PMI | Africa IRS (AIRS) Project

Indoor Residual Spraying (IRS 2) Task Order Six

SENEGAL 2017 FINAL ENTOMOLOGICAL MONITORING REPORT

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SENEGAL 2017 FINAL ENTOMOLOGICAL MONITORING REPORT

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ACRONYMS

AIRS	Africa Indoor Residual Spraying Project
CDC	Centers for Disease Control and Prevention
CSP	Circumsporozoite
ELISA	Enzyme-Linked Immuno-Sorbent Assay
FST	Faculty of Sciences and Technology
HBR	Human Biting Rate
HLC	Human Landing Catch
IRD	Indoor Resting Densities
IRS	Indoor Residual Spraying
KDR	Knock Down Resistance
LEVP	Laboratory of Vector and Parasite Ecology
NMCP	National Malaria Control Program
PCR	Polymerase Chain Reaction
PMI	President's Malaria Initiative
PSC	Pyrethrum Spray Catch
UCAD	University of Cheikh Anta Diop
WHO	World Health Organization

EXECUTIVE SUMMARY

This report presents key findings of entomological monitoring conducted during the dry season (January to March) and the rainy season (August-November) for the 2017 campaign. The IRS campaign was conducted between June 30 and July 23, 2017. In each of the four indoor residual spraying (IRS) districts (Nioro, Malem Hodar, Koungheul, and Koupentoum), two sentinel sites were added in each district to the four the team used for monitoring in 2016. The additional two sites were from neighboring districts that did not receive IRS (external control). Therefore, for each IRS district, there were two sprayed hot spots, two unsprayed non-hot spot (internal control), and the two unsprayed non-hot spot external controls.

Vector monitoring during the dry season (before IRS) resulted in very low catches of *Anopheles* in three of the four districts. However, in Nioro, the team collected 2,620 *Anopheles*, with the majority being *An. funestus* s.l. Post-IRS, *An. gambiae* s.l. was the main species collected in three sites, with *An. funestus* s.l. continuing to be the predominant species in Nioro.

Cone bioassay of walls (mud and cement) sprayed with pirimiphos-methyl (Actellic CS 300) produced mean mortality rates greater than the World Health Organization (WHO) threshold of 80 percent for between three to five months after spraying.

The peak biting rates were recorded in September for Nioro and Koumpentoum, in October for Malem Hodar, and in November for Koungheul. In general, *An. gambiae* s.l. biting rates were low even in the rainy season, with the highest being <2 bites per person per night. The mean biting rates of *An. gambiae* s.l. in IRS districts were consistently low in the unsprayed external control sites. Indoor resting densities (IRDs) of *An. gambiae* s.l. were highest during the rainy season, with a peak in Nioro districtof 5.9 females per room per day in September. The mean biting rate of *An. funestus* in Nioro was higher than for *An. gambiae* s.l. with a peak in October (4 bites per person per night). Indoor resting densities (IRDs) of *An. funestus* s.l. were highest during the dry season, reaching a peak in March at 14.9 per room per day.

The average anthropophily rate was low in sprayed areas and in their control sites, with a mean anthropophily rate of 0.27 (208/775). Horses were the main blood-source of *An. gambiae* s.l. females in both sprayed sites and their internal and external controls and represent 41% of the blood meals (318/775). The anthropophily rate of *An. gambiae* s.l. in unsprayed districts was high in Velingara (0.91; 40/44) and in Kedougou (0.92; 35/38).

The presence of infective females in sprayed villages was noted in all districts except Koungheul. In general the number of *Anopheles* collected was low in all sprayed sites and internal control sites, resulting in only one or two positive mosquitoes per site and making accurate determination of sporozoite rates difficult. In Nioro, two infective *An. funestus* s.l. were collected in sprayed sites and controls, indicating this species is involved in transmission despite the low anthropophily rate of 0.09 in sprayed areas and 0.11 in unsprayed areas of Nioro. In Velingara and Kedougou, the sporozoite rates were approximately 1 to 2%, although the number tested was also small.

The results of WHO susceptibility assays showed resistance of *Anopheles gambiae* s.l. to all three pyrethroid insecticides tested in the majority of the districts in Senegal. For pirimiphos-methyl 0.25%, the vector populations were fully susceptible in all current IRS sites, but resistance occurred in Pikine (Dakar suburb) and Diourbel. The fact that susceptibility to bendiocarb and deltamethrin in IRS districts is confined to pockets may indicate that IRS with an organophosphate has limited the development of resistance to other insecticide classes.

Overall, there was evidence that IRS had an impact in terms of vector biting rates and resting densities. However, the low general vector densities and high zoophily rate of *An. arabiensis* and *An. funestus* s.l. across the majority of Senegal, even in unsprayed areas, makes it difficult to quantify the degree of impact in terms of disease transmission. In Velingara and Kedougou, the vector trends are different and highly anthropophilic *An. gambiae*. predominate.

1. INTRODUCTION

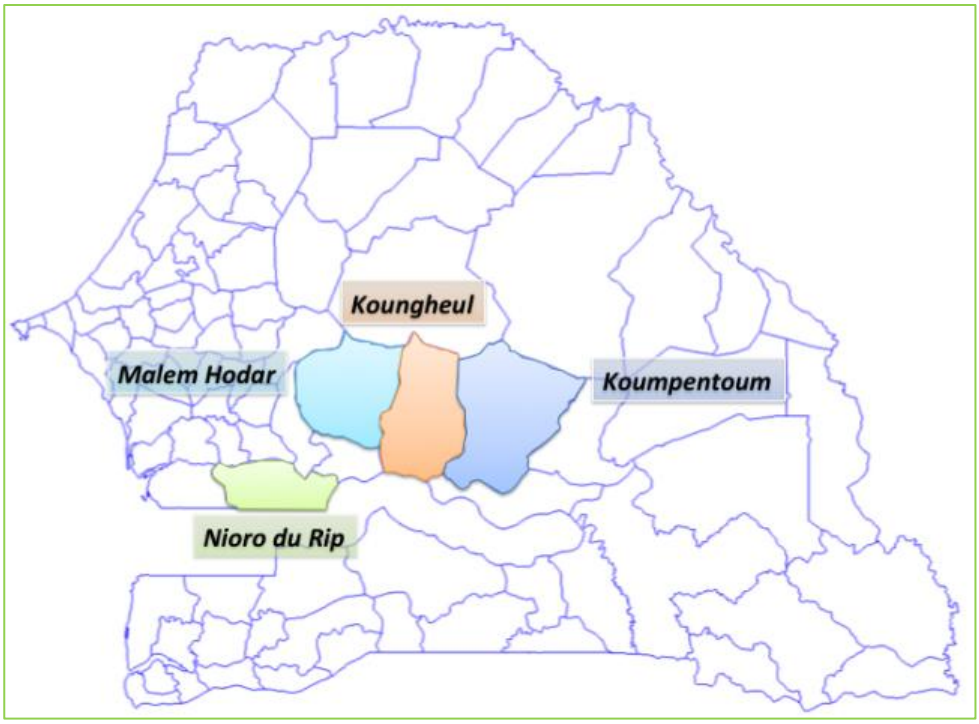
In Senegal, IRS implementation began as a pilot in three health districts (Velingara, Nioro and Richard-Toll) in 2007. Based on the results achieved, the National Malaria Control Program (NMCP) and President's Malaria Initiative (PMI) decided to expand IRS to new districts in 2010 (Guinguineo, Malem Hodar, and Koumpentoum). Since 2013, Africa IRS (AIRS)/Senegal has been implementing IRS in four districts (with Nioro replacing Velingara in 2015).

In 2015, AIRS started implementing IRS in Koumpentoum, Koungheul, Malem Hodar, and Nioro (Figure 1) with a new strategy. The strategy targets health posts with high malaria incidence (> 15 cases/1,000 inhabitants) known as hot spots inside the health district. This led to a change in entomological monitoring, with the number of sentinel sites per district reduced (from five to four in IRS districts). The entomology monitoring frequency increased to monthly. For a third year, the campaign focused on 2015 hot spots.

The Laboratory of Vector and Parasite Ecology (LEVP) of the Faculty of Science and Technology (FST) at the University of Cheikh Anta Diop (UCAD) in Dakar, in collaboration with NMCP, has been implementing entomological monitoring activities in Senegal since 2007. Since 2015, while LEVP continued the implementation of entomological monitoring activities, it has been a subcontractor under the PMI AIRS Project.

The main results of the dry season and rainy season for the 2017 campaign are presented in this report.

Figure 1. Geographical locations of the PMI-Senegal IRS districts sprayed for the 2017 campaign



2. METHODOLOGY

2.1 DISTRICTS AND SENTINEL VILLAGES FOR THE 2017 CAMPAIGN

In each of the four IRS districts, four sentinel sites were used for monitoring in 2017 In Nioro, Malem Hodar and Koungheul districts, two sentinel sites were malaria hot-spot villages (sprayed) and two were non-hot spot villages (unsprayed). For each IRS district, we selected an additional two sites from neighboring districts that did not receive IRS (external control).

Classification of sites as hotspot or non-hot spot was originally based on 2013 data and has since been updated by NMCP based on 2015 health facility data. Therefore, some unsprayed non-hot spot villages have since been re-classified as hot-spots. Therefore, in Koumpentoum the four sentinel sites were all hot spots based on 2015 classification (all sprayed).

In Koungheul the former internal controls Touba Ali Benda and Nguerane boumack were sprayed in 2017 and were replaced by Sam Diebel (Fass thieckene health post) and Ko Soce (Keur mandoumbe health post).

TABLE 1: SENTINEL VILLAGES SELECTED IN IRS DISTRICTS AND THEIR CONTROL, AUGUST - NOVEMBER 2017

District	Status	Health Post	Sentinel Villages	Geographical coordinates	
				Latitude	Longitude
Nioro	Hot Spots	Darou Salam	Bamba Diakhatou	14.08069°	16.04251°
		Thila Grand	Ndramé Ndimb	13.604914°	-15.963954°
	Non-Hot Spots	Paos Koto	Paos Koto	13.783977°	-15.801159°
		Medina Sabakh	Camara	13°38'17.6''	15°57'48.2''
Ndofane	Control for Nioro	Tawa Mboudaye	Tawa Mboudaye	13°58'31.6''	16°12'15.5''
		Darou Mbitteyene	Darou Mbitteyene	13°59'01.5''	16°08'11.9''
Koumpentoum	Hot Spots	Koumpentoum	Village 1	13.909582°	-14.503577°
		Méréto	Koumaré	13.905140°	-14.372731°
		Kouthiaba	Kouthiaba	14.177377°	-14.454830°
		Syll Serigne Malick	Syll Serigne Malick	14°12.341'	-14°32.506'
Tambacounda	Control for Koumpentoum	Koussanar	Koussanar	13.864912°	-14.080138°
		Sinthiou Malem	Ly Counda	13.791756°	-13.839031°
Koungheul	Hot Spots	Ida Mouride	Ida Mouride	13.988108°	-14.681809°
		Saly Escale	Pakala	13.831722°	-14.937530°
	Non-Hot Spots	Fass thieckene	Sam Diebel	13.90672°	014.78555°
		Keur mandoumbe	Ko Soce	13.84771°	014.85147°
Kaffrine	Control for Koungheul	Djokoul	Wey Naan	13.980534°	-15.219800°
		Ngodibo	Pété	14.096960°	-15.452728°
Malem Hodar	Hot Spots	Maka Belal	Maka Belal	14.109558°	-15.234244°
		Tip Saloum	Tip Saloum	14.18189°	15.24248°
	Non-Hot Spots	Dianké Souf	Dianké Souf	14.228570°	-15.334641°
		Ndiote Seane	Ndiote Mor Coumba	14.420000°	-15.178220°
Kaffrine	Control for Malem Hodar	Ngodibo	Pété	14.096960°	-15.452728°
		Kathiote	Thiamene Kathiote	13.56952°	-15.23827°

Key Terminology
Sprayed = hotspot village sprayed with Actellic CS in 2017.
Internal control = low transmission unsprayed village within the same district as the sprayed site

collect larvae for susceptibility tests. The geographical locations of sentinel sites are represented in Figure 2.

The sentinel sites in unsprayed districts for entomology surveillance in Senegal are the same as the previous except in Velingara, where the team collected additional data in October on vector behavior from the south east of Senegal. There malaria transmission is higher.

Figure 2. Geographical locations of districts with entomological monitoring

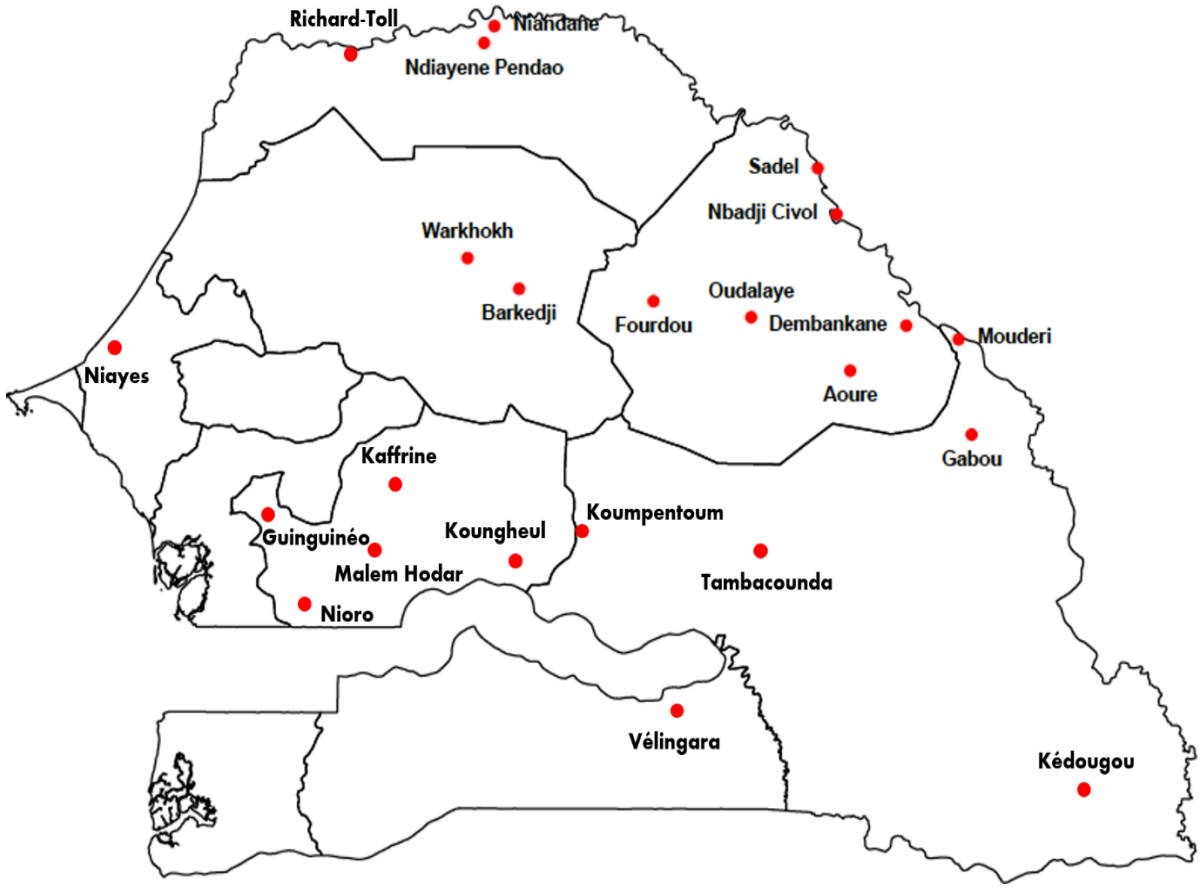

Richard-Toll
Ndiayene Pendao
Sadel
Nbadji Civol
Warkhokh
Barkedji
Oudalaye
Fourdou
Dembankane
Mouderi
Niayes
Aoure
Gabou
Kaffrine
Koumpentoum
Guinguinéo
Koungheul
Malem Hodar
Tambacounda
Nioro
Vélingara
Kédougou

TABLE 2: SENTINEL VILLAGES SELECTED IN UNSPRAYED DISTRICTS

Health district	Sentinel villages	Entomological activities	Frequency
Northern and East Central Districts			
Niayes	Ngadiaga, Ndiambalo, Thiaye, Touba Taw Fekh, and Beer	HLC indoors/outdoors, indoor PSC, parity rates, susceptibility tests.	Once every two months
Richard-Toll	Mbagame, Rosso Béthio, Ndiandiou, Maka Diama, Taba Darou Salam, Mallé, Gnith, Ronkh, Khor, and Reynabé	HLC indoors/outdoors, indoor PSC, parity rates, susceptibility tests	Once during the rainy season
Linguere*	Barkedji and Ouarkhokh	HLC indoors/outdoors, indoor PSC, parity rates	
Podor*	Ndiayène Pendao and Niandane	HLC indoors/outdoors, indoor PSC, parity rates	
Ranerou*	Oudalaye and Fourdou	HLC indoors/outdoors, indoor PSC, parity rates	
Matam*	Sadel and Nabadji Ciwol	HLC indoors/outdoors, indoor PSC, parity rates	
Kanel*	Haouré and Dembankané	HLC indoors/outdoors, indoor PSC, parity rates	
Bakel*	Gabou and Moudéry	HLC indoors/outdoors, indoor PSC, parity rates	
Niakhar	Niakhar	Susceptibility testing	Once
Diourbel	Keur Mbaye Sarr	Susceptibility testing	Once
Pikine et Guédiawaye	(Flooded areas in suburbs of Dakar)	Susceptibility testing	Once
Southern Districts			
Tambacounda	Koussanar¥, Lycounda¥, and Badi	HLC indoors/outdoors, indoor PSC, parity rates	Once per month
Ndoffane	Tawa Mboudaye¥ and Darou Mbitéyène¥	HLC indoors/outdoors, indoor PSC, parity rates	
Kaffrine	Pété¥, Thiamène Cathiote¥, and Wey	HLC indoors/outdoors, indoor PSC, parity	
Malem Hodar	Malem Thiérigne¥	HLC indoors/outdoors, indoor PSC, parity	
Kédougou	Tomboronkoto and Bandafassi	HLC indoors/outdoors, indoor PSC, parity rates,	Once every two months
Vélingara	Madina Dianguet and Nemataba	HLC indoors/outdoors, indoor PSC, parity rates, susceptibility tests	
Guinguineo	Guinguineo city	Susceptibility testing	Once

¥External control villages of IRS districts * Districts of Senegal River Valley

2.2 INSECTICIDES SPRAYED

PMI-AIRS sprayed pirimiphos-methyl (Actellic CS 300) at 1g/m^2 in the districts of Koungheul, Koumpentoum, Malem Hodar, and Nioro. Table 3 illustrates spraying and testing dates.

TABLE 3: IRS TREATMENT DATES AND TIMING OF CONE BIOASSAY IN THE IRS SENTINEL VILLAGES IN 2017

District	Sentinel Site	Date of spray	1st bioassay	2nd bioassay	3rd bioassay	4th bioassay	5th bioassay
Koungheul	Pakala	16/07/2017	05/08	09/09	10/10	17/11	12/12
	Ida Mouride	19/07/2017	04/08	08/09	09/10	16/11	11/12
Malem Hodar	Makka Bella	05/07/2017	05/08/2017	09/09	10/10	16/11	11/12
	Tip Saloum	05/07/2017	08/08/2017	15/09	11/10	17/11	12/12
Koumpentoum	Koumaré	14/07/2017	08/08	13/09	15/10	17/11	13/12
	Village 1	07/07/2017	07/08	12/09	14/10	16/11	14/12
	Kouthiaba	21/07/2017	09/08	14/09	16/10	18/11	15/12
	Syll Sérigne Malick	08/07/2017	10/08	15/09	17/10	19/11	17/12
Nioro	Bamba Diakhatou	30/07/2017	05/08	14/09	23/10	16/11	13/12
	Ndramé Ndimb	30/07/2017	06/08	15/09	25/10	17/11	14/12

2.3 EFFECTIVENESS OF INDOOR RESIDUAL SPRAYING (IRS)

UCAD conducted cone bioassays for quality assurance after spraying with a susceptible strain of *An. gambiae* s.l. in the four IRS districts (Koumpentoum, Malem Hodar, Koungheul, and Nioro). The target was to conduct the cone bioassay within one week of IRS. However, the same team conducted bioassays in all sites so there were delays that meant conducting bioassays one to four weeks after IRS. Treatment effectiveness in Malem Hodar, Koungheul, and Nioro was determined in 10 sprayed residential rooms selected in two treated villages (five per village), with two untreated control rooms (one per village). In Koumpentoum, the team tested 20 rooms. The choice of rooms in the villages was done by lottery (drawing numbers from a tin) and selected rooms were repeatedly tested each month during monitoring.

Cone bioassays were performed in each room according to WHO standard protocols. Female mosquitoes of a susceptible strain of *Anopheles coluzzii* (originally from Cameroon) reared at the insectary (Research Institute for Development, Institut Pasteur of Dakar, and Parasite Vector Control Service) were used for this purpose. Three cones were placed on each wall and 10

mosquitoes were exposed in each cone. The location of the cones on the walls changed slightly each month as it was noted that tape used for attaching cones removed part of the wall surface when removed. For the negative controls, three cones were fixed to a piece of untreated white paper and then attached to an untreated wall. Mortality of test mosquitoes was recorded 24 hours after exposure, with Abbott's correction implemented if mortality was between 5 percent and 20 percent in the negative controls. The IRS treatment was considered effective if the mortality was greater than 80 percent, in accordance with WHO guidelines.

2.4 Monitoring Vector Dynamics

The team sampled vector populations by i) indoor collections in homes by pyrethrum spray catch (PSC) and ii) night time human landing catch (HLC) indoors and outdoors.

The project carried out collections of indoor resting mosquitoes by PSC in sprayed and control districts, in 10 rooms per village per month. In each village, HLC was conducted for two consecutive nights in three houses by six people per night located indoors and outdoors (two humans per house). The same houses were used each month for both PSCs and HLCs.

In the field, the project team morphologically identified (genus / species) collected specimens. A sub-sample of host-seeking females was dissected for the determination of parity rate. Blood-fed females were individually preserved in micro-tubes for determination of blood meal source. All captured females were individually conserved in micro-tubes for laboratory analysis (species identification, infection and knock down resistant (*kdr*) gene detection, etc).

2.5 WHO susceptibility tests

The project carried out insecticide susceptibility tests in four sprayed districts and in selected unsprayed districts. Adult females two to five days old that were collected from the wild as larvae were used for testing.

Insecticides tested

The tests were carried out in WHO-test cylinders with papers impregnated with diagnostic concentrations of the following insecticides:

Pyrethroids:

- Deltamethrin 0.05%
- Permethrin 0.75%
- Alphacypermethrin 0.05%

Organophosphates:

- Pirimiphos-methyl 0.25%

Carbamates:

- Bendiocarb 0.1%

The project team exposed mosquitoes to treated papers for one hour and recorded mortality after 24 hours post-exposure.

For each insecticide, at least 100 mosquitoes were tested in four replicates of 25. An accompanying negative control was always tested. The basis of the interpretation of the results based on WHO 2013 guidelines is in the table below.

Susceptibility status	WHO threshold	Additional threshold	Observations
Susceptible	98-100%	98-100%	Susceptibility confirmed
Resistant	Less than 98%	90-98%	Resistance suspected
		Less than 90%	Resistance confirmed

2.6 CENTERS FOR DISEASE CONTROL AND PREVENTION (CDC) BOTTLE BIOASSAYS FOR RESISTANCE INTENSITY MONITORING

The project carried out insecticide resistance intensity tests from September through October in Nioro and Kédougou districts and Richard Toll and Dakar suburbs (Pikine and Guediawaye). Female *An. gambiae* s.l. from wild larvae that were two to five days old were used for these tests. Two molecules were tested; deltamethrin in all sites and bendiocarb in Dakar suburbs.

The specimens were exposed to a diagnostic time of 30 minutes and the tests were corrected with Abbott's formula when control mortality was between three percent and 10%. The interpretation of the results is based on WHO criteria for susceptibly tests.

2.7 LABORATORY MOLECULAR ANALYSES

Sporozoite rate

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

Blood meal source

From *An. gambiae* s.l. collected by PSC, the origin of blood meals was determined by the direct ELISA method described by Beier et al. (1986). The anthropophily rate was determined by the ratio of the number of blood meals taken from humans over the number of meals identified. The same was done for the different animal hosts tested.

Species identification

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

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 95% confidence interval was calculated for infection rates of *Plasmodium falciparum*.

3. RESULTS

3.1 RESIDUAL EFFECTIVENESS OF PIRIMIPHOS-METHYL IRS AGAINST A SUSCEPTIBLE STRAIN OF *ANOPHELES COLUZZII* IN CONE BIOASSAY

Cone bioassay of walls (mud and cement) sprayed with pirimiphos-methyl (Actellic CS 300) produced mean mortality rates greater than the WHO threshold of 80 percent three to five months after spraying (Table 4).

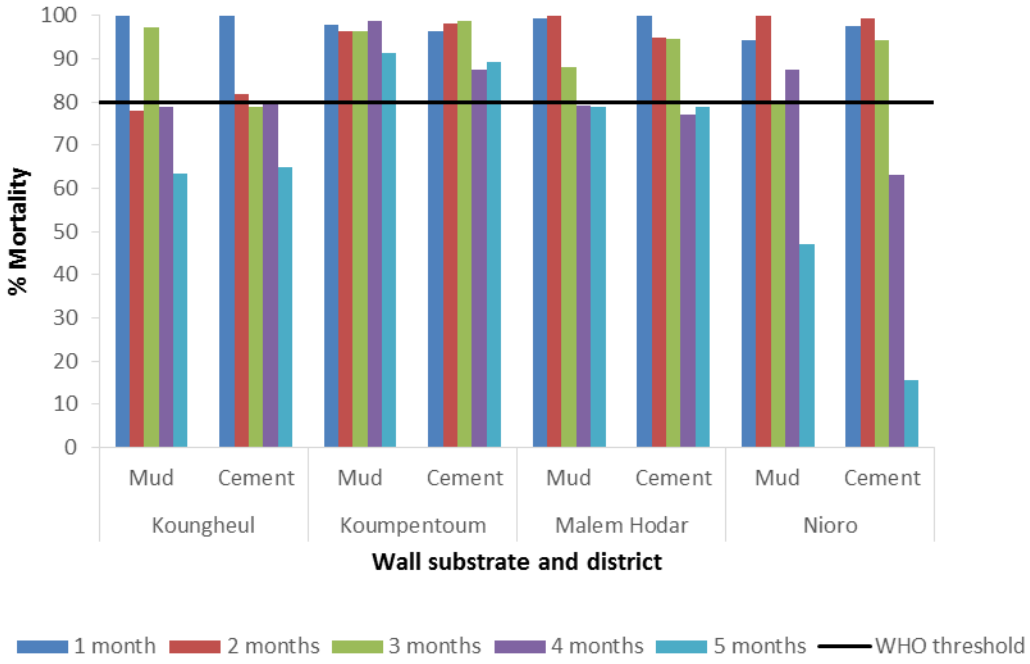
TABLE 4: EFFECTIVENESS OF PIRIMIPHOS-METHYL (ACTELLIC CS 300) ON MUD AND CEMENT WALLS AGAINST A SUSCEPTIBLE INSECTARY STRAIN OF *AN. GAMBIAE* S.S. IN IRS DISTRICTS

Districts		Mortality Rate %				
		1 month	2 months	3 months	4 months	5 months
Koumpentoum	Control	2.5% (3/120)	5.8% (7/121)	4.2% (5/120)	3.3% (4/120)	0.8% (1/122)
	Exposed	97.3% (584/600)	97.7%* (619/633)	97.9% (600/613)	82.3% (494/600)	90.1% (548/608)
Koungheul	Control	3.3% (2/61)	6.3% (4/64)	1.6% (1/62)	1.6% (1/62)	3.2% (2/63)
	Exposed	100% (314/314)	79.6%* (249/308)	87.8% (259/295)	84.7% (271/320)	65.4% (200/306)
Malem Hodar	Control	3.2% (2/62)	10.9% (7/64)	3.1% (2/64)	0% (0/64)	0% (0/61)
	Exposed	99.7% (316/317)	97.5%* (306/313)	91.3% (285/312)	78.2% (244/312)	78.8% (242/307)
Nioro	Control	6.9% (5/72)	5.5% (4/73)	4.2% (3/71)	0% (0/71)	1.4% (1/71)
	Exposed	97.2%* (332/341)	99.4%* (371/373)	92.9% (325/350)	65.5% (224/342)	18.8% (66/352)

*Adjusted mortality by Abbot's formula
1 month = August; 2 month = September; 3 months = October; 4 months = November; 5 months = December.
Nb. T0- Due to delays, cone bioassay for quality assurance was approximately 1 month after spraying.

The residual effect according the nature of the wall seemed to be the same except in Nioro where lower residual efficacy was seen on cement (Fig 3). In Nioro, the decrease in mortality was more significant in one of the two sentinel villages and results were different according to individual houses.

Figure 3. Residual efficacy of Actellic 300CS (pirimiphos-methyl) sprayed on mud and cement walls in Koungheul, Koumpentoum, Malem Hodar, and Nioro. Cone bioassay with susceptible insectary reared *An. coluzzii* for 30 minutes, with mortality recorded 24 hours later.



In Koungheul, 765 *An. gambiae* s.s. were exposed on mud walls and 621 on cement walls to monitor the effectiveness of pirimiphos-methyl (Actellic CS 300). Mortality was 100% for all rooms tested one month after treatment. Mortality was greater than 80% on cement and on mud walls four months after spraying (Annex 1a).

In Koumpentoum, 3,054 *An. gambiae* s.s. were exposed. The sprayed walls were effective after five months with a mortality of 91.3% on mud walls and 89.3% on cement walls (Annex 1b).

In Malem Hodar, 779 *An. gambiae s.s.* were exposed to mud walls compared with 782 for cement. Mortality was greater than 80% for three months on mud and cement, with mortality >78% after five months (Annex 1c).

In Nioro, 1,758 *An. gambiae s.s.* were exposed to the walls (177 on mud walls compared to 1,581on cement). The insecticide remained effective three months after spraying (Annex 1d).

3.2 Vector Population Dynamics in IRS Districts

3.2.1 Composition of species

Mosquito collections conducted in the dry season (January to March) resulted in very low catch size in Koumpentoum, Koungheul, and Malem Hodar (<15 *Anopheles* at each site). However, in

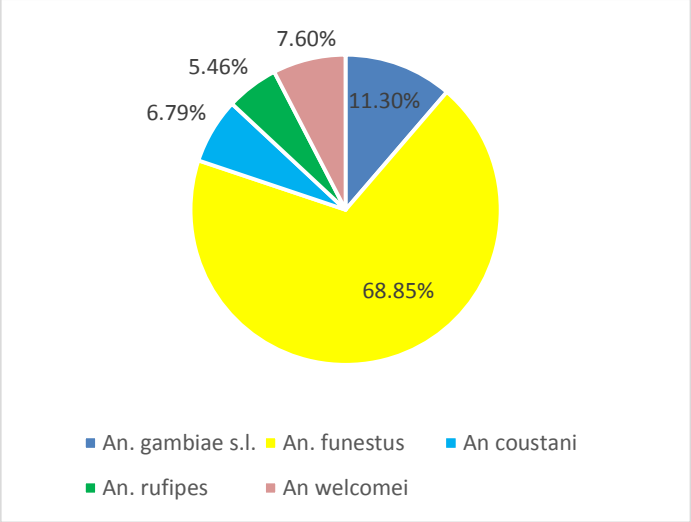
Nioro the team collected 2,620 *Anopheles*, with 69% being *An. funestus* s.l. and only 11% *An. gambiae* s.l. (Figure 4a).

Between August and November 2017, after spraying, *Anopheles gambiae* s.l. was the main species group caught in resting collections by PSC and biting collections by HLC in the districts of Koumpentoum, Koungheul, and Malem Hodar. In Nioro district, *An. funestus* s.l. continued to be the predominant species, accounting for 55% of all *Anopheles* collected (Fig. 5). Other *Anopheles* that were collected included *An. pharoensis* and *An. rufipes* in all IRS districts except Koumpentoum.

Figure 4: Species composition of *Anopheles* caught by HLC and PSC in IRS districts during pre (January to March) and Post (August to November) IRS 2017.

Nioro

a) Pre –IRS n=2,620



b) Post –IRS n= 2,063

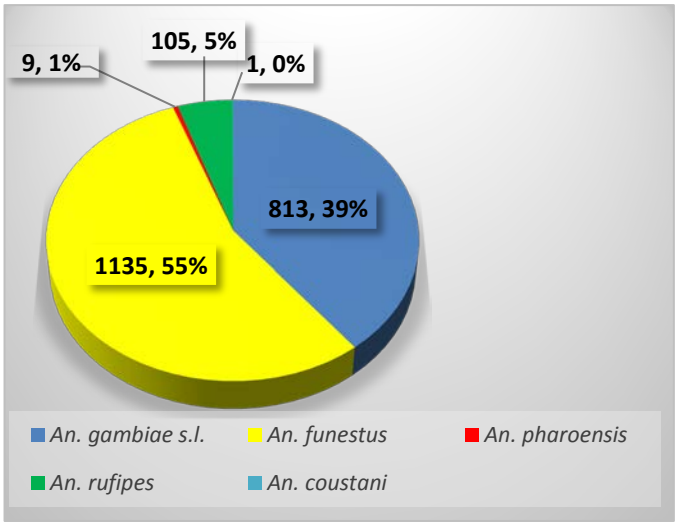


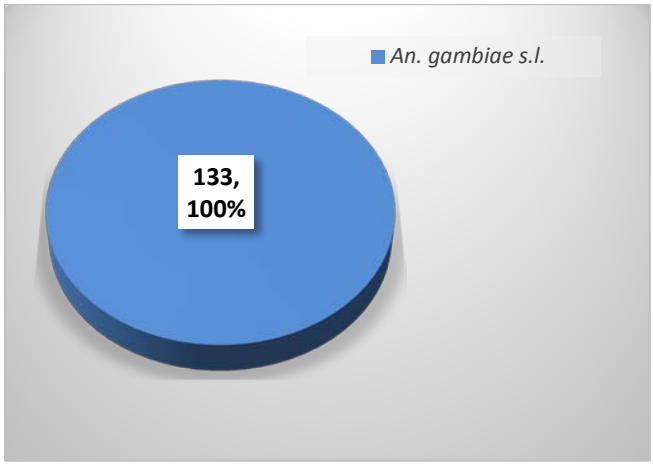
Figure 5: Species composition of *Anopheles* caught by HLC and PSC in IRS districts Post IRS (August to November 2017).

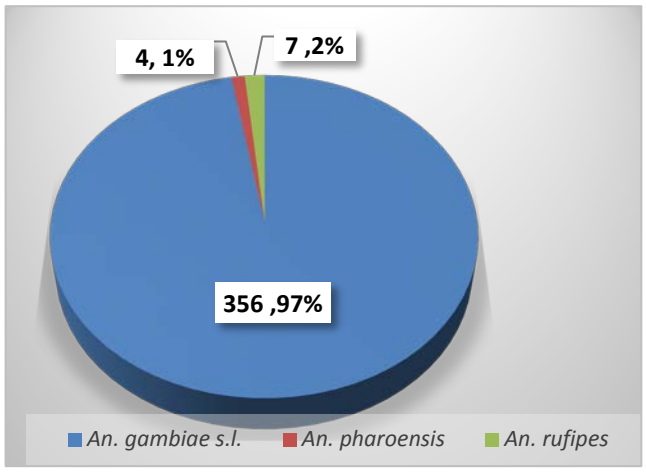
Koumpentoum

a) Post –IRS, August to November 2017, n=133

Koungheul

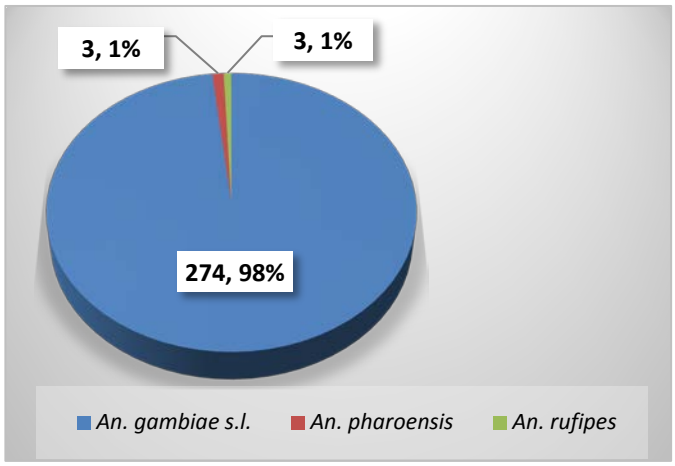
b) Post –IRS, August to November 2017, n=367





Malem Hodar

c) Post –IRS, August to November 2017, n=280



3.2.2 HUMAN BITING RATE (HBR)

3.2.2.1 HUMAN BITING RATE AT DISTRICT LEVEL (SPRAYED AND UNSPRAYED VILLAGES COMBINED)

Vector populations of *An. gambiae* s.l. increased during the rainy season, with the highest biting rate in Nioro district and lowest in Koumpentoum. The peak biting rates were recorded in September for Nioro and Koumpentoum, in October for Malem Hodar, and in November for Koungheul (Fig5). In general, *An. gambiae* s.l. biting rates were low even in the rainy season, with the highest being <2 bites per person per night.

In Nioro, *An. funestus* s.l. was also present with a much higher biting rate than *An. gambiae s.l.* in the wet and rainy season (Fig 6).

Figure 5: Human biting rate of *Anopheles gambiae* s.l. in IRS districts (sprayed and unsprayed villages combined)

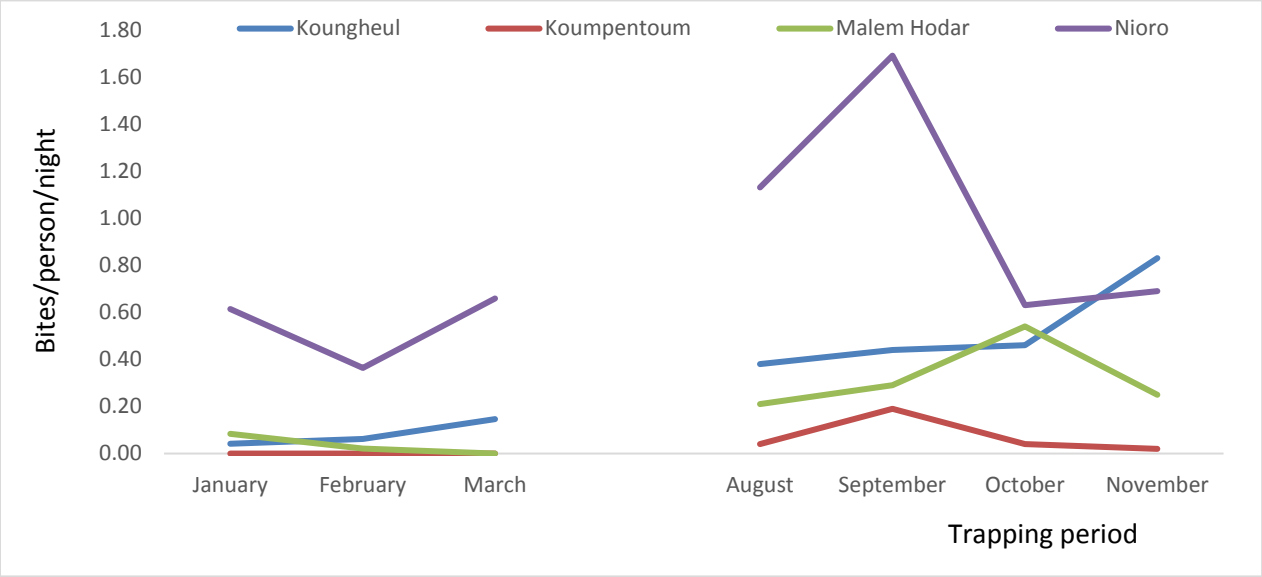
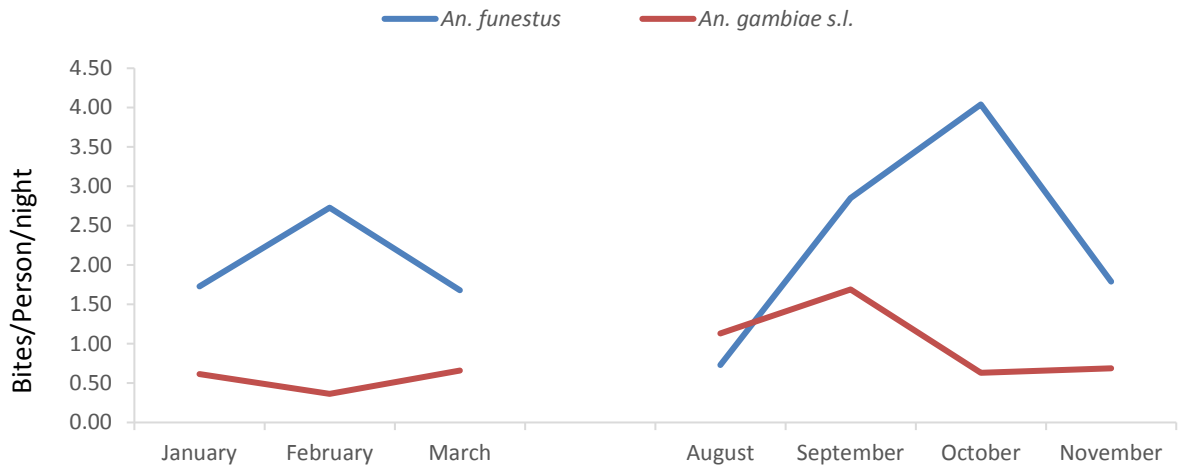


Figure 6: Human biting rate of *Anopheles gambiae* s.l. and *Anopheles funestus* s.l. in the Nioro district (sprayed and unsprayed villages combined).



3.2.2.2 RATIO OF INDOOR AND OUTDOOR BITING MEASURED BY HUMAN LANDING CATCH

TABLE 5: ENDOPHAGY INDEX OF VECTORS IN IRS DISTRICTS

		Pre IRS (Jan- March)	Post IRS (August to November)

Districts	Species	Indoor	Outdoor	**Endophagy**	Indoor	Outdoor	**Endophagy**	
Koungheul	*An. gambiae* s.l.	3	9	**25.0%**	45	56	**44.6%**	NS
Koumpentoum	*An. gambiae* s.l.	0	8	**0.0%**	4	10	**28.6%**	NS
Malem Hodar	*An. gambiae* s.l.	2	7	**22.2%**	35	27	**56.5%**	NS
Nioro	*An. gambiae* s.l.	35	37	**48.6%**	89	109	**44.9%**	NS
	An. funestus	135	141	**48.9%**	158	294	**35.0%**	p<0.05

In all districts there was statistically a similar proportion of indoor and outdoor biting by *An. gambiae* s.l. *An. funestus* s.l. in Nioro was significantly exohagic.

3.2.2.3 Human biting rates (HBR) in sprayed hot spots, unsprayed hot spots (external controls), and unsprayed low transmission villages (internal controls).

The HBR was predicted to be far greater in the unsprayed hot spots (external controls) than in sprayed hot spots or unsprayed low transmission villages (internal controls). This expected pattern was observed in Koumpentoum and Nioro (Fig 7). In Koungheul and Malem Hodar, the mean biting rate was low (<1 bite per person per night) every month regardless of whether the villages were hot spots, low transmission, unsprayed, or sprayed. *An. funestus* s.l. was present in all areas and at all trapping periods with a higher density in the external control of Nioro (Fig 8). The combination of all IRS districts showed a consistently higher biting rate of *An. gambiae* s.l. in the unsprayed external control sites (Fig 9).

Figure 7: *Anopheles gambiae* s.l. HBR in sprayed hot spots, unsprayed hot spots (external controls), and unsprayed low transmission villages (internal controls)

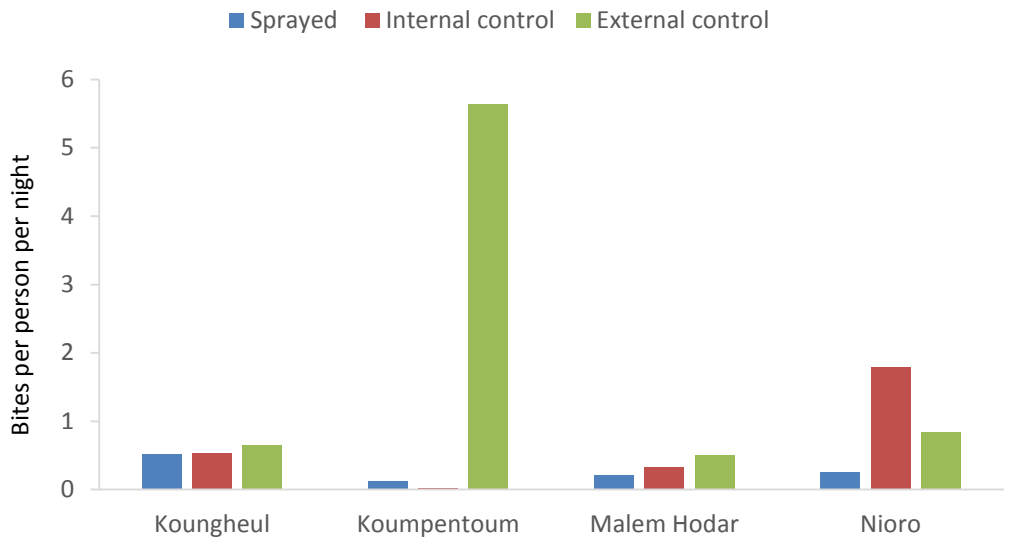


Figure 8: *Anopheles gambiae* s.l. and *An. funestus* HBR in Nioro district and its controls.

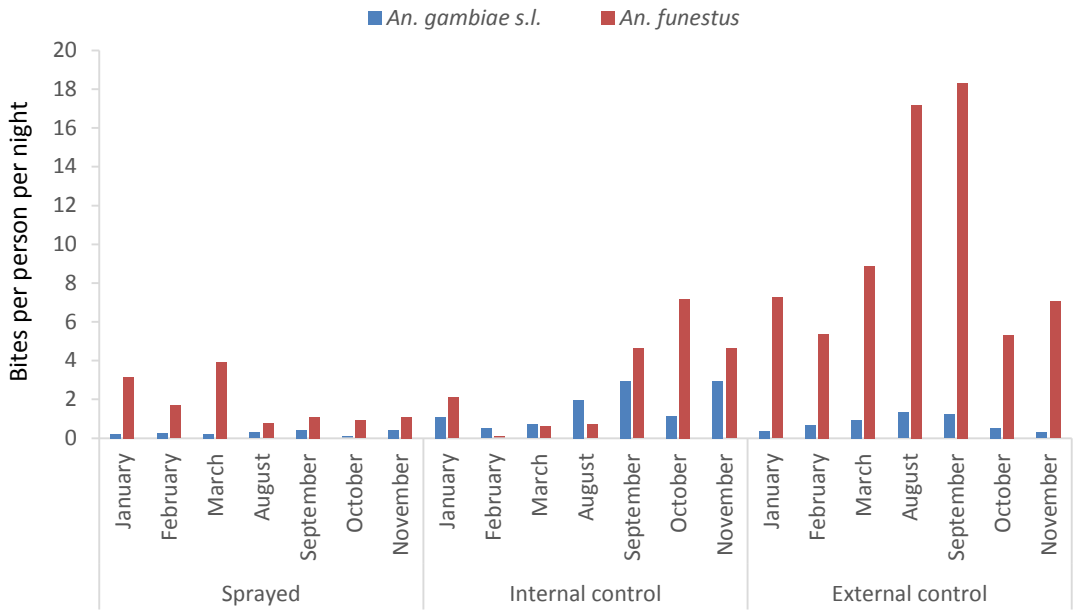
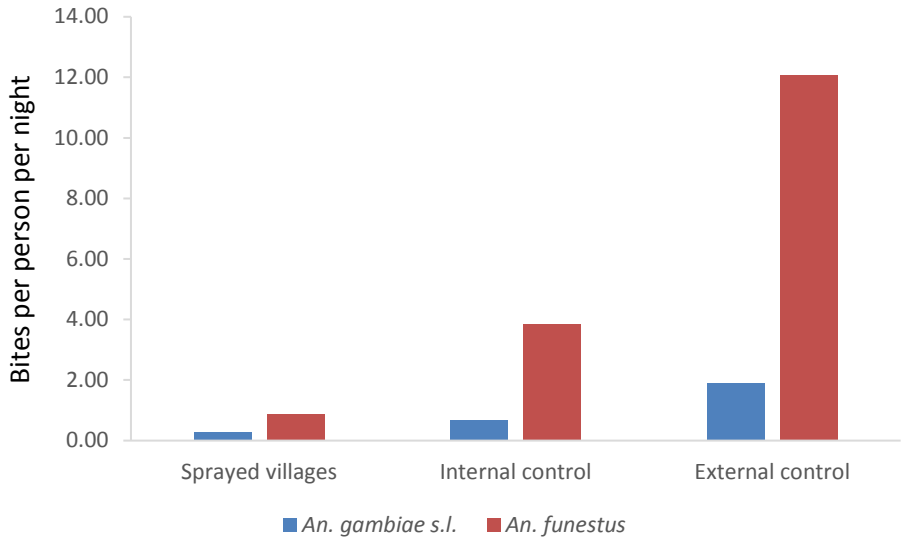


Figure 9: *Anopheles gambiae* s.l. HBR in sprayed hot spots, unsprayed hot spots (external controls), and unsprayed low transmission villages (internal controls) combined all IRS districts



3.2.3 INDOOR RESTING DENSITY (IRD)

3.2.3.1 INDOOR RESTING DENSITY AT DISTRICT LEVEL BY PSC (SPRAYED AND UNSPRAYED VILLAGES COMBINED)

The *An. gambiae* s.l. Indoor Resting Density (IRD) was zero in three districts between January and March, with Nioro the only site with *An. gambiae* s.l. collected in the dry season (Figure 10). During the rainy season, the IRD continued to be greater in Nioro than the other three districts, with a peak in September at 5.9 *An. gambiae* s.l. per room per day (Fig 10).

The percentage of blood-fed females was generally 50-60%, with very few unfed and the remainder being half-gravid or gravid (Table 6).

Figure 10: Indoor resting density of *Anopheles gambiae* s.l. in IRS districts (including sprayed and unsprayed villages).

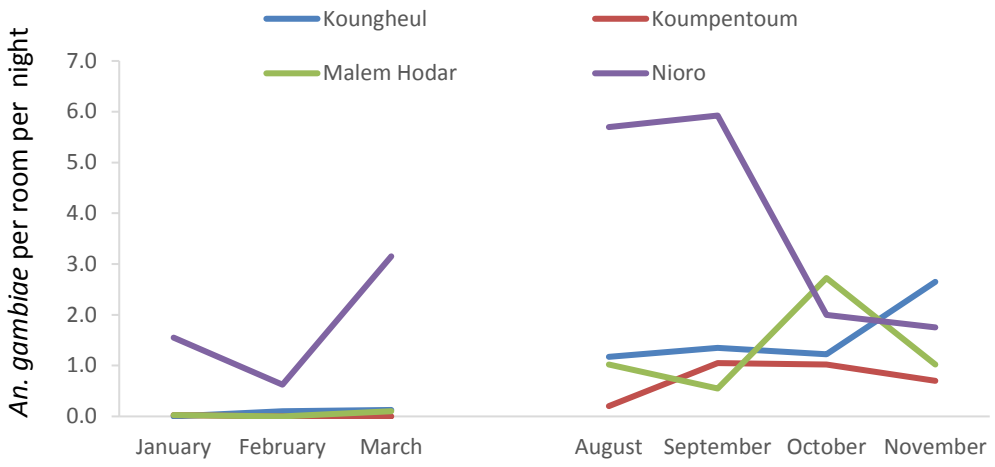


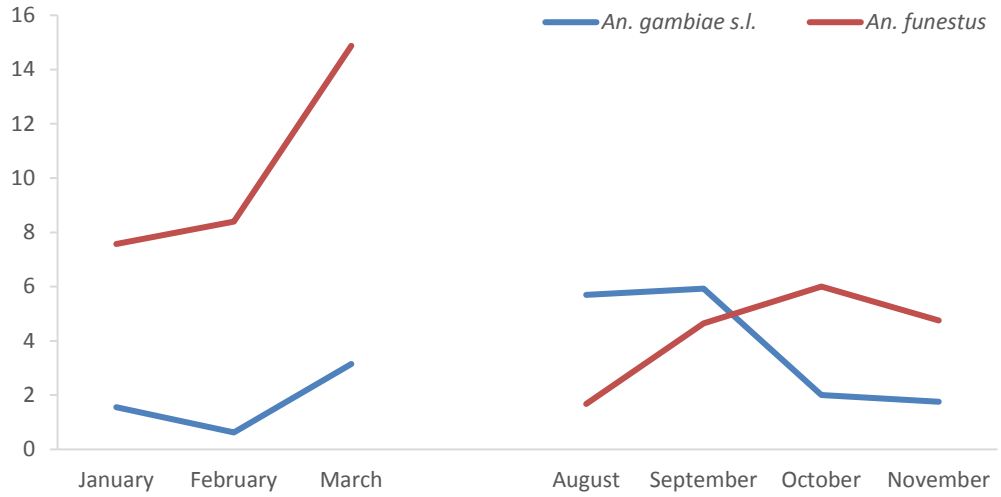
TABLE 6: ABDOMINAL STATUS OF INDOOR RESTING *AN. GAMBIAE*S.L. IN IRS DISTRICTS (SPRAYED AND UNSPRAYED VILLAGES COMBINED).

District	Unfed	Blood-fed	Half gravid	Gravid	Total
Koungheul	1 (0.4%)	113 (44.1%)	79 (30.9%)	63 (24.6%)	256
Koumpentoum	3 (2.5%)	66 (55.5%)	18 (15.1%)	32 (26.9%)	119
Malem Hodar	9 (4.2%)	142(66 :7%)	29 (13.6%)	33 (15.5%)	213
Nioro	45 (7.3%)	377 (61.3%)	125 (20.3%)	68 (11.1%)	615

Nioro was the only site where the team collected considerable densities of *An. funestus* s.l. by PSC. IRDs of *An. funestus* s.l. were highest during the dry season, reaching a peak in March at 14.9 per room per day.

IRDs were relatively high during the rainy season, especially in August to September for *An. gambiae* s.l., with 5.9 females per room. For *An. funestus,* the rainy season peak was in October at 6.0 females per room. (Fig. 11).

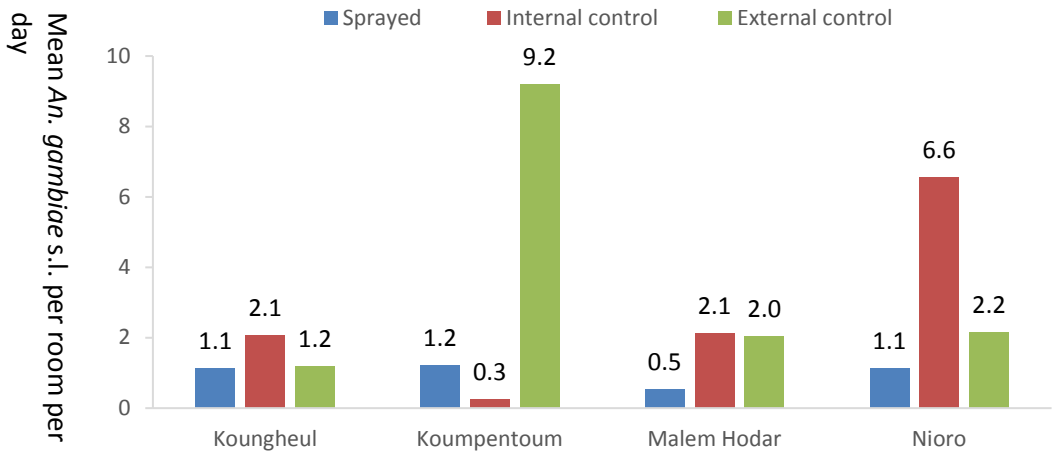
Figure 11: Comparison of *Anopheles gambiae,* s.l. and *Anopheles funestus* s.l. in Nioro district



3.2.3.2 Indoor Resting Densities in sprayed hot spots, unsprayed hot spots (external controls) and unsprayed low transmission villages (internal controls)

The IRD of *An. gambiae* s.l. was greater in external control sites (unsprayed hot spots) only in Koumpentoum. In Nioro it was higher in the internal control villages (unsprayed low transmission) (Figure 12). Resting densities were similarly low in sprayed villages and unsprayed controls in Koungheul and Malem Hodar. Mean IRDs were less than two *An. gambiae* s.l. per room in all sprayed villages.

Figure 12: *Anopheles gambiae* s.l. IRDs for sprayed hot spots, unsprayed hot spots and low transmission villages



3.2.4 PARITY RATE

3.2.4.1 PARITY RATE AT DISTRICT LEVEL

Data for all districts showed no difference in parity rates between sprayed and internal control sites, but the parity rate was higher in external control areas ($p<0.05$) (Table 7).

TABLE 7. *AN. GAMBIAE* S.L. PARITY RATE IN SPRAYED AREAS AND THEIR CONTROLS

District	Sprayed sites	Internal control	External control
Koungheul	50% (15/30)	69.7% (23/33)	73% (27/37)
Koumpentoum	75% (6/8)	100% (1/1)	83.5% (278/333)
Malem Hodar	46,7% (7/15)	75% (21/28)	69.4% (25/36)
Nioro	53.3% (8/15)	44.2% (46/104)	68.1% (32/47)
All districts	**52.9 (36/68)**	**54.8 (91/166)**	**79.9 (362/453)**

3.3 UNSPRAYED DISTRICTS

3.3.1 COMPOSITION OF SPECIES

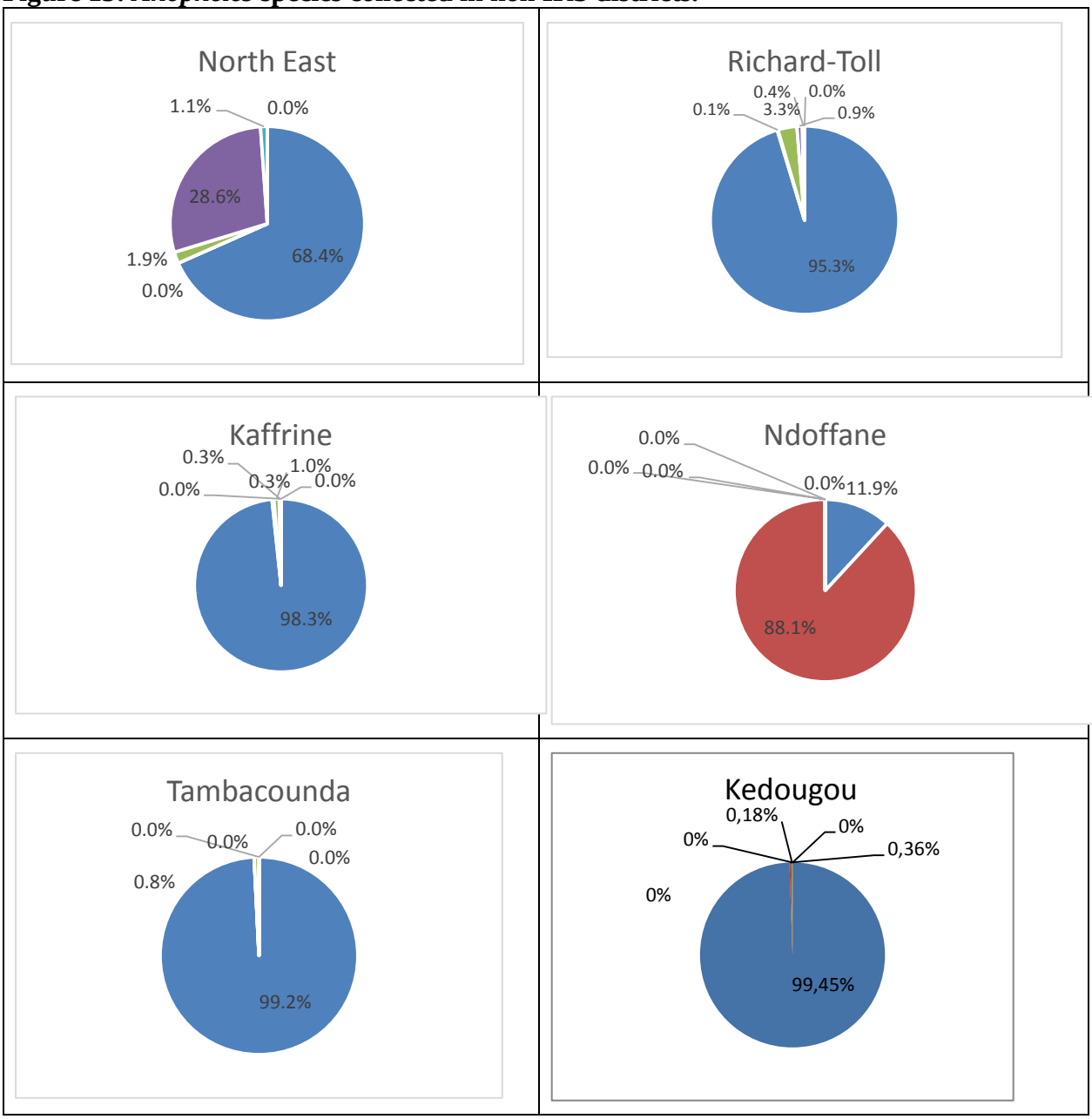
Six species of *Anopheles* were collected in non-IRS districts (Table 8). In all districts, *An. gambiae* s.l. was the predominant species except in Ndoffane, where *An. funestus* s.l. represented 88.1% of the collected *Anopheles* (Fig 13).

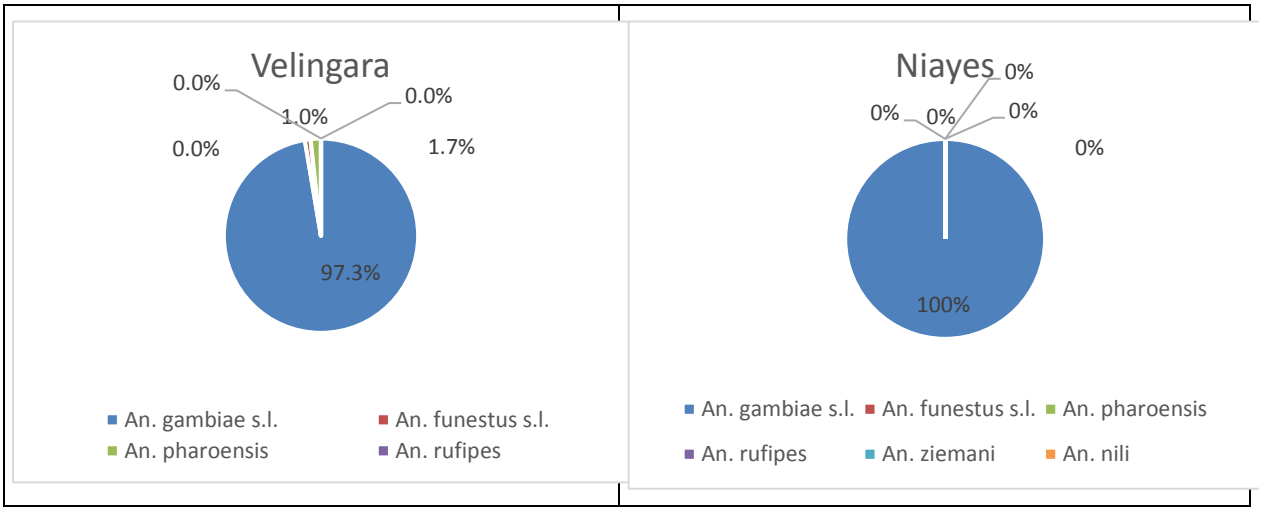
TABLE 8: *ANOPHELES* SPECIES IN NON-IRS DISTRICTS

	Kedougou	Ndoffane	Niayes	Richard-Toll	Kaffrine	Tambacounda	Velingara	North Est

An.gambiae s.l.	2,189	253	150	731	295	1,277	511	1,520
An. funestus s.l.	8	1,878	0	1	1	0	5	0
An. pharoensis	0	1	0	25	3	10	9	42
An. rufipes	0	0	0	7	1	0	0	636
An. ziemani	0	0	0	3	0	0	0	25
An. nili	4	0	0	0	0	0	0	0

Figure 13: *Anopheles* species collected in non IRS districts.





3.3.2 HUMAN BITING RATE AND INDOOR RESTING DENSITY OF *ANOPHELES GAMBIAE* S.L. IN NON-IRS DISTRICTS

The densities of human biting *An. gambiae* s.l. were higher in the south of the country (Kedougou, Velingara, and Tambacounda). In other districts, the HBR was low and generally less than two bites/person/night. Despite the high biting rates in the south of the country, the IRD was generally very low except in Tambacounda (9.2 females per room) (Table 9). Except in Niayes, approximately half of all *An. gambiae* s.l. collected resting indoors were blood-fed (Table 10).

TABLE 9: HBR RATE AND IRD OF *ANOPHELES GAMBIAE* S.L. IN NON-IRS DISTRICTS

	HLC (HBR)					PSC (IRD)				
	August	September	October	November	Mean	August	September	October	November	Mean
Kedougou	-	40.17	-	1.12	20.65	-	0.85	-	0.30	0.58
Ndoffane	1.33	1.21	0.54	0.29	0.84	4.20	1.85	1.80	0.75	2.15
Niayes	0.04	-	0.04	-	0.04	1.16	-	1.80	-	1.48
Richard-Toll	-	-	-	-	-	-	6.97	-	-	6.97
Kaffrine	0.25	1.03	0.64	0.39	0.58	1.53	1.83	0.83	2.87	1.77
Tambacounda	0.88	13.83	4.88	2.96	5.64	2.55	7.00	13.55	13.70	9.20
Velingara	6.63	-	11.33	-	8.98	2.25	-	1.75	-	2.00
North Est		1.7		0.7	1.2		4.5		5.3	4.9

Spray Catches **HBR**: Human Bite Rate **IRD**: Indoor Resting Density **HLC**: Human Landing Catches **PSC**: Pyrethrum

TABLE 10: ABDOMINAL STATUS OF *AN. GAMBIAE* S.L. COLLECTED BY PSC IN UNSPRAYED DISTRICTS

District	Unfed	Blood-fed	Half gravid	Gravid	Total
Kaffrine	8 (3.8%)	94 (44.3%)	70 (33.0%)	40 (18.9%)	212
Tambacounda	6 (0.8%)	414 (56.3%)	1 (0.1%)	315 (42.8%)	736
Ndoffane	0 (0.0%)	82 (47.7%)	58 (33.7%)	32 (18.6%)	172
Richard-Toll	73 (10.0%)	413 (56.5%)	64 (8.8%)	181 (24.8%)	731
Niayes	7 (4.7%)	44 (29.7%)	35 (23.6%)	62 (41.9%)	148
Velingara	5 (6.3%)	50 (62.5%)	3 (3.8%)	22 (27.5%)	80
Kedougou	3 (13.0%)	14 (60.9%)	3 (13.0%)	3 (13.0%)	23

3.4 SUSCEPTIBILITY TESTS OF MALARIA VECTORS TO INSECTICIDES

3.4.1 WHO SUSCEPTIBILITY TESTS WITH IMPREGNATED PAPERS

For each insecticide, the project tested at least 100 mosquitoes in four replicates of 25. The mortality rates of the exposed samples were validated by using untreated controls. The basis for interpreting the results is in the table below (WHO, 2016).

Status	Interpretation threshold	Additional analysis threshold	Observations
Susceptible	98-100%	98-100%	Confirmed susceptibility
Resistant	Less than 98%	90-98%	Resistance to be confirmed
		Less than 90%	Confirmed resistance

The results of WHO tube assays (Table 11 and Figure 15) showed resistance of *Anopheles gambiae* s.l. to all three pyrethroid insecticides tested in the majority of the districts. However, in three of four IRS districts, the team recorded susceptibility to deltamethrin. In Koumpentoum, an IRS site, the team recorded full susceptibility to permethrin, deltamethrin, and alphacypermethrin. The frequency of resistance to all pyrethroids (<40% mortality) was particularly high in the Dakar suburbs and Kedougou.

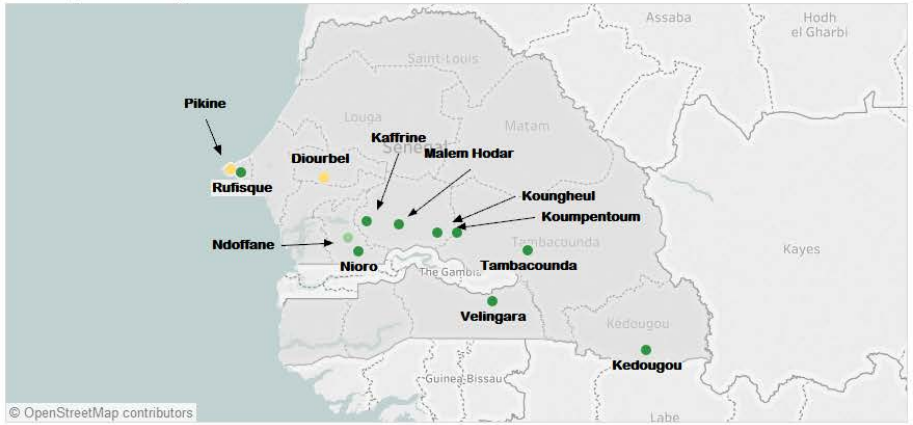
For pirimiphos-methyl 0.25%, the vector populations were fully susceptible in all current IRS sites, but resistance was recorded in Pikine (Dakar suburb) and Diourbel. *An. gambiae* were resistant to bendiocarb in most sites, with high frequency resistance in the Dakar suburbs and Diourbel. However, susceptibility was recorded in IRS districts of Koumpentoum and Koungheul and possible resistance in Nioro.

The finding of pockets of susceptibility to bendiocarb and deltamethrin in IRS districts may be an indication that IRS with an organophosphate has limited the development of resistance to other insecticide classes.

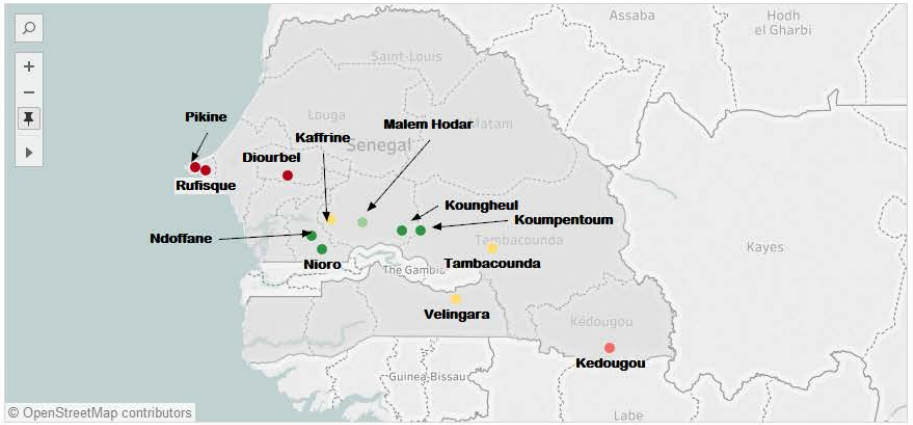
Fig 15: Insecticide susceptibility map in Senegal (2017).

Insecticide Susceptibility Map For Senegal Entomological Sentinel Sites (2017)

Pirimiphos-methyl



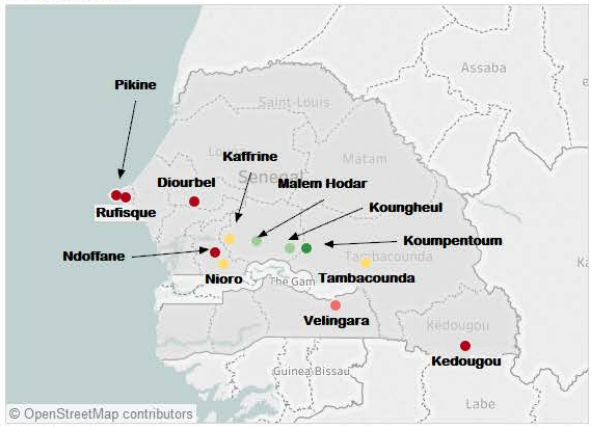
Bendiocarb



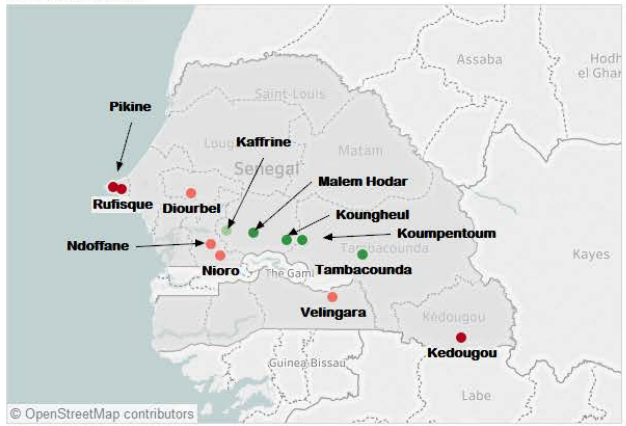
Mortality Rate

- 0-40% Mortality
- 41-65% Mortality
- 66-90% Mortality
- 91-97% Mortality
- 98-100% Mortality

Permethrin



Deltamethrin



Alphacypermethrin

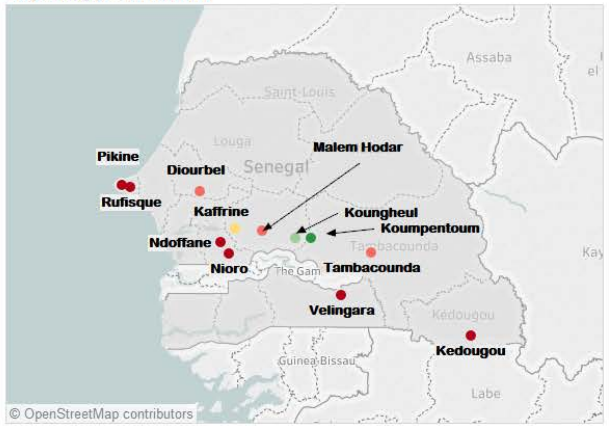


TABLE11: SUSCEPTIBILITY TEST RESULTS FOR *ANOPHELES GAMBIAE* S.L. 24 HOURS AFTER ONE HOUR EXPOSURE TO WHO DIAGNOSTIC DOSES OF FIVE INSECTICIDES.

Districts	Deltamethrin 0.05%			Permethrin 0.75%			Alphacypermethrin 0,05%			Pirimiphos-methyl 0.25%			Bendiocarb 0.1%		
	No tested	Dead 24h	% Mortality	No tested	Death 24h	%	No tested	Death 24h	%	No tested	Death 24h	%	No tested	Death 24h	%
	IRS districts														
Koungheul	102	101	**98.9***	103	100	**96.8***	101	96	**94.5***	100	100	**100**	106	106	**100**
Koumpentoum	153	149	**97,4**	150	150	**100**	133	131	**98,5**	148	148	**100**	143	143	**100**
Malem Hodar	102	100	**98**	102	98	**96**	103	61	**59.2**	106	106	**100**	104	98	**94.2**
Nioro	112	45	**40.2**	105	85	**81**	-	-	**-**	100	100	**100**	107	107	**100**
	IRS District controls														
Ndoffane	112	45	**40.2**	112	29	**25.8**	107	23	**21.5**	108	100	**92.6**	111	109	**98.2**
Kaffrine	101	94	**93**	105	88	**83,8**	103	80	**77,7**	113	113	**100***	104	78	**72,3***
Tambacounda	120	117	**98**	126	96	**76,2**	141	77	**54,6**	112	112	**100**	119	97	**79,5***
	Central districts														
Diourbel	234	125	**53,4**	238	24	**10**	126	55	**43,7**	215	177	82,3	247	91	**36,8**
	Dakar and its suburbs														
Pikine	129	50	**39**	116	10	**9**	127	22	**17,3**	120	95	**79,1**	121	14	**3,9***
Rufisque	117	32	**27**	117	11	**9**	230	12	**5,2**	127	126	99,2	218	58	**26,6**
	Southern districts														
Velingara	108	70	**65**	124	65	**52,4**	-	-	**-**	105	104	**99**	104	85	**81,7**
Kedougou	125	44	**35**	116	9	**7,8**	125	25	**20**	101	100	**99**	137	70	**51,1**

*Corrected mortality

Resistance intensity with the CDC bottle test

In the work plan CDC resistance intensity assays were planned using ×1, ×2, ×5, and ×10 the diagnostic dose of a pyrethroid and a carbamate in selected sites of high resistance. In WHO susceptibility tests, there were several sites with high frequency resistance to deltamethrin. However, the ×1 diagnostic dose of deltamethrin in bottle bioassays killed >90% of *An. gambaie* s.l. in all sites except Kedougou. The low intensity of resistance in CDC bottle bioassays in the Dakar suburbs and Kedougou was seemingly at odds with the high frequency resistance recorded in WHO cylinder tests. The same was true with bendiocarb, as 100% mortality was recorded in all sites except one using ×1 times the diagnostic dose. In 2018, VectorLink Senegal will conduct intensity bioassays using WHO filter papers to avoid any differences due to type of test or field treatment of bottles.

TABLE 12: RESISTANCE INTENSITY OF *AN. GAMBIAE* S.L. USING CDC BOTTLE BIOASSAYS

Districts	Deltamethrin 1× 30min			Deltamethrin 2× 30min			Bendiocarb 1× 30min		
	No tested	Death 24h	% Mortality	No tested	Dead 24h	% Mortality	No tested	Dead 24h	% Mortality
Koungheul	103	100	**97**	**-**	**-**	**-**	104	104	**100**
Koumpentoum	112	106	**94.6**	**-**	**-**	**-**	112	112	**100**
Malem Hodar	111	111	**100**	**-**	**-**	**-**	111	111	**100**
Ndoffane	105	104	**99**	**-**	**-**	**-**	57	57	**100**
Kaffrine	108	102	**94.4**	**-**	**-**	**-**	104	104	**100**
Tambacounda	99	96	**97**	**-**	**-**	**-**	111	111	**100**
Kedougou	106	93	**87.7**	113	113	**100**	93	88	**94.6**
Rufisque	102	97	**95.1**	101	101	**100**	203	203	**100**
Pikine	102	98	**96.1**	96	92	**95.8**	120	120	**100**

3.5 LAB ANALYSES

3.5.1 BLOOD FEEDING IN IRS DISTRICTS AND PAIRED UNSPRAYED CONTROLS

Table 13 shows the results of blood meal source identification from endophilic *An. gambiae* s.l. females collected by PSC in sprayed districts. The average anthropophilic rate was low in sprayed areas and in their control sites, with a mean anthropophily rate of 0.27 (208/775). Horses were the main blood-source of *An. gambiae* s.l. females in both sprayed sites and their internal and external controls and represent 41% of the blood meals (318/775).

The anthropophily rate of *An. funestus* s.l. was very low in Nioro (sprayed areas and controls), with a mean of 14.5% (33/227) fed on humans. Horses were the main blood-meal source of *An. funestus* s.l. in Nioro, accounting for 38% (87/227) of blood meals.

Unsprayed districts

The anthropophily rate of *An. gambiae* s.l. in unsprayed districts was high in Velingara (0.91; 40/44) and in Kedougou (0.92; 35/38). In Niayes and Richard Toll, the anthopophily rate for *An. gambiae* s.l. was <0.25.

TABLE 13: ORIGIN OF BLOOD MEAL AND ANTHROPOPHILIC RATE OF *AN. GAMBIAE* S.L. IN SPRAYED SENTINEL SITES AND THEIR INTERNAL AND EXTERNAL CONTROLS (AUGUST TO NOVEMBER 2017)

Species	Districts	Localities	N	T	ND	Monospecific					MIX H/A	MIX A/A	IA
						H	B	S	C	Ho			
An. gambiae s.l	Koungheul	Sprayed	49	46	2	13	7	2	0	18	2	2	0.34
		Internal control	64	64	3	25	1	1	0	26	4	5	0.48
		External control	38	36	1	7	1	1	0	18	1	7	0.23
	Koumpentoum	Sprayed	66	65	2	6	12	4	0	22	12	7	0.28
		External control	417	218	18	51	28	7	0	80	18	16	0.35
	Malem Hodar	Sprayed	28	28	2	3	3	2	0	13	1	4	0.15
		Internal control	114	85	4	33	17	0	0	29	1	1	0.42
		External control	80	79	4	13	4	1	0	40	4	12	0.23
	Nioro	Sprayed	60	29	4	4	2	2	0	14	0	4	0.16
		Internal control	316	67	12	6	13	2	0	31	0	3	0.11
		External control	87	58	4	3	9	5	0	27	1	9	0.07

An. funestus	Nioro	Sprayed	58	25	3	2	3	1	0	15	0	1	0.09
		Internal control	292	66	12	6	20	2	0	17	0	9	0.11
		External control	369	136	5	17	18	6	0	55	8	28	0.19

N = number of blood-fed mosquitoes collected, T = number tested, 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

TABLE 14: ANTHROPOPHILY RATE IN UNSPRAYED DISTRICTS

(AUGUST TO NOVEMBER 2017)

Species	Districts	N	T	ND	Monospecific					MIX H/A	MIX A/A	IA
					H	B	S	C	Ho			
An. gambiae s.l	Velingara	54	48	4	39	0	2	0	1	1	1	0.91
	Niayes	25	25	10	2	4	3	0	4	1	1	0.2
	Richard Toll	413	39	2	7	4	0	0	17	1	8	0.21
	Kédougou	43	43	5	35	2	0	0	1	0	0	0.92
An. funestus	Kédougou	3	3	0	0	1	1	0	0	1	0	0.33
An. pharoensis	Richard Toll	17	7	0	2	0	0	0	4	0	1	0.28
An. rufipes	Richard Toll	3	2	0	0	1	0	0	1	0	0	0

N = number of blood-fed mosquitoes collected, T = number tested, 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

3.5.2 VECTOR SPOROZOITE RATES

IRS districts

Table 15 presents the results of sporozoite ELISA for mosquitoes collected by HLC in the sprayed sentinel sites and their paired untreated controls (internal and external). The presence of infective females was noted in sprayed villages in all districts except Koungheul. In general, the number of *Anopheles* we collected was low in all sprayed sites and internal control sites, resulting in only one or two positive mosquitoes per site. This made accurate determination of sporozoite rates difficult. For the IRS districts (sprayed and internal control), sporozoite positive mosquitoes were 6 *An. gambiae* s.l. and 1 *An. funestus* in September and 1 *An. gambiae* s.l in October. In the external controls there were 3 sporozoite positive *An. gambiae* s.l. (2 in September and 1 in October) and 1 *An. funestus* in November.

In Nioro, there were 1 infective *An. funestus* s.l. collected in sprayed sites and 1 in external control, indicating the species is involved in transmission despite the low anthropophhily rate. In Velingara and Kedougou, the sporozoite rates were approximately 1-2%.

TABLE 15: SPOROZOITE RATE OF *AN. GAMBIAE* S.L AND *AN. FUNESTUS* IN SPRAYED DISTRICTS AUGUST – NOVEMBER 2017

	Districts	Sprayed				Internal control				External control			
		Collected	Tested	Positive	CSPI	Collected	Tested	Positives	CSPI	Collected	Tested	Positives	CSPI
An. gambiae s.l.	Koungheul	50	49	0	0	51	51	1	0.02	62	62	0	0
	Malem Hoddar	20	18	1	0.056	42	42	1	0.024	48	46	0	0
	Koumpentoum	14	14	1	0.071					541	162	3	0.019
	Nioro	25	21	2	0.095	173	65	1	0.015	82	64	0	0
An. funestus	Nioro	84	29	1	0.034	368	79	0	0	1161	180	1	0.006

TABLE 16: SPOROZOITE RATE OF FEMALES COLLECTED BY HLC IN UNSPRAYED DISTRICTS AUGUST – NOVEMBER 2017

Districts	*An. gambiae* s.l			*An. funestus*			*An. nili*			*An. pharoensis*		
	Tested	Positives	CSPI	Tested	Positives	CSPI	Tested	Positives	CSPI	Tested	Positives	CSPI
Vélingara	146	2	0.014	1	0	0	0	0	0	3	0	0
Niayes	2	0	0	0	0	0	0	0	0	0	0	0
Kédougou	236	4	0.017	2	0	0	4	0	0	0	0	0

3.5.3 ENTOMOLOGICAL INOCULATION RATES

EIR was very low in IRS districts and in their controls (Table 17). It was in unsprayed districts located in the south east of the country (Velingara and Kedougou) where EIR was higher (Table 18).

Table 17: Entomological inoculation rate (ib / h / n) of malaria vectors from HLC in IRS districts, August - November 2017

Districts	Localities	AR (b/h/n)	CSPI	EIR (ib/h/n)
Koungheul	Hot spot	0.52	0	**0**
	Internal control	0.53	0.02	**0.0106**
	External control	0.65	0	**0**
Koumpentoum	Hot spot	0.07	0.071	**0.005**
	External control	5.64	0.019	**0.107**
Malem Hodar	Hot spot	0.21	0.056	**0.012**
	Internal control	0.33	0.024	**0.008**
	External control	0.50	0	**0.000**
Nioro	Hot spot	0.26	0.034	**0.009**
	Internal control	1.8	0	**0.000**
	External control	0.84	0.006	**0.005**
Nioro	Hot spot	0.88	0.034	**0.030**
	Internal control	3.83	0	**0.000**
	External control	12.09	0.006	**0.073**

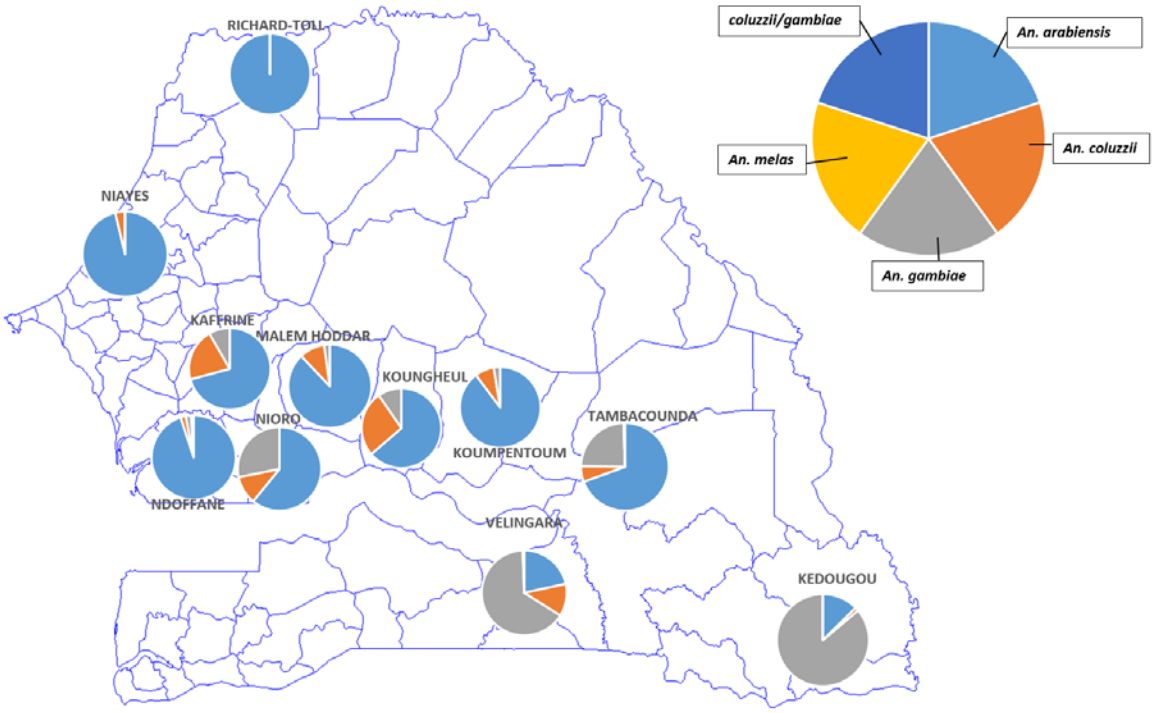
Table 18: Entomological inoculation rate (ib / h / n) of malaria vectors from HLC in unsprayed districts, August - November 2017

Districts	AR	CSPI	EIR
Vélingara	**8.98**	0.014	**0.126**
Niayes	**0.04**	0	**0.000**
Richard Toll	**-**	-	
Kédougou	**20.65**	0.017	**0.351**

3.5.4 *ANOPHELES GAMBIAE* SIBLING SPECIES COMPOSITION

Figure 15 shows species percentage of *An. gambiae* composition by location. *An. arabiensis* is predominant with a regressive gradient from the north to the south of the country, where *An. gambiae s.s.* has higher density. Detailed results of the distribution by collection method are in the annexes.

Figure 15: Proportion of the different species of the *An. gambiae* complex in the districts



3.5.5 KDR 1014F AND 1014S FREQUENCY

Lab analysis for the determination of KDR gene frequency is ongoing.

4. Conclusion

Overall, there was evidence that IRS had an impact on vector biting rates and resting densities. However, the low general vector densities and high zoophily rate of *An. arabiensis* and *An. funestus* s.l. across the majority of Senegal--even in unsprayed areas--make it difficult to quantify the degree of impact in terms of disease transmission. In Velingara and Kedougou, the vector trends are different, and highly anthropophilic *An. gambiae* predominate.

ANNEXES

Annex 1: Bioassays

Annex 1a: Effectiveness of pirimiphos-methyl (Actellic CS 300) on mud and cement walls against a susceptible insectary strain of *An. gambiae* s.s. in the District of Koungheul (August to November 2017)

Time after spraying		Mud					Cement					Total				
		1 month	2 months	3 months	4 months	5 months	1 month	2 months	3 months	4 months	5 months	1 month	2 months	3 months	4 months	5 months
Exposed	IRS	157	155	143	158	152	157	153	152	162	154	314	308	295	320	306
	Control	30	33	31	31	32	31	31	31	31	31	61	64	62	62	63
KD 30'	IRS	29	3	4	7	7	35	1	3	14	3	64	4	7	21	10
	Control	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Mortality 24 h	IRS	157	124	139	141	100	157	125	120	130	100	314	249	259	271	200
	Control	2	3	0	0	2	0	1	1	1	0	2	4	1	1	2
Mortality rate 24 h (%)	IRS	**100***	**78***	**97.2**	**89.2**	**63.5***	**100**	**81.7**	**78.9**	**80.2**	**64.9**	**100**	**79.6***	**87.8**	**84.7**	**65.4**
	Control	6.7	9.1	0	0	6.25	0	3.2	3.2	3.2	0	3.3	6.3	1.6	1.6	3.17

Annex 1b: Effectiveness of pirimiphos-methyl (Actellic CS 300) on mud and cement walls against a susceptible insectary strain of *An. gambiae* s.s. in the District of Koumpentoum (August to November 2017).

Time after spraying		Mud					Cement					Total				
		1 month	2 months	3 months	4 months	5 months	1 month	2 months	3 months	4 months	5 months	1 month	2 months	3 months	4 months	5 months
Exposed	IRS	240	255	243	240	242	360	378	370	360	366	600	633	613	600	608
	Control	90	91	90	90	92	30	30	30	30	30	120	121	120	120	122
KD 30'	IRS	142	135	70	56	42	257	184	97	75	84	399	319	167	131	126
	Control	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Mortality 24 h	IRS	235	247	234	179	221	349	372	366	315	327	584	619	600	494	548
	Control	0	5	4	4	1	3	2	1	0	0	3	7	5	4	1
Mortality rate 24 h (%)	IRS	**97.9**	**96.4***	**96.3**	**74.6**	**91.3%**	**96.5***	**98.3***	**98.9**	**87.5**	**89.3%**	**97.3**	**97.7***	**97.9**	**82.3**	**90.1%**
	Control	0	5.5	4.4	4.4	1.1%	10	6.7	3.3	0	0.0%	2.5	5.8	4.2	3.3	0.8%

Annex 1c: Effectiveness of pirimiphos-methyl (Actellic CS 300) on mud and cement walls against a susceptible insectary strain of *An. gambiae* s.s. in the District of Malem Hodar (August to November 2017)

Time after spraying		Mud					Cement					Total				
		1 month	2 months	3 months	4 months	5 months	1 month	2 months	3 months	4 months	5 months	1 month	2 months	3 months	4 months	5 months
Exposed	IRS	159	155	160	154	151	158	158	152	158	156	317	313	312	312	307
	Control	0	0	0	0	0	62	64	64	64	61	62	64	64	64	61
KD 30'	IRS	8	9	24	1	2	37	2	39	0	1	45	11	63	1	3
	Control	0	0	0	0	0	0	4	0	0	0	0	4	0	0	0
	IRS	158	155	141	122	119	158	151	144	122	123	316	306	285	244	242

Mortality 24 h	Control	0	0	0	0	0	2	7	2	0	0	2	7	2	0	0
Mortality rate 24 h (%)	IRS	**99.4**	**100**	**88.1**	**79.2**	**78.8**	**100**	**95***	**94.7**	**77.2**	**78.8**	**99.7**	**97.5***	**91.3**	**78.2**	**78.8**
	Control	0	0	0	0	0	3.2	10.9	3.1	0	0	3.2	10.9	3.1	0	0

Annex 1d: Effectiveness of pirimiphos-methyl (Actellic CS 300) on mud and cement walls against a susceptible insectary strain of *An. gambiae* s.s. in the District of Nioro (August to November 2017)

Time after spraying		Mud					Cement					Total				
		1 month	2 months	3 months	4 months	5 months	1 month	2 months	3 months	4 months	5 months	1 month	2 months	3 months	4 months	5 months
Exposed	IRS	38	37	34	32	36	303	336	316	310	316	341	373	350	342	352
	Control	72	73	71	71	71	0	0	0	0	0	72	73	71	71	71
KD 30'	IRS	37	6	0	0	2	179	40	34	9	11	216	46	34	9	13
	Control	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Mortality 24 h	IRS	36	37	27	28	17	296	334	298	196	49	332	371	325	224	66
	Control	5	4	3	0	1	0	0	0	0	0	5	4	3	0	1
Mortality rate 24 h (%)	IRS	**94.3**	**100**	**79.4**	**87.5**	**47.2**	**97.7**	**99.4**	**94.3**	**63.2**	**15.5**	**97.2**	**99.4**	**92.9**	**65.5**	**18.75**
	Control	6.9	5.5	4.2	0	1.4	0	0	0	0	0	6.9	5.5	4.2	0	1.4

Annex 2: Vector Dynamic

Annex 2a: Specific composition of anopheline fauna according to the sampling method in sprayed districts.

Districts	Species	HLC			RC	Total
		Indoor	Outdoor	Total		
Koungheul	*An. gambiae s.l.*	45	56	101	256	357
	An. pharoensis	2	1	3	1	4
	An. rufipes	0	0	0	6	6
Koumpentoum	*An. gambiae s.l.*	4	10	14	119	133
Malem Hodar	*An. gambiae s.l.*	35	27	62	213	275
	An. pharoensis	2	1	3	0	3
	An. rufipes	0	0	0	2	2
Nioro	*An. gambiae s.l.*	89	109	198	615	813
	An. funestus	158	294	452	683	1135
	An. pharoensis	2	6	8	1	9
	An. rufipes	0	0	0	105	105
	An. coustani	0	0	0	1	1
TOTAL	*An. gambiae s.l.*	173	202	375	1203	1578
	An. funestus	158	294	452	683	1135
	An. pharoensis	6	8	14	2	16
	An. rufipes	0	0	0	113	113
	An. coustani	0	0	0	1	1

HLC: Human Landing Catches; RC: Resting Collect

Annex 2b: Human biting rate and indoor resting density of *Anopheles gambiae s.l.* and *Anopheles funestus* females in IRS districts (sprayed and unsprayed villages combined).

Districts	Species		HLC (HBR)														RC (IRD)				
			Indoor					Outdoor					Total									
			A	S	O	N	T	A	S	O	N	T	A	S	O	N	T	A	S	O	N	T
Koungheul	***An. gambiae* s.l.**	HN/Room	24	24	24	24	96	24	24	24	24	96	48	48	48	48	192	40	40	40	40	160
		Number	5	12	14	14	45	13	9	8	26	56	18	21	22	40	101	47	54	49	106	256
		HBR/IRD	**0.21**	**0.5**	**0.58**	**0.58**	**0.47**	**0.54**	**0.38**	**0.33**	**1.08**	**0.58**	**0.38**	**0.44**	**0.46**	**0.83**	**0.53**	**1.18**	**1.35**	**1.23**	**2.65**	**1.6**
Koumpentoum		HN/Room	24	24	24	24	96	24	24	24	24	96	48	48	48	48	192	40	40	40	40	160
		Number	0	3	1	0	4	2	6	1	1	10	2	9	2	1	14	8	42	41	28	119

	***An. gambiae* s.l.**	**HBR/IRD**	**0**	**0.13**	**0.04**	**0**	**0.04**	**0.08**	**0.25**	**0.04**	**0.04**	**0.1**	**0.04**	**0.19**	**0.04**	**0.02**	**0.07**	**0.2**	**1.05**	**1.03**	**0.7**	**0.74**
Malem Hodar	***An. gambiae* s.l.**	HN/Room	24	24	24	24	96	24	24	24	24	96	48	48	48	48	192	40	40	40	40	160
		Number	8	9	12	6	35	2	5	14	6	27	10	14	26	12	62	41	22	109	41	213
		HBR/IRD	**0.33**	**0.38**	**.05**	**0.25**	**0.36**	**0.08**	**0.21**	**0.58**	**0.25**	**0.28**	**0.21**	**0.29**	**0.54**	**0.25**	**0.32**	**1.03**	**0.55**	**2.73**	**1.03**	**1.33**
Nioro	***An. gambiae* s.l.**	HN/Room	24	24	24	24	96	24	24	24	24	96	48	48	48	48	192	40	40	40	40	160
		Number	23	45	10	11	89	31	36	20	22	109	54	81	30	33	198	228	237	80	70	615
		HBR/IRD	**0.96**	**1.88**	**0.42**	**0.46**	**0.93**	**1.29**	**1.5**	**0.83**	**0.92**	**1.14**	**1.13**	**1.69**	**0.63**	**0.69**	**1.03**	**5.7**	**5.93**	**2**	**1.75**	**3.84**
	An. funestus	HN/Room	24	24	24	24	96	24	24	24	24	96	48	48	48	48	192	40	40	40	40	160
		Number	15	51	56	36	158	20	86	138	50	294	35	137	194	86	452	67	186	240	190	683
		HBR/IRD	**0.63**	**2.13**	**2.33**	**1.5**	**1.65**	**0.83**	**3.58**	**5.75**	**2.08**	**3.06**	**0.73**	**2.85**	**4.04**	**1.79**	**2.35**	**1.68**	**4.65**	**6**	**4.75**	**4.27**

HLC: Human Landing Catches; HBR: Human Biting Rate; HN: Human/Night RC: Resting Collect IRD: Indoor Resting Density
A: August; S: September; O: October; N: November; T: Total

Annex 2c: Endophagy of *An. gambiae* s.l. in IRS districts and their control

Villages	**Koungheul**			**Koumpentoum**			**Malem Hodar**			**Nioro**		
	Indoor	Outdoor	**Endophagy**	Indoor	Outdoor	**Endophagy**	Indoor	Outdoor	**Endophagy**	Indoor	Outdoor	**Endophagy**
Sprayed villages	27	23	**54**	4	8	**33.3**	13	7	**65.0**	15	10	**60.0**

Internal control	18	33	**35.3**	0	2	**0**	22	20	**52.4**	74	99	**42.8**
External control	36	26	**58.1**	293	248	**54.2**	30	18	**62.5**	47	34	**58.0**
Total	81	82	**49.7**	297	258	**53.5**	65	45	**59.1**	136	143	**48.7**

Annex 2d: Human Biting Rate of *Anopheles gambiae*, s.l. and *Anopheles funestus* in sprayed villages and their controls

Districts	Species		Sprayed villages					Internal control					External control				
			A	S	O	N	T	A	S	O	N	T	A	S	O	N	T
Koungheul	***An. gambiae* s.l.**	HN	24	24	24	24	96	24	24	24	24	96	24	24	24	24	96
		Number	13	14	9	14	50	5	7	13	26	51	4	30	22	6	62
		HBR	**0.54**	**0.58**	**0.38**	**0.58**	**0.52**	**0.21**	**0.29**	**0.54**	**1.08**	**0.53**	**0.17**	**1.25**	**0.92**	**0.25**	**0.65**
Koumpentoum	***An. gambiae* s.l.**	HN	24	24	24	24	96	24	24	24	24	96	24	24	24	24	96
		Number	2	7	2	1	12	0	2	0	0	2	21	332	117	71	541
		HBR	**0.08**	**0.29**	**0.08**	**0.04**	**0.13**	**0**	**0.08**	**0**	**0**	**0.02**	**0.88**	**13.83**	**4.88**	**2.96**	**5.64**
Malem Hodar	***An. gambiae* s.l.**	HN	24	24	24	24	96	24	24	24	24	96	24	24	24	24	96
		Number	3	5	7	5	20	7	9	13	5	32	8	19	10	11	48
		HBR	**0.13**	**0.21**	**0.29**	**0.21**	**0.21**	**0.29**	**0.38**	**0.54**	**0.21**	**0.33**	**0.33**	**0.79**	**0.42**	**0.46**	**0.50**
Nioro	***An. gambiae* s.l.**	HN	24	24	24	24	96	24	24	24	24	96	24	24	24	24	96
		Number	7	10	3	10	25	47	71	27	71	173	32	29	13	7	81
		HBR	**0.29**	**0.42**	**0.13**	**0.42**	**0.26**	**1.96**	**2.96**	**1.13**	**2.96**	**1.8**	**1.33**	**1.21**	**0.54**	**0.29**	**0.84**
	An. funestus	HN	24	24	24	24	96	24	24	24	24	96	24	24	24	24	96
		Number	18	26	22	26	84	17	111	172	111	368	412	452	128	169	1161
		HBR	**0.75**	**1.08**	**0.92**	**1.08**	**0.88**	**0.71**	**4.63**	**7.17**	**4.63**	**3.83**	**17.17**	**18.83**	**5.33**	**7.04**	**12.09**

Annex 2e: Indoor Resting Density of *Anopheles gambiae, s.l.* and *Anopheles funestus* in sprayed villages and their control in IRS districts

Districts	Species		Sprayed villages					Internal control					External control				
			A	S	O	N	T	A	S	O	N	T	A	S	O	N	T
Koungheul	*An. gambiae* s.l.	NP	20	20	20	20	80	20	20	20	20	80	20	20	20	20	80
		Number	14	28	15	33	90	33	26	34	73	166	20	37	16	23	96
		IRD	**0.70**	**1.40**	**0.75**	**1.65**	**1.13**	**1.65**	**1.30**	**1.70**	**3.65**	**2.08**	**1.0**	**1.85**	**0.80**	**1.15**	**1.20**
Koumpentoum	*An. gambiae* s.l.	NP	20	20	20	20	80	20	20	20	20	80	20	20	20	20	80
		Number	8	39	23	28	98	0	3	18	0	21	51	140	271	274	736
		IRD	**0.40**	**1.95**	**1.15**	**1.40**	**1.23**	**0**	**0.15**	**0.90**	**0**	**0.26**	**2.55**	**7.0**	**13.55**	**13.70**	**9.20**
Malem Hoddar	*An. gambiae* s.l.	NP	20	20	20	20	80	20	20	20	20	80	20	20	20	20	80
		Number	7	8	17	11	43	34	14	92	30	170	33	47	9	74	163
		IRD	**0.35**	**0.40**	**0.85**	**0.55**	**0.54**	**1.70**	**0.70**	**4.60**	**1.50**	**2.13**	**1.65**	**2.35**	**0.45**	**3.70**	**2.04**
Nioro	*An. gambiae* s.l.	NP	20	20	20	20	80	20	20	20	20	80	20	20	20	20	80
		Number	36	15	16	24	91	192	222	64	46	524	84	37	36	15	172
		IRD	**1.80**	**0.75**	**0.80**	**1.20**	**1.14**	**9.60**	**11.10**	**3.20**	**2.30**	**6.55**	**4.20**	**1.85**	**1.80**	**0.75**	**2.15**
	An. funestus	NP	20	20	20	20	80	20	20	20	20	80	20	20	20	20	80
		Number	4	17	44	48	113	63	169	196	142	570	325	87	102	203	717
		IRD	**0.20**	**0.85**	**2.20**	**2.40**	**1.41**	**3.15**	**8.45**	**9.80**	**7.10**	**7.13**	**16.25**	**4.35**	**5.10**	**10.15**	**8.96**

NP: number of houses; IRD: Indoor Resting Density ; A: August; S: September; O: October; N: November; T: Total

Annex 2f: Reproductive status of *Anopheles gambiae, s.l.* female collected by PSC

District	Status	August				September				October				November				Total			
		Go	Gr	SGr	AJ	Go	Gr	SGr	AJ	Go	Gr	SGr	AJ	Go	Gr	SGr	AJ	Go	Gr	SGr	AJ
Koungheul	Sprayed	21	9	17	0	18	7	29	0	34	6	9	0	40	41	24	1	**113**	**63**	**79**	**1**
Koumpentoum	Sprayed	5	3	0	0	22	4	15	1	26	12	1	2	13	13	2	0	**66**	**32**	**18**	**3**
Malem Hoddar	Sprayed	30	3	6	2	17	1	2	2	74	21	13	1	21	8	8	4	**142**	**33**	**29**	**9**
Nioro	Sprayed	131	20	59	18	167	33	23	14	46	7	21	6	33	8	22	7	**377**	**68**	**125**	**45**
Kaffrine	Unsprayed	18	9	18	1	25	9	21	0	12	0	12	1	39	22	19	6	**94**	**40**	**70**	**8**
Tambacounda	Unsprayed	25	26	0	0	99	40	1	0	156	115	0	0	134	134	0	6	**414**	**315**	**1**	**6**
Ndoffane	Unsprayed	35	29	20	22	22	1	14	0	16	2	18	0	9	0	6	0	**82**	**32**	**58**	**0**
Richard-Toll	Unsprayed	-	-	-	-	413	181	64	73	-	-	-	-	-	-	-	-	**413**	**181**	**64**	**73**
Niayes	Unsprayed	25	13	19	1	-	-	-	-	19	49	16	6	-	-	-	-	**44**	**62**	**35**	**7**
Vélingara	Unsprayed	29	9	3	4	-	-	-	-	21	13	0	1	-	-	-	-	**50**	**22**	**3**	**5**
Kédougou	Unsprayed	-	-	-	-	8	3	3	3	-	-	-	-	6	0	0	0	**14**	**3**	**3**	**3**

Annex 2g: Parity Rate (% parous females) of *Anopheles gambiae*, s.l. and *Anopheles funestus* in IRS district

Month	Koungheul			Koumpentoum			Malem Hodar			Nioro					
	Anonpheles gambiae s.,l.			*Anonpheles gambiae s.,l.*			*Anonpheles gambiae s.,l.*			*Anonpheles gambiae s.,l.*			*Anonpheles funestus*		
	TC	TD	PR	TC	TD	PR	TC	TD	PR	TC	TD	PR	TC	TD	PR
August	18	8	25% (2/8)	2	1	0% (0/1)	10	7	29% (2/7)	54	36	33% (12/36)	35	25	48% (12/25)
September	21	14	43% (6/14)	9	7	86% (6/7)	14	9	78% (7/9)	81	50	60% (30/50)	137	83	54% (45/83)
October	22	15	80% (12/15)	2	1	50% (1/2)	26	19	68.4% (13/19)	30	14	42.8% (6/14)	194	102	64.7% (66/102)
November	40	26	69.2% (18/26)	1	0	0 %	12	8	75% (6/8)	33	19	31.6% (6/19)	86	64	26.6% (17/64)
Total	**101**	**63**	**60.3% (38/63)**	**14**	**9**	**50% (7/14)**	**62**	**43**	**65.1% (28/43)**	**198**	**119**	**45.4% (54/119)**	**452**	**274**	**51.1% (140/274)**

TC: Total Collected; TD: Total Dissected; PR: Parity Rate

Annex 2h: Parity rate in IRS districts and their controls

Districts	Species	Sprayed villages			Internal control			External control		
		TC	TD	PR	TC	TD	PR	TC	TD	PR
Koungheul	*An. gambiae* s.l.	50	30	50% (15/30)	51	33	69.7% (23/33)	62	37	73% (27/37)
Koumpentoum	*An. gambiae* s.l.	12	8	75% (6/8)	2	1	100% (1/1)	541	333	83.5% (278/333)
Malem Hoddar	*An. gambiae* s.l.	20	15	46.7% (7/15)	42	28	75% (21/28)	48	36	69.4% (25/36)
Nioro	*An. gambiae* s.l.	25	15	53.3% (8/15)	173	104	44.2% (46/104)	81	47	68.1% (32/47)
	An. funestus	84	58	29.3% (17/58)	368	216	56.9% (123/216)	1161	669	61.6% (412/669)

Annex 2i: Specific composition of anopheline fauna according to the sampling method and parity rates in unsprayed districts.

Districts	Species	HLC	PSC	Total	Parity*		
					TC	TD	PR
Kedougou	*An. gambiae* s.l.	991	23	1014	991	329	83.6% (275/329)
	An. funestus	1136	47	1183	1136	265	75.8% (201/265)
	An. nili	4	0	4	-	-	-
Ndoffane	*An. gambiae* s.l.	81	172	253	81	47	68.1% (32/47)

	An. funestus	1161	717	1878	1161	669	61.6% (412/669)
	An. pharoensis	1	0	1	-	-	-
Niayes	*An. gambiae* s.l.	2	148	150	-	-	-
Richard-Toll	*An. gambiae* s.l.	-	731	731	-	-	-
	An. funestus	-	1	1	-	-	-
	An. pharoensis	-	25	25	-	-	-
	An ziemani	-	3	3	-	-	-
	An rufipes	-	7	7	-	-	-
Kaffrine	*An. gambiae* s.l.	83	212	295	83	55	72.7% (40/55)
	An. funestus	0	1	1	-	-	-
	An. pharoensis	3	0	3	-	-	-
	An rufipes	0	1	1	-	-	-
Tambacounda	*An. gambiae* s.l.	541	736	1277	541	333	83.5% (278/333)
	An. pharoensis	10	0	10	-	-	-
Vélingara	*An. gambiae* s.l.	431	80	511	431	243	83.1% (202/243)

	An. funestus	3	2	5	-	-	-
	An. pharoensis	9	0	9	-	-	-

* Parturity was evaluated only for *An. gambiae, s.l.* and *An. funestus*.

Annex 2j: Human Biting Rate and Indoor Resting Density of *Anopheles gambiae* s.l. in unsprayed districts

Districts		HLC (HBR)															PSC (IRD)				
		Indoor					Outdoor					Total									
		A	S	O	N	T	A	S	O	N	T	A	S	O	N	T	A	S	O	N	T
Kedougou	HN/R	-	12	-	12	24	-	12	-	12	24	-	24	-	24	48	-	20	-	20	40
	Number	-	475	-	12	487	-	489	-	15	504	-	964	-	27	991	-	17	-	6	23
	HBR/IRD	**-**	**39.58**	**-**	**1**	**20.29**	**-**	**40.75**	**-**	**1.25**	**21**	**-**	**40.17**	**-**	**1.12**	**20.65**	**-**	**0.85**	**-**	**0.30**	**0.58**
Ndoffane	HN/R	12	12	12	12	48	12	12	12	12	48	24	24	24	24	96	20	20	20	20	80
	Number	25	12	8	2	47	7	17	5	5	34	32	29	13	7	81	84	37	36	15	172
	HBR/IRD	**2.08**	**1.00**	**0,67**	**0,17**	**0.98**	**0.58**	**1.42**	**0.42**	**0.42**	**0.71**	**1.33**	**1.21**	**0.54**	**0.29**	**0.84**	**4.20**	**1.85**	**1.80**	**0.75**	**2.15**
Niayes	HN/R	12	-	12	-	24	12	-	12	-	24	24	-	24	-	48	50	-	50	-	100
	Number	1	-	0	-	1	0	-	1	-	1	1	-	1	-	2	58	-	90	-	148
	HBR/IRD	**0,08**	**-**	**0**	**-**	**0.04**	**0**	**-**	**0.08**	**-**	**0.04**	**0.04**	**-**	**0.04**	**-**	**0.04**	**1.16**	**-**	**1.80**	**-**	**1.48**
Richard-Toll	HN/R	-	-				-	-				-	-				-	110	-	-	110
	Number	-	-				-	-				-	-				-	767	-	-	767
	HBR/IRD	-	-				-	-				-	-	-	-	-	**-**	**6.97**	-	-	**6.97**
Kaffrine	HN/R	18	18	18	18	72	18	18	18	18	72	36	36	36	36	144	30	30	30	30	120
	Number	7	21	15	4	47	2	16	8	10	36	9	37	23	14	83	46	55	25	86	212
	HBR/IRD	0,39	**1,17**	**0,83**	**0,22**	**0.65**	**0.11**	**0.89**	**0.44**	**0.56**	**0.50**	**0.25**	**1.03**	**0.64**	**0.39**	**0.58**	**1.53**	**1.83**	**0.83**	**2.87**	**1.77**
Tambacounda	HN/R	12	12	12	12	48	12	12	12	12	48	24	24	24	24	96	20	20	20	20	80
	Number	8	195	57	33	293	13	137	60	38	248	21	332	117	71	541	51	140	271	274	736
	HBR/IRD	**0,67**	**16,25**	**4,75**	**2,75**	**6.10**	**1.08**	**11.42**	**5.00**	**3.17**	**5.17**	**0.88**	**13.83**	**4.88**	**2.96**	**5.64**	**2.55**	**7.00**	**13.55**	**13.70**	**9.20**
Velingara	HN/R	12	-	12	-	24	12	-	12	-	24	24	-	24	-	48	20	-	20	-	40
	Number	82	-	155	-	237	77	-	117	-	194	159	-	272	-	431	45	-	35	-	80
	HBR/IRD	**6.83**	**-**	**12.92**	**-**	**9.88**	**6.42**	**-**	**9.75**	**-**	**8.08**	**6.63**	**-**	**1133**	**-**	**8.98**	**2.25**	**-**	**1.75**	**-**	**2.00**

HLC: Human Landing Catches **PSC**: Pyrethrum Spray Catches **HBR**: Human Bite Rate **IRD**: Indoor Resting Density

A: August; S: September; O: October; N: November; T: Total

Annex 2k: HBR rate and IRD of A*nopheles gambiae* s.l. in non-IRS districts

	HLC (HBR)					PSC (IRD)*				
	August	September	October	November	Mean	August	September	October	November	Mean
Kedougou	-	40.17	-	1.12	20.65	-	0.85	-	0.30	0.58
Ndoffane	1.33	1.21	0.54	0.29	0.84	4.20	1.85	1.80	0.75	2.15
Niayes	0.04	-	0.04	-	0.04	1.16	-	1.80	-	1.48
richard-Toll	-	-	-	-	-	-	6.97	-	-	6.97
Kaffrine	0.25	1.03	0.64	0.39	0.58	1.53	1.83	0.83	2.87	1.77
mbacounda	0.88	13.83	4.88	2.96	5.64	2.55	7.00	13.55	13.70	9.20
Velingara	6.63	-	11.33	-	8.98	2.25	-	1.75	-	2.00

HLC: Human Landing Catches **PSC**: Pyrethrum Spray Catches **HBR**: Human Bite Rate **IRD**: Indoor Resting Density

Annex 3: Lab results

Annex 3a: Number of specimens identified by PCR and distribution according to the different species of the *An. gambiae* complex (HLC and PSC)

Districts	n	species				
		An. arabiensis	*An. coluzzii*	*An. gambiae*	*An. melas*	AC/AG
Koungheul	135	86	36	13	0	0
Koumpentoum	39	35	3	1	0	0
Malem Hoddar	91	80	9	2	0	0
Nioro	110	67	12	31	0	0
Kaffrine	96	68	20	8	0	0
Ndoffane	99	94	2	2	1	0
Niayes	27	26	1	0	0	0
Kédougou	238	30	3	205	0	0
Tambacounda	226	157	13	55	0	1
Vélingara	171	37	21	112	1	0
Richard-Toll	31	31	0	0	0	0
Total	1263	711	120	429	2	1

AC/AG = hybride *coluzzii/gambiae*

Annex 3b: Number of specimens identified by PCR and distribution according to the different species of the *An. gambiae* complex (HLC)

Ditricts	n	species				
		An. arabiensis	*An. coluzzii*	*An. gambiae*	*An. melas*	AC/AG
Koungheul	97	66	19	12	0	0
Koumpentoum	14	13	0	1	0	0
Malem Hoddar	44	38	4	2	0	0
Nioro	48	32	3	13	0	0
Kaffrine	58	41	9	8	0	0
Ndoffane	61	57	1	2	1	0
Niayes	2	2	0	0	0	0
Kédougou	210	26	3	181	0	0
Tambacounda	107	62	9	35	0	1
Vélingara	143	34	16	92	0	0
Richard-Toll	0	0	0	0	0	0

Total	784	371	64	346	1	1

AC/AG = hybride *coluzzii/gambiae*

Annex 3c: Number of specimens identified by PCR and distribution according to the different species of the *An. gambiae* complex (PSC)

Districts	n	species				
		An. arabiensis	*An. coluzzii*	*An. gambiae*	*An. melas*	AC/AG
Koungheul	38	20	17	1	0	0
Koumpentoum	25	22	3	0	0	0
Malem Hoddar	47	42	5	0	0	0
Nioro	62	35	9	18	0	0
Kaffrine	38	27	11	0	0	0
Ndoffane	38	37	1	0	0	0
Niayes	25	24	1	0	0	0
Kédougou	28	4	0	24	0	0
Tambacounda	119	95	4	20	0	0
Vélingara	28	3	5	20	0	0
Richard-Toll	31	31	0	0	0	0
Total	479	340	56	83	0	0

AC/AG = hybride *coluzzii/gambiae*

Annex 3d: Number of specimens identified by PCR and distribution of the different species of the *An. gambiae* complex according to the sprayed villages and their unsprayed internal and external controls (HLC and PSC)

District		**n**	***An. arabiensis***	***An. coluzzii***	***An. gambiae***	***An. melas***	***AC/AG***
Malem Hodar	Hot spot	26	23	3	0	0	0
	Internal control	65	57	6	2	0	0
	External control	66	47	15	4	0	0
Koungheul	Hot spot	65	40	22	3	0	0
	Internal control	70	46	14	10	0	0
	External control	64	46	11	7	0	0
Koumpentoum	Hot spot	35	32	2	1	0	0
	Internal control	4	3	1	0	0	0
	External control	226	157	13	55	0	1
Nioro	Hot spot	29	15	5	9	0	0
	Internal control	81	52	7	22	0	0
	External control	99	94	2	2	1	0
Total		**830**	**612**	**101**	**115**	**1**	**1**

Annex 3e: Number of specimens identified by PCR and distribution of the different species of the *An. gambiae* complex according to the sprayed villages and their unsprayed internal and external controls (HLC)

District		n	*An. arabiensis*	*An. coluzzii*	*An. gambiae*	*An. melas*	*AC/AG*
Malem Hodar	Hot spot	13	13	2	0	0	0
	Internal control	31	31	2	2	0	0
	External control	31	31	6	4	0	0
Koungheul	Hot spot	49	49	16	3	0	0
	Internal control	48	48	3	9	0	0
	External control	46	46	6	7	0	0
Koumpentoum	Hot spot	12	12	0	1	0	0
	Internal control	2	2	0	0	0	0
	External control	107	107	9	35	0	1
Nioro	Hot spot	10	10	1	2	0	0
	Internal control	38	38	2	11	0	0
	External control	61	61	1	2	1	0
Total		**448**	**322**	**48**	**76**	**1**	**1**

Annex 3f: Number of specimens identified by PCR and distribution of the different species of the *An. gambiae* complex according to the sprayed villages and their unsprayed internal and external controls (PSC)

District		n	*An. arabiensis*	*An. coluzzii*	*An. gambiae*	*An. melas*	*AC/AG*
Malem Hodar	Hot spot	13	12	1	0	0	0
	Internal control	34	30	4	0	0	0
	External control	35	26	9	0	0	0
Koungheul	Hot spot	16	10	6	0	0	0
	Internal control	22	10	11	1	0	0
	External control	18	13	5	0	0	0

Koumpentoum	Hot spot	23	21	2	0	0	0
	Internal control	2	1	1	0	0	0
	External control	119	95	4	20	0	0
Nioro	Hot spot	19	8	4	7	0	0
	Internal control	43	27	5	11	0	0
	External control	38	37	1	0	0	0
Total		**382**	**290**	**53**	**39**	**0**	**0**

Annex 3g: : Origin of blood meal and anthropophilic rate of An. gambiae s.l. in sprayed sentinel sites and their internal and external controls

Species	Districts	Localities				Simple meal					Mix meal with Human							Mix meal with Animal				IA
			N	T	ND	H	B	S	C	Ho	H/B	H/S	H/Ho	H/S/Ho	H/B/Ho	H/B/S	H/B/C	B/S	B/Ho	S/Ho	B/S/Ho	
An. gambiae s.l	Koungheul	Hot spot	49	46	2	13	7	2	0	18	0	0	2	0	0	0	0	1	0	1	0	0.341
		Internal control	64	64	3	25	1	1	0	26	1	1	2	0	0	0	0	2	2	0	0	0.475
		External control	38	36	1	7	1	1	0	18	0	0	1	0	0	0	0	4	1	1	1	0.229
	Koumpentoum	Hot spot	56	55	2	4	12	4	0	14	0	0	11	0	0	1	0	2	4	1	0	0.302
		Internal control s	10	10	0	2	0	0	0	8	0	0	0	0	0	0	0	0	0	0	0	0.200
		External control	417	218	18	51	28	7	0	80	3	5	9	1	0	0	0	4	4	8	0	0.345
	Malem Hodar	Hot spot	28	28	2	3	3	2	0	13	1	0	0	0	0	0	0	1	2	1	0	0.154
		Internal control	114	85	4	33	17	0	0	29	0	0	1	0	0	0	0	0	1	0	0	0.420
		External control	80	79	4	13	4	1	0	40	0	0	4	0	0	0	0	8	1	3	1	0.227
	Nioro	Hot spot	60	29	4	4	2	2	0	13	0	0	0	0	0	0	0	2	1	1	0	0.160
		Internal control	316	67	12	6	13	2	0	31	0	0	0	0	0	0	0	0	3	0	0	0.109
		External control	87	58	4	3	9	5	0	27	0	1	0	0	0	0	0	7	1	1	0	0.074
An. funestus	Nioro	Hot spot	58	25	3	2	3	1	0	15	0	0	0	0	0	0	0	0	0	0	1	0.091
		Internal control	292	66	12	6	20	2	0	17	0	0	0	0	0	0	0	1	7	1	0	0.111
		External control	369	136	5	17	18	5	0	55	2	3	3	0	0	0	0	9	15	3	1	0.191