM	Monthly	X (Control)
Koungheul	Ida Mouride and Pakala	HLC, PSC, and IRM	Monthly	X
Kaffrine	Thiamène Kathiote	HLC, PSC, and IRM	Monthly	X (Control)
Malem Hodar	Maka Belal and Tip Saloum	HLC, PSC, and IRM	Monthly	X
Diofior	Simal and Palmarin Diakahnor	HLC, PSC, and IRM	Every two months	
Niakhar***	Toucar and Kothiokh	HLC, PSC	Twice in rainy season and once in dry season	
Sudano-Guinean				
Dianke Makha	Gouta and Soutouta	HLC, PSC, and IRM	Monthly	
Kédougou	Tomboronkoto and Bandafassi	HLC, PSC, and IRM	Monthly	
Vélingara	Madina Dianguet and Bonkonto	HLC, PSC, and IRM	Monthly	
Oussouye***	Mlomp and Kajilon	HLC, PSC	Every two months	

* In Richard Toll, HLC was carried out in two sites (Ross Bethio and Maka Diama) and PSC in all nine sentinel sites.
**These two districts are in Niayes, an ecological area where the water table outcrops and the main economic activities of people are market gardens. PSC was conducted in all sites except Thiaye and Ndiambalo.
***Entomological activities in these districts are supported by the Global Fund, but are included in this report at the request of the national-level NMCP.

Diourbel District in central Senegal (Sudano-Sahelian zone) is a particular area of concern for malaria control. According to the NMCP annual epidemiological bulletin, in 2016, malaria incidence in Diourbel was among the highest in the country, more than 25 cases per 1,000 inhabitants. In 2017, the district had the highest incidence (49.2) with the majority of cases recorded at the Keur Serigne Mbaye Sarr health post (3,026 malaria cases and 124.5 cases per 1,000 inhabitants).[1] Therefore, in 2018, additional surveillance was done in this area to include identification of breeding sites, along with routine HLC and PSC collection and IRM.

2.2 Routine Vector Surveillance

Entomological vector bionomics was conducted in 24 districts using: i) indoor PSCs in houses and ii) indoor and outdoor HLCs in selected houses (Table 2).

Table 2: Longitudinal Monitoring Adult Mosquito Collection Methods

Collection Method	Time	Frequency	Sample*
PSC	7:00 am to 9:00 am	Once per site per collection time point	10 houses per site
HLC	8:00 pm to 6:00 am	Two consecutive nights per site per collection time point	3 houses per site

**Same houses sampled each month to the greatest extent possible.*

For PSC, a white cloth/sheets were placed on the floor from wall to wall in sampled houses. The teams sprayed the commercial pyrethroid insecticide Hewelon in the house after closing windows, doors, and any other openings. All food and drinking water were covered or removed from the house before spraying. After a 10-minute knock-down period, the sheets were collected.

Six collectors per night (two per house) performed HLCs 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 outdoors. The collectors switched between indoors and outdoors on an hourly basis. The collectors used flashlights and a tubing aspirator to collect mosquitoes that landed on their legs before the mosquitoes could bite. The teams transferred the mosquitoes hourly to paper cups covered with a mosquito net. Temperature and relative humidity were recorded hourly during the collection period. The same houses were used each month for both PSCs and HLCs.

The following indicators in Table 3 were calculated based on the number of mosquitoes collected through each collection method:

Table 3: Summary of Indicators by Collection Method

Collection Method	Indicator	Definition
PSC	Indoor resting density	# mosquitoes / house / day
	% of fed females	# fed mosquitoes / Total collected by PSC
HLC	HBR	# bites / person / night
	Parity rate	Percentage of parous mosquitoes
	Exophagic rate	Percentage of mosquitoes biting outside
	Endophagic rate	Percentage of mosquitoes biting inside

Note: HBR=human biting rate

All mosquitoes collected were morphologically identified (genus / species) in the field using adult identification keys (Gillies and De Meillon, 1968). A sub-sample of malaria vectors were ovary-dissected to determine the parity rate. Blood-fed females collected using PSC were individually preserved in micro-tubes for later determination of blood meal source in the laboratory. All vectors collected using HLC were individually preserved in micro-tubes for laboratory analysis (species identification, infection and knock down resistant (*kdr-west and kdr-east*) gene detection).

[1] NMCP, decembre_2017, Bulletin de surveillance sentinelle du paludisme au Sénégal

2.3 INSECTICIDE SUSCEPTIBILITY TESTS

Vector susceptibility tests against the insecticides used in IRS and LLINs were conducted (Table 4).

TABLE 4: LIST OF INSECTICIDES TESTED AND DOSES

Class	Insecticide	Diagnostic Dose*		
		1x	5x	10x
Pyrethroid	Deltamethrin	0.05%	0.25%	0.50%
	Permethrin	0.75%	3.75%	7.50%
	Alpha-cypermethrin	0.05%	0.25%	0.50%
Synergist	PBO	4%		
Organophosphate	Pirimiphos-methyl	0.25%		
Carbamate	Bendiocarb	0.10%		
Neonicotinoid	Clothianidin	13.2 mg/m^2		
Pyrrole	Chlorfenapyr	12.5, 50, 100, and 200 µg/b		

**There is currently no diagnostic dose determined for clothianidin or chlorfenapyr.*

Fifteen localities were sampled for larvae and the resulting 3–5-day-old adult female *An. gambiae* s.l. emerging from the larvae were used for susceptibility tests. As sufficient larvae could not be found, tests were not done in two districts (Niakhar and Oussouye). All insecticides except chlorfenapyr were tested using the World Health Organization (WHO) method. The CDC bottle assay method was used for chlorfenapyr. The WHO tests were conducted using insecticide-impregnated papers and kits to test the susceptibility status of each locality vectors against all the selected insecticides at diagnostic doses and resistance intensities. For each insecticide, at least 100 mosquitoes were tested in four replicates of 25 females per tube. The mosquitoes were aspirated into holding tubes for one hour to check for any moribund before exposure. Four sets of exposure tubes were prepared by lining the insecticide impregnated paper into the tubes. The holding tubes and exposure tubes were later attached and mosquitoes were gently blown from the holding tubes to the exposure tubes for one hour. After the hour of contact, the mosquitoes were gently transferred to the holding tubes and provided with a 10% sugar solution until the 24h mortality reading. Two additional tube containing 25 mosquitoes each were set as negative controls for each test.

Synergist assays, using PBO, were also conducted with pyrethroids, using the same method, with pre-exposure of the mosquitoes to the synergist for one hour before exposure to the insecticides. Mosquitoes were exposed to the impregnated papers for one hour and the mortality was recorded 24 hours after exposure.

The susceptibility status of each colony at diagnostic concentration of each insecticide was interpreted based on WHO 2016 resistance criteria listed below[2].

WHO Threshold	Susceptibility Status
98–100%	Susceptibility confirmed
90–98%	Resistance suspected
Less than 90%	Resistance confirmed

When resistance to the diagnostic concentration of pyrethroids was observed, intensity of resistance was identified using the WHO susceptibility test (5x and 10x). The results were also interpreted in accordance with the WHO criteria of low, moderate, and high resistance intensity[3].

[2] WHO, Test procedures for insecticide resistance monitoring in malaria vector mosquitoes (Second edition), 2016

[3] [3]WHO, Test procedures for insecticide resistance monitoring in malaria vector mosquitoes (Second edition), 2016

Resistance Intensity	Mortality at 5x	Mortality at 10x
Low resistance	98–100%	--
Modereat resistance	<98%	98–100%
High resistance	--	<98%

Clothianidin-impregnated papers were prepared locally following a protocol designed by PMI VectorLink using the formulated product used for IRS (SumiShield® 50WG, 50% active ingredient). 20 ml of distilled water was diluted with 26.4 mg of SumiShield® 50WG to create a solution of clothianidin; 2 ml of the solution were used to impregnate each 12x15 cm Whatman filter paper and left to dry overnight. Once the papers were prepared, the testing procedure followed the WHO method described above, with mortality recorded every 24 hours, up to seven days post exposure or until 100 percent mortality was recorded. If 100% mortality was recorded before seven days, the tubes were kept for an additional day to confirm mortality.

In the absence of diagnostic concentration and time, the chlorfenapyr test was conducted using susceptible *An. coluzzii* mosquitoes and wild mosquitoes emerging from larval collection. Several concentrations of the insecticide were tested (12.5, 25, 50, 100, and 200 µg/bottle) for 60 minutes of exposure time and mortality was recorded every 24 hours for 72 hours post exposure.

The tests were corrected with Abbott's formula when control mortality was between 5 percent and 10 percent.

2.4 Laboratory Molecular Analyses

Molecular analyses were conducted by LEVP and the Research Institute for Development. The mosquito samples were appropriately preserved and sent to the laboratories to determine the sporozoite infection rate, the blood meal source, the vector species, and characterization of the resistance mechanisms.

2.4.1 Sporozoite rate

The circumsporozoite enzyme-linked immuno-sorbent assay (CSP ELISA) described by Burkot et al. (1984) and slightly modified by Wirtz et al., (1987) was used to determine the sporozoite infection rate of *An. gambiae* s.l. collected by HLC. The sporozoite index (SI) was presented as a percentage of the specimens carrying the *Plasmodium falciparum* CSP antigen over the total number of specimens tested with ELISA.

The entomological inoculation rate (EIR) is an average number of infective bites per person per night, and is the product of the sporozoite index (SI) and the human biting rate (HBR). The EIR determines the intensity of the malaria transmission in a given locality.

2.4.2 Blood meal source

The blood meal source of the fed *An. gambiae* s.l. collected using PSC was determined by the direct ELISA method described by Beier et al. (1986). The anthropophilic rate was determined by the ratio females that were human-fed to the number of fed females tested. The same was done for the different animal hosts tested.

2.4.3 Species identification

The molecular identification of *An. gambiae* sibling species was performed on a subsample of living and dead female mosquitoes from susceptibility tests as well as HLC and PSC collections. The molecular identification was performed by polymerase chain reaction (PCR) according to the protocol of Wilkins et al. (2006).

2.4.4 Characterization of resistance mechanisms

The *kdr-west* and *kdr-east* and acetylcholinesterase (*Ace-1*) mutations were characterized on subsamples of dead and alive mosquitoes used in the susceptibility tests. The protocol described by Huynh et al. (2007) was used to perform the different PCR analyses. The frequency of the mosquitoes carrying the resistance allele was described per locality.

2.5 Statistical Analysis

Homogeneity tests of percentages were carried out by the standard test of $\chi 2$ with the threshold of significance set at 0.05. The results of the entomological collections in all sites were pooled and are presented per geographical area of the country.

3. RESULTS

3.1 VECTOR POPULATION DYNAMICS

3.1.1 ENTOMOLOGICAL PARAMETERS ACROSS ALL SITES

In all geographical areas, *An. gambiae* s.l. was the main species caught using both HLC and PSC (Table 5, Figure 2). *An. funestus* s.l. was present and nearly as predominant as *An. gambiae* s.l. only in the Sudanese zone, specifically, only in Nioro and Ndoffane districts, where *An. funestus* s.l. made up 67.4 percent and 57.0 percent, respectively, of all *anopheles* collected (Annex B). In the Sahelian zone, *An. gambiae* s.l. was the only species encountered, whereas *An. pharoensis* and *An. rufipes* were collected in all geographical areas except the Sahelian. *An. coustani* and *An. welcomei* were found in the Sudanese zone and *An. nili* in the Sudano-Guinean region.

FIGURE 2: MAP OF SPECIES DISTRIBUTION OF *ANOPHELES* CAUGHT BY HLC AND PSC

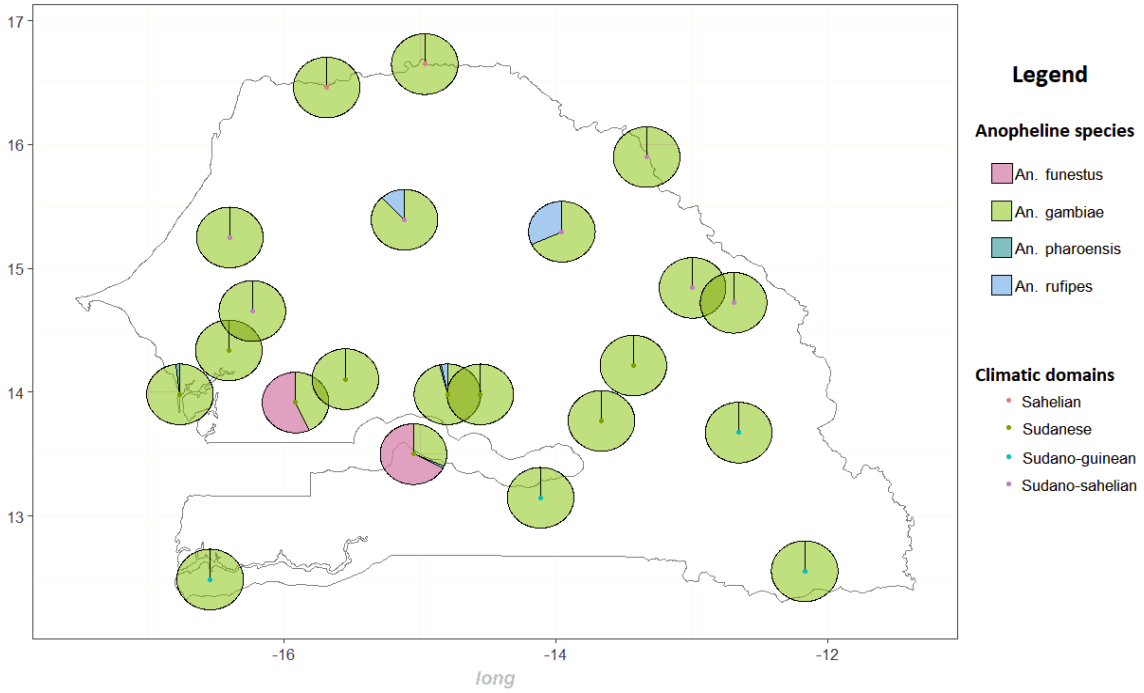


TABLE 5: SPECIES COMPOSITION OF *ANOPHELES* CAUGHT BY HLC AND PSC

	HLC					PSC					Total				
Geographic area	**Sahelian**	**Sudano-Sahelian**	**Sudanese**	**Sudano-Guinean**	**Total**	**Sahelian**	**Sudano-Sahelian**	**Sudanese**	**Sudano-Guinean**	**Total**	**Sahelian**	**Sudano-Sahelian**	**Sudanese**	**Sudano-Guinean**	**Total**
An. gambiae s.l.	15	673	759	10712	12159	64	978	4044	476	5562	79 (100%)	1651 (93.33%)	4803 (58.1%)	11188 (99.71%)	17721
An. pharoensis	0	1	10	11	22	0	0	2	0	2	0	1 (0.06%)	12 (0.15%)	11 (0.10%)	24
An. Rufipes	0	0	4	0	4	0	117	29	5	151	0	117 (6.61%)	33 (0.40%)	5 (0.04%)	155
An. funestus	0	0	1319	1	1320	0	0	2095	5	2100	0	0	3414 (41.3%)	6 (0.05%)	3420
An. coustani	0	0	1	0	1	0	0	1	0	1	0	0	2 (0.02%)	0	2
An. welcomei	0	0	1	0	1	0	0	0	0	0	0	0	1 (0.01%)	0	1
An. nili	0	0	0	10	10	0	0	0	0	0	0	0	0	10 (0.09%)	10
Total	**15**	**674**	**2094**	**10734**	13517	64	1095	6171	486	7816	**79**	**1769**	**8265**	**11220**	**21333**

3.1.2 INDOOR RESTING DENSITY AND ABDOMINAL STATUS OF *AN. GAMBIAE* S.L. COLLECTED BY PSC

The *An. gambiae* s.l. indoor resting density (IRD) was higher in the Sudano-Guinean area (26.30 females/house) than in the Sudano-Sahelian (9.03 females/house) and Sudanese zones (4.13 females/house). The peaks of the IRD were observed during the middle of the rainy season (September to October) (Figure 3). Table 6 shows that the percentage of blood-fed mosquitoes was higher than the gravid females in all geographic areas.

TABLE 6: ABDOMINAL STATUS OF INDOOR RESTING *AN. GAMBIAE* S.L.

Geographic Area	Blood Fed	Gravid	Half Gravid	Unfed	Total
Sahelian	46 (72%)	10 (16%)	6 (9%)	2 (3%)	64
Sudano-Sahelian	601(61%)	243 (25%)	114 (12%)	20 (2%)	978
Sudanese	2634 (65%)	744 (18%)	523 (13%)	143 (4%)	4044
Sudano-Guinean	276 (58%)	101 (21%)	57 (12%)	42 (9%)	476

3.1.3 PARITY RATE OF *AN. GAMBIAE* S.L. COLLECTED BY HLC

In Sudanese and Sudano-Guinean areas, the parity rate increased progressively during rainy season – from .44 in July to .68 in October the Sudanese area, and from 3.7 in July to 8.2 in December in Sudano-Guinean area (Fig 3). In the Sudano-Sahelian area, the parity rate was low and stable during the whole rainy season period and increased at the beginning of the dry season (December).

3.1.4 HBR AND ENDOPHAGIC RATE

An. gambiae s.l. density increased during the rainy season, with the highest HBR observed in August and September in all areas (Figure 3). In the Sahelian area, the only field visit was done in September. A second visit was to be supported by the NMCP through Global Fund, but did not happen. A visit in the dry season is planned for March 2019.

The highest HBR was recorded in September in the Sudano-Guinean area (62.11 bites/person/night). The next highest was in August in the Sudano-Sahelian area (6.0 bites/person/night). The HBR was higher in the Sudano Sahelian area than in the Sudanese area, which can be explained by the high vector density in Diourbel.

Outside of the Sahelian area, which had a small number of vectors collected, the exophagic behavior was most evident in Sudanese-Sahelian area compared to other zones of the country (Table 7).

TABLE 7: ENDOPHAGIC RATE OF VECTORS BY GEOGRAPHIC AREA

	Sahelian	Sudano- Sahelian	Sudanese	Sudano-Guinean
Indoor	5	311	383	5488
Outdoor	10	362	372	5224
Rate	**0.33**	**0.46**	**0.51**	**0.51**
Chi-square test	Not Significant *p = 0.197*	Significant *p = 0.049*	Not Significant *p = 0.689*	Significant *p = 0.011*

FIGURE 3: HUMAN BITING RATE, INDOOR RESTING DENSITY AND PARITY RATE OF *AN. GAMBIAE* S.L. BY GEOGRAPHIC AREAS

**For the Sahelian zone, mosquitoes were collected only in September 2018. For the Sudano-Guinean zone, IRD and parity multiplied by 10 to allow for comparison with HBR.*

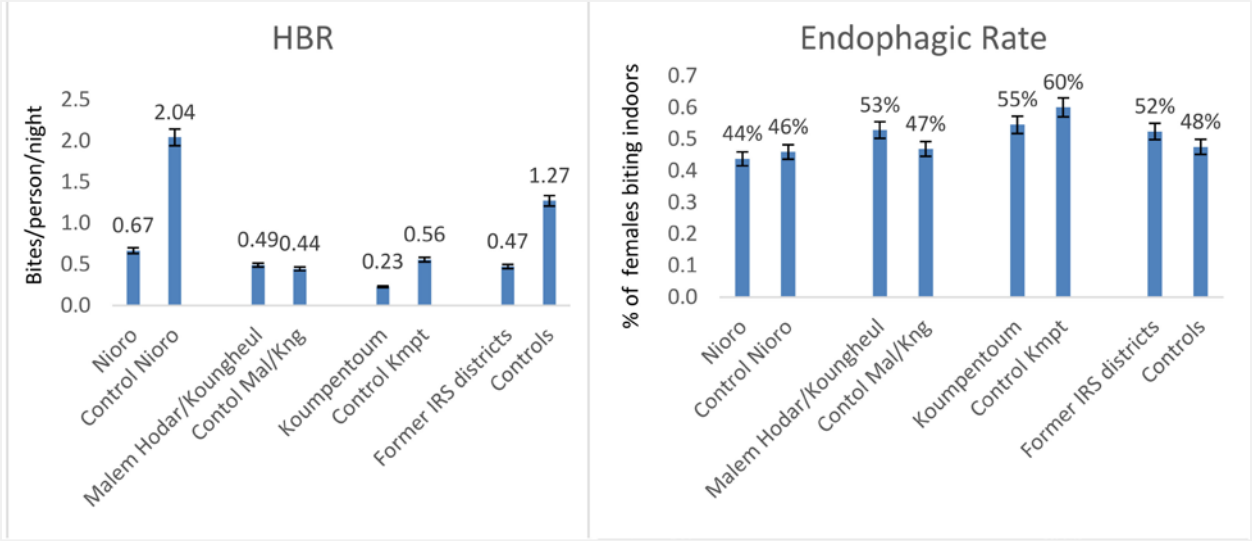
3.2 ENTOMOLOGICAL PARAMETERS OF THE FORMER IRS DISTRICTS AND CONTROLS

This section describes the results from entomological indicators collected through routine monitoring in former IRS districts (Malem Hodar, Koungheul, Koumpentoum, and Nioro) and controls (Kaffrine and Tambacounda).

3.2.1 HBR AND ENDOPHAGIC RATE

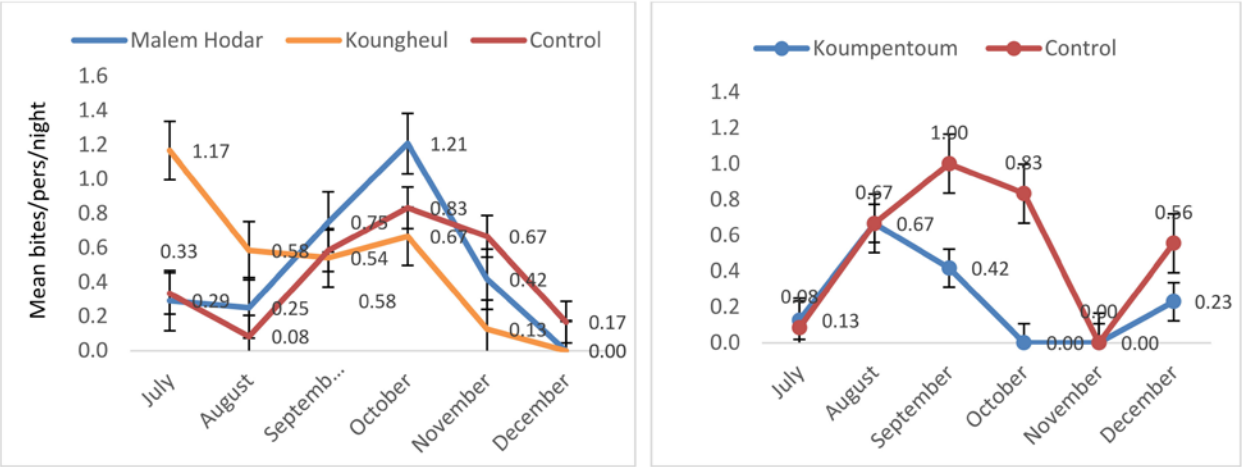
The HBR of *An. gambiae* s.l. remained low (less than 1.0 bite/ person/ night) in all IRS districts and controls except in the Nioro control site, where the HBR was 2.04 bites per person per night (Figure 4 and annex C). The endophagic rate was similar in former IRS districts compared with their controls, but a slight tendency toward exophagic behavior was registered in Nioro and its control Figure 4 an annex D).

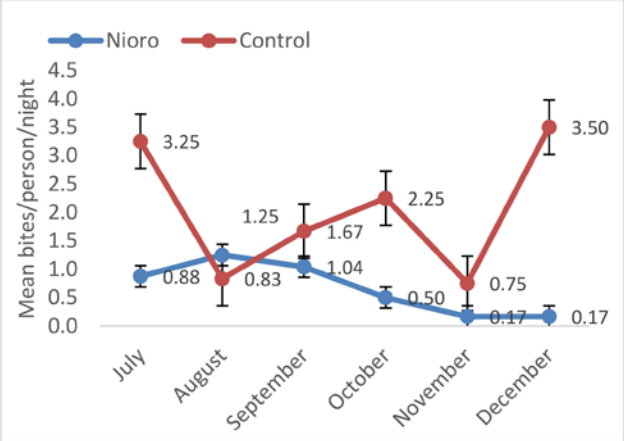
FIGURE 4: MEAN HUMAN BITING RATE AND ENDOPHAGIC INDEX OF *AN. GAMBIAE* S.L. IN FORMER IRS DISTRICTS AND CONTROLS

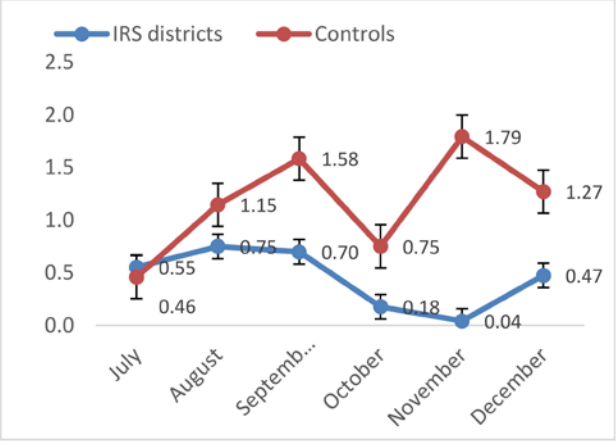


By combining all IRS districts, HBR was higher in control area than in sprayed zone (Figure 5). By district, HBR in control districts was higher at the pic of the density (September and October).

FIGURE 5: MEAN HUMAN BITING RATE OF *AN. GAMBIAE* S.L. IN FORMER IRS DISTRICTS AND CONTROLS







3.2.2 IRD AND ABDOMINAL STATUS OF *AN. GAMBIAE* S.L. COLLECTED BY PSC

Results showed that IRD was significantly higher in control districts except in Koumpentoum control (Figures 6 and annex E7). The distribution of the abdominal status was similar between former IRS district and their controls with overall a higher percentage of fed females (Table 7).

FIGURE 6: AVERAGE IRD OF *AN. GAMBIAE* S.L. IN FORMER IRS DISTRICTS AND CONTROLS

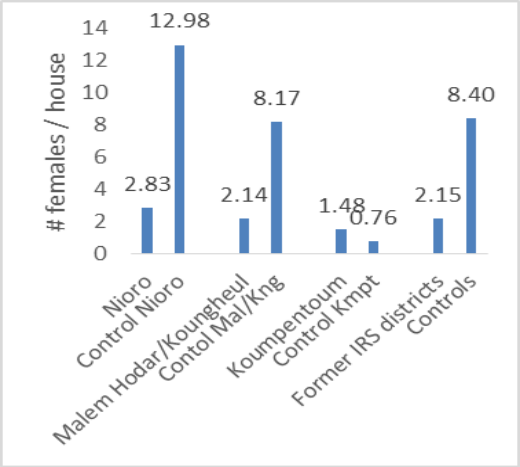
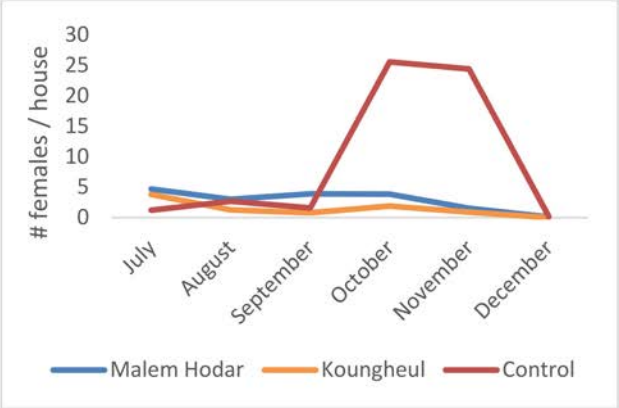
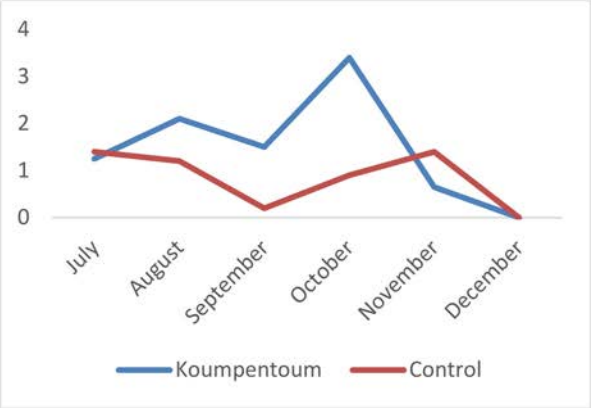


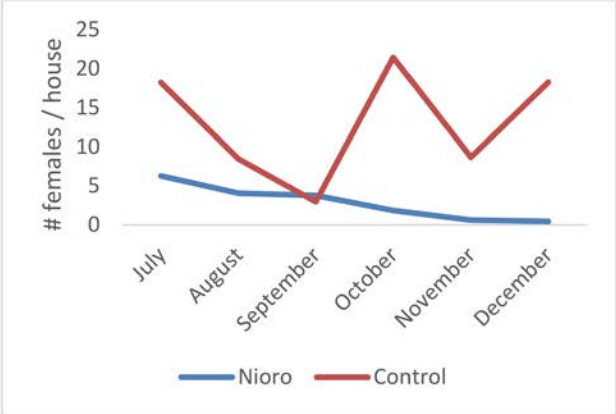
TABLE 7: ABDOMINAL STATUS OF INDOOR RESTING *AN. GAMBIAE* S.L. IN FORMER IRS DISTRICTS AND CONTROLS

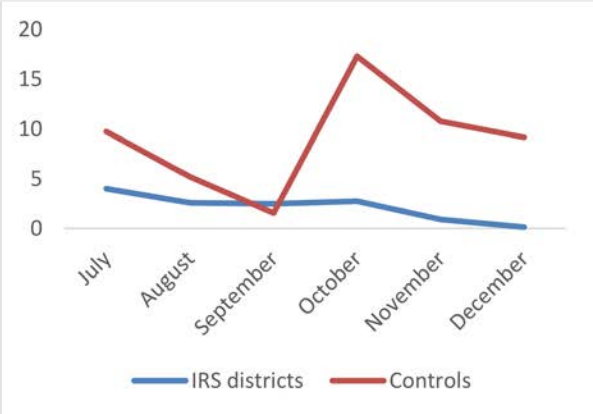
	Blood Fed	Gravid	Half Gravid	Unfed	Total
Nioro	212 (63%)	73 (22%)	42 (12%)	12 (4%)	339
Ndoffane	973 (62%)	389(25%)	145 (9%)	51 (3%)	1558
Malem Hodar	209 (61%)	49 (14%)	55 (16%)	28 (8%)	341
Koungheul	79 (46%)	51 (29%)	42 (24%)	1 (1%)	173
Kaffrine	302 (53%)	64 (11%)	163 (28%)	43 (8%)	572
Koumpentoum	65 (37%)	57 (32%)	54 (30%)	2 (1%)	178
Tambacounda	24 (45%)	12 (23%)	16 (30%)	1 (2%)	53
Total former IRS districts	565 (55%)	230 (22%)	193 (19%)	43 (4%)	1031
Total controls	1299 (60%)	465 (21%)	324 (15%)	95 (4%)	2183

FIGURE 7: MEAN IRD OF *AN. GAMBIAE* S.L. IN FORMER IRS DISTRICTS AND CONTROLS









3.2.3 PARITY RATE OF *AN. GAMBIAE* S.L. COLLECTED BY HLC

Except in Nioro and Ndoffane, the parity rate was high in both former IRS districts and their control zones, and higher in IRS districts area than in their controls (Annex F)

3.2.4 ENTOMOLOGICAL PARAMETERS OF *AN. FUNESTUS* S.L.

An. funestus s.l. were found in high density only in the Nioro and Ndoffane sites. Seventy-six percent of *An. funestus* s.l. from HLCs were collected in Ndoffane, 24 percent in Nioro. PSC collections yielded a similar breakdown, 71 percent from Ndoffane and 29 percent from Nioro (Table 8). HBRs and IRDs were higher in Ndoffane than in Nioro, though a low parity rate was found in both districts (Figures 8-10). *An. funestus* was shown to be exophagic: less than 35 percent of mosquitoes collected via HLC were collected outdoors and exophilic (percentage of fed females more than 66 percent) in Nioro and Ndoffane (Table 9).

TABLE 8: PROPORTION OF *AN. FUNESTUS* IN NIORO COMPARED TO NDOFFANE

		July	August	September	October	November	December	Total
Nioro	HLC	27	72	70	83	28	32	312 (24%)
	PSC	46	72	78	64	149	198	607 (29%)
	Total	**73 (10%)**	**144 (18%)**	**148 (33%)**	**147 (28%)**	**177 (50%)**	**230 (47%)**	**919 (27%)**
Ndoffane	HLC	146	325	178	139	80	101	969 (76%)
	PSC	546	330	127	235	94	156	1488 (71%)
	Total	**692 (90%)**	**655 (82%)**	**305 (67%)**	**374 (72%)**	**174 (50%)**	**257 (53%)**	**2457 (73%)**
Total *An. funestus* collected	HLC	173	397	248	222	108	133	1281
	PSC	592	402	205	299	243	354	2095
	Total	765	799	453	521	351	487	3376

FIGURE 8: MEAN HUMAN BITING RATE OF *AN. FUNESTUS* IN NIORO AND NDOFFANE

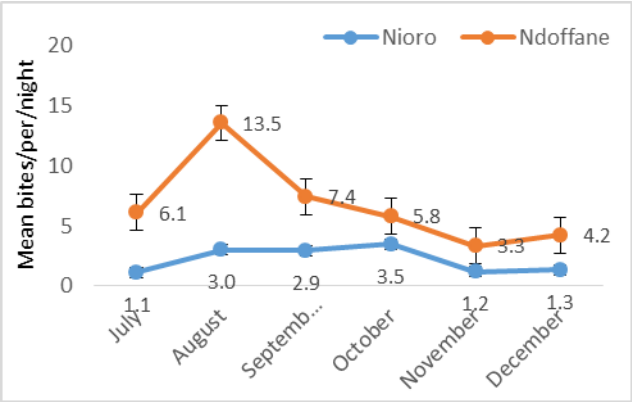


FIGURE 9: INDOOR RESTING DENSITY OF *AN. FUNESTUS* IN NIORO AND NDOFFANE

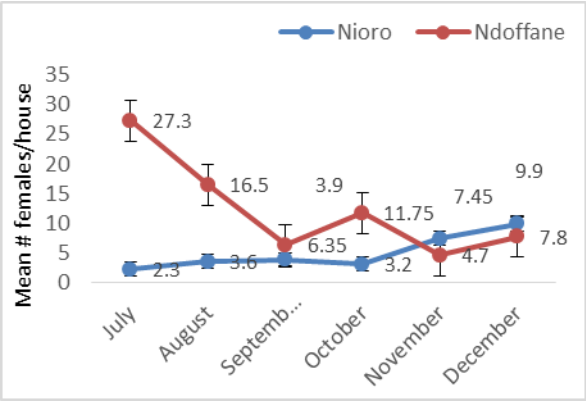


FIGURE 10: PARITY RATE OF *AN. FUNESTUS* COLLECTED THROUGH HLC IN NIORO AND NDOFFANE

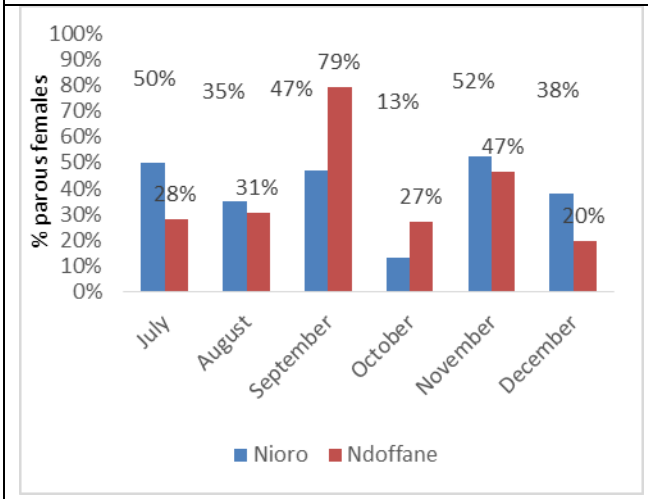


TABLE 9: ENDOPHAGIC INDEX AND ABDOMINAL STATUS OF *AN. FUNESTUS* COLLECTED IN NIORO AND NDOFFANE

	Ndoffane	Nioro
Indoor	359	108
Outdoor	610	204
Endophagic Index	**0.37**	**0.35**

Abdominal Status		**B Fed**	**Gr**	**HGr**	**Unfed**	**Total**
Nioro	Effective	397	121	59	30	607
	%	65%	20%	10%	5%	
Ndoffane	Effective	977	319	101	91	1488
	%	66%	21%	7%	6%	
Total	Effective	1374	440	160	121	2095
	%	66%	21%	8%	6%	

3.3 DIOURBEL ENTOMOLOGICAL SURVEILLANCE

The composition of *Anopheles* collected were 99.9% *An. gambiae* s.l. and .1% *An. pharoensis*. The HBR was high in all reported months, an average 3.95 bite/person/night. It was highest in August and September as well as December, which is the end of the rainy season (Figure 11 and annex G). Overall, the proportions of endophagic and exophagic of *An. gambiae* s.l. were similar. Furthermore, the IRD was high at the beginning of the rainy season and peaked in September (3.7 females/house). Overall, the proportions of blood-fed and gravid females of *An. gambiae* s.l. were similar (Table 10). The parity was low throughout the monitoring period except at the end of the rainy season (94 percent).

FIGURE 11: MEAN HUMAN BITING RATE AND ENDOPHAGIC RATE OF *AN. GAMBIAE* S.L. IN DIOURBEL DISTRICT

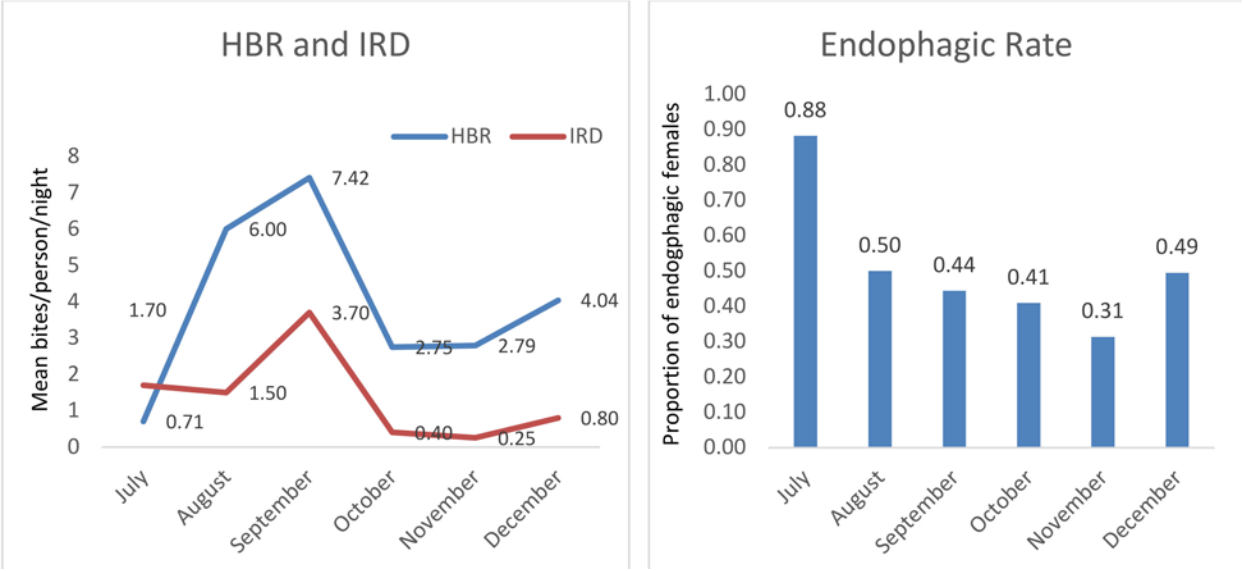


TABLE 10: ABDOMINAL STATUS OF *AN. GAMBIAE* S.L. IN DIOURBEL DISTRICT

	Blood Fed	Gravid	Half Gravid	Unfed	Total
Number Collected	59	42	61	5	167
%	35%	25%	37%	3%	

As part of the surveillance, main potential breeding sites for malaria vectors were identified and geo-localized in Keur Serigne Mbaye Sarr, and six other close-by neighborhoods in July 2018 (Table 11). Larvae were sampled from identified breeding sites by drying rainwater early in the dry season. Keur Serigne Mbaye Sarr was the only neighborhood where a high density of *An. gambiae* s.l. larvae was collected. Monitoring of positive breeding sites from July 2018 to January 2019 showed that the ravine with permanent water in the Keur Serigne Mbaye Sarr area was responsible for the presence of *An. gambiae* s.l. during the dry season.

TABLE 11: POTENTIAL BREEDING SITES IDENTIFIED IN KEUR S. MBAYE SARR AND SURROUNDING NEIGHBORHOODS, DIOURBEL DISTRICT, JANUARY 2019

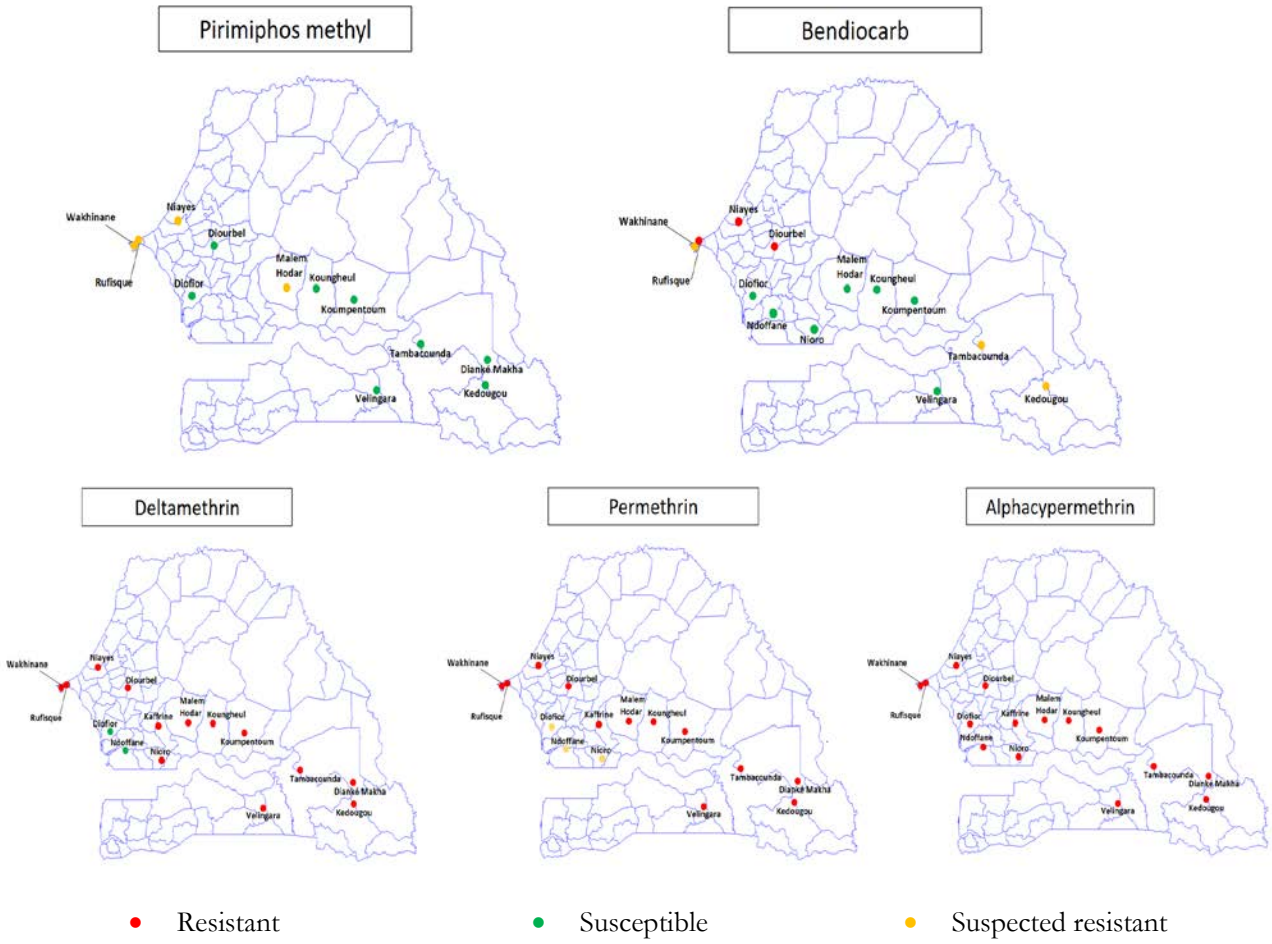
Neighborhood	**N° Breeding Site**	**C.GPS** Longitude and latitude	**Nature** Permanent, semi-permanent, Temporary	**Feature** Natural/ Anthropic Zone and / or flooded houses	**Observations** Very Positive (++) / Positive (+) / Negative (-)
Keur Serigne Mbaye Sarr	**1**	N 14°39'03,41" W16°13'39,14"	**Ravine with water constantly**	**Natural**	**++ with floating vegetation**
	2	N 14°39'14,1" W16°12'34,2"	Ravine with semi-permanent water	Natural	- drying up of water
	3	N14°39'09" W16°13'47,7"	Semi-permanent rainwater	Anthropic	- drying up of water
	4	N14°39'12,5" W16°16'48,5"	Semi-permanent rainwater	Anthropic	- drying up of water
Keur Cheikh Anta Mbacke	**1**	N14°39'35,6" W16°14'19,7"	Semi-permanent rainwater	Natural	- drying up of water
	2	N14°39'0" W16°14'0"	Temporary rainwater	Anthropic	- drainage drying
	3	N14°39'17,9" W16°14'24,3"	Semi-permanent rainwater	Zone and / or flooded houses	- pumping water
	4	N14°39'32,3" W16°14'52,9"	Temporary rainwater	Natural	- drying up of water
	5	N14°39'0" W16°14'0"	Temporary rainwater	Natural	- drying up of water
Keur Thierno Kandji	**1**	N14°38'47,3" W16°14'09,9"	Semi-permanent rainwater	Zone and / or flooded houses	- pumping water
	2	N14°38'41,7" W16°14'17,6"	Semi-permanent rainwater	Zone and / or flooded houses	- pumping water
	3	N14°38'49,1" W16°14'01,6"	Semi-permanent rainwater	Natural	- drying up of water
Médinatoul (Abattoir)	**1**	N14°39'14,1" W16°13'38,8"	Semi-permanent rainwater	Natural	- drying up of water
Route de Touba	**1**	N14°38'01,6" W16°12'33,7"	Permanent water pond	Natural	- drainage drying
Touba Sérasse	**1**	N14°38'14,5" W16°12'5,05"	Semi-permanent	Natural	- drying up of water
Sessène	**1**	N14°38'37,8" W16°14'36,4"	Permanent water	Natural	- water catchment area
	2	N14°38'31,7" W16°14'37,7"	Semi-Permanent rainwater	Natural	- drying up of water

3.4 Susceptibility Tests of Malaria Vectors to Insecticides

3.4.1 Insecticide resistance testing using the WHO method

The results showed resistance of *An. gambiae* s.l. to all three pyrethroid insecticides tested in the majority of the districts, with the exception of two districts of the Sudanese area (Dioffior and Ndoffane), where the team recorded susceptibility to deltamethrin (Figure 12). For pirimiphos-methyl 0.25%, the vector populations were fully susceptible in the majority of the districts, with the exception of the suburbs of Dakar where probable resistance was found. *An. gambiae* was resistant to bendiocarb in most sites of the Sudano-Sahelian area (Dakar suburbs and Diourbel), and probable resistance was found in cotton-growing areas (Tambacounda and Kédougou).

Figure 12: Insecticide Susceptibility, 2018



3.4.2 Resistance intensity using WHO method

In 2018, PMI VectorLink Senegal conducted intensity bioassays using WHO filter papers to avoid any differences due to type of test or field treatment of bottles. The three pyrethroid insecticides tested (deltamethrin, permethrin, and alpha-cypermethrin) showed high resistance in Dakar surbubs and in Kédougou. In the other districts, the intensity varied (Table 12).

TABLE 12: PERCENT MORTALITY OF *AN. GAMBIAE* S.L. AT DIFFERENT INTENSITIES OF INSECTICIDES, WHO METHOD

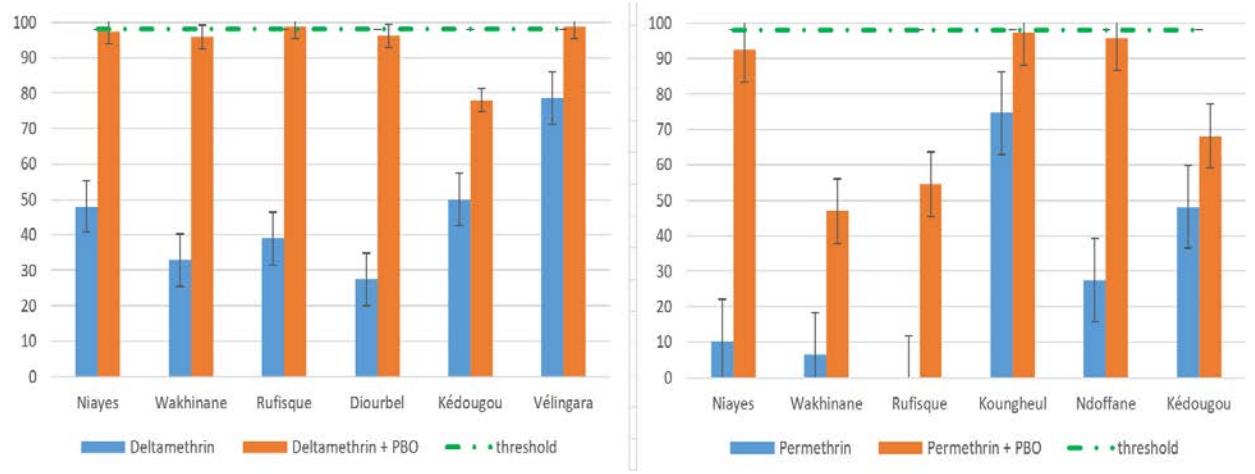
Districts	Deltamethrin				Permethrin				Alpha-cypermethrin			
	1 X	5X	10X	Intensity	1 X	5X	10X	Intensity	1 X	5X	10X	Intensity
Wakhinane	67.5	90.4	90.5	**High**	6.89	85.6	95.5	**High**	1.9	-	-	**High***
Rufisque	15.9	87.5	91	**High**	8.8	41.2	85.0	**High**	8.9	-	-	**High***
Niayes	60.2	86.4	92.03	**High**	27.6	83.8	94.0	**High**	25.0	-	-	**High***
Diourbel	54.1	88	98.9	**Moderate**	45.9	81.8	92.2	**High**	47.7	-	-	**High***
Nioro	77.0	99.1	na	**Low**	90.0	100	na	**Low**	75.9	-	-	**Moderate***
Ndoffane	98.2	na	na	**-**	90.5	97.0	na	**Moderate***	65	-	-	**Moderate***
Diofior	100	na	na	**-**	94.1	100	na	**Low**	81.3	91.0	98.0	**Moderate**
Kaffrine	69.3	80.0	99.1	**Moderate**	50,9	93,8	100	**Moderate**	72.3	-	-	**Moderate***
Malem Hodar	64.7	95.1	100	**Moderate**	66,1	87.0	93.6	**High**	5.5	85.9	-	**Moderate***
Koungheul	72.8	100	na	**Low**	61,9	87.1	100	**Moderate**	78.3	95.9	97.9	**High**
Koumpentoum	79.0	96.1	-	**Moderate***	66	82.4	-	**Moderate***	77.7	91.3	-	**Moderate***
Tambacounda	87.4	-	-	**Moderate***	87.9	94.3	-	**Moderate***	89.0	-	-	**Moderate***
Vélingara	56.7	82.5	96.1	**High**	31.4	80.9	100.0	**Moderate**	34.2	85.9	-	**Moderate***
Dianké Makha	18.8	91.6	-	**Moderate***	25.0	63.1	-	**High***	43.1	-	-	**High***
Kédougou	65.4	61.4	84.7	**High**	29.0	71.0	93.2	**High**	53.2	55.0	-	**High***

***Test not completed for all concentrations due to a lack of sufficient numbers of mosquitoes.**

3.4.3 SYNERGIST TESTING USING THE WHO METHOD

Synergist tests with PBO were done with both pyrethroid insecticides in four sites. The presence of a synergist contributed consistently to a decrease in resistance to both pyrethroids but especially to deltamethrin versus permethrin (Figure 13).

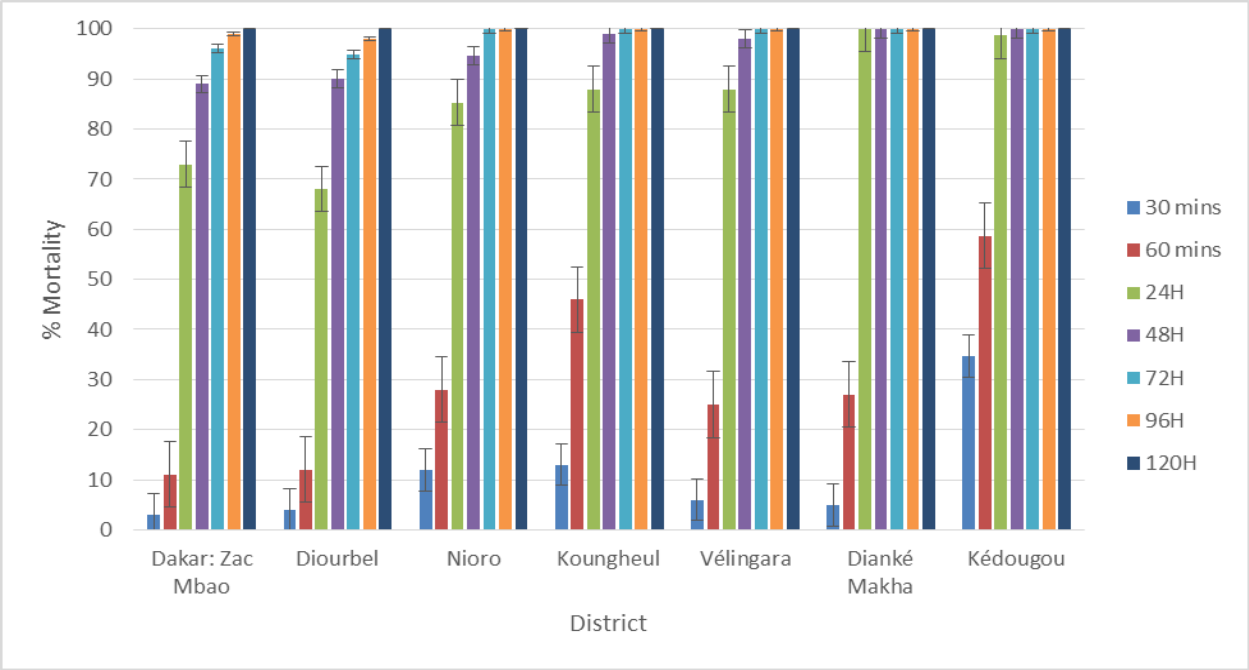
FIG 13: MORTALITY OF *AN. GAMBIAE* S.L. EXPOSED TO DELTAMETHRIN AND PERMETHRIN WITH OR WITHOUT PRE-EXPOSURE TO PBO USING WHO PAPERS



3.4.4 INSECTICIDE SUSCEPTIBILITY TEST WITH CLOTHIANIDIN

An. gambiae s.l. showed to be susceptible to clothianidin in all districts where they were tested. 100% mortality was reached before the seventh day in all sites (Fig 14).

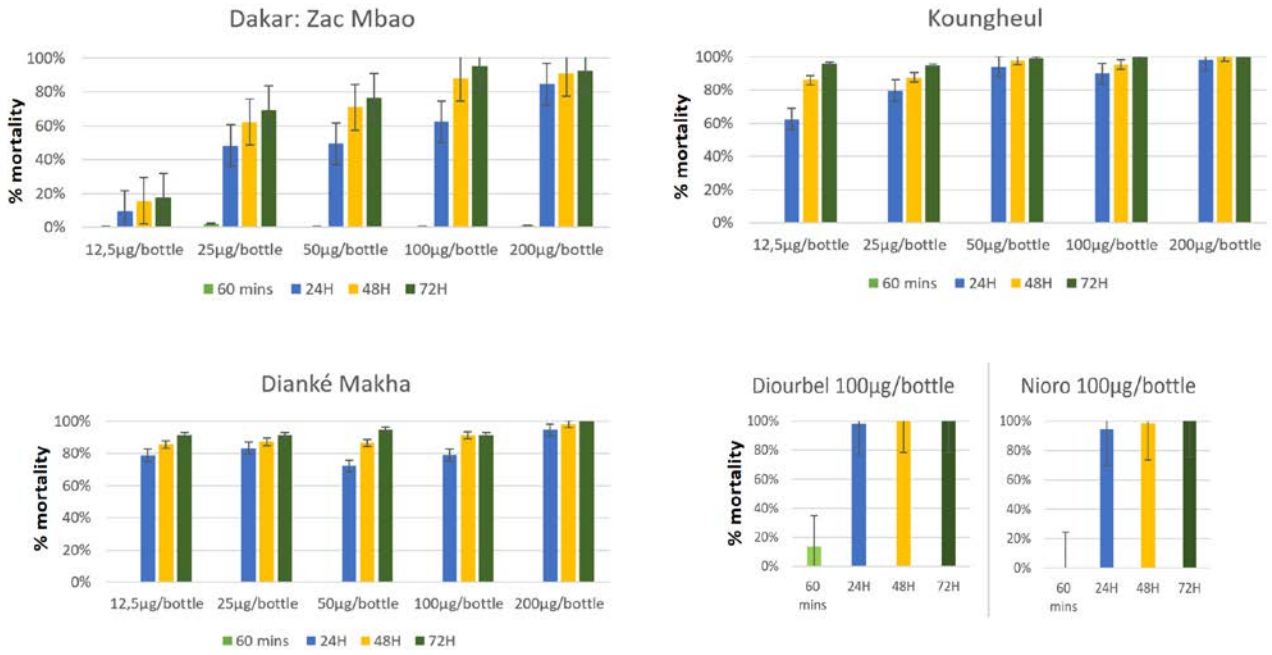
FIGURE 14: MORTALITY OF *AN. GAMBIAE* S.L. EXPOSED TO CLOTHIANIDIN



3.4.5 INSECTICIDE SUSCEPTIBILITY TEST WITH CHLORFENAPYR

Results in Figure 15 show resistance of *An. gambiae* s.l. to chlorfenapyr in the suburb of Dakar, even with the higher concentration (200 µg/bottle). *An. gambiae* s.l. populations tested with chlorfenapyr showed susceptibility with 50 µg/bottle in Koungheul (Sudanese area) but only with the higher concentration (200 µg/bottle) in Dianke Makha (Sudano-Guinean area). In Diourbel (Sudano-Sahelian area) and in Nioro (Sudanese area), only one concentration (100 µg/bottle) could be tested due to a shortage of mosquitoes. Susceptibility of *An. gambiae* s.l. was determined at this concentration.

FIGURE 15: MORTALITY OF *AN. GAMBIAE* S.L. EXPOSED TO CHLORFENAPYR



3.5 LABORATORY ANALYSES

3.5.1 BLOOD MEAL ANALYSIS

Table 13 shows the results of blood meal source identification of *An. gambiae* s.l. females collected using PSC, by district. The average anthropophilic rate was low in Sahelian (50 percent), Sudano-Sahelian (24 percent), and Sudanese areas (14 percent). In the Sudano-Guinean zone, the anthropophilic rate was more than 52 percent. In Sahelian and Sudano-Sahelian areas, cows and horses were the main blood source of *An. gambiae* s.l. females. In the Sudanese area, horses were the main blood source, representing 52 percent of the total analyzed.

The anthropophilic rate of *An. funestus* s.l. was low in Nioro and Ndoffane, with only 20 percent that fed on humans. Horses were the main blood source of *An. funestus* s.l. in these districts, accounting for 49 percent of blood meals.

3.5.2 VECTOR SPOROZOITE RATES

Infected females were found in the Sudano-Sahelian area (Diourbel), in the Sudanese area (Tambacounda, Koungheul, Nioro, and Ndoffane), and in all three districts of Sudano-Guinean (Table 14). In Nioro and Ndoffane, where *An. gambiae* s.l. and *An. funestus* were sympatric, these two species were found to be infected. Infected females were collected across all months with the exception of December and January (Table 15). Other *Anopheles* vectors with low densities (12 *An. nili*, 11 *An. pharoensis,* and 01 *An. rufipes*) were tested; all were negative.

3.5.3 ENTOMOLOGICAL INOCULATION RATES

Table 16 presents the nightly EIR from July 2018 to January 2019, which is the product of the HBR and the SI. EIR showed that transmission occurred indoors and outdoors in all geographic areas but was higher in Sudano-Guinean area (0.42 to 1.61 infected bite per person per night).

TABLE 13: ORIGIN OF BLOOD MEAL AND ANTHROPOPHILIC RATE OF *AN. GAMBIAE* S.L. AND *AN. FUNESTUS* S.L.

Species	District	N	T	U	Monospecific					Mix H/A				Mix A/A					AR
					H	B	S	C	Ho	HB	HHo	HS	HBS	SHo	SC	CHo	BS	BHo	
An. gambiae s.l.	Podor	20	12	3	1	2	1	ND	0	0	0	0	ND	0	ND	ND	3	2	**0.083**
	Richard Toll	43	32	14	0	7	0	ND	9	0	0	1	ND	0	ND	ND	1	0	**0.031**
	Bakel	195	59	15	2	25	3	ND	10	0	0	0	ND	0	ND	ND	3	1	**0.034**
	Matam	128	39	6	0	5	2	ND	18	0	1	0	ND	0	ND	ND	5	2	**0.026**
	Kanel	82	44	9	6	17	4	ND	1	2	1	1	ND	1	ND	ND	1	1	**0.227**
	Ranerou	91	65	23	8	14	5	ND	7	0	2	0	ND	0	ND	ND	4	2	**0.154**
	Linguere	20	18	3	5	1	3	ND	2	0	2	0	ND	1	ND	ND	1	0	**0.389**
	Niayes	34	34	7	5	8	3	0	7	0	0	0	0	1	0	0	3	0	**0.147**
	Diourbel	59	58	12	16	9	5	0	9	0	0	1	0	1	0	0	4	1	**0.293**
	Kaffrine	296	152	23	11	12	0	0	92	0	2	0	0	2	0	1	6	3	**0.086**
	Malem Hodar	204	204	25	22	25	6	0	103	0	1	2	0	4	0	0	13	3	**0.123**
	Koungheul	78	77	5	11	3	9	0	29	0	1	1	0	7	0	0	9	2	**0.169**
	Nioro	211	123	13	15	10	2	0	63	0	2	0	0	0	0	0	17	1	**0.138**
	Ndoffane	972	192	51	19	15	10	0	78	0	2	0	1	5	0	0	10	1	**0.115**
	Koumpentoum	62	61	14	16	5	6	0	11	0	1	2	0	2	0	0	3	1	**0.311**
	Tamba	22	22	3	6	1	3	0	7	0	0	0	0	2	0	0	0	0	**0.273**
	Vélingara	136	135	29	70	6	8	1	8	1	0	4	0	3	0	0	5	0	**0.556**
	Kédougou	34	33	7	17	1	0	0	2	0	2	1	0	0	0	0	3	0	**0.606**
	Diankhé Makha	80	79	19	40	1	5	0	12	0	0	1	0	1	0	0	0	0	**0.519**
An. funestus s.l.	Nioro	395	232	38	28	20	12	0	115	0	1	1	0	2	1	0	9	5	**0.129**
	Ndoffane	969	152	8	39	12	6	0	57	0	4	3	0	3	0	0	19	1	**0.303**

Note: N = number of blood-fed mosquitoes collected, T = number tested, U = Unamplified, ND = not determined, H = human, B = bovine, S = sheep, C = chicken, Ho = Horse, Mix H/A = human and animal, Mix A/A = animal and animal

Sahelian Sudano - Sahelian Sudanese Sudano-Guinean

TABLE 14: SPOROZOITE RATE OF *AN. GAMBIAE* S.L. AND *AN. FUNESTUS* S.L.

District	*An. gambiae* s.l				*An. funestus* s.l.			
	C	T	P	SI	C	T	P	SI
Podor	13	13	0	0	-	-	-	-
Richard Toll	2	2	0	0	-	-	-	-
Bakel	24	24	0	0	-	-	-	-
Matam	11	11	0	0	-	-	-	-
Kanel	24	24	0	0	-	-	-	-
Ranerou	26	26	0	0	-	-	-	-
Linguere	7	7	0	0	-	-	-	-
Niayes	7	7	0	0	-	-	-	-
Diourbel	580	225	4	**0,017**	-	-	-	-
Kaffrine	31	30	0	0	-	-	-	-
Malem Hodar	70	68	0	0	-	-	-	-
Koungheul	80	80	3	**0,037**	-	-	-	-
Koumpentoum	33	32	0	0	-	-	-	-
Tambacounda	40	39	1	**0,025**	-	-	-	-
Ndoffane	295	137	1	**0,007**	969	221	2	**0,009**
Nioro	96	80	1	**0,012**	350	210	1	**0,004**
Vélingara	2522	292	7	**0,023**	-	-	-	-
Diankhé Makha	2350	119	4	**0,033**	-	-	-	-
Kédougou	4437	257	5	**0,019**	4	4	0	0

C= Total collected; T = Total tested; P = Positives; SI = Sporozoite Index

TABLE 15: SPOROZOITE RATE OF FEMALES COLLECTED BY HLC BY MONTH

District	July		August		September		October		November		December		January	
	Tested	Positive	Tested	Positive	Tested	Positive	Tested	Positive	Tested	Positive	Tested	Positive	Tested	Positive
	***An. funestus* s.l.**													
Ndoffane	31	0	41	1	35	1	40	0	47	0	-	-	27	0
Nioro	26	0	40	0	38	0	45	1	28	0	-	-	32	0
	***An. gambiae* s.l.**													
Diourbel	27	0	60	2	60	1	18	1	0	0	-	-	60	0
Koungheul	28	0	14	0	13	1	21	1	3	1	-	-	1	0
Tambacounda	8	0	1	0	8	0	12	0	10	1	-	-	0	0
Ndoffane	29	0	19	0	25	0	18	1	13	0	-	-	33	0
Nioro	21	0	18	0	21	0	12	1	4	0	-	-	4	0
Dianke Makha	-	-	-	-	59	3	-	-	60	1	-	-	-	-
Kédougou	58	0	60	0	56	2	51	3	16	0	16	0	-	-
Velingara	58	1	58	4	58	0	60	2	42	0	16	0	-	-

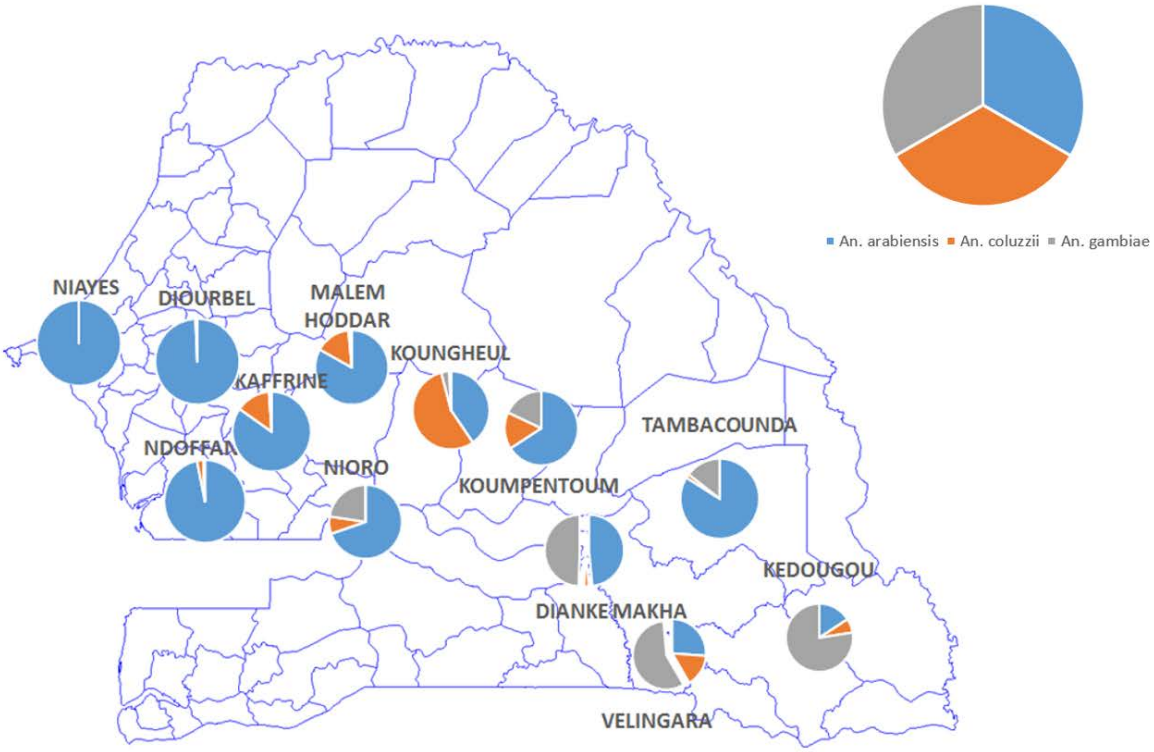
TABLE 16: NIGHTLY EIR OF MALARIA VECTORS COLLECTED USING HLC

Geographic Zone	Species	District	Indoor			Outdoor			Total		
			HBR	SI	EIR	HBR	SI	EIR	HBR	SI	EIR
Sahelian	*An. gambiae* s.l	Podor	0.33	-	-	0.75	-	-	0.54	-	-
		Richard Toll	0.08	-	-	0.08	-	-	0.08	-	-
Sudano-Sahelian		Bakel	1.00	-	-	1.00	-	-	1.00	-	-
		Matam	0.42	-	-	0.50	-	-	0.46	-	-
		Kanel	1.00	-	-	1.00	-	-	1.00	-	-
		Ranerou	1.08	-	-	1.08	-	-	1.08	-	-
		Linguere	0.17	-	-	0.25	-	-	0.29	-	-
		Niayes	0.028	-	-	0.17	-	-	0.097	-	-
		Diourbel	3.63	0.02	**0.09**	4.42	0.02	**0.106**	4.03	0.017	**0.07**
Sudanese		Kaffrine	0.41	-	-	0.44	-	-	0.43	-	-
		Malem Hodar	0.58	-	-	0.39	-	-	0.49	-	-
		Koungheul	0.58	0.05	**0.03**	0.53	0.03	**0.01**	0.55	0.037	**0.02**
		Nioro	0.58	-	-	0.75	0.02	**0.02**	0.67	0.012	**0.008**
		Ndoffane	1.87	0.02	**0.04**	2.22	-	-	2.05	0.0073	**0.01**
		Koumpentoum	0.25	-	-	0.21	-	-	0.23	-	-
		Tamba	0.66	-	-	0.44	0.06	**0.03**	0.56	0.025	**0.01**
Sudano-Guinean		Diankhé Makha	46.91	0.04	**1.87**	51	0.03	**1.53**	48.95	0.033	**1.61**
		Vélingara	18.72	0.02	**0.37**	16.31	0.03	**0.49**	17.51	0.024	**0.42**
		Kédougou	35.12	0.03	**1.05**	26.5	0.01	**0.26**	30.81	0.019	**0.58**
	An. funestus	Nioro	2.02	-	-	2.83	0.01	**0.03**	2.43	0.0048	**0.01**
		Ndoffane	4.98	0.01	**0.06**	8.47	0.01	**0.101**	6.73	0.009	**0.06**

3.5.4 *AN. GAMBIAE* S.L. SPECIES COMPOSITION

Figure 16 shows species composition of *An. gambiae* s.l. per site. *An. arabiensis* is predominant in the North but the proportion gradually decreased from North to South of the country and inversely for *An. gambiae* s.s. showing higher density in the South.

FIGURE 16: PROPORTION OF *AN. GAMBIAE* S.L. ACROSS DISTRICTS



From 460 *An. funestus* s.l. females collected through HLC and PSC and tested by PCR for molecular identification, 98% (449) were successfully amplified and identified as *An. funestus* s.s. in the districts of Nioro and Ndoffane. Further investigation is needed to understand why 2% didn't amplify (Table 17).

TABLE 17: NUMBER OF *AN. FUNESTUS S.L.* FEMALES IDENTIFIED BY PCR

	Tested	Amplified	September	October	November
Ndoffane	211	204	71	63	70
Nioro	249	245	72	93	80
Total	460	449	143	156	150

4. CONCLUSION

Overall, no change was observed in vector distribution, behavior and susceptibility to insecticides tested compared to the previous years' data. *An. gambiae* s. l. remained the predominant species for *Anopheles* fauna and *An. funestus* still have a higher density in Nioro and Ndoffane. In the past, *An. pharoensis* was predominant in Richard Toll area, and *An. funestus* was present in the north of the country as well. Neither of these species was collected in the north this year. Additional investigations should be done in these areas to determine if this year's finding confirms the current trend. Biting rate of *An. gambiae* s.l. in IRS sites was very low in both 2017 (0.49 bites/person/night) when sites were sprayed and 2018 (0.47 bites/person/night) when unsprayed. There was also not significant difference of the sporozoite rate in both years of collection (2.69% in 2017 vs 2.00% in 2018, p-value=0.4). Indoor resting density (1.88 females/house in 2017 vs. 2.15 in 2018) and parity rate (0.54 in 2017 vs 0.62 in 2018) increased slightly when unsprayed in 2018. However, an increase in malaria transmission was not observed between the previous IRS year and the first unsprayed year with 0.013 infected bites/person/night in 2017 and 0.009 infected bites/person/night in 2018 respectively. The same trends were observed with *An. funestus*, except the parity rate, which decreased from 0.51 in 2017 to 0.38 in 2018 (Annex I).

Both LLINs and IRS are eligible as malaria vector control tools in all areas of the country, but there may be a need for complementary tools targeting exophilic or exophagic vectors (*An. funestus*) or larval stages in certain areas such as Diourbel District. In the southeast of the country, the vectors bite more indoors than outdoors but despite the endophagic trends, showed an exophilic tendency; which could limit the effectiveness of IRS in the region.

Pirimiphos-methyl remains effective, except in Dakar suburbs (Wakhinane, Rufisque, and Niayes) and in the former IRS district of Malem Hodar, although the new insecticides tested, especially clothianidin, look to be promising for future malaria vector interventions. Resistance in the Dakar suburbs is likely due to the high pollution and excessive use of domestic insecticide while in Malem Hodar is likely the result of pirimiphos methyl as the insecticide for IRS. For the next IRS campaign, pirimiphos-methyl could be used in areas that have not been sprayed for at least two years and clothianidin-based products, such as SumiShield® and Fludora™ Fusion, could be considered for use in all other areas. However, because Fludora™ Fusion is a combination of deltamethrin and clothianidin and resistance was found to deltamethrin in most districts, further testing is needed to determine whether this product would be an effective option for IRS. When considering procurement of LLINs, synergist tests showed that PBO + deltamethrin may be more appropriate than PBO + permethrin LLINs.

Even though IRS and LLINs could be implemented in Diourbel, the urban status of the district may pose as a constraint (Senegal AIRS EOSR 2017, NMCP 2019) in achieving full coverage as there tends to be lower acceptance of these interventions in urban areas. The feasibility of larval breeding site management needs to be determined, but could contribute to decreasing transmission.

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ANNEXES

ANNEX A: GEOGRAPHICAL COORDINATES FOR SENTINEL VILLAGES SELECTED IN FORMER IRS DISTRICTS AND CONTROLS

DISTRICT	HEALTH POST	SENTINEL VILLAGES	GEOGRAPHICAL COORDINATES	
			Latitude	Longitude
Nioro	Darou Salam	Bamba Diakhatou	14.08069°	-16.04251°
	Thila Grand	Ndramé Ndimb	13.604914°	-15.963954°
Ndofane	Tawa Mboudaye	Tawa Mboudaye	13°58'31.6"	16°12'15.5"
	Keur Soce	Sagnanème	14°01'45"5	16°04'28"1
Koumpentoum	Koumpentoum	Village 1	13.909582°	-14.503577°
	Kouthiaba	Kouthiaba	14.177377°	-14.454830°
Tambacounda	Koussanar	Koussanar	13.864912°	-14.080138°
Koungheul	Ida Mouride	Ida Mouride	13.988108°	-14.681809°
	Saly Escale	Pakala	13.831722°	-14.937530°
Kaffrine	Djokoul	Wey Naan	13.980534°	-15.219800°
	Ngodibo	Pété	14.096960°	-15.452728°
Malem Hodar	Maka Belal	Maka Belal	14.109558°	-15.234244°
	Tip Saloum	Tip Saloum	14.18189°	-15.24248°

ANNEX B: SPECIFIC COMPOSITION OF *ANOPHELINE* FAUNA ACCORDING TO THE SAMPLING METHOD

Anopheles sp	Sahelian		Sudano-Sahelian							Sudanese									Sudano-Guinean			
	Richard Toll	Podor	Matam	Kanel	Ranerou	Linguere	Bakel	Niayes	Diourbel	Niakhar	Dioffior	Kaffrine	Malem Hodar	Koungheul	Nioro	Ndoffane	Koumpentoum	Tambacounda	Diankemakhan	Kédougou	Velingara	Oussouye
An. gambiae s.l.	46	33	139	106	236	59	219	156	736	858	88	604	411	247	435	1856	211	93	2531	4488	2534	1635
	100%	100%	100%	100%	68.41%	88.06%	100%	100%	99.86%	99.54%	98%	100%	99.5%	95.7%	30.7%	43.0%	99.1%	100%	99.72%	99.64%	99.6%	99.94%
An. pharoensis	0	0	0	0	0	0	0	0	1	0	2	0	1	3	5	0	1	0	3	2	6	1
	0%	0%	0%	0%	0%	0%	0%	0%	0.14%	0.00%	2%	0%	0.2%	1.2%	0.4%	0.0%	0.5%	0%	0.12%	0.04%	0.2%	0.06%
An. rufipes	0	0	0	0	109	8	0	0	0	4	0	0	1	8	19	0	1	0	4	1	0	0
	0%	0%	0%	0%	31.59%	11.94%	0%	0%	0.00%	0.46%	0%	0%	0.2%	3.1%	1.3%	0.0%	0.5%	0%	0.16%	0.02%	0.0%	0.00%
An. funestus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	957	2457	0	0	0	3	3	0
	0%	0%	0%	0%	0%	0%	0%	0%	0%	0.0%	0%	0%	0.0%	0%	67.4%	57.0%	0.0%	0%	0.00%	0.07%	0.1%	0.00%
An. coustani	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0
	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0.0%	0%	0.1%	0.0%	0.0%	0%	0.00%	0.00%	0.0%	0.00%
An. wecomei	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0.0%	0%	0.1%	0.0%	0.0%	0%	0.00%	0.00%	0.0%	0.00%
An. nili	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10	0	0
	0%	0%	0%	0%	0%		0%	0%	0%	0%	0%	0%	0.0%	0%	0.0%	0.0%	0.0%	0%	0%	0.22%	0%	0%
Total	**46**	**33**	**139**	**106**	**345**	**67**	**219**	**156**	**737**	**862**	**90**	604	413	258	1419	4313	213	93	2538	4504	2543	1636
Total Collected	79		1651							8265									11221			
An gambiae	79		1651							4803									11188			
An. pharoensis	0		1							12									11			
An. rufipes	0		117							33									5			
An. funestus	0		0							3414									6			
An. coustani	0		0							2									0			
An. wecomei	0		0							1									0			
An. nili	0		0							0									10			

HLC: Human Landing Catches; RC: Resting Collect

ANNEX C: HUMAN BITING RATE OF *AN. GAMBIAE* S.L. IN FORMER IRS DISTRICTS AND CONTROLS

		July	Aug	Sept	Oct	Nov	Dec	Total
Nioro	# person nights	24	24	24	24	24	24	144
	# *An. gambiae* s.l.	21	30	25	12	4	4	96
	HBR	**0.88**	**1.25**	**1.04**	**0.50**	**0.17**	**0.17**	**0.67**
Ndoffane	# person nights	24	24	24	24	24	24	144
	An. gambiae s.l.	78	20	40	54	18	84	294
	HBR	**3.25**	**0.83**	**1.67**	**2.25**	**0.75**	**3.50**	**2.04**
Malem Hodar	# person nights	24	24	24	24	24	24	144
	# *An. gambiae* s.l.	7	6	18	29	10	0	70
	HBR	**0.29**	**0.25**	**0.75**	**1.21**	**0.42**	**0.00**	**0.49**
Koungheul	# person nights	24	24	24	24	24	24	144
	# *An. gambiae* s.l.	28	14	13	16	3	0	74
	HBR	**1.17**	**0.58**	**0.54**	**0.67**	**0.13**	**0.00**	**0.51**
Kaffrine	# person nights	12	12	12	12	12	12	72
	# *An. gambiae* s.l.	4	1	7	10	8	2	32
	HBR	**0.33**	**0.08**	**0.58**	**0.83**	**0.67**	**0.17**	**0.44**
Koumpentoum	# person nights	24	24	24	24	24	24	144
	# *An. gambiae* s.l.	4	3	16	10	0	0	33
	HBR/	**0.17**	**0.13**	**0.67**	**0.42**	**0.00**	**0.00**	**0.23**
Tambacounda	# person nights	12	12	12	12	12	12	72
	# *An. gambiae* s.l.	9	1	8	12	10	0	40
	HBR	**0.75**	**0.08**	**0.67**	**1.00**	**0.83**	**0.00**	**0.56**
Total former IRS districts	# person nights	96	96	96	96	96	96	576
	# *An. gambiae* s.l.	60	53	72	67	17	4	273
	HBR	**0.63**	**0.55**	**0.75**	**0.70**	**0.18**	**0.04**	**0.47**
Total controls	# person nights	48	48	48	48	48	48	288
	# *An. gambiae* s.l.	91	22	55	76	36	86	366
	HBR	**1.90**	**0.46**	**1.15**	**1.58**	**0.75**	**1.79**	**1.27**

ANNEX D: ENDOPHAGIC RATE OF *AN. GAMBIAE* S.L. IN FORMER IRS DISTRICTS AND CONTROLS

Endophagic Rate		July	August	September	October	November	December	Total
Nioro	Indoor	10	15	11	4	0	2	42
	Outdoor	11	15	14	8	4	2	54
	Rate	**0.48**	**0.50**	**0.44**	**0.33**	**0.00**	**0.50**	**0.44**
Ndoffane	Indoor	33	10	12	26	9	45	135
	Outdoor	45	10	28	28	9	39	159
	Rate	**0.42**	**0.50**	**0.30**	**0.48**	**0.50**	**0.54**	**0.46**
Malem Hodar	Indoor	3	5	7	22	5	0	42
	Outdoor	4	1	11	7	5	0	28
	Rate	**0.43**	**0.83**	**0.39**	**0.76**	**0.50**	**-!**	**0.60**
Koungheul	Indoor	15	8	7	9	2	0	41
	Outdoor	13	6	6	7	1	0	33
	Rate	**0.54**	**0.57**	**0.54**	**0.56**	**0.67**	**-**	**0.55**
Kaffrine	HN/house	1	1	2	5	5	1	15
	Effective	3	0	5	5	3	1	17
	HBR/IRD	**0.25**	**1.00**	**0.29**	**0.50**	**0.63**	**0.50**	**0.47**
Koumpentoum	Indoor	1	2	10	5	0	0	18
	Outdoor	3	1	6	5	0	0	15
	Rate	**0.25**	**0.67**	**0.63**	**0.50**	**-**	**-**	**0.55**
Tambacounda	Indoor	7	0	4	8	5	0	24
	Outdoor	2	1	4	4	5	0	16
	Rate	**0.78**	**0.00**	**0.50**	**0.67**	**0.50**	**-!**	**0.60**
Total former IRS districts	Indoor	29	30	35	40	7	2	143
	Outdoor	31	23	37	27	10	2	130
	Rate	**0.48**	**0.57**	**0.49**	**0.60**	**0.41**	**0.50**	**0.52**
Total controls	Indoor	41	11	18	39	19	46	174
	Outdoor	50	11	37	37	17	40	192
	Endophagic Rate	**0.45**	**0.50**	**0.33**	**0.51**	**0.53**	**0.53**	**0.48**

ANNEX E: INDOOR RESTING DENSITY OF *AN. GAMBIAE* S.L. IN FORMER IRS DISTRICTS AND CONTROLS

		July	August	September	October	November	December	Total
Nioro	# Houses	20	20	20	20	20	20	120
	# Collected	125	81	75	37	12	9	339
	IRD	**6.25**	**4.05**	**3.75**	**1.85**	**0.60**	**0.45**	**2.83**
Ndoffane	# Houses	20	20	20	20	20	20	120
	# Collected	364	169	59	428	173	365	1558
	IRD	**18.20**	**8.45**	**2.95**	**21.40**	**8.65**	**18.25**	**12.98**
Malem Hodar	# Houses	20	20	20	20	20	20	120
	# Collected	94	60	78	77	30	2	341
	IRD	**4.70**	**3.00**	**3.90**	**3.85**	**1.50**	**0.10**	**2.84**
Koungheul	# Houses	20	20	20	20	20	20	120
	# Collected	76	25	16	38	18	0	173
	IRD	**3.80**	**1.25**	**0.80**	**1.90**	**0.90**	**0.00**	**1.44**
Kaffrine	# Houses	10	10	20	10	10	10	70
	# Collected	12	27	31	256	244	2	572
	IRD	**1.20**	**2.70**	**1.55**	**25.60**	**24.40**	**0.20**	**8.17**
Koumpentoum	# Houses	20	20	20	20	20	20	120
	# Collected	25	42	30	68	13	0	178
	IRD	**1.25**	**2.10**	**1.50**	**3.40**	**0.65**	**0.00**	**1.48**
Tambacounda	# Houses	10	10	20	10	10	10	70
	# Collected	14	12	4	9	14	0	53
	IRD	**1.40**	**1.20**	**0.20**	**0.90**	**1.40**	**0.00**	**0.76**
Total former IRS districts	# Houses	80	80	80	80	80	80	480
	# Collected	320	208	199	220	73	11	1031
	IRD	**4.00**	**2.60**	**2.49**	**2.75**	**0.91**	**0.14**	**2.15**
Total controls	# Houses	40	40	60	40	40	40	260
	# Collected	390	208	94	693	431	367	2183
	IRD	**9.75**	**5.20**	**1.57**	**17.33**	**10.78**	**9.18**	**8.40**

ANNEX F: PARITY RATE OF *AN. GAMBIAE* S.L. IN FORMER IRS DISTRICTS AND CONTROLS

		July	August	September	October	November	December	Total
Nioro	Total dissected	17	19	19	10	1	4	70
	Parous	5	0	6	3	0	2	16
	Parity rate	**0.29**	**0.00**	**0.32**	**0.30**	**0.00**	**0.50**	**0.23**
Ndoffane	TD	145	61	10	49	44	15	324
	Parous	44	11	1	33	6	5	100
	Parity rate	**0.30**	**0.18**	**0.10**	**0.67**	**0.14**	**0.33**	**0.31**
Malem Hodar	Total dissected	6	6	18	28	9	0	67
	Parous	6	3	11	27	8	0	55
	Parity rate	**1.00**	**0.50**	**0.61**	**0.96**	**0.89**	**0**	**0.82**
Koungheul	Total dissected	17	7	1	6	1	0	32
	Parous	17	6	1	6	1	0	31
	Parity rate	**1.00**	**0.86**	**1.00**	**1.00**	**1.00**	**-**	**0.97**
Kaffrine	Total dissected	4	1	6	8	8	2	29
	Parous	3	1	1	5	7	0	17
	Parity rate	**0.75**	**1.00**	**0.17**	**0.63**	**0.88**	**0.00**	**0.59**
Koumpentoum	Total dissected	3	3	7	8	0	0	21
	Parous	2	0	5	8	0	0	15
	Parity rate	**0.67**	**0.00**	**0.71**	**1.00**	**-**	**-**	**0.71**
Tambacounda	Total dissected	6	0	6	7	10	0	29
	Parous	6	0	6	6	9	0	27
	Parity rate	**1.00**	**-**	**1.00**	**0.86**	**0.90**	**-**	**0.93**
Total former IRS districts	Total dissected	43	35	45	52	11	4	190
	Parous	30	9	23	44	9	2	117
	Parity rate	**0.70**	**0.26**	**0.51**	**0.85**	**0.82**	**0.50**	**0.62**
Total controls	Total dissected	155	62	22	64	62	17	382
	Parous	53	12	8	44	22	5	144
	Parity rate	**0.34**	**0.19**	**0.36**	**0.69**	**0.35**	**0.29**	**0.38**

ANNEX G: HUMAN BITING RATE, ENDOPHAGY INDEX, INDOOR RESTING DENSITY, AND PARITY RATE OF *AN. GAMBIAE* S.L. IN DIOURBEL

	July	August	September	October	November	December	Average
# person nights	24	24	24	24	24	24	144
# collected	17	144	178	66	67	97	569
HBR	**0.71**	**6.00**	**7.42**	**2.75**	**2.79**	**4.04**	**3.95**
# Collected Indoor	15	72	79	27	21	48	262
# Collected Outdoor	2	72	99	39	46	49	307
Endophagy Index	**0.88**	**0.50**	**0.44**	**0.41**	**0.31**	**0.49**	**0.46**
Total Rooms	20	20	20	20	20	20	120
Total Collected	34	30	74	8	5	16	167
IRD	**1.7**	**1.5**	**3.7**	**0.4**	**0.25**	**0.8**	**1.4**
# Dissected	16	71	132	46	50	80	395
# Parous	5	19	15	8	14	75	136
Parity Rate	**0.31**	**0.27**	**0.11**	**0.17**	**0.28**	**0.94**	**0.17**

ANNEX I: ENTOMOLOGICAL INDICATORS FOR *AN. GAMBIAE S.L.* AND *AN. FUNESTUS* IN SPRAYED SITES (2017) COMPARED TO UNSPRAYED (2018)

	Human Biting Rate	Indoor Resting Density	Parity Rate	Anthropophilic Rate	Sporozoites Rate	EIR
An. gambiae s.l.						
2017	0.49	1.88	0.54	0.28	0.027	0.013
2018	0.47	2.15	0.62	0.16	0.020	0.009
Ratio	0.96	1.14	1.15	0.57	0.74	0.69
An. funestus s.l.						
2017	2.40	4.27	0.51	0.09	0.009	0.021
2018	2.20	9.90	0.38	0.13	0.005	0.011
Ratio	0.92	2.32	0.74	1.44	0.56	0.52