



PRESIDENT'S MALARIA INITIATIVE



PMI | Africa IRS (AIRS) Project
Indoor Residual Spraying (IRS 2) Task Order Six

**SENEGAL 2016 ENTOMOLOGICAL
LABORATORY SUPPLEMENT**

SUBMITTED: MARCH 02, 2018

Recommended Citation: The President's Malaria Initiative (PMI)/Africa Indoor Residual Spraying (AIRS) Project. March 2018. Senegal 2016 Supplemental Laboratory Data. Bethesda, MD. PMI Africa IRS (AIRS) Project Indoor Residual Spraying (IRS 2) Task Order Six, Abt Associates Inc.

Contract No. and Task order: GHN-I-00-09-00013-00 & AID- OAA-TO-14-00035

Submitted to: United States Agency for International Development/PMI

Abt Associates Inc. | 4550 Montgomery Avenue | Suite 800 North
| Bethesda, Maryland 20814 | T. 301.347.5000 | F. 301.913.9061
| www.abtassociates.com



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ACRONYMS

AIRS	Africa Indoor Residual Spraying Project
CDC	Centers for Disease Control and Prevention
CSP	Circumsporozoite
CSPI	Circumsporozoite protein index
EIR	Entomological Inoculation Rate
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 Resistant
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
USAID	United States Agency for International Development
VGSC	Voltage Gated Sodium Channel
WHO	World Health Organization

EXECUTIVE SUMMARY

Laboratory data for samples the team collected in 2016 showed that *An. arabiensis* was the predominant malaria vector found across most of Senegal, with exceptions in the south of the country in Kedougou and Velingara. In those areas, *An. gambiae* s.s. was most common during the rainy season. In general, the anthropophily index was low for *An. arabiensis*, with many having taken blood-meals from horses and cattle. In Kedougou (0.97) and in Velingara (0.93), the anthropophily index was high, with most *An. gambiae* having fed on humans. Sporozoite rates were very low in indoor residual spraying (IRS) districts and areas where *An. arabiensis* were dominant. In southern sites where *An. gambiae* were common, sporozoite rates were 0.6% in Kedougou and 1.1% in Velingara.

1. METHODS

1.1 MOSQUITO SAMPLING PERIOD & IRS TIMING

IRS was conducted in the villages of health posts where residual malaria transmission has been reported with malaria incidence greater than 15 confirmed cases per 1000 inhabitants. The teams sprayed pirimiphos-methyl (Actellic CS 300) at 1g/m² in the districts of Koungheul, Koumpentoum, Malem Hodar, and Niore in July and early August 2016. Rainfall in IRS districts of Senegal is usually between June and October, with the dry season lasting 7 months, between November and May. *An. gambiae* and *An. funestus* were sampled in IRS and unsprayed sentinel sites from August 2016 to December 2016. Molecular analysis of these samples is included in this report.

1.2 LABORATORY MOLECULAR ANALYSES

Species identification

The molecular identification of *An. gambiae* sibling species was performed on a subsample of living and dead female mosquitoes from susceptibility tests, human landing catch (HLC) and pyrethrum spray catch (PSC). The molecular identification was performed by polymerase chain reaction (PCR), following the protocol of Wilkins et al. (2006). The number of specimens tested per site is presented in Annex 1A.

Blood meal source

The team used the direct enzyme-linked immuno-sorbent assay (ELISA) method described by Beier et al. (1986) to determine the origin of blood meals in *An. gambiae* s.l. collected by PSC. The team determined the anthropophily rate by the ratio of the number of blood meals taken from humans over the number of meals identified. The same ratio was used for the different animal hosts tested.

Sporozoite rate

To determine the sporozoite infection rate of *An. gambiae* s.l. collected by HLC, the team used the circumsporozoite (CSP) ELISA described by Burkot et al., (1984) and slightly modified by Wirtz et al., (1987). Infection rates were determined as a percentage by the ratio of the specimens carrying the *Plasmodium falciparum* CSP antigen over the total number of specimens tested by ELISA.

Voltage gated sodium channel (VGSC) resistance mutations

The Vgsc-1014F (knock down resistance (KDR) West) and 1014S (KDR East) mutations were respectively identified according to the protocols of Martinez-Torres et al. (1998) and Ranson et al. (2000).

2. RESULTS

SUPPLEMENTARY LABORATORY DATA FOR 2016 IRS CAMPAIGN

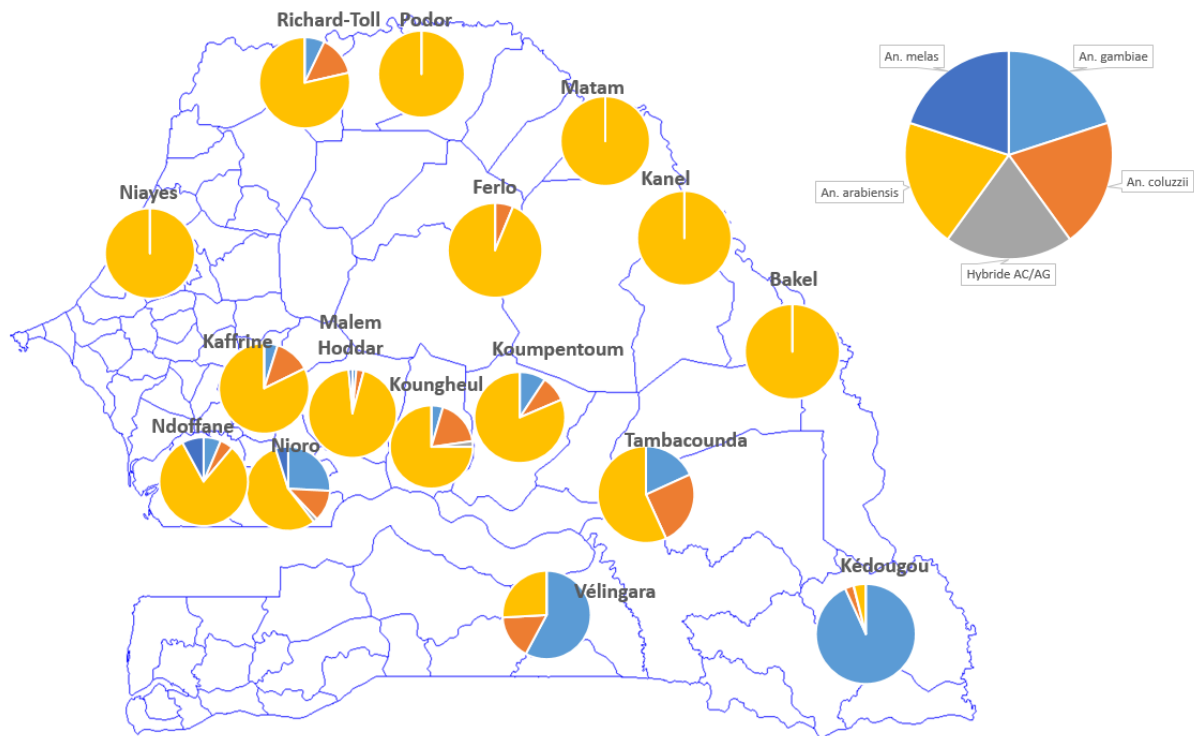
2.1 *ANOPHELES GAMBIAE* SIBLING SPECIES COMPOSITION

Figure 1 shows the percentage species composition by location according to PCR results. In the southern (Velingara) and south-east (Kedougou) districts of the country, a large predominance of *An. gambiae* was noted from specimens collected during the rainy season, and a higher frequency of *An. arabiensis* was recorded in the dry season. Everywhere else, *An. arabiensis* remained the predominant species of the *An. gambiae* complex in all seasons (See Annex 1).

The presence of *An. coluzzi* was noted in all districts (except for Niayes due to very low numbers). The frequency was higher in the southern half of the country, notably in the districts of Nioro, Kédougou, Tambacounda, and Vélingara (Figure 1).

Species PCR was not done for *An. funestus* s.l. found only in Nioro and N dofane. In Senegal, PCR in previous years showed the presence of only *An. funestus* s.s.

Figure 1: Proportion of the different species of the *An. gambiae* complex in the districts



Annex 1 shows detailed results of identification of *An. gambiae* s.l. captured in the sprayed districts and their internal and external controls during the dry and rainy seasons. With a large predominance of *An. arabiensis* in both sprayed sites and untreated controls, insecticide treatment did not appear to have an impact on the composition of the *An. gambiae* complex.

For laboratory identified *An. gambiae* s.l. from susceptibility tests the proportion dead in susceptibility tests was 84% *An. arabiensis*, 2% *An. coluzzii* and 14% *An. gambiae*. A similar proportion was found for mosquitoes that survived exposure; 79% *An. arabiensis*, 2% *An. coluzzii* and 18% *An. gambiae*.

2.2 BLOOD MEAL ANALYSIS

2.2.1 IRS DISTRICTS AND PAIRED UNSPRAYED CONTROLS

Figures 2-6 show the results of blood meal source identification from endophilic *An. gambiae* s.l. and *An. funestus* s.l. females collected by PSC in sprayed districts. More detailed data are in Annex 2. In all districts where IRS was conducted, the proportion of malaria vectors that blood-fed on humans was low. The anthropophily index was 0.41 in Kougheul, 0.30 in Koumpentoum, 0.27 in Malem Hodar, and 0.24 in Nioro for *An. gambiae* s.l., and 0.27 in Nioro for *An. funestus* s.l. The blood feeding sources were similar for sprayed areas and unsprayed controls (see Annex 2). The most common blood meal source was horses in all sites, with cattle also being a common animal blood meal source.

Figure 2. *An. gambiae* s.l. blood meals from Kougheul collected by PSC (n=263).

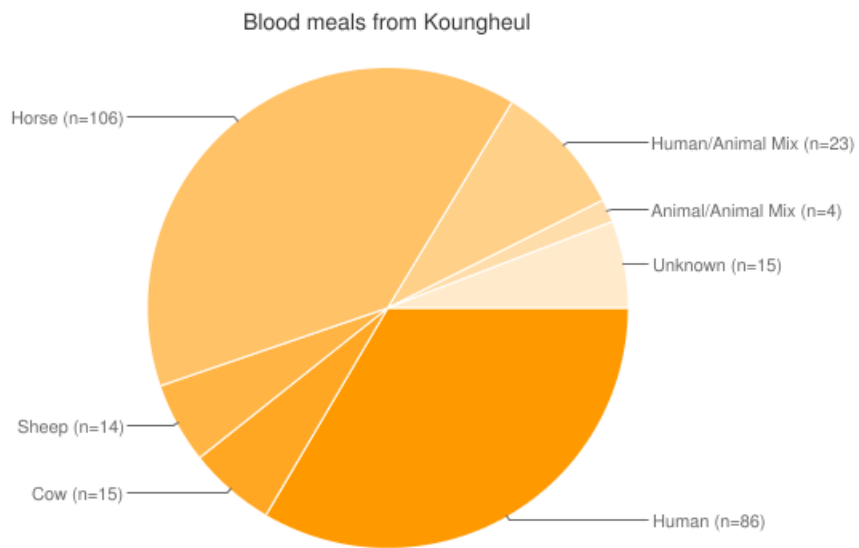


Figure 3. *An. gambiae* s.l. blood meals from Koumpentoum collected by PSC (n=366).

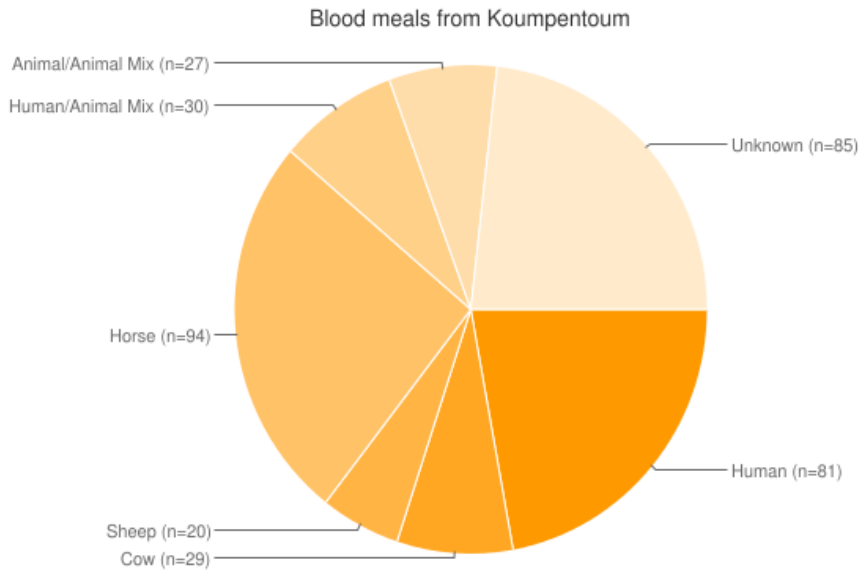


Figure 4. *An. gambiae* s.l. blood meals from Malem Hodar collected by PSC (n=339).

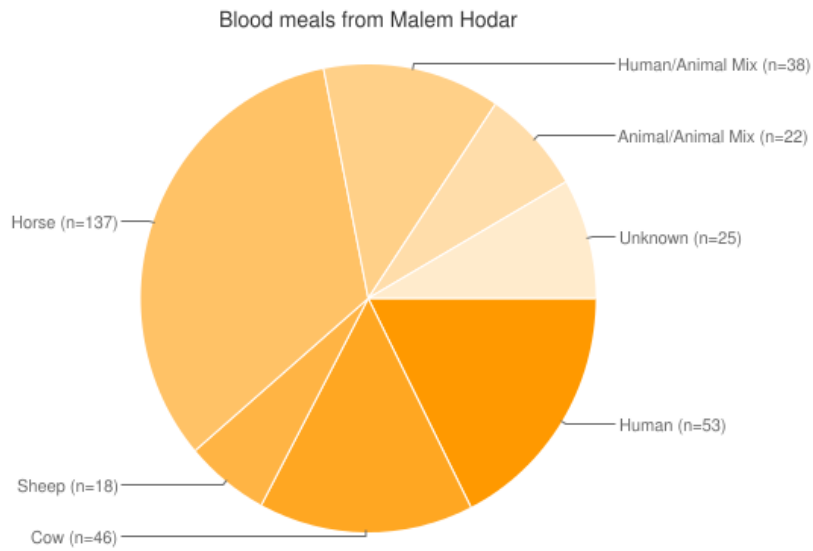


Figure 5. *An. gambiae* s.l. blood meals from Nioro collected by PSC (n=506).

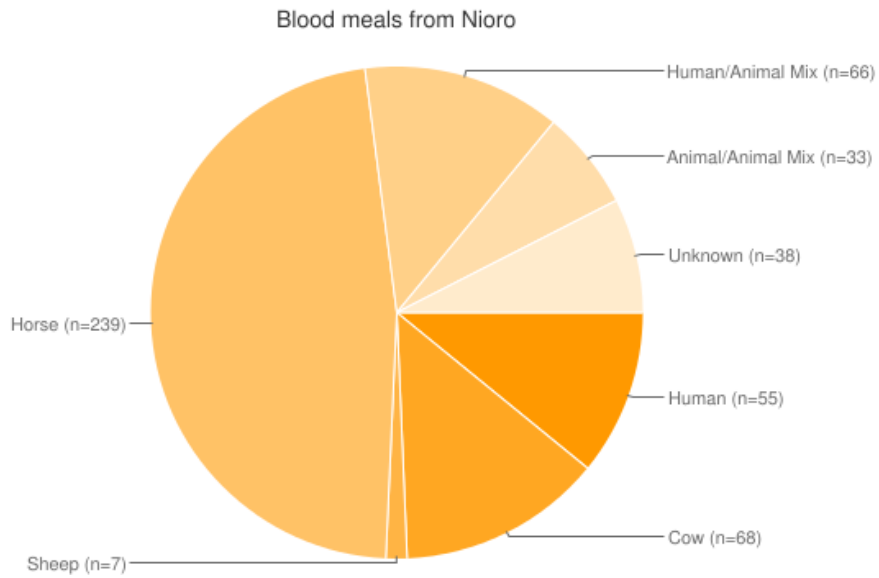
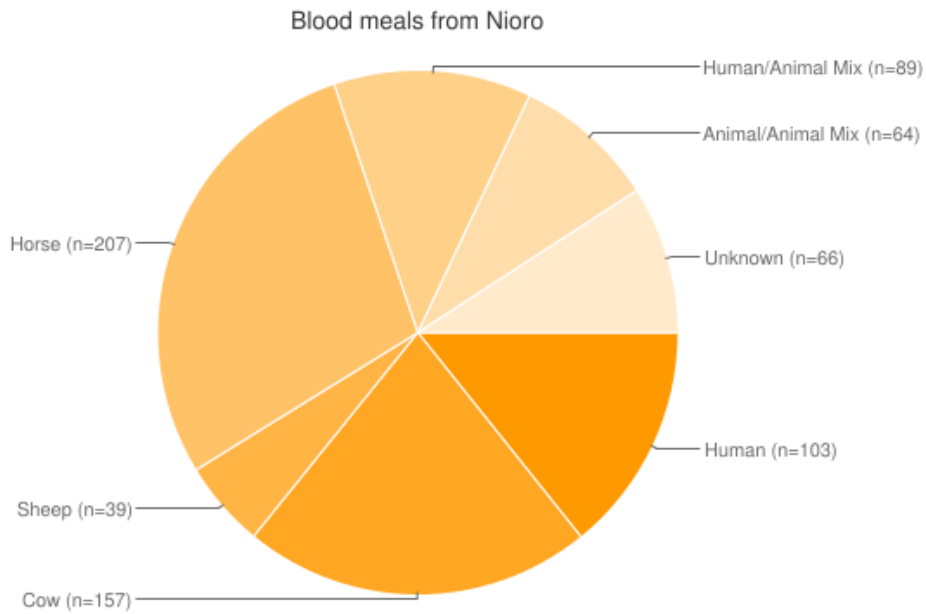


Figure 6. *An. funestus* s.l. blood meals from Nioro collected by PSC (n=725).



2.2.2 UNSPRAYED DISTRICTS

In the southern areas of Velingara and Kedougou, the anthropophily index of *An. gambiae* s.l. was high at 0.93 and 0.97, respectively. In northern sites of Richard Toll and Niayes, the anthropophily index was low and similar to the IRS districts.

TABLE 1: BLOOD MEAL ANALYSIS OF ANOPHELES COLLECTED BY PSC IN UNSPRAYED DISTRICTS.

Species	Districts	N	T	U	Single source					MIX	MIX	IA
					H	C	S	Ch	Ho	H/A	A/A	
<i>An. gambiae</i> s.l.	Velingara	46	46	3	40	0	2	0	1	0	0	0.93
	Niayes	22	22	3	10	2	0	0	7	0	0	0.53
	Richard Toll	151	54	7	11	10	5	0	16	4	1	0.32
	Kédougou	97	95	6	85	3	0	0	0	1	0	0.97
<i>An. funestus</i> s.l.	Velingara	9	9	3	0	5	0	0	0	1	0	0.17
	Niayes	4	3	0	1	1	1	0	0	0	0	0.33
	Richard Toll	1	0	0	0	0	0	0	0	0	0	0
<i>An. pharoensis</i>	Richard Toll	30	11	2	3	2	0	0	4	0	0	0.33

N= TOTAL BLOOD-FED, T= NUMBER TESTED, U= UNKNOWN, H= HUMAN, C= COW, S= SHEEP, CH= CHICKEN, HO= HORSE, H/A= MIXED HUMAN/ANIMAL, A/A = MIXED ANIMAL/ANIMAL
IA = INDEX OF ANTHROPOPHILY

2.3 VECTOR SPOROZOITE RATES

IRS districts

Table 2 presents the results of sporozoite ELISA for *Anopheles* collected by HLC in the sprayed sentinel sites and their paired untreated controls (internal and external). Light trap catches (CDC / LT) were only conducted in Nioro for comparison with HLC. In general, the sporozoite rate was low regardless of spray status, with only 12 sporozoite positives detected from 2,760 tested (0.43%). Infected *An. gambiae* s.l. in IRS areas were only detected in Malem Hodar and Nioro districts, where sporozoite rates were 4.5% (2/44) and 1.2% (1/83) respectively.

Unsprayed districts

For unsprayed districts, only *An. gambiae* s.l. were found to be sporozoite positive, with infection rates of 1.10% (4/362) and 0.6% (8/1329) in Velingara and Kedougou, respectively (Table 3).

2.4 ENTOMOLOGICAL INOCULATION RATE (EIR)

IRS districts

The number of *Anopheles* collected in sprayed districts by HLC was low; therefore, an accurate estimate of sporozoite rates and entomological inoculation rate (EIR) could not be determined. In general, the biting rate and sporozoite rate were very low at all sites, with negligible nightly EIR. See Annex 3 for details.

Unsprayed districts

The EIR between August and December was approximately 0.09 infectious bites per night in Velingara and 0.28 infectious bites in Kédougou. (Table 2)

TABLE 2: EIR OF ANOPHELES GAMBIAE COLLECTED BY PSC IN UNSPRAYED DISTRICTS (AUGUST TO DECEMBER 2016).

District	Indoor			Outdoor			Average		
	AR	CSPI	EIR	AR	CSPI	EIR	AR	CSPI	EIR
	(b/h/n)		(ib/h/n)	(b/h/n)		(ib/h/n)	(b/h/n)		(ib/h/n)
Vélingara	6.75	0.013	0.09	8.58	0.011	0.09	7.67	0.012	0.09
Niayes	0.03	0	0.00	0.06	0	0.00	0.04	0	0.00
Richard Toll	0.88	0	0.00	0.88	0	0.00	0.88	0	0.00
Kédougou	50.25	0.007	0.35	44.08	0.004	0.18	47.17	0.006	0.28

TABLE 3: SPOROZOITE RATE OF AN. GAMBIAE S.L. AND AN. FUNESTUS ACCORDING COLLECTING METHOD IN SPRAYED DISTRICTS AUGUST 2016 – DECEMBER 2016

Districts	Methods	Sprayed				Internal control				External control				
		Collected	Tested	Positives	CSPI	Collected	Tested	Positives	CSPI	Collected	Tested	Positives	CSPI	
<i>An. gambiae s.l.</i>	Koungheul	HLC	113	119	0	0.000	38	38	0	0.000	132	38	0	0.000
	Malem Hodar	HLC	45	44	2	0.045	66	68	0	0.000	144	68	0	0.000
	Koumpentoum	HLC	37	34	0	0.000	12	12	0	0.000	467	12	4	0.333
	Nioro	HLC& CDC	118	74	1	0.014	211	146	2	0.014	176	141	2	0.014
<i>An. funestus</i>	Nioro	HLC& CDC	635	415	0	0.000	450	309	0	0.000	595	604	1	0.002

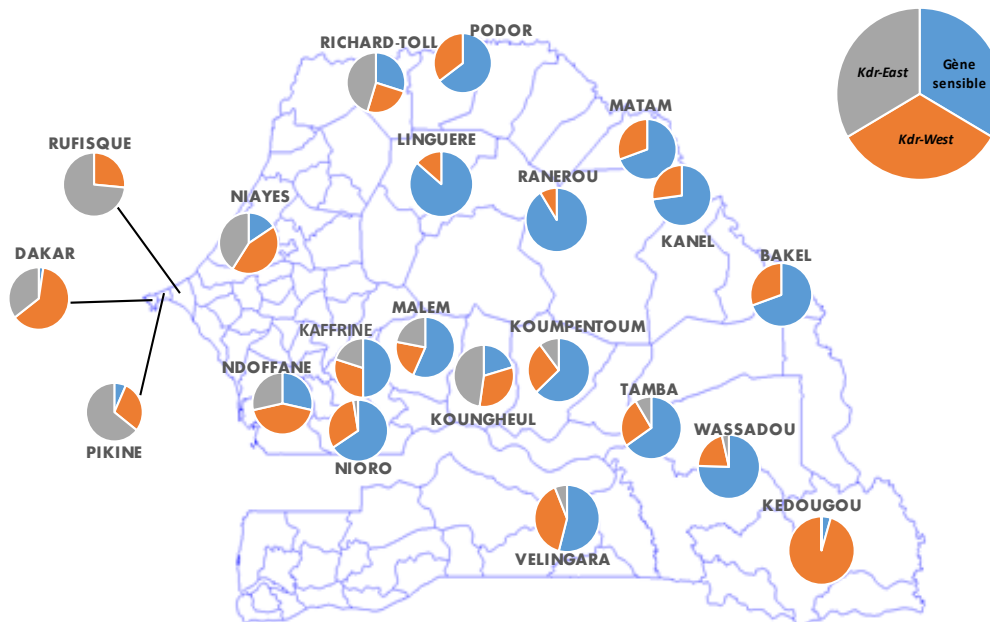
TABLE 4: SPOROZOITE RATE OF FEMALES COLLECTED BY HLC IN UNSPRAYED DISTRICTS AUGUST 2016 – DECEMBER 2016

Districts	<i>An. gambiae s.l.</i>			<i>An. pharoensis</i>		
	Tested	Positives	CSPI (%)	Tested	Positives	CSPI (%)
Velingara	362	4	1.10	2	0	0
Niayes	3	0	0	-	-	-
Richard Toll	13	0	0	112	0	0
Kedougou	1329	8	0.6	0	0	0
Total	1707	12	0.007	114	0	0

2.5 VGSC 1014F AND 1014S FREQUENCY

Figure 7 shows the allelic frequency of mutations *vgsc*-1014F (*kdr-w*) and *vgsc*-1014S (*kdr-e*) in the different districts.

Figure 7: Nationwide allele frequencies of VGSC mutations.



Both the VGSC 1014S and 1014F mutations were present in Senegal, with different frequencies in different locations. The highest frequencies of VGSC mutations were in Kedougou, where VGSC-1014F was found at close to fixation. In the Dakar suburbs, few wild type alleles were detected, but both VGSC 1014F and 1014S were present. In the departments of Pikine and Rufisque (including Bargny), the frequencies of the VGSC-1014S mutation were higher than that of the VGSC-1014F, which predominates in the city of Dakar. In Niayes, both mutations were present at relatively similar frequencies.

In IRS districts (Koungheul, Koumpentoum, Malem Hodar, and Nioro), both mutations were present at varying frequency. Wild type alleles remained at a relatively high frequency in Koumpentoum, Malem Hodar, and Nioro.

In the districts of the Senegal River Valley (Matam, Kanel and Bakel) and Ferlo (Ran  rou and Lingu  re), only the VGSC-1014F mutation was detected. The allelic frequency ranged from 21% (Kanel) to 35% (Podor). In Ferlo, the allelic frequencies of the VGSC-1014F mutation were relatively low, especially in Ran  rou district (9%). In Richard-Toll district, the VGSC-1014F and 1014S mutations were very common in F1 females exposed to insecticides (73%).

3. ANNEX

ANNEX 1A: NUMBER OF SPECIMENS IDENTIFIED BY PCR AND DISTRIBUTION ACCORDING TO THE DIFFERENT SPECIES OF THE AN. GAMBIAE COMPLEX (AUGUST-DECEMBER 2016)

Districts	Total identified	Species				
		<i>A. arabiensis</i>	<i>An. coluzzii</i>	<i>An. gambiae</i>	<i>An. melas</i>	AC/AG
Koungheul	44 (14)	33 (14)	8	2	-	1
Koumpentoum	43 (9)	35 (9)	4	4	-	-
Malem Hoddar	72 (50)	68 (47)	2 (1)	1 (1)	1 (1)	-
Nioro	125 (46)	69 (34)	15 (2)	33 (7)	6	2 (1)
Kaffrine	62 (29)	51 (29)	8	3	-	-
Ndoffane	63 (20)	51 (16)	3	4 (2)	5 (2)	-
Niayes	3	3	-	-	-	-
Kédougou	720(20)	28 (13)	20	671 (7)	1	-
Tambacounda	132 (41)	75 (28)	33 (5)	24 (8)	-	-
Vélingara	166 (13)	43 (6)	27 (2)	96 (5)	-	-
Richard-Toll	13	11	2	1	-	-
Bakel	47 (1)	47 (1)	-	-	-	-
Matam	5	5	-	-	-	-
Podor	38 (9)	38 (9)	-	-	-	-
Linguere	28 (4)	26 (4)	2	-	-	-
Total	1561 (256)	583 (210)	124 (10)	839 (30)	13 (3)	3 (1)

() = Dry season

AC/AG = hybrid *coluzzii/gambiae*

ANNEX 1B: 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 (AUGUST-DECEMBER 2016)

Method	District		Number tested (Total)	Did not amplify	<i>An. gambiae</i>	<i>An. coluzzii</i>	Hybrid AC/AG	<i>An. arabiensis</i>	<i>An. melas</i>
HLC	Malem Hodar	Hot Spots	20 (45)	0	0	1	0	19	0
		Internal C.	29 (71)	0	1	1	0	26	1
		External C.	45 (150)	3	3	3	0	36	0
	Koungheul	Hot Spots	38 (125)	4	1	6	1	26	0
		Internal C.	11 (38)	1	1	2	0	7	0
		External C.	44 (132)	1	0	5	0	38	0
	Koumpentoum	Hot Spots	34 (37)	1	3	4	0	26	0
		Internal C.	12 (12)	2	1	0	0	9	0
		External C.	143 (467)	11	24	33	0	75	0
	Nioro	Hot Spots	42 (131)	1	15	6	1	19	0
		Internal C.	65 (217)	1	13	7	1	41	2
		External C.	55 (179)	6	3	1	0	41	4
CDC-LT		Hot Spots	3 (3)	0	2	0	0	0	1
		Internal C.	19 (64)	3	2	2	0	9	3
		External C.	16 (36)	2	1	2	0	10	1
Total			576 (1707)	36	70	73	3	382	12

ANNEX 2: ORIGIN OF BLOOD MEAL AND ANTHROPOPHILIC RATE OF *AN. GAMBIAE* S.L. IN SPRAYED SENTINEL SITES AND THEIR INTERNAL AND EXTERNAL CONTROLS (AUGUST TO DECEMBER 2016).

Species	Districts	Localities	N	T	F	Monospecific					MIX H/A	MIX A/A	IA	
						H	B	S	C	Ho				
<i>An. gambiae</i> s.l.	Koungheul	Hot spot	68	64	9	11	9	3	0	31	1	0	0.218	
		Internal control	42	42	4	8	5	2	0	20	2	1	0.263	
		External control	213	157	2	67	1	9	0	55	20	3	0.561	
	Koumpentoum	Hot spot	63	36	1	12	6	1	0	12	3	1	0.429	
		Internal control	23	13	0	4	0	1	0	7	1	0	0.385	
		External control	443	316	83	65	23	18	0	75	26	26	0.391	
	Malem Hodar	Hot spot	94	90	7	16	14	4	0	36	9	4	0.301	
		Internal control	75	71	5	14	19	4	0	22	5	2	0.287	
		External control	209	177	13	22	14	10	0	79	24	15	0.280	
	<i>An. funestus</i> s.l.	Nioro	Hot spot	150	90	3	5	8	0	0	57	8	9	0.149
			Internal control	405	265	17	26	28	4	0	123	54	13	0.322
			External control	144	63	2	7	21	0	0	25	0	1	0.114
Hot spot			134	92	7	14	8	2	0	38	18	5	0.376	
Internal control			266	119	8	9	41	5	0	28	16	12	0.225	
External control			284	106	11	14	27	5	0	41	3	5	0.178	

N= Total blood-fed, T= tested, F= Failed to identify host, H= Human, B= Bovine, S= Sheep, C= Chicken, Ho= Horse, H/A= Human/Animal mix, A/A= Animal mix, IA = Index of anthropophily

ANNEX 3A: ENTOMOLOGICAL INOCULATION RATE (IB/H/N) OF VECTORS COLLECTED BY HLC ACCORDING TO THE LOCALITY AND THE TIME IN IRS DISTRICTS (AUGUST2016-DECEMBER 2016)

Species	IRS Districts	Locality	Indoor			Outdoor		
			AR (b/h/n)	CSPI	EIR (ib/h/n)	AR (b/h/n)	CSPI	EIR (ib/h/n)
<i>An. gambiae</i> s.l	Koungheul	Hot spot	0.63	0	0	0.66	0	0
		Internal control	0.21	0	0	0.17	0	0
		External control	0.79	0	0	0.58	0	0
	Koumpentoum	Hot spot	0.20	0	0	0.17	0	0
		Internal control	0.09	0	0	0.03	0	0
		External control	1.73	0.008	0.014	2.59	0.017	0.044
	Malem Hodar	Hot spot	0.20	0.1	0.021	0.26	0	0
		Internal control	0.36	0	0	0.37	0	0
		External control	0.63	0	0	0.75	0	0
	Nioro	Hot spot	0.56	0.028	0.016	0.82	0	0
		Internal control	1.32	0.027	0.036	1.38	0	0
		External control	1.20	0.026	0.031	1.27	0	0
<i>An. funestus</i>	Nioro	Hot spot	3.28	0	0	3.72	0	0
		Internal control	1.62	0	0	1.71	0	0
		External control	6.30	0.002	0.013	5.41	0	0

ANNEX4 : NUMBER OF MOSQUITOES AND ALLELIC FREQUENCY OF THE MUTATIONS L1014F (KDR-W) AND L1014S (KDR-E) IN *AN. GAMBIAE* S.L ACCORDING TO THE DISTRICTS (AUGUST -DECEMBER 2017)

Districts	N	SS	Genotypes									
			KDR-w			Allelic frequency (%)		KDR_e			Allelic frequency (%)	
			RwRw	RwRe	RwS	S	Rw	ReRe	ReRw	ReS	S	Re
Sprayed (center)												
Koungheul	44	-	1	25	1	20.45	31.81	-	25	17	20.45	47.73
Koumpentoum	58	25	2	6	21	62.93	26.72	2	6	2	62.93	10.34
Malèm Hodar	59	19	-	8	17	56.78	21.19	3	8	12	56.78	22.03
Nioro	57	23	-	5	26	65.79	31.58	-	5	3	65.79	2.63
Unsprayed												
Ndoffane	51	7	1	27	15	28.43	43.14	1	27	-	28.43	28.43
Kaffrine	52	16	1	12	17	50	29.81	3	12	3	50	20.19
Tambacounda	52	20	2	2	21	65.38	25.96	-	2	7	65.38	8.65
Wassadou	56	34	5	1	12	75.45	20.91	-	1	3	75.45	3.64
Kédougou	55	1	51	-	3	4.55	95.45	-	-	-	4.55	0
Vélingara	49	15	6	5	22	54.08	39.80	-	5	1	54.08	6.12
Niayes	60	1	2	33	15	15.83	43.33	7	33	2	15.83	40.83
Dakar	41	-	19	11	2	2.44	62.20	9	11	-	2.44	35.37
Pikine	45	1	12	2	-	6.67	28.89	26	2	4	6.67	64.44
Rufisque	51	-	5	17	-	0	26.47	29	17	-	0	73.53
Richard-Toll	42	2	2	17	-	26.92	26.92	-	17	21	26.92	46.15
Podor	212	64	1	-	147	64.86	35.14	-	-	-	-	-
Matam	294	122	7	-	165	69.56	30.44	-	-	-	-	-
Kanel	48	22	-	-	26	72.92	27.08	-	-	-	-	-
Bakel	69	27	-	-	42	69.57	30.43	-	-	-	-	-
Ranéroù	85	70	-	-	15	91.18	8.82	-	-	-	-	-
Linguère	69	50	-	-	19	86.23	13.77	-	-	-	-	-

