PMI | Africa IRS (AIRS) Project

Indoor Residual Spraying (IRS 2) Task Order Six

MALI: ENTOMOLOGICAL MONITORING OF 2016 IRS ACTIVITIES

FINAL REPORT

MARCH 2017

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AIRS MALI
ANNUAL ENTOMOLOGICAL MONITORING REPORT

JANUARY 2016 – DECEMBER 2016

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ACRONYMS

AIRS	Africa Indoor Residual Spraying
CDC	Centers for Disease Control and Prevention
DDT	Dichlorodiphenyltrichloroethane
EIR	Entomological Inoculation Rate
ELISA	Enzyme-Linked Immunosorbent Assay
HLC	Human Landing Catch
IR	Insecticide Resistance
IRS	Indoor Residual Spraying
LLINS	Long lasting Insecticidal Nets
KDR	Knock Down Rate
LLIN	Long Lasting Insecticide-Treated Net
MFO	Mixed Function Oxidase
NMCP	National Malaria Control Program
PBO	Piperonyl Butoxide
PCR	Polymerase Chain Reaction
PMI	United States President's Malaria Initiative
PSC	Pyrethrum Spray Catch
SI	Sporozoite Index
SMC	Seasonal Malaria Chemoprevention
TO	Task Order
USAID	United States Agency for International Development
WHO	World Health Organization

EXECUTIVE SUMMARY

The residual efficacy of pirimiphos-methyl (Actellic 300 CS) sprayed on walls in Koulikoro, Fana and Barouéli was two months for mud, cement and painted cement and three months for mud and Kaolin (according to World Health Organization (WHO) criteria of > 80% Mortality). This is similar to the residual duration of 3 months which was reported during the 2015 spray campaign using the same formulation. Despite the relatively short residual duration, mortality remained >60% for 5 months in 2016.

WHO tube tests revealed full susceptibility to pirimiphos-methyl (0.25%) in all surveyed sites except Niono (a non-IRS site) where further testing is needed to confirm the result. Possible resistance to bendiocarb (0.1%) was recorded in Bougouni and Niono (non-IRS sites), with full susceptibility noted in all others sites. Resistance to DDT (4%), permethrin (0.75%) and deltamethrin (0.05%) was widespread throughout southern and central Mali. The intensity of resistance was generally higher to permethrin than deltamethrin. The intensity of resistance to these two pyrethroids is strong as there were survivors in all sites to 10X the diagnostic dosage. The partial implication of MFOs in resistance to pyrethroids was also observed in synergist assays.

Morphological identification of *Anopheles* vectors collected from human landing catch, pyrethrum spray catch and CDC light trap indicated that *An. gambiae* s.l. was the predominant vector (>96%) followed by *An. pharoensis, An. rufipes, and An. ziemanni*. The molecular data have revealed *An. coluzzii* as the most common species followed by *An. gambiae*, *gambiae/coluzzii* (hybrids) and *An. arabiensis*. However, quality assurance tested at CREC, Benin indicated 20% discordance in species identification. Although indoor resting densities were lower in IRS sites compared to control sites throughout the monitoring period, a peak of 10 *An. gambiae* s.l./house/day was observed in Koulikoro (IRS site 1) in September (2 months after spraying). In Bla, a former IRS site, vectors densities were high over the 5 month monitoring period with a peak of 34 *An. gambiae* s.l./house/day in August.

The human biting rates were also generally lower in IRS sites compared to control sites with peaks obtained in August and September both indoors and outdoors. Cumulative post-IRS data shows a human rate of 6.1 bites/human/night in IRS sites compared with11.9 b/h/n in control sites. The sporozoite index was not significantly lower in IRS site (2.1%, CI: 0.8-3.4) than in control sites (2.9%, CI: 1.5-4.3). The entomological inoculation rate (EIR) was lower in IRS sites (7.03 infected bites/human/5 months) than control sites (45.33 ib/human/5 months), equating to an 84.5% reduction. Given the lack of sufficient baseline data (only 1 month due to the short vector season) before IRS implementation, we cannot be certain that any differences in entomological indices between control and IRS areas were due to IRS. As the EIR is relatively high in IRS areas, additional control measures should be strengthened.

I. INTRODUCTION

In 2008, the PMI AIRS program conducted IRS with a pyrethroid insecticide in Bla and Koulikoro districts, with Barouéli District added in 2011. By 2012, pyrethroid resistance was detected and led to a switch to a carbamate insecticide in all IRS districts. In 2014, resistance to carbamates was detected in some regions and the program switched to an organophosphate insecticide. In 2016, PMI supported IRS in two districts, Barouéli and Koulikoro, and participated in the UNITAID-funded NGenIRS

Project, which supported spraying an additional district of Fana using an organophosphate product, Actellic 300CS. IRS occurred at the start of the seasonal rains in July, being timed to provide maximum impact during the peak transmission period of the year.

The 2016 campaign started on July 9, with 228,672 structures sprayed and 788,922 persons protected in the three districts. To evaluate the quality and the impact of IRS, entomological monitoring was conducted in seven sites (3 sprayed and 3 paired unsprayed control areas as well as the former IRS site of Bla).

1.1 SPECIFIC AIMS

To determine:

- Vector species composition
- Vector indoor resting densities
- Vector biting rates, times and location (indoor or outdoor)
- Sporozoite index and Entomological Inoculation Rate (EIR)
- Vector parity rates post IRS
- Vector blood meal origin
- Comparability of CDC light trap vs human landing catch
- Quality of spraying and insecticide decay rate

Susceptibility levels of *An. gambiae* s.l. to the four classes of insecticides in the 14 selected sentinel sites located in central and southern Mali, resistance intensity and frequency of resistance mechanisms.

All of these data will enable the National Malaria Control Program (NMCP) and the various partners involved in vector control in Mali to make decisions on the type of strategies (type of nets to be distributed to populations, type of insecticide to be used for IRS) to be implemented.

2. METHODOLOGY

2.1 STUDY AREA

2.1.1 IRS ENTOMOLOGICAL SURVEILLANCE SITES

AIRS Mali collected data on key entomological indicators from seven surveillance sites located in the regions of Koulikoro and Ségou (Table 1).The region of Koulikoro covers 90,120 km², had 2.4 million inhabitants in 2009 and is irrigated by several rivers. Agricultural production includes dry cereals, rice and cash crops (peanut, sesame, cotton, sorrel).

The region of Ségou (central Mali) covers 64,947km², had 2.3 million inhabitants in 2009, and has a semi-arid climate with an average annual rainfall of 513 mm. The presence of several rivers allows irrigated crops including cotton, millet, sorghum, maize, rice, fonio, wheat, peanut, dry cereals, and livestock farming.

Three of the seven entomology monitoring sites were located in the IRS targeted districts; the other three were located in adjacent unsprayed areas and served as control areas, with an additional site that is a former IRS site (Bla) (Figure 1).

• Koulikoro (IRS site 1) was compared to Kati (control site 1)

• Fana (IRS site 2) was compared to Dioila (control site 2)

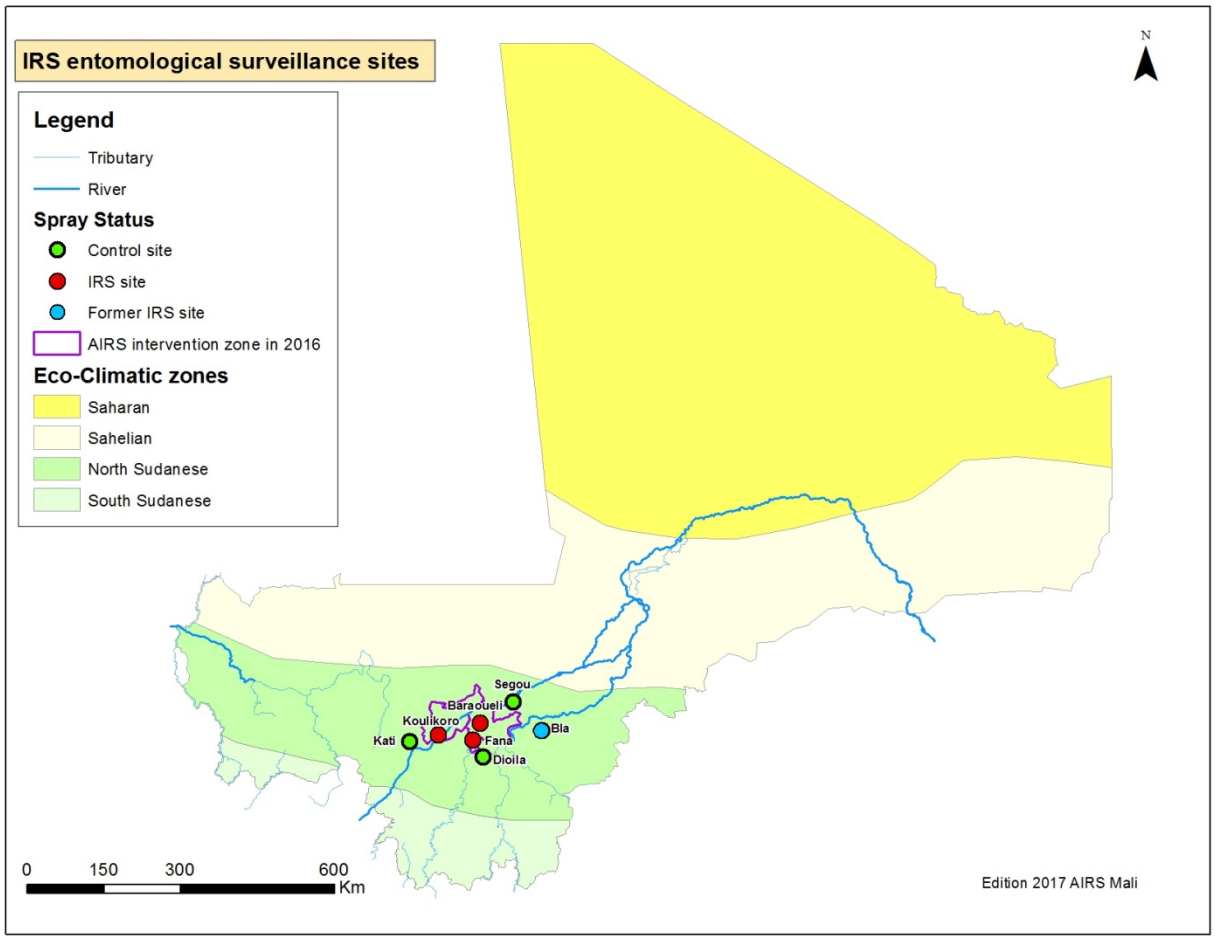
• Barouéli (IRS site 3) was compared to Segou (control site 3)

Data were collected in Bla (Former IRS site) to assess the impact of stopping IRS. The last year of IRS in Bla was 2014.

TABLE I: IRS ENTOMOLOGICAL SURVEILLANCE SITES

Region	District	Health Area	Site (village)	Spray Status	Geographic Zone
Koulikoro	Kati	N'gabakoro-droit	Sala	Non-sprayed (control)	Northern Sudanese
	Koulikoro	Tienfala	Tienfala	Sprayed	
	Fana	Fana central	Guana	Sprayed	
	Dioila	Dioila central	Kola	Non-sprayed (control)	
Segou	Baroueli	Tigui	Diaka Were	Sprayed	
	Segou	Zambougou	Kegnebougou	Non-sprayed	
	Bla	Touna	Djina	Former IRS site	

FIGURE 1: ECOCLIMATIC MAP OF MALI SHOWING THE IRS ENTOMOLOGICAL SURVEILLANCE SITES.



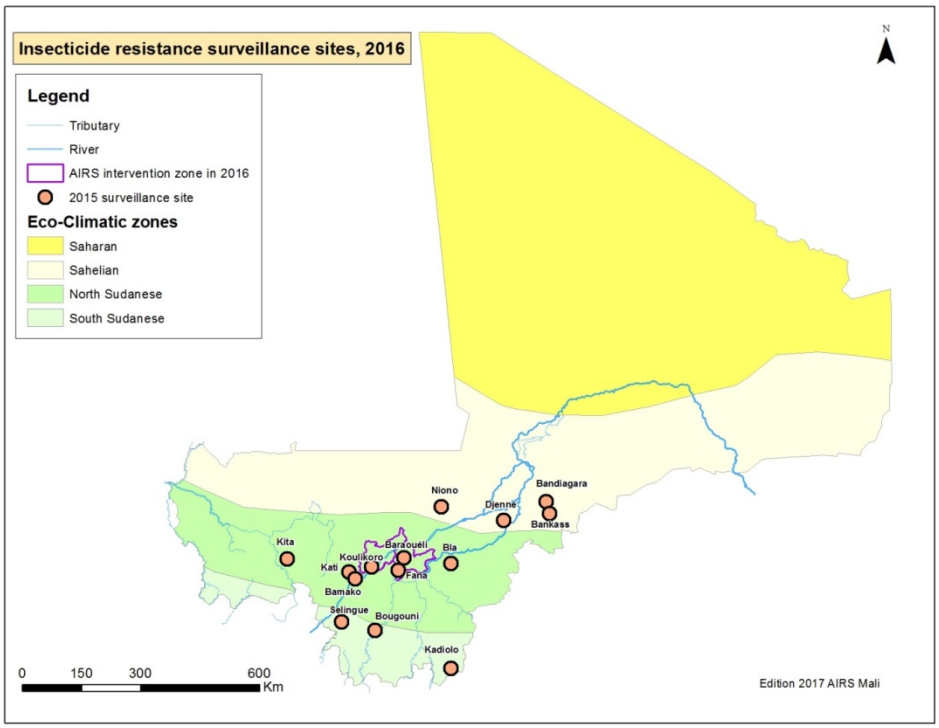
2.1.2 NATIONWIDE SITES FOR INSECTICIDE RESISTANCE MAPPING

Insecticide resistance testing was conducted in 14 monitoring sites, the majority of which have been used for several years. These 14 sites include the 3 IRS target sites mentioned above and 11 other sentinel sites selected for various reasons (Table 2). This was the fourth national-level insecticide resistance survey, with previous monitoring implemented in 2012, 2014 and 2015. By using the same sites, mosquito resistance trends can be monitored over time. Geographical locations of each of the14 sites are shown in Figure 2.

TABLE 2: SURVEILLANCE SITES USED FOR INSECTICIDE RESISTANCE MAPPING

Region	District	Village	Reason for Selection	Geographic Zone
Kayes	Kita	Fourgna Berda/ Banfara	Intense use of insecticides for agriculture	Northern Sudanese
Koulikoro	Koulikoro	Tienfala	IRS campaign area	
	Fana	Gouana	IRS campaign area	Northern Sudanese
	Kati	Baguineda	Areas where LLINs were distributed in 2012 and 2014 and are used. Significant use of irrigation. Use of insecticides to control simulium	Northern Sudanese
Segou	Niono	Sokourani/ Toumakoro	Significant use of irrigation. LLIN universal coverage in 2015	Sahelian Flooded
	Bla	Tia, Touna	LLIN universal coverage in 2015	Northern Sudanese
	Baroueli	Bouadie/Tigui	IRS campaign area. LLINs universal coverage in 2015	
Sikasso	Bougouni	Massabla/Dalabani	Intense use of insecticides for agriculture LLIN universal coverage in 2015	South Sudanese
	Yanfoila	Selingue	Significant use of irrigation. LLIN universal coverage in 2015	South Sudanese Sahelian Flooded
	Kadiolo	Kadiolo	Intense use of insecticides for agriculture LLIN universal coverage in 2015	South Sudanese
Mopti	Badiangara	Badiangara	Non-use of insecticides, traditional agriculture (limited use of herbicide) LLIN universal coverage in 2014	Sahelian
	Bankass	Bankass		Sahelian Flooded
	Djenne	Gomitogo/Djenne central/ Wono/ Edugu Were		
District de Bamako	Commune IV	Dicoroni Para	Areas where LLINs have been distributed and are used. LLINs universal coverage in 2015	Northern Sudanese Suburban

FIGURE 2: ECOCLIMATIC MAP OF MALI SHOWING THE INSECTICIDE RESISTANCE SURVEILLANCE SITES



2.2 HUMAN LANDING CATCH AND CDC LIGHT TRAP

Human landing catches (HLC) and CDC light traps (CDC-LT) were set up from 06:00 pm to 06:00 am for two consecutive nights each month per site. These two collection methods were carried out before (early July) and after (August to December) IRS in four different houses (two for HLC and two for CDC light traps) randomly selected at each site to evaluate the biting location (Indoor/Outdoor), biting time and changes in biting behavior of *Anopheles*.

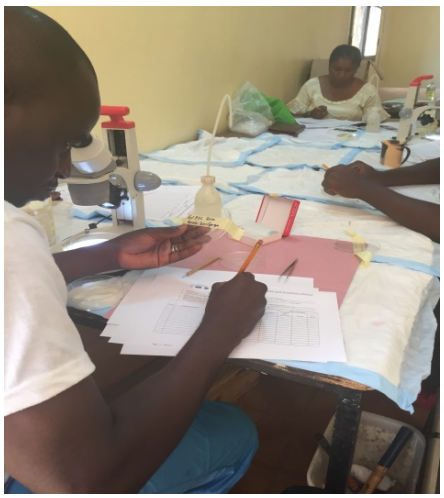
Human landing catches were conducted according to the conventional methodology, with a person sitting with exposed legs and feet collecting any landing mosquitoes using an aspirator and a flashlight (figure 3). One person sat inside and a second person sat outside each of the two selected houses for the mosquito collections. These collections allowed us to determine the level of *P. falciparum* transmission risk in each site by the calculation of the frequency of infected bites received by a human.

One CDC light trap installed about 1.5 m above the ground inside and outside each of two different houses was also used for sampling mosquitoes. Inside houses, the trap was placed next to an untreated mosquito net with a sleeper (Figure 4). Outside, an awake collector sat next to the trap as bait. Dry ice was not used due to lack of availability. For HLC and CDC light traps, the collectors were randomly rotated between the houses during the collection nights to adjust the variations in the attractiveness of mosquitoes. These two sampling methods were used in order to see if they had comparable performances. Volunteers involved in the collection and supervision of the work were provided with malaria prophylaxis. The captured mosquitoes were identified the following morning as *Anopheles* or culicine based on the Gillies & de Meillon (1968) determination key. The *Anopheles* vectors were dissected to determine the parity rate and then stored in 70% ethanol (Figure 5). The heads and thorax of these *Anopheles* specimens were used for sporozoite screening by the ELISA CSP method (Beier, 2002). The abdomens were used for molecular characterization (PCR for species identification of *An. gambiae* s.l. complex).

FIGURE 3: INDOOR LANDING CATCH, KATI

FIGURE 4: INDOOR CDC LIGHT TRAP, FANA

FIGURE 5: MOSQUITOES WERE IDENTIFIED, OVARY DISSECTIONS CONDUCTED AND STORAGE OF LABELLED SAMPLES PREPARED IN THE FIELD LAB (SIMPLE ROOM), KOULIKORO DISTRICT.



2.3 PYRETHRUM SPRAY CATCH (PSC)

Each month, PSC was carried out in 20 houses in each site in order to sample indoor resting mosquitoes. Ten houses per day were visited during two consecutive days from 7:00 a.m. to 12:00 a.m. (collections could not be conducted earlier as people are asleep). Before the start of PSC, all the occupants, animals and food were removed from the houses. All the openings present in each house were blocked with fabric. White canvas was then spread out to cover the entire floor and the remaining furniture. Thereafter, a collector first sprayed from outside the eaves if there are any and after, the inside of the houses with Spritex aerosol containing tetramethrin 0.3% and permethrin 0.03%. After spraying, the door remained closed for 10-15 minutes. The collectors gently removed the canvas from the houses and then collected mosquitoes that were knocked-down (Figure 6). Mosquitoes were put in carefully labeled petri dishes (Figure 7). They were transported to the field lab for morphological identification as *Anopheles* and culicine and separated by sex. Abdominal status (unfed, fed, half gravid and gravid) of all female *Anopheles* was determined. A subset of samples of *Anopheles gambiae* s.l. were preserved in 1.5 ml labeled Eppendorf tubes containing 70% ethanol for further processing in molecular lab.

FIGURE 6: MOSQUITOES COLLECTION BY PSC, BLA KATI

FIGURE 7: MOSQUITOES CAUGHT BY PSC,

2.4 Quality of spray insecticide and decay rate

To assess the longevity of pirimiphos methyl on the walls, cone bioassays were carried out with mosquitoes of the susceptible *An. gambiae* Kisumu strain in 3 districts: Koulikoro (10 houses), Barouéli (6 houses) and Fana (3 houses) according to the World Health Organization (WHO, 2006) protocol. During the evaluation, the walls encountered were made of cement, painted cement, mud (most common wall materials) or mud and kaolin. During bioassays, 10-15 non blood-fed females Anopheles aged 2 to 5 days were introduced into each of 3 cones fixed at three different heights (0.5 m, 1 m and 1.5 m) on the sprayed wall (Figure 8). Mosquitoes were exposed for 30 minutes and then transferred into different labeled cups and provided cotton swabs soaked in a10% sugar solution.

The knock-down was recorded after 30 minutes of exposure and mortality after 24 hours. Mortality rates were corrected with the Abbott's formula when the control mortality was between 5 and 20%.

FIGURE 8: CONE TESTS ON A CEMENT WALL, KOULIKORO DISTRICT.

2.5 MOSQUITO LARVAE COLLECTIONS AND REARING

From August to December 2016, the 14 monitoring sites selected for insecticide resistance mapping were surveyed by the AIRS Mali team for larval collection. Larvae and pupae of An. gambiae were collected from breeding sites using dippers (Figure 9). Larvae and pupae were sorted by genus and brought back to the field rearing room until the adult stage (Figure 10). There were not enough larvae to test all insecticides in Niono district.

FIGURE 9: MOSQUITO LARVAE COLLECTION

FIGURE 10: FIELD INSECTARY

2.6 WHO SUSCEPTIBILITY TUBE TESTS

Four batches of 20-25 non blood-fed females *Anopheles*, aged 2 to 5 days were used for the susceptibility tube tests (Figure 11) according to the World Health Organization protocol (WHO, 2013). These tests were performed with deltamethrin (0.05%), permethrin (0.75%), DDT (4%), bendiocarb (0.1%) and pirimiphos methyl (0.25%). Knock-down was recorded in time intervals (10, 15, 20, 30, 40, 50, 60 and 80 minutes). After 60 minutes of exposure, the mosquitoes were transferred into the observation tubes and fed with a 10% sugar solution. Mortality was recorded at 24 hours. A subsample of alive and dead mosquitoes were preserved in 1.5 ml eppendorf tubes containing silica gel for molecular analyses (PCR species, *kdr* 1014F, *kdr* 1014S and *Ace 1R*) in collaboration with the Laboratory of Applied Molecular Biology (LBMA) of the University of Bamako. The screening of the *Ace1R* mutation was performed only on mosquitoes that survived exposure to bendiocarb (carbamate) or pirimiphos-methyl (organophosphate).

FIGURE 11: WHO SUSCEPTIBILITY TESTS, FANA DISTRICT

2.7 PYRETHROID RESISTANCE INTENSITY ASSAY AND RESISTANCE ENZYME OXIDASES

The resistance intensity to pyrethroid insecticides was assessed in each of the 14 sites covered by the resistance mapping study. For this purpose, four pre-measured vials containing concentrated permethrin or deltamethrin were rinsed 4 times with 500 µl of acetone and then transferred to a flask to which acetone was added so as to have 50 ml of permethrin or Deltamethrin 1X, 2X, 5X and 10X (respectively 21.5 µg/ml, 43 µg/ml, 107.5 µg/ml and 215 µg/ml), the diagnostic dose being provided by CDC, Atlanta. The insecticide solutions were then stored at 4 °C in the dark.

The CDC bottle bioassays were then carried out with 250 ml Wheaton bottles coated with 1 ml of the diluted insecticide solution. A bottle coated with acetone was used as a control. A mouth aspirator was

used to introduce 20 to 26 females aged 3 to 5 days into 250 ml Wheaton bottles coated with insecticide (Figure 12). The number of dead or alive mosquitoes was recorded by time interval (15, 30, 35, 40, 45, 60, 75, 90, 105 and 120 min). The mortality rate at diagnostic time of 30 minutes was determined.

The implication of mixed function oxidases (MFOs: metabolic enzyme) in the resistance of the populations of *An. gambiae* s.l. to pyrethroids was determined by pre-exposure to Piperonyl Butoxide (PBO) 100 µg / ml of four batches of 20 to 26 non-blood fed females *Anopheles* aged 3 to 5 days for one hour. After that, mosquitoes were exposed to permethrin 1X or deltamethrin 1X. A control exposing mosquitoes to a bottle coated with acetone was also performed. The mortality rates recorded at the diagnostic time of 30 minutes for the tests with and without PBO were compared for each site. The room temperature and humidity were recorded during each test.

FIGURE 12: PYRETHROID RESISTANCE INTENSITY ASSAY WITH CDC BOTTLE TEST, BOUGOUNI DISTRICT

2.8 MOLECULAR CHARACTERIZATION OF *AN. GAMBIAE*; PCR SPECIES, *KDR* 1014F / 1014S AND *ACE1R*

A subsample of female *Anopheles* was sent to LBMA for vector molecular characterization and to CREC, Benin for quality assurance. For quality assurance, in each insecticide resistance monitoring site, legs of the twenty first mosquitoes of the 50 tested at LBMA for species identification, molecular forms of *An gambiae* s.l. and *kdr* 1014F/1014S were tested at CREC for the same analyzes. This means that for one mosquito, two results (one from LBMA and one from CREC) are available. The same PCR protocols were used by both labs (CREC and LBMA). Thus, females *Anopheles* were analyzed by PCR according to the protocol described by Santolamazza *et al.*, 2008. This method allows both identification of *Anopheles* species and molecular form discrimination. The L1014F and L1014S *kdr* mutations were respectively identified according to the protocol of Martinez-Torres *et al.* (1998) and Ranson *et al.* (2000). The detection of the *Ace 1R* mutation was done performed on the protocol described by Weill *et al.* (2004).

2.9 DATA ANALYSIS

For PSC, the mean density of *An. gambiae* s.l. was determined by the following way:

- Total number of vectors collected by species / Total number of houses surveyed.

The human biting rate (HBR) was determined from HLC using the formula:

- Total number of vectors collected / Total number of human nights.

The parity rate of the collected specimens of *An. gambiae* s.l. was calculated using the formula: (Total number of parous vectors/total number of vectors dissected) x100.

The sporozoite index was calculated as follows: (Total number of infected vectors / Total number of vectors tested with ELISA) x100.

For the susceptibility tests, the resistance status of each site was determined on the basis of the WHO, 2013 criteria. For this, when the mortality rate is below 90%, the mosquito population is said to be resistant. When the mortality rate is between 90% and 97%, there is a suspicion of resistance. Beyond 98%, the mosquito population is considered as susceptible.

Z-test for difference in proportions was used to compare sporozoite index, parous and mortality rate between two populations.

3. RESULTS

3.1 VECTOR SPECIES COMPOSITION

Six different *Anopheles* species were encountered; namely *An. gambiae* s.l., *An. pharoensis*, *An. rufipes*, *An. funestus* s.l., *An. coustani* and *An. ziemanni*. The most predominant was *An. gambiae* s.l. which accounted for more than 96% of the collected mosquitoes irrespective of the sampling method (Table 3). After combining data of the three collection methods (HLC, PSC and CDC-LT), only 84 *An. pharoensis*, 10 *An. rufipes*, 3 *An. funestus*, 2 *An. coustani* and 1 *An. ziemanni* were captured.

Molecular species identification results are presented in figures 13-17. From the three adult mosquito sampling methods, a maximum of 30 specimens of *An. gambiae s.l* from each site were analyzed monthly by PCR for species identification. The results show that molecular forms, *An. coluzzii* and *An. gambiae* were found in sympatry in all the 7 surveyed sites. Overall, out of the 804 specimens of *An. gambiae* s.l. which were tested, 66.6% (n=536) were *An. coluzzii*, 16% (n=129) were *An. gambiae*, 10% (n=80) hybrids (*gambiae/coluzzii*), 0.4% (n=3) *An. arabiensis*, and 7.0% did not amplify (n=56) (Figure 13). More details are given in Annex A, Table A1.

The proportion related to one or the other species varies depending on the site (Figures 14, 15, 16 and 17). Overall, *An. coluzzii* was predominant in all sites (Figures 14,16,17) except for Fana and Dioila (Figure 15) where it was found in similar proportions with *An. gambiae* (p>0.05).

TABLE 3: VECTOR SPECIES COMPOSITION PER SAMPLING METHODS IN ALL SITES, JULY-DECEMBER 2016

Sampling methods	Species	Koulikoro (IRS site1)	Kati (Control site 1)	Fana (IRS site 2)	Dioila (control site 2)	Barouéli (IRS site3)	Ségou (control site 3)	Bla (former IRS)	Total
HLC	*An. gambiae s.l*	550 (97.2%)	970 (96.2%)	272 (100%)	492 (99.8%)	0	92 (97.9%)	105 (95.5%)	2481 (97.6%)
	An. pharoensis	16 (2.8%)	38 (3.8%)	0	1 (<1%)	0	2 (2.1%)	2 (1.8%)	59 (2.3%)
	An. rufipes	0	0	0	0	0	0	2 (1.8%)	2 (<1%)
	An. funestus	0	0	0	0	0	0	1 (<1%)	1 (<1%)

PSC	*An. gambiae s.l*	505 (100%)	1225 (100%)	250 (98.8%)	531 (100%)	110 (95.7%)	528 (97%)	1426 (95%)	4575 (97.9%)
	An. rufipes	0	0	2 (<1%)	0	5 (4.3%)	13 (2.4%)	71 (4.7%)	91 (1.9%)
	An. pharoensis	0	0	1 (<1%)	0	0	2 (<1%)	1 (<1%)	4 (<1%)
	An. funestus	0	0	0	0	0	1 (<1%)	1 (<1%)	2 (<1%)
	An. coustani	0	0	0	0	0	0	1 (<1%)	1 (<1%)
CDC Light trap	*An. gambiae s.l*	124 (97.6%)	397 (95.6%)	90 (97.8%)	88 (100%)	5 (100%)	71 (97.2%)	41 (95.3%)	816 (96.8%)
	An. pharoensis	2 (<1%)	18 (4.4%)	0	0	0	1 (1.4%)	0	21 (2.5%)
	An. rufipes	1 (<1%)	0	0	0	0	1 (1.4%)	2 (4.7%)	4 (<1%)
	An. ziemanni	0	0	1 (1.1%)	0	0	0	0	1 (<1%)
	An. coustani	0	0	1 (1.1%)	0	0	0	0	1 (<1%)

FIGURE13: *AN. GAMBIAE* S.L. SAMPLING SPECIES PCR, KOULIKORO, FANA, BAROUÉLI, KATI, DIOILA, SÉGOU AND BLA, JULY-DECEMBER 2016.

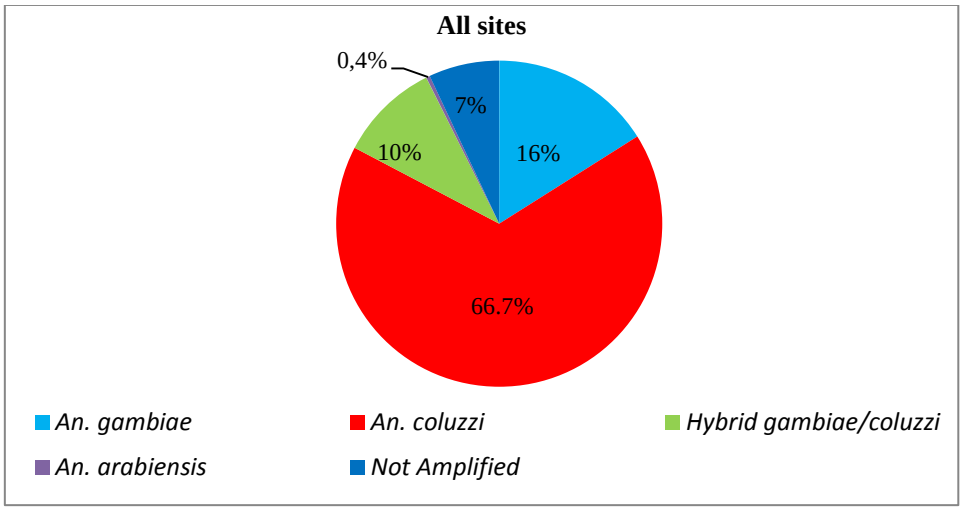


FIGURE 14: *AN. GAMBIAE* S.L. SAMPLING SPECIES PCR, KOULIKORO, KATI, JULY-DECEMBER 2016.

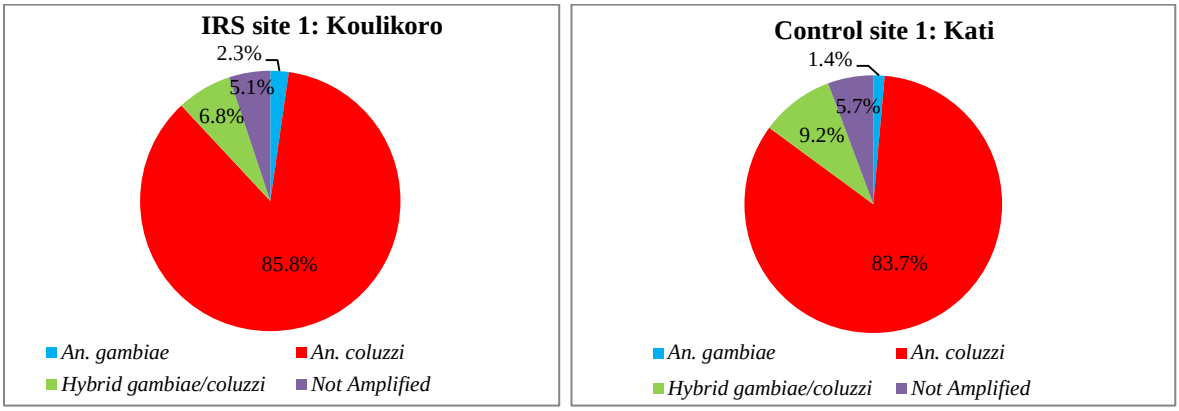


FIGURE 15: *AN. GAMBIAE* S.L. SAMPLING SPECIES PCR, FANA, DIOILA, JULY-DECEMBER 2016.

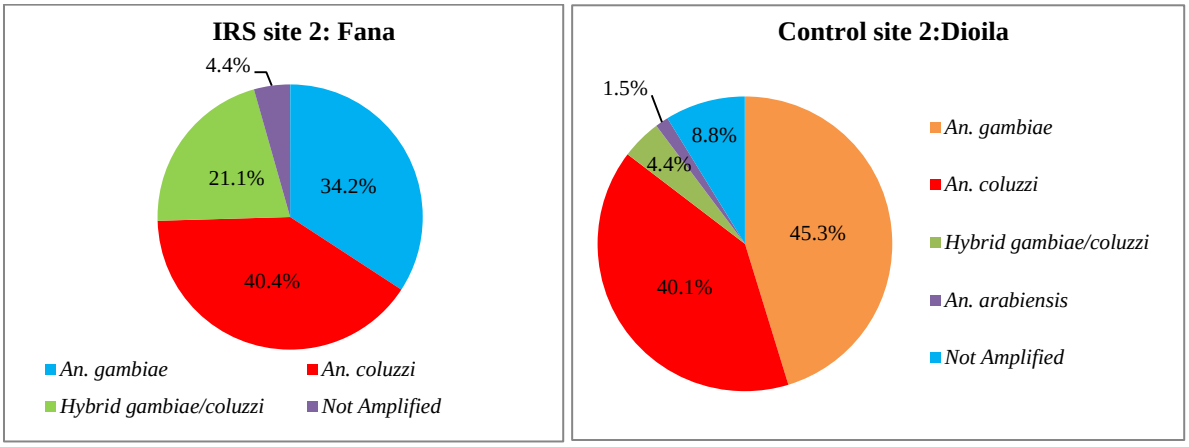


FIGURE 16: *AN. GAMBIAE* S.L. SAMPLING SPECIES PCR, BAROUÉLI, SÉGOU, JULY-DECEMBER 2016.

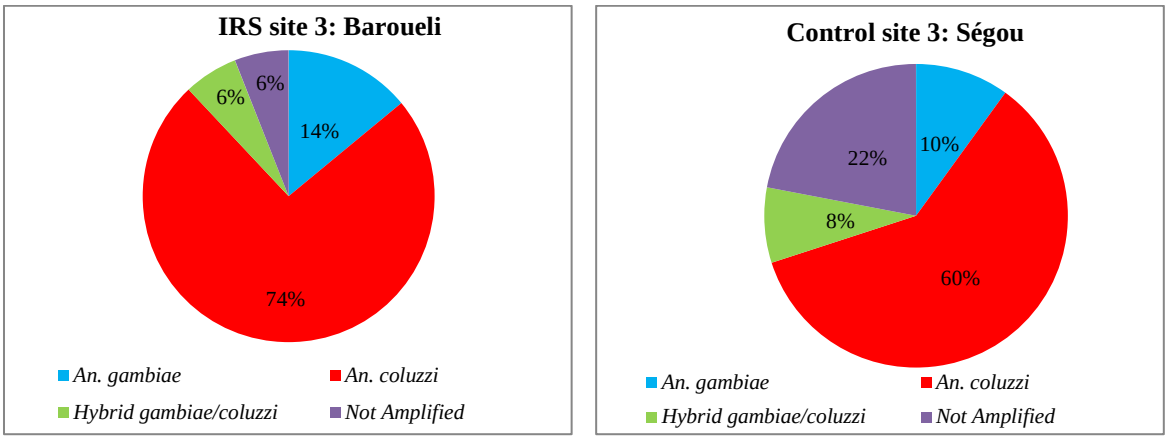
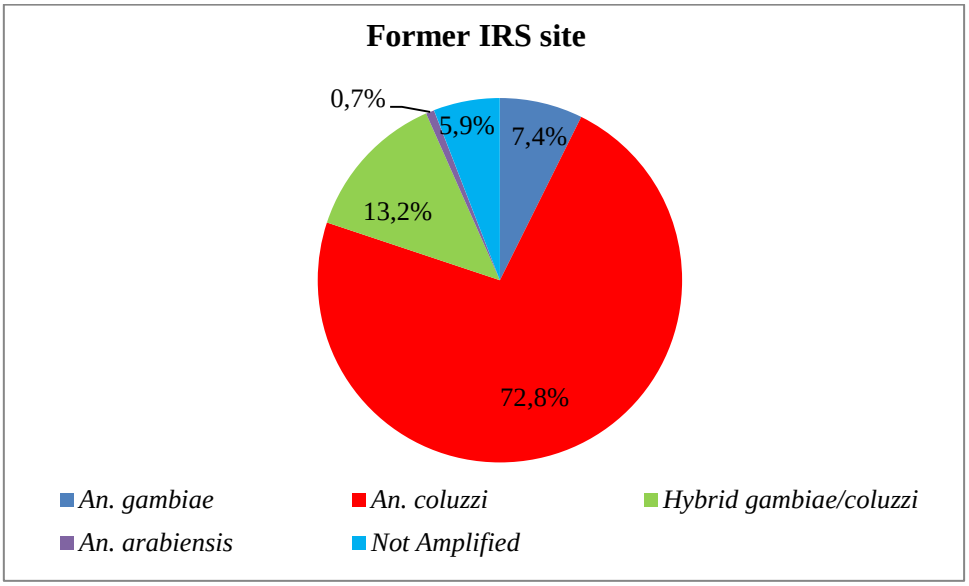


FIGURE 17: *AN. GAMBIAE* S.L. SAMPLING SPECIES PCR, BLA, JULY-DECEMBER 2016.



3.2 VECTOR INDOOR RESTING DENSITY

After IRS implementation (mid-July), the highest densities of indoor resting vectors were recorded in August and September with numbers reaching a peak of 32.8 *An. gambiae* s.l./house/day in Kati (control site 1), 7.9 *An. gambiae* s.l./house/day in Dioila (control site 2) as well in Ségou (control site 3) and 17.1 *An. gambiae* s.l./house/day in Bla (Former IRS site) (Figures 18-20). During the peak densities of control sites, the three IRS sites displayed lower densities of vectors with peaks of 11, 3 and 2 *An. gambiae* s.l./house/day respectively in Koulikoro (IRS site 1), Fana (IRS site 2) and Barouéli (IRS site 3). Furthermore, from July to October, the mean indoor resting density of *An. gambiae* s.l. is higher in the 3 control sites combined than in the 3 IRS sites combined (Figure 21). Overall the indoor resting densities were lower in IRS sites, but in Koulikoro (IRS site 1) up to 10 *An. gambiae* per house per day were collected just 2 months after spraying. More details are given in Annex B, Table B1.

FIGURE 18: MEAN INDOOR RESTING DENSITY (PSC) OF *AN. GAMBIAE* S.L. BY MONTH, KOULIKORO (IRS SITE 1) AND KATI (CONTROL SITE 1), JULY-DECEMBER 2016.

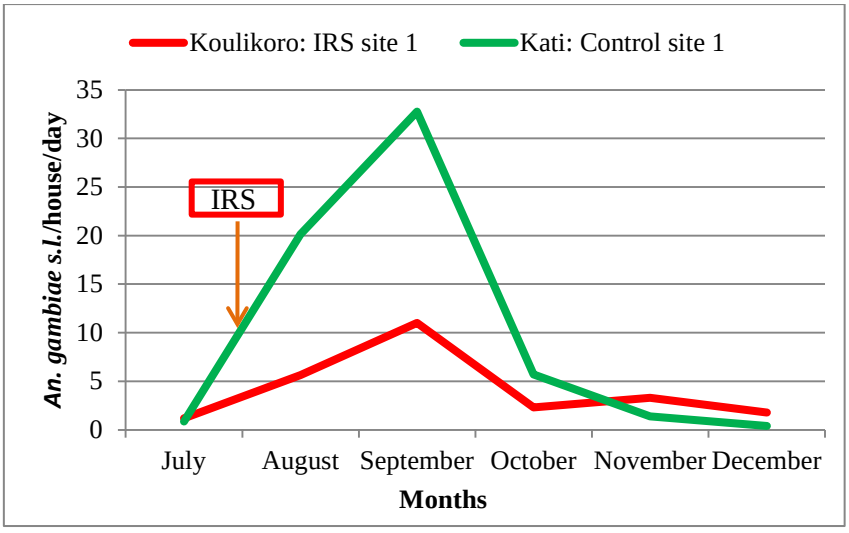


FIGURE 19: MEAN INDOOR RESTING DENSITY (PSC) OF *AN. GAMBIAE* S.L. BY MONTH, FANA (IRS SITE 2) AND DIOILA (CONTROL SITE 2), JULY-DECEMBER 2016

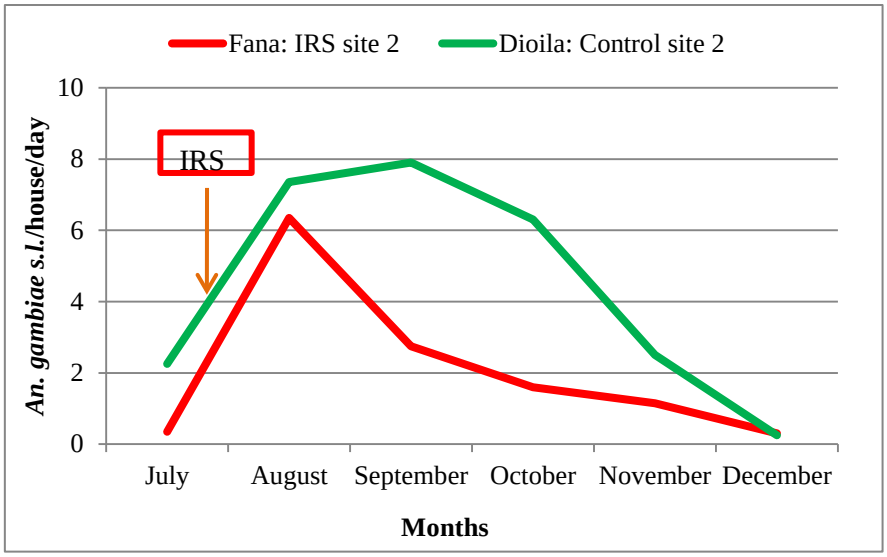


FIGURE 20: MEAN INDOOR RESTING DENSITY (PSC) OF *AN. GAMBIAE* S.L. BY MONTH; BAROUÉLI (IRS SITE 3), SÉGOU (CONTROL SITE 3) AND BLA (FORMER IRS SITE), JULY-DECEMBER 2016

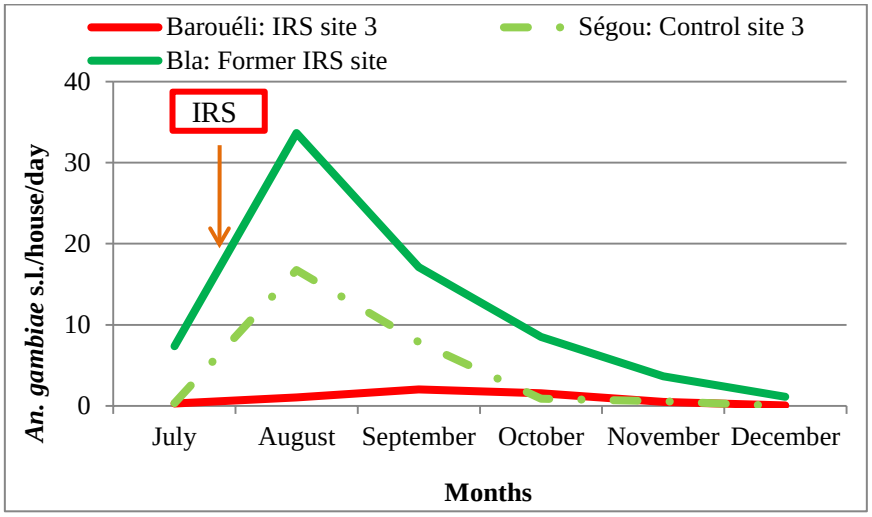
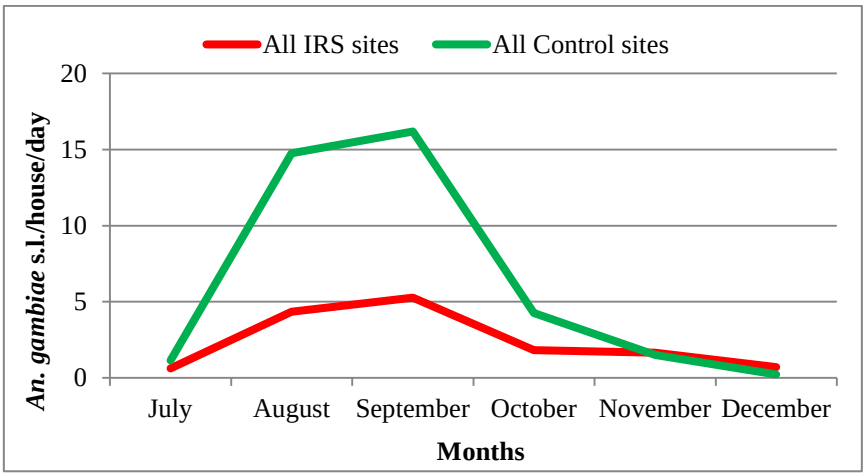


FIGURE 21: MEAN INDOOR RESTING DENSITY (PSC) OF *AN. GAMBIAE* S.L. BY MONTH, ALL IRS SITES (KOULIKORO, FANA AND BAROUÉLI), ALL CONTROL SITES (KATI, DIOILA AND SÉGOU), JULY-DECEMBER 2016.



3.3 VECTOR BITING RATES

Peak biting was in August and September regardless of spray status and then declined between October and December. In IRS sites, the peaks obtained were respectively 24 and 28 bites/human/night (b/h/n) (indoors and outdoors) in Koulikoro (IRS site 1) and 36 and 14b/h/n in Fana (IRS site 2) (Figures 22&23). No *An. gambiae* s.l. were found in Barouéli (IRS site 3) (Figures 24). The highest biting rates were recorded in control sites and in the former IRS site: 60 and 52 b/h/n in Kati (Control site 1), 34 and 35 b/h/n in Dioila (Control site 2), 12 and 8 b/h/n in Ségou (Control site 3) and 9 and 10 b/h/n in Bla (Former IRS site) (Figures 22-23 & 24).While the human biting rate was generally lower in sprayed areas the nightly human biting rates were still very high in August and September both indoors and outdoors (Figures 25). Table 4 summarizes the monthly human biting rates before and after spraying. More details are provided in annex C, Tables C1 and C2.

FIGURE 22: *AN. GAMBIAE* S.L. HUMAN BITING RATE, KOULIKORO AND KATI, JULY-DECEMBER 2016

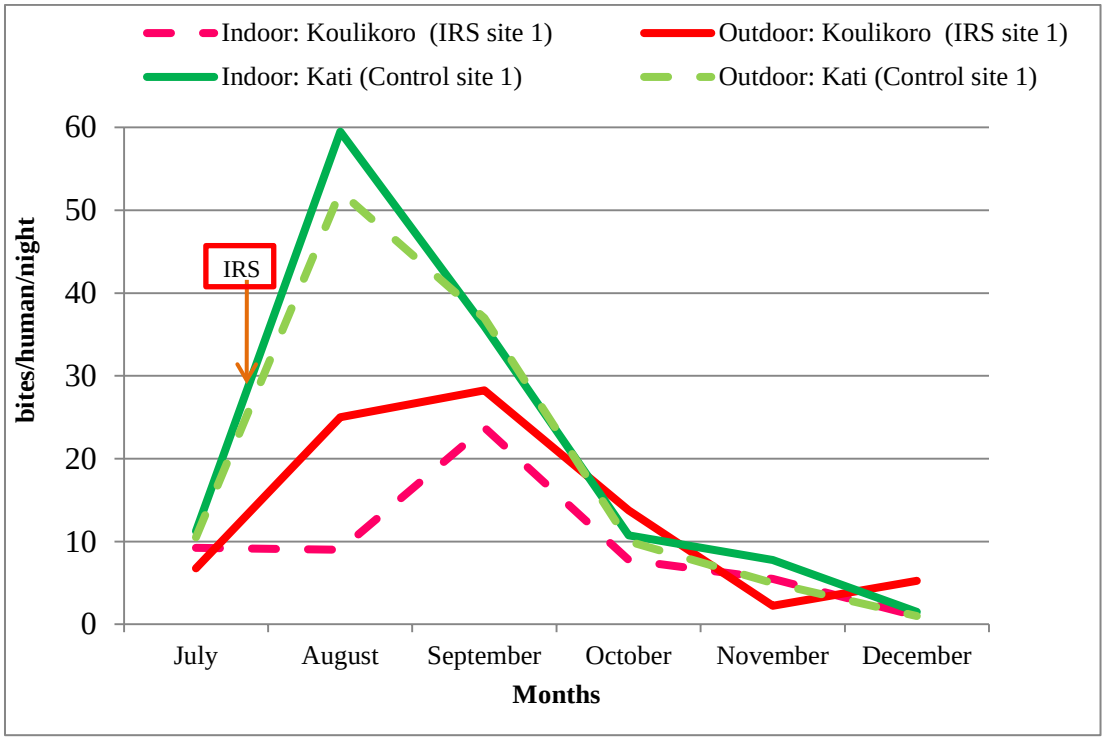


FIGURE 23:*AN. GAMBIAE* S.L. HUMAN BITING RATE, FANA AND DIOILA, JULY-DECEMBER 2016

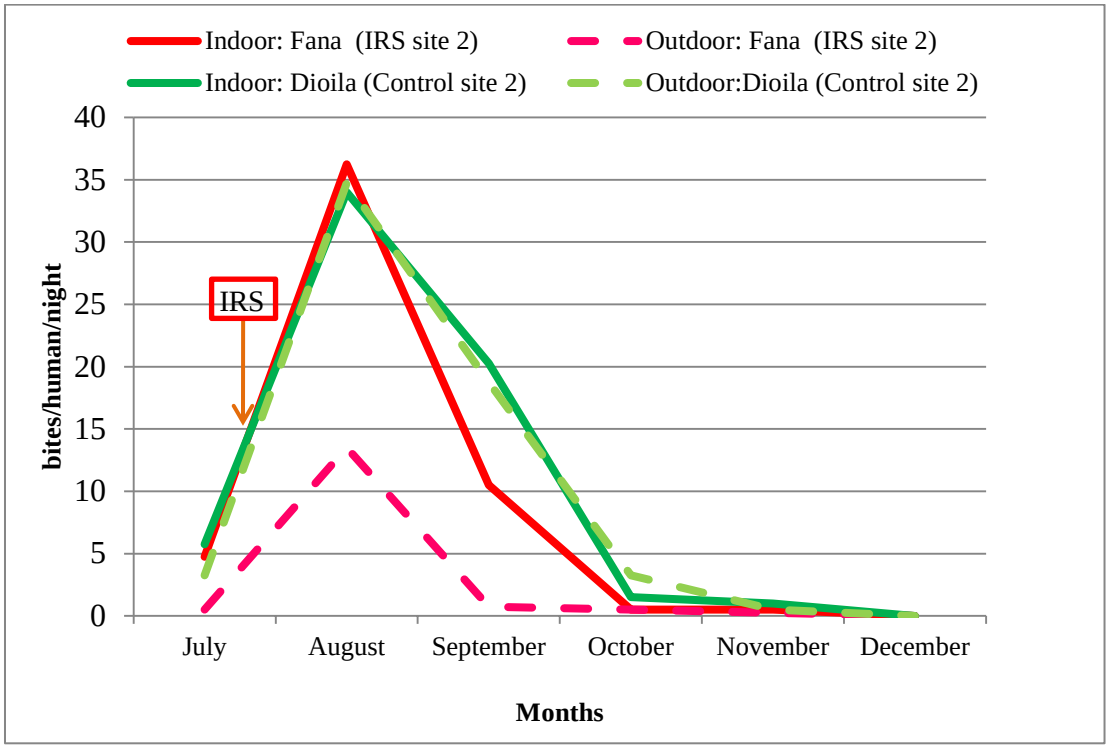


FIGURE 24: *AN. GAMBIAE* S.L. HUMAN BITING RATE; BAROUÉLI, SÉGOU AND BLA, JULY-DECEMBER 2016

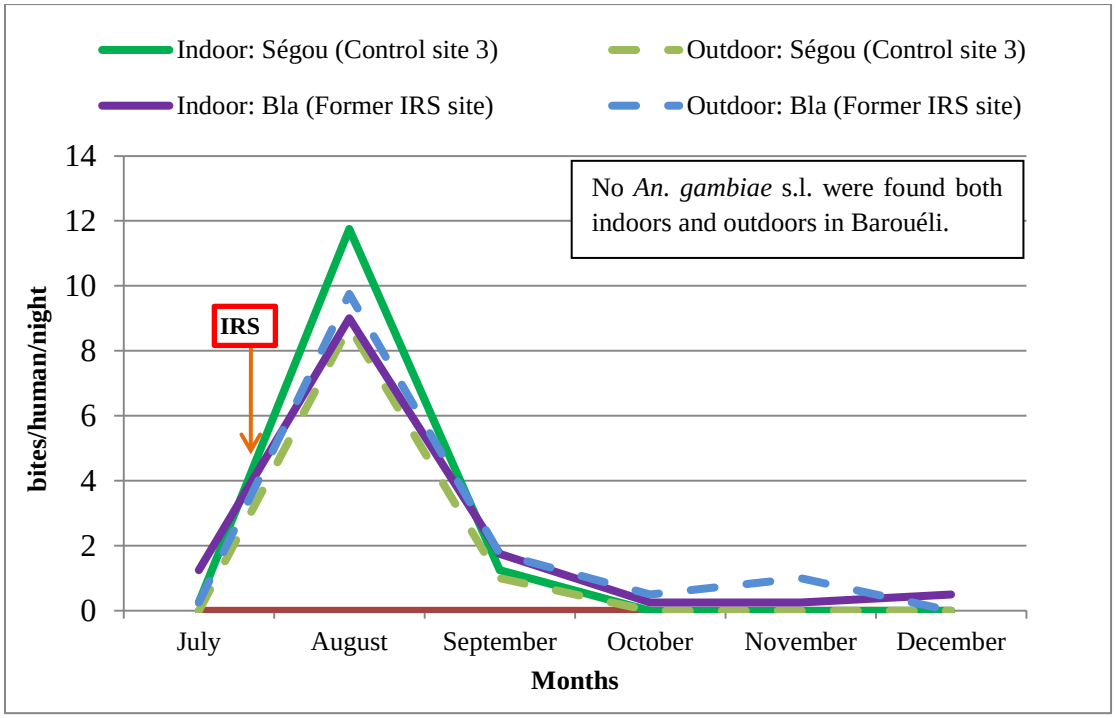


FIGURE 25: *AN. GAMBIAE* S.L. MEAN HUMAN BITING RATE FOR ALL IRS SITES (KOULIKORO, FANA AND BAROUÉLI), AND ALL CONTROL SITES (KATI, DIOILA AND SÉGOU), JULY-DECEMBER 2016.

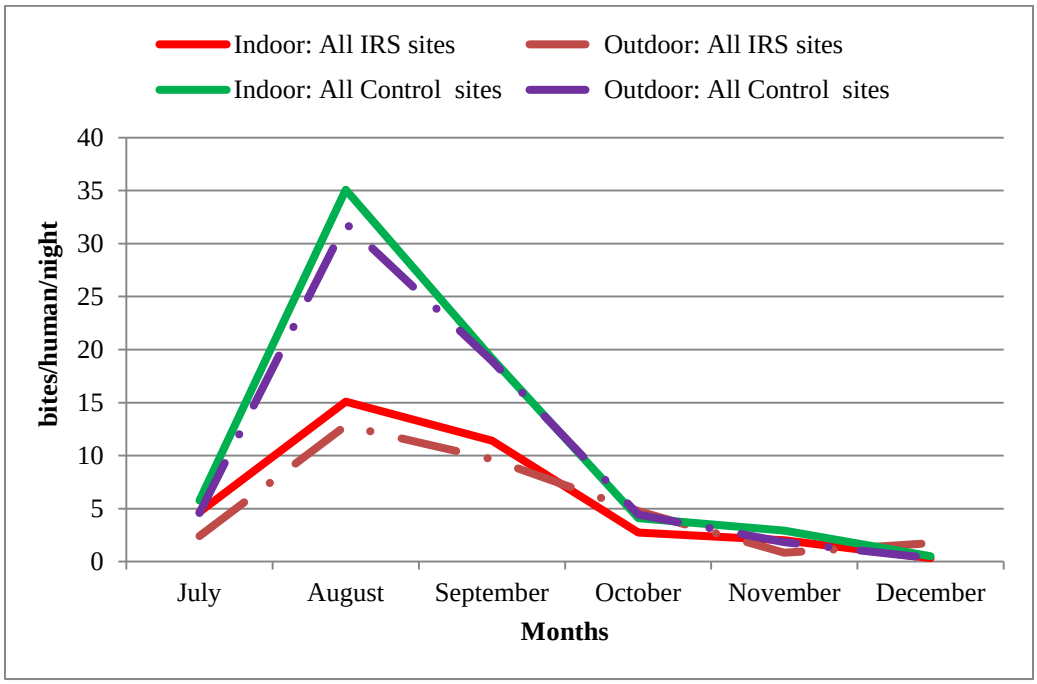


TABLE 4: MONTHLY HUMAN BITING RATES FOR ALL SITES BEFORE AND AFTER IRS.

	Pre IRS		Post IRS					
	HBR (b/h/n)		HBR (b/h/n)					
SITES	July		August	September	October	November	December	Mean
IRS site 1 : Koulikoro	8		17	26	10.8	3.9	3.1	12.2
Control site 1 : Kati	10.9		55.9	36.5	10.4	6.4	1.3	22.1
IRS site 2 : Fana	2.6		26	5.6	0.5	0.4	0	6.5
Control site 2 : Dioila	4.5		34.4	19.5	2.4	0.8	0	11.4
IRS site 3 : Baroueli	0		0	0	0	0	0	0
Control site 3 : Ségou	0.1		10.3	1.1	0	0	0	2.3
Former IRS site : Bla	0.8		9.4	1.8	0.4	0.6	0.3	2.5
Grand Total : IRS sites	3.5		14.3	10.5	3.8	1.4	1	6.1
Grand Total : Control sites	5.2		33.5	19	4.3	2.4	0.4	11.9

HBR: human biting rate, b/h/n: bite/human/night

3.4 VECTOR BITING TIMES

Figures 26-29 display the same tendency in terms of biting times for *An. gambiae* s.l., in IRS and control sites. *An. gambiae* s.l. appears to bite predominantly late at night with a similar proportion indoors and outdoors, with little biting before 10pm. The biting times are not presented for Baroueli, Segou and Bla as <100 *An. gambiae* s.l. were caught. More details are available in Annex D, Tables D1 & D2.

FIGURE 26: *AN. GAMBIAE* S.L., HOURLY BITING RATES (N=225 INDOORS, N=325 OUTDOORS), KOULIKORO (IRS SITE1), JULY-DECEMBER 2016.

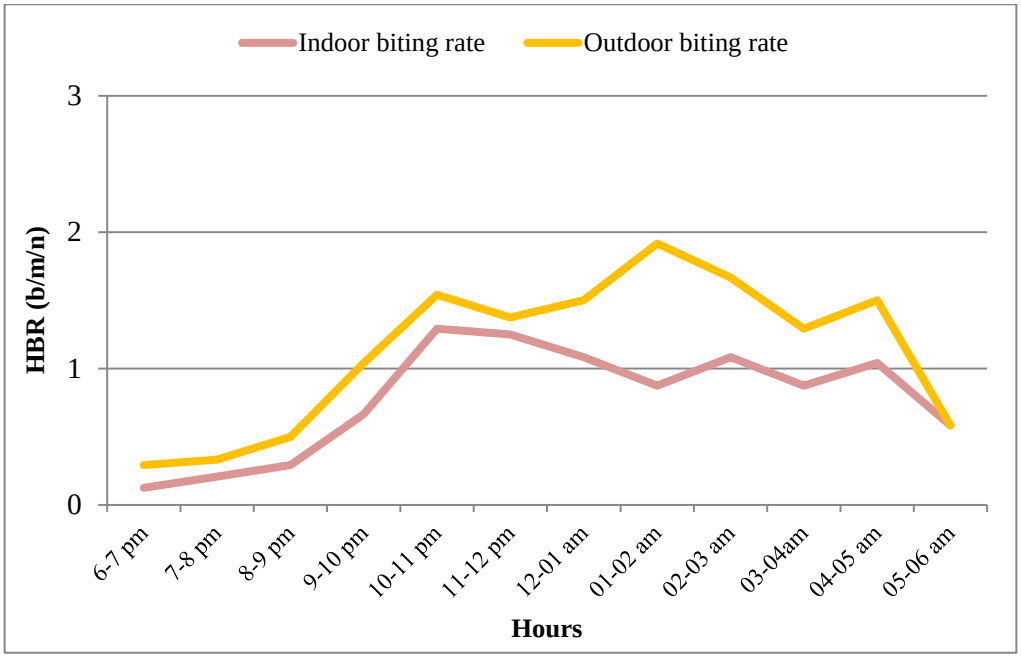


FIGURE 27: *AN. GAMBIAE* S.L., HOURLY BITING RATES (N=507 INDOORS, N=463 OUTDOORS), KATI (CONTROL SITE 1), JULY-DECEMBER 2016.

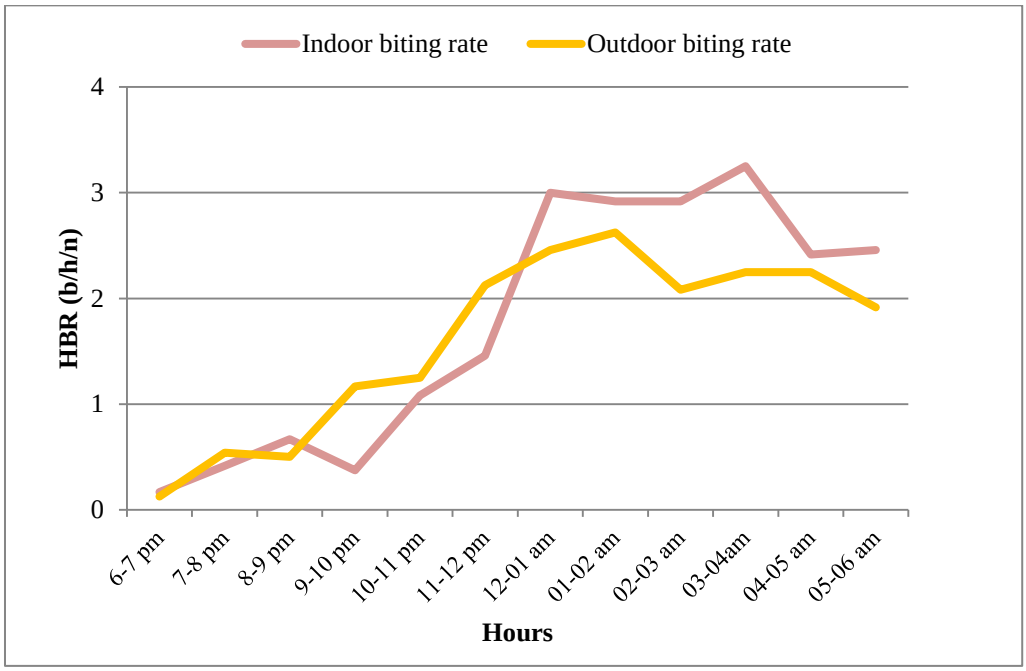


FIGURE 28: *AN. GAMBIAE* S.L., HOURLY BITING RATES (N= 210 INDOORS, N= 62 OUTDOORS), FANA (IRS SITE 2), JULY-DECEMBER 2016.

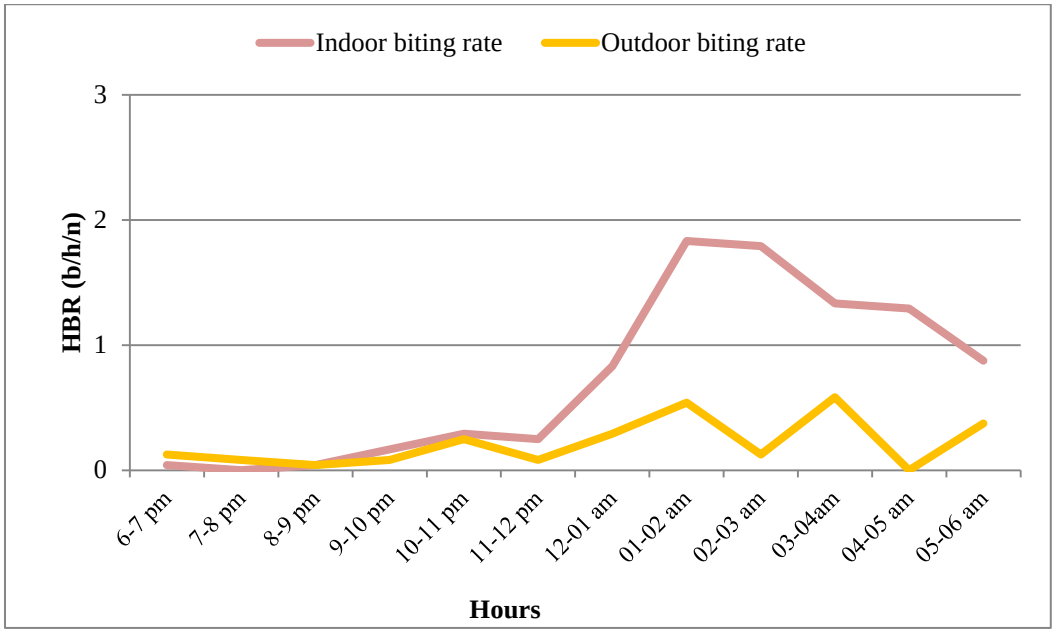
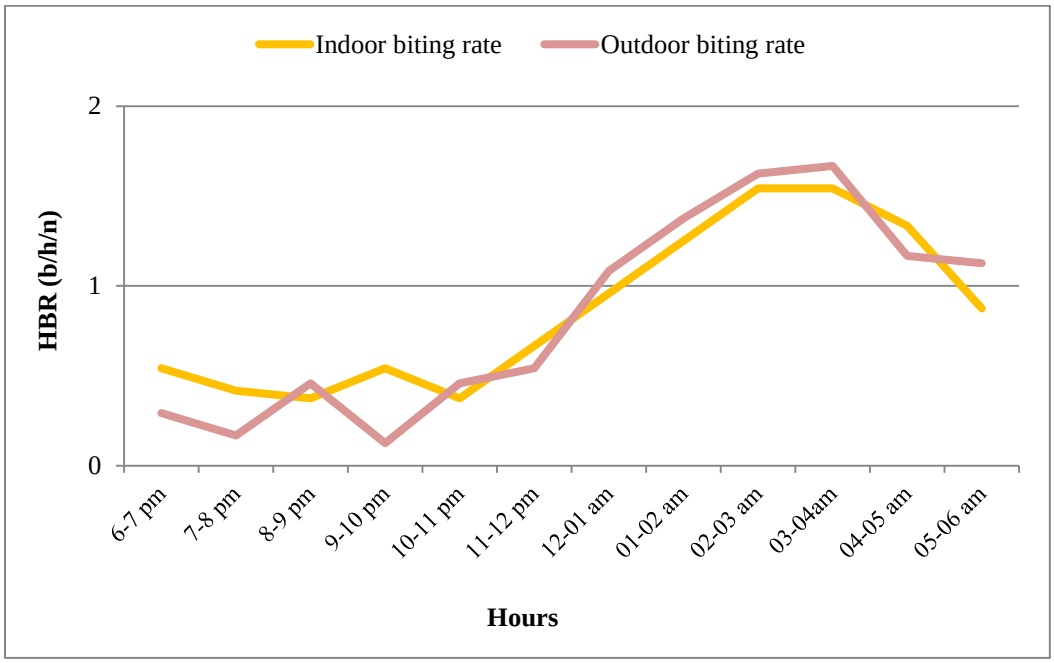


FIGURE 29: *AN. GAMBIAE* S.L., HOURLY BITING RATES (N=250 INDOORS, N=242 OUTDOORS), DIOILA (CONTROL SITE 2), JULY-DECEMBER 2016.



3.5 BITING LOCATION

The frequency of *An. gambiae* s.l. bites indoors and outdoors in each site is presented in Table 5 (more details are provided in annex C, Table C1 & C2). Prior to IRS, biting rates in *An. gambiae* s.l. were similar indoors and outdoors at all sites except for Fana where there was more biting indoors (IRS site2). As pre-IRS sampling was only for 1 time point, any differences may be due to sampling bias. Post-IRS, biting behavior in *An. gambiae* s.l. was variable according to each IRS site (Koulikoro, Fana

& Barouéli). But, after accumulating the data, biting rates appear to be similar indoors and outdoors in IRS sites and control sites. In Barouéli no mosquito was found irrespective of the period (Pre and Post-IRS).

TABLE 5: *AN. GAMBIAE* S.L., MEAN HUMAN BITING RATE INDOORS AND OUTDOORS, JULY-DECEMBER 2016.

Period/site	Indoor	Outdoor	In:Out Ratio
Pre-IRS			
Koulikoro: IRS site 1	9.3	6.8	0.6 : 0.4
Fana: IRS site 2	4.8a	0.5b	0.9 : 0.1
Baroueli : IRS site 3	0	0	n/a
TOTAL: IRS sites	4.7a	2.4b	0.7 : 0.3
Kati: control site 1	11.3	10.5	0.5 : 0.5
Dioila: control site 2	5.8a	3.3a	0.6 : 0.4
Segou: control site 3	0.3a	0a	1.0 : 0.0
TOTAL: Control sites	5.8a	4.6a	0.6: 0.4
Bla: Former IRS site	1.3a	0.3a	0.8: 0.2
Post- IRS			
Koulikoro: IRS site 1	9.4a	14.9b	0.4 : 0.6
Fana: IRS site 2	9.6a	3.0b	0.8 : 0.2
Baroueli: IRS site 3	0	0	n/a
TOTAL: IRS sites	6.3a	6.0a	0.5 : 0.5
Kati: control site 1	23.1a	21.1a	0.5 : 0.5
Dioila: control site 2	11.4a	11.5a	0.5 : 0.5
Segou: control site 3	2.6a	2.0a	0.6 : 0.4
TOTAL: Control sites	12.4a	11.5a	0.5 : 0.5
Bla: Former IRS site	2.4a	2.6a	0.5 : 0.5

HBR indoor and outdoor of a same site with different superscript are statistically significant from each other. n/a: result not available

3.6 SPOROZOITE INDEX (SI)

Table 6 shows the monthly variation of sporozoite index in all IRS and control sites. More details about the number of specimens of *An. gambiae* s.l. tested for the presence of Plasmodium falciparum antigen using ELISA CSP and the number of mosquitoes found positive are mentioned in Annex E, Table E1.

Overall, for the entire monitoring period, no significant difference is noted between the SI of the IRS sites combined (2.1%, CI: 0.8-3.4) and the one of control sites combined (2.9%, CI: 1.5-4.3) (p=0.5).

TABLE 6: SI, MONTHLY COLLECTIONS, ALL 7 SITES

	Pre IRS	Post IRS						
	SI : % (N)	SI : % (N)						
SITES	July	August	September	October	November	December	Mean	CI
IRS site 1 : Koulikoro	0% (68)	0% (60)	0% (60)	2.5% (80)	3.3% (60)	2.3% (44)	1.6% (304)	[0.2-3.1]
IRS site 2 : Fana	0% (26)	0% (60)	3.4% (59)	5 % (20)	4.5% (22)	20% (5)	3% (166)	[0.4-5.6]
IRS site 3 : Baroueli	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Control site 1 : Kati	0% (123)	0% (50)	1.7% (58)	0% (70)	0% (60)	0% (13)	0.4% (251)	[0-1.2]
Control site 2 : Dioila	3% (33)	3.3% (60)	11.7% (60)	5.7% (70)	0% (30)	0% (3)	5.8% (223)	[2.8-8.9]
Control site 3 : Ségou	0% (6)	3.3% (60)	2% (50)	n/a	n/a	n/a	2.7% (110)	[0-5.8]
Former IRS site : Bla	5.3% (94)	0% (60)	3.3% (60)	0% (69)	0% (60)	0% (13)	0.8% (262)	[0-1.8]
Grand Total : IRS sites	0% (94)	0% (120)	1.6% (119)	3% (100)	3.7% (82)	4.1% (49)	2.1% (470)	[0.8-3.4]
Grand Total : Control sites	0.6% (162)	2.3% (170)	5.3% (168)	2.9% (140)	0% (90)	0% (16)	2.9% (584)	[1.5-4.3]

N: number of mosquitoes tested for Elisa CSP

3.7 ENTOMOLOGICAL INOCULATION RATE (EIR)

EIR is of paramount importance as it shows the transmission risk of *Plasmodium falciparum*. More details are provided in Annex F, Table F1. Post-IRS, the peak of transmission was reached in all sites in September-October. During this period, the peak was of 8.1 ib/human/month in Koulikoro (IRS site 1) while it was 18.6 ib/h/month in Kati (control site 1) ($p<0.05$). At Fana (IRS site 2), the recorded peak of EIR was 5.7 ib/h/month against 68.3 in Dioila (Control site 2) ($p<0.05$). No mosquitoes were collected in Barouéli (IRS site 3) and the highest EIR in Ségou (control site 3) was 10.2 ib/h/month while it was of 1.8 ib/h/month in Bla (former IRS site) (Table 7). Due to sampling limitations in terms of number of mosquitoes tested monthly for sporozoite rate and number of houses used to monitor biting rates, the monthly EIR is likely to be highly variable and EIR over 5 months should give a more accurate measure. Overall, post-IRS, there was a lower EIR in IRS sites combined at 7.03 ib/h per 5 months compared to control sites combined at 45.33 ib/h per 5 months ($p<0.05$). Overall the EIR was 84.5% lower in IRS sites than unsprayed control sites.

TABLE 7: MONTHLY EIR FOR ALL 7 SITES BEFORE AND AFTER IRS.

	Pre IRS	Post IRS					Post IRS
	Monthly EIR	Monthly EIR					Total EIR (ib/h/5m)
SITES	July	August	September	October	November	December	Total
IRS site 1 : Koulikoro	0	0	0	8.1	3.9	2.1	14.1
Control site 1 : Kati	0	0	18.6	0	0	0	18.6
IRS site 2 : Fana	0	0	5.7	0.8	0.5	0	7
Control site 2 : Dioila	4.1	34.1	68.3	4.1	0	0	106.5
IRS site 3 : Baroueli	0	0	0	0	0	0	0
Control site 3 : Ségou	0	10.2	0.7	0	0	0	10.9
Former IRS site : Bla	1.2	0	1.8	0	0	0	1.8
Grand Total : IRS sites	0	0	1.9	2.97	1.47	0.7	7.04
Grand Total : Control sites	1.37	14.77	29.2	1.37	0	0	45.34

EIR: Entomological inoculation rate, ib/h/5m: infected bites/human per 5months

3.8 PARITY RATE

Unfed and freshly fed specimens of *An. gambiae* s.l. collected by HLC, PSC and CDC- LT were dissected Pre and Post-IRS to determine the parity rate. Prior to IRS, parity rate was similar in Koulikoro (IRS site 1) and Kati (control site 1) (p=0.26). The few specimens of *An. gambiae* s.l. that were dissected in IRS sites 2 and 3 do not allow for a good comparison with control sites 2 and 3. In Bla, a former IRS site, it was about 93.2% [86.8-99.6]. However, after combining Pre-IRS data, no significant difference was observed between parity rates of IRS sites combined and control sites combined (p=0.81) (Table 8).

After IRS, the parity rate was higher in Koulikoro (IRS site 1) compared to Kati (control site 1) (p<0.001). The same trend was recorded for Barouéli (IRS site 3) compared with Ségou and Bla (control sites 3 & former IRS site) (p<0.05). The combined post-IRS data shows a higher parity rate in IRS sites (70.6%; 95% CI: 66.8-74.4) than in control sites (51.8%; 95%CI: 48.9-54.7) (p<0.001) (Table 8, Details are in Annex G: Table G1). There is no biological explanation for this difference as IRS is expected to reduce the parity rate in sprayed areas.

TABLE 8: *AN. GAMBIAE* S.L. PARITY RATE (HLC, PSC, CDC-LT) PRE AND POST-IRS, JUNE-DECEMBER

	N Dissected	N Parous	Parity (%)	Confidence Interval 95%
Pre IRS				
Koulikoro : IRS site 1	38	21	55.3	[39.5-71.1]
Fana: IRS site 2	7	6	85.7	[59.8-100]
Barouéli: site 3	1	1	100	-
Total IRS sites	**46**	**28**	**60.9**	**[46.8-75]**
Kati: Control site 1	64	28	43.8	[31.6-55.9]
Dioila: Control site 2	33	28	84.8	[72.6-97.1]
Ségou : Control site 3	5	4	80	[44.9-100]
Total Contol sites	**102**	**60**	**58.8**	**[49.3-68.4]**
Bla : Former IRS site	59	55	93.2	[86.8-99.6]
Post IRS				
Koulikoro : IRS site 1	353	288	81.6	[77.5-85.6]
Fana: IRS site 2	180	85	47.2	[39.9-54.5]
Barouéli: site 3	18	16	88.9	[74.4-100]
Total IRS sites	**551**	**389**	**70.6**	**[66.8-74.4]**
Kati: Control site 1	638	292	45.8	[41.9-49.6]
Dioila: Control site 2	338	192	56.8	[51.5-62.1]
Ségou : Control site 3	164	107	65.2	[58-72.5]
Total Contol sites	**1140**	**591**	**51.8**	**[48.9-54.7]**
Bla : Former IRS site	179	94	52.5	[45.2-59.8]

N: number of *Anopheles gambiae* s.l.

3.9 BLOOD MEAL ORIGIN

The details on monthly human and bovine blood meal index in each site are given in table 9. Overall, *An. gambiae* s.l. fed more on humans (10.5%) in control sites as compared to IRS sites (2%) (Details are in Annex H, Table H1). The vast majority of samples were negative for presence of human or bovine blood according to the ELISA method used. This either indicates that *An. gambiae* s.l. was mostly feeding on other animal species or that the method was not sufficiently sensitive or inadequate storage of samples. Training of LBMA staff will be conducted this year as part of a regional laboratory training program.

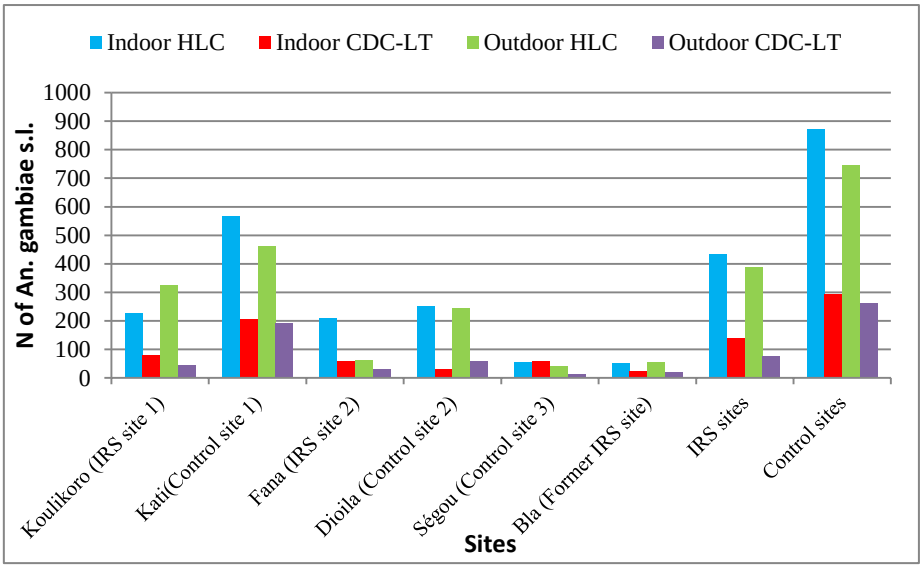
TABLE 9: HUMAN AND BLOOD MEAL INDEX, ALL SITES

SITES	N Tested	Human + (%)	Bovine + (%)	Human/Bovine + (%)	Others (%)
IRS site 1 : Koulikoro	50	0	0	0	100
IRS site 2 : Fana	50	6	0	0	94
IRS site 3 : Baroueli	50	0	0	0	100
Control site 1 : Kati	50	4	0	0	96
Control site 2 : Dioila	50	18	6	2	74
Control site 3 : Ségou	50	12	4	4	80
Control site 4 : Bla	50	8	26	2	64
Grand Total : IRS sites	150	2	0	0	98
Grand Total : Control sites	200	10.5	9	2	78.5

3.10 COMPARISON OF HUMAN LANDING CATCH AND CDC LIGHT TRAP (CDC-LT)

Figure 30 shows the number of *An. gambiae* s.l. collected by HLC and CDC-LT in all sites both indoors and outdoors from July to December over 48 collection nights. Data collected shows that HLC caught more mosquitoes than CDC-LT with a significant difference in all sites except Ségou and Bla where similar proportions of *An. gambiae* s.l. were obtained with the two sampling methods. After cumulating data, CDC-LT managed to collect only 26.6% (219/822) and 35.8% (556/1554) of the total number of *An. gambiae* s.l. that were collected by HLC respectively in IRS sites and Control sites. Based on this finding, biting rates will be determined in 2017 using only HLC. With CDC-LT, indoor collections were better than outdoor collections (Figure 32).

FIGURE 30: NUMBER OF *AN. GAMBIAE* S.L. COLLECTED INDOORS AND OUTDOORS BY HLC AND CDC-LT IN ALL SITES, ALL SITES, JULY-DECEMBER 2016



3.11 QUALITY OF SPRAYING AND INSECTICIDE DECAY RATE

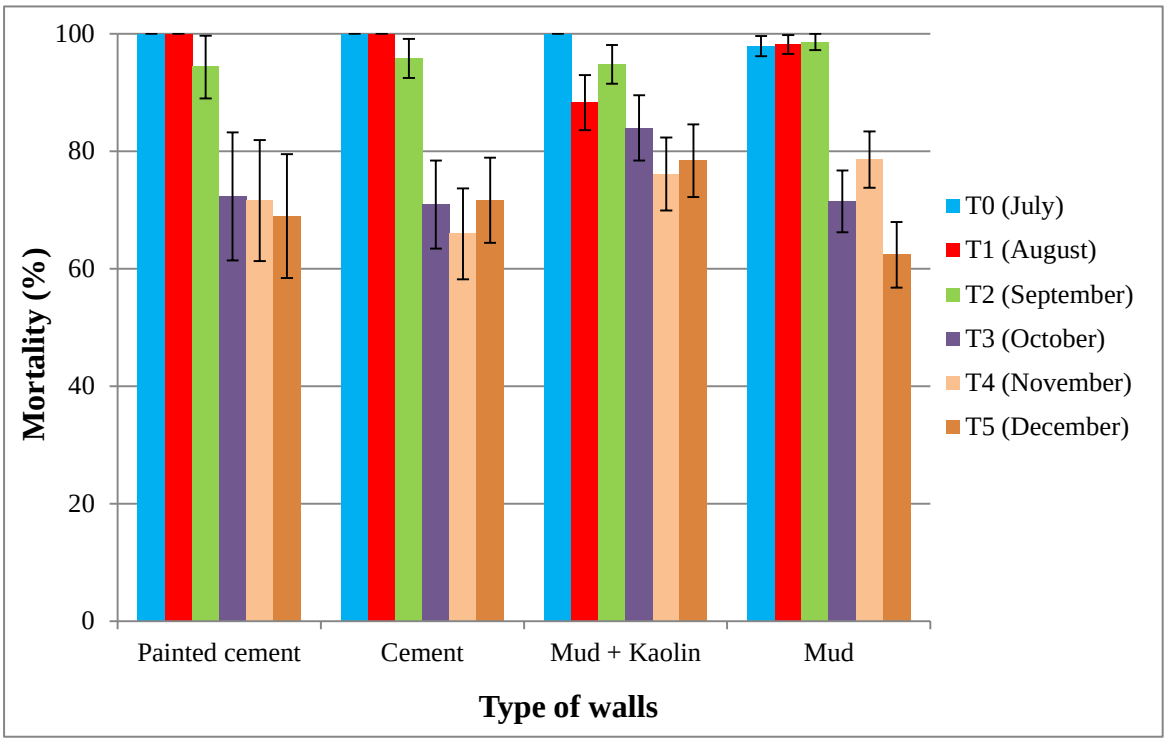
At the beginning of July 2016, AIRS Mali sprayed Koulikoro, Baroueli, and Fana districts with pirimiphos-methyl (Actellic 300 CS). Bioassays were performed one to two days after IRS, following World Health Organization (WHO) procedures. The results showed good-quality spraying in all three districts. There was no difference in the mortality rates (97.8 to 100%) of mosquitoes exposed to the sprayed walls, irrespective of their type (Mud, Mud + Kaolin, Cement, painted cement) at three different heights (Table 10).

TABLE 10: RESULTS OF QUALITY ASSURANCE CONE BIOASSAY TESTING FOLLOWING IRS (JULY 2016)

Cone Position	No. Structures	No. Mosquitoes	No. Mosquitoes Knocked Down 30 Min	% Knock Down 30 Min	No. Mosquitoes Knocked Down 60 Min	% Knock Down 60 Min	No. Mosquitoes Dead after 24 Hrs.	% Observed Mortality
Top	19	224	55	24.6%	158	70.5%	219	97.8%
Middle	19	222	33	14.9%	164	75.7%	222	100%
Bottom	19	226	58	25.7%	164	72.6%	225	99.6%
Total test	**57**	**672**	**146**	**21.7%**	**486**	**72.3%**	**666**	**99.1%**

The mortality rates which were obtained according to each type of wall and testing months are showed by Figure 31. Mortality was >80% for two months on painted cement, cement, and mud. For mud + kaolin mortality was >80% for three months. Despite being below the WHO threshold, mortality was >60% for 5 months on all surfaces and may have continued to provide some degree of control (More details are given in Annex I, Table I1).

FIGURE 31: WHO CONE TEST RESULTS, *AN. GAMBIAE* S.L. KISUMU MORTALITY AFTER 30 MINUTES EXPOSURE TO PIRIMIPHOS-METHL, KOULIKORO, FANA AND BAROUÉLI.



3.12 VECTOR SUSCEPTIBILITY TO INSECTICIDES

3.12.1 PIRIMIPHOS-METHYL 0.25% (ORGANOPHOSPHATE)

In 2016, full susceptibility was observed in all sites except for Niono (area of significant use of irrigation and LLIN universal coverage in 2015), where high resistance was surprisingly noted (Table 11). Repeat testing is needed in Niono to confirm these results and also to confirm the quality of insecticide papers by testing against susceptible *An. gambiae* Kisumu. More details are provided in Annex J, Table J1.

TABLE 11: MORTALITY OF *AN. GAMBIAE* S.L. TESTED WITH 0.25% PIRIMIPHOS-METHYL IN WHO SUSCEPTIBILITY TESTS IN 2014, 2015 AND 2016.

District (Site)	2014 % Mortality	2015 % Mortality	2016 % Mortality
Koulikoro	100%	100%	100%
Kati	100%	100%	100%
Bamako	100%	100%	100%
Bla	100%	100%	99%
Baroueli	100%	100%	100%
Niono	99%	100%	49%
Selingue	100%	100%	100%
Bougouni	100%	100%	100%
Kadiolo	100%	100%	100%
Djenne	100%	100%	100%
Bandigara	100%	100%	100%
Bankass	100%	100%	100%
Kita	n/a	n/a	100%
Fana	n/a	n/a	100%
Mean	100%	100%	97%

n/a: no data

3.12.2 PYRETHROID INSECTICIDES (PERMETHRIN 0.75% AND DELTAMETHRIN 0.05%)

Taking into account the mean mortality rates, vectors appeared to be more resistant to permethrin 0.75% than to deltamethrin 0.05% in 2015 and 2016 (Table 12). For permethrin 0.75%, mortality rates varied from 11% to 74% in 2016. For deltamethrin 0.05%, the mean mortality rate increased from 35% in 2014 to 64% in 2015, then remained at the same level (64%) in 2016 (Table 12). Based on WHO 2013 criteria; populations of *An. gambiae* s.l. from all sites were found to be resistant to permethrin 0.75% in 2016 (See Annex J, Table J2). The situation was the same with deltamethrin 0.05% except for Kita and Bankass where possible resistance was observed (Table 12). More details are shown in Annex J, Table J3.

TABLE 12: MORTALITY OF *AN. GAMBIAE* S.L. TESTED WITH 0.75% PERMETHRIN AND 0.05% DELTAMETHIN IN WHO SUSCEPTIBILITY TESTS IN 2014, 2015 AND 2016.

	Permethrin 0.75%			Deltamethrin 0.05%		
District (site)	2014 % Mortality	2015 % Mortality	2016 % Mortality	2014 % Mortality	2015 % Mortality	2016 % Mortality
Kita	19%	65%	74%	64%	98%	95%
Koulikoro	6%	14%	19%	15%	49%	85%
Kati	25%	7%	6%	18%	38%	65%
Bamako	1%	63%	50%	6%	79%	50%
Bla	47%	83%	41%	14%	64%	64%
Baroueli	25%	21%	11%	31%	66%	81%
Niono	11%	49%	n/a	42%	58%	20%
Selingue	72%	57%	33%	42%	56%	40%
Bougouni	61%	85%	67%	77%	79%	76%
Kadiolo	13%	54%	21%	43%	63%	47%
Djenne	21%	19%	14%	40%	41%	28%
Bandiagara	32%	22%	58%	30%	45%	89%
Bankass	54%	87%	64%	37%	90%	93%
Fana	n/a	n/a	41%	n/a	n/a	52%

Mean	30%	48%	38%	35%	64%	64%

n/a: no data

3.12.3 BENDIOCARB 0.1% (CARBAMATE)

In 2012, after performing testing to bendiocarb, suspected resistance in populations of *An. gambiae* s.l. was found in 3 sites, namely Bougouni, Kadiolo (areas of cotton production and high use of insecticide) and Bla, an IRS site at the time. In the 8 remaining surveyed sites full susceptibility was recorded (Table 13). In 2016, 14 sites were surveyed. Data obtained showed full susceptibility in 12 sites and possible resistance in 2 sites (Niono and Bougouni) (Table 13). More details are provided in Annex J, Table J4.

TABLE 13: MORTALITY OF *AN. GAMBIAE* S.L. TESTED WITH 0.1% BENDIOCARB IN WHO SUSCEPTIBILITY TESTS IN 2012, 2014, 2015 AND 2016.

Districts (Site)	2012	2014	2015	2016
	% Mortality	% Mortality	% Mortality	% Mortality
Koulikoro	98%	100%	100%	99%
Kati	100%	100%	100%	98%
Bamako	100%	96%	98%	98%
Bla	88%	94%	100%	100%
Baroueli	98%	97%	99%	98%
Niono	100%	100%	100%	94%
Selingue	-	99%	100%	100%
Bougouni	85%	86%	100%	92%
Kadiolo	78%	92%	94%	100%
Djenne	100%	99%	100%	100%
Bandigara	100%	100%	100%	100%
Bankass	100%	100%	100%	100%
Kita	n/a	n/a	n/a	100%
Fana	n/a	n/a	n/a	100%
Mean	95%	97%	99%	99%

n/a: no data

3.12.4 DDT (ORGANOCHLORINE)

As in previous years of insecticide resistance monitoring (2012, 2014 and 2015), *An. gambiae* s.l. was highly resistant to DDT in 2016 despite the fact that DDT was not used in public health nor for agricultural purpose. In 2016, the lowest and the highest mortality rate recorded was respectively 3% in Koulikoro and 37% in Bandiagara after testing populations of *An. gambiae* s.l. collected from 13 sites (Annex J, Table J5). As there is widespread resistance to DDT and no prospect of public health use, DDT resistance will not be monitored in 2017.

TABLE 14: MORTALITY OF *AN. GAMBIAE* S.L. TESTED WITH 4% DDT IN WHO SUSCEPTIBILITY TESTS, 2012, 2014, 2015 AND 2016

District (Site)	2012	2014	2015	2016
	% Mortality	% Mortality	% Mortality	% Mortality
Koulikoro	11%	4%	37%	3%
Kati	17%	1%	7%	4%
Bamako	18%	7%	24%	17%
Bla	48%	6%	41%	21%
Baroueli	13%	16%	15%	11%
Niono	44%	5%	7%	n/a
Selingue	n/a	13%	30%	13%
Bougouni	28%	9%	68%	12%

Kadiolo	12%	6%	40%	10%
Kita	n/a	n/a	n/a	22%
Djenne	42%	19%	31%	11%
Bandiagara	76%	14%	28%	37%
Bankass	47%	25%	55%	30%
Fana	n/a	n/a	n/a	11%
Mean	32%	10%	32%	16%

n/a: no data

3.13 RESISTANCE INTENSITY ASSAY

Table 15 shows results obtained after exposing *An. gambiae* s.l. from the 14 surveyed sites to permethrin and deltamethrin at 1X, 2X, 5X and 10X diagnostic dosages to assess the intensity of insecticide resistance. Mortality rates of populations of *An. gambiae* s.l. exposed to permethrin 10X ranged from 28% to 93% in all sites. With deltamethrin 10X, *An. gambiae* s.l. showed a mortality rate varying from 53% to 91%. Overall, resistance to pyrethroids is quite intense at all sites with some slight differences according to insecticides and sites. The intensity of resistance was higher with permethrin than with deltamethrin in most sites. No significant difference in pyrethroid resistance intensity were observed between 2014, 2015 and 2016 (Table 16) indicating that the strength of resistance has remained stable.

TABLE 15: IR INTENSITY ASSAY RESULTS OF *AN. GAMBIAE* S.L. TESTED AGAINST PERMETHRIN AND DELTAMETHRIN IN 2016.

	Perm 1X		Perm 2X		Perm 5X		Perm 10X		Delta 1X		Delta 2X		Delta 5X		Delta 10X	
SITES	%	N	%	N	%	N	%		%	N	%	N	%	N	%	N
DJENNE	17	100	27	100	27	99	28 R	93	38	98	43	100	60	101	53R	98
BANDIAGARA	19	104	44	104	49	104	69 R	104	38	104	53	104	87	104	78R	104
BANKASS	42	104	71	104	81	104	75 R	104	43	100	52	100	74	102	83R	102
KOULIKORO	34	100	53	100	92	100	91 SR	100	2	100	60	96	84	101	81R	100
FANA	18	73	30	104	36	92	66 R	79	21	100	50	94	48	100	76R	91
BAROUELI	3	66	26	94	30	102	93 SR	100	39	72	52	83	71	97	89R	104
BAMAKO	34	100	20	100	36	100	74 R	100	84	100	82	100	80	100	90SR	100
KATI	78	100	75	100	86	100	91 SR	100	84	100	92	100	61	100	88R	100
BLA	20	104	44	104	88	100	88 R	101	64	101	62	96	74	101	88R	104
SELINGUE	4	100	17	100	29	99	44 R	100	23	100	36	99	51	100	64R	100
BOUGOUNI	9	80	18	97	17	100	83 R	93	43	80	85	100	92	95	88R	104
NIONO	16	104	26	78	n/a	n/a	93 SR	103	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
KITA	47	100	35	100	64	100	64 R	100	62	100	63	100	77	100	71R	100
KADIOLO	7	100	22	100	43	100	61R	80	55	80	69	80	90	80	91SR	80

%: Mortality percentage; N: number tested; R: resistance; SR: suspected resistance; n/a - results not available

TABLE 16: MORTALITY OF *AN. GAMBIAE* S.L. TESTED WITH 10 TIMES THE DIAGNOSTIC DOSE OF PERMETHRIN OR DELTAMETHRIN, BOTTLE BIOASSAYS; 2014, 2015 AND 2016

	Permethrin			Deltamethrin		
Sites	**2014**	**2015**	**2016**	**2014**	**2015**	**2016**
	10X	**10X**	**10X**	**10X**	**10X**	**10X**
Koulikoro	40%	66%	91	77%	94%	81%
Bamako	81%	82%	74	87%	86%	90%
Bla	73%	45%	88	91%	84%	88%
Baroueli	86%	60%	93	100%	91%	89%
Niono	95%	80%	93	49%	76%	n/a

Selingue	87%	63%	44	67%	64%	64%
Bougouni	51%	55%	83	68%	76%	88%
Kadiolo	64%	69%	61	61%	84%	91%
Kati	n/a	n/a	91	n/a	n/a	88%
Djenne	94%	65%	28	100%	95%	53%
Bandigara	19%	92%	69	93%	88%	78%
Bankass	80%	84%	75	86%	84%	83%
Kita	n/a	n/a	64	n/a	n/a	71%
Fana	n/a	n/a	66	n/a	n/a	76%
Mean	**70%**	**69%**	**73%**	**80%**	**84%**	**80%**

n/a – no data

3.14 MIXED FUNCTION OXIDASES RESISTANCE

Results in Table 17 shows that MFOs are partially implicated in phenotypic resistance to permethrin in two sites (Bamako and Barouéli), deltamethrin in four sites (Bankass, Kita, Fana & Bla) and to both in five sites (Djénné, Koulikoro, Sélingué, Kadiolo and Bougouni). Although pre-exposure of *An. gambiae* s.l. to PBO significantly increased mortality rates after exposure to permethrin and deltamethrin in most sites, full susceptibility was not reached.

TABLE 17: 2016 MIXED FUNCTION OXIDASE RESISTANCE, 30 MINUTES EXPOSURE, BOTTLE BIOASSAY.

Sites	Permethrin				Deltamethrin			
	Insecticide only		+ PBO		Insecticide only		+ PBO	
	%	N	%	N	%	N	%	N
DJENNE	17R	100	38*R	94	38R	98	69*R	91
BANDIAGARA	19R	104	25R	104	38R	104	40R	104
BANKASS	42*R	104	30R	104	43R	100	71*R	104
BAMAKO	34R	100	75*R	100	84R	100	83R	100
KATI	78R	100	75R	100	84R	100	88R	100
NIONO	16R	104	n/a	n/a	n/a	n/a	n/a	n/a
KITA	49R	100	56R	100	62R	100	82*R	100
KOULIKORO	34R	100	69*R	100	2R	100	51*R	100
FANA	18R	73	21R	100	21R	100	47*R	100
BAROUELI	3R	66	36*R	100	39R	72	46R	100
BLA	20R	104	26R	104	64R	101	86*R	102
SELINGUE	4R	100	49*R	77	23R	100	69*R	104
KADIOLO	7R	100	60*R	80	55R	80	74*R	80
BOUGOUNI	9R	80	31*R	80	43R	80	73*R	80

%; Mortality percentage; N: number tested; R: resistance;

* $p<0.05$ (Z-test for difference in proportions). The control mortality for each test was <5%.

3.15 VECTOR SPECIES IDENTIFICATION

Overall, 687 specimens of *An. gambiae* s.l. (47-50 mosquitoes per site) from the 14 insecticide resistance surveillance sites were analyzed by PCR to determine the species composition. Specimens were from larval collections that were subsequently tested as adults in WHO susceptibility bioassays. *An. coluzzii* was the predominant species in ten of the sites, followed by *An. gambiae* in three sites. In Kita, *An. arabiensis*, *An. gambiae* s.s. *and* hybrids (*coluzzii* x *gambiae*) were in similar proportion.

The overall frequencies were 56% *An. coluzzii*, 23% *An. gambiae*, 13% *An. coluzzii* x *gambiae* hybrids, and 6% *An. arabiensis* (Table 18). 2% of the tested samples failed to be amplified

TABLE 18: *AN GAMBIAE* S.L. SIBLING SPECIES COMPOSITION FROM INSECTICIDE RESISTANCE MONITORING TESTS IN 14 SURVEILLANCE SITES.

			An. arabiensis		*An. gambiae*		*An*		Hybrid		Total	
Regions	**Sites**						*coluzzii*		coluzzii & gambiae		Non Amplified	
		Total tested	**N**	%	**N**	%	**N**	%	**N**	%	**N**	%
Kayes	Kita	50	14	28	15	30	7	14	14	28	0	0
Koulikoro	Koulikoro	50	0	0	5	10	43	86	2	4	0	0
	Fana	50	7	14	24	48	16	32	3	6	0	0
	Kati	50	0	0	4	8	42	84	1	2	3	6
	Total	150	7	5	33	22	101	67	6	4	3	2
Segou	Niono	50	0	0	0	0	36	72	11	22	3	6
	Bla	50	0	0	7	14	41	82	1	2	1	2
	Baroueli	50	8	16	28	56	7	14	5	10	2	4
	Total	150	8	5	35	23	84	56	17	11	6	4
Sikasso	Bougouni	50	0	0	36	72	11	22	3	6	0	0
	Selingue	50	0	0	0	0	28	56	19	38	3	6
	Kadiolo	50	0	0	15	30	33	66	2	4	0	0
	Total	150	0	0	51	34	72	48	24	16	3	2
Mopti	Bandiagara	50	0	0	15	30	33	66	2	4	0	0
	Bankass	50	12	24	10	20	20	40	7	14	1	2
	Djenne	50	0	0	1	2	39	78	10	20	0	0
	Total	150	12	8	26	17	92	61	19	13	1	0.7
Bamako	Bamako IV	50	0	0	2	4	37	74	11	22	0	0
	Grand Total	**700**	**41**	6	**162**	23	**393**	56	**91**	13	13	2

3.16 VECTOR RESISTANCE MECHANISMS (KDR 1014 F, 1014S, AND ACE1R)

The 1014F *kdr* mutation was detected in mosquitoes that were tested with 0.75% permethrin and 0.05% deltamethrin (the same specimens that were used for species identification). Overall, the 1014F *kdr* mutation was detected in 602 out of 687 tested specimens of *An. gambiae* s.l. (Details are in Table 20 & Annex K, Tables K1, K2, K3, K3, K4 & K5). The mutation was widespread in the 14 insecticide resistance monitoring sites in 2016. Cumulated data of the 14 sites showed a low frequency of *kdr* 1014F in *An. arabiensis* which was 20% [10.5-34.1]. Within *An. coluzzii*, *An. gambiae* s.s. and hybrids of *coluzzii* x *gambiae;* the average frequency was similar and ranged from 85% to 89% (Table 20).

The *kdr* 1014S mutation was quite rare in most sites. Only a few homozygous resistant (RR) and heterozygous genotypes (RS) were found at Fana, Bandiagara, Bla and Kadiolo, giving an average frequency of 3% in *An. arabiensis* and 6% in *An. gambiae s.s.* (Table 21).

Only those mosquitoes that survived exposure to bendiocarb and pirimiphos-methyl were screened for *Ace1*^{R} mutation in 5 sites (Table 19). Results obtained showed a frequency ranging from 0% in Bla

and Bamako to 62% [35.8-83.7] in Bougouni, a site where suspected resistance to bendiocarb was observed. In Bougouni, agriculture with intense use of insecticides is practiced.

TABLE 19: *AN GAMBIAE* S.L., *ACE1*^{R} MUTATION ALLELIC FREQUENCIES IN ALIVE MOSQUITOES TO BENDIOCARB AND PIRIMIPHOS-METHYL FROM 5 INSECTICIDE RESISTANCE MONITORING SITES.

		Genotypes			
Sites	Total	RR	SS	RS	f(*Ace1*^{R})
Baguineda	2	0	1	1	0.25
Bougouni	8	3	1	4	0.62
Bla	1	0	1	0	0
Djicorini	2	0	2	0	0
Niono	17	0	15	2	0.05
Grand Total	30	3	20	7	0.22

TABLE 20: *AN. GAMBIAE* S.L., *KDR* 1014F MUTATION ALLELIC FREQUENCIES IN INSECTICIDE RESISTANCE MONITORING SITES.

			An. arabiensis				*An. gambiae*				*An coluzzii*				Hybrids *coluzzii and gambiae*			
Sites	Total amplified	Total Non Amplified	RR	SS	RS	f(*kdr*)	RR	SS	RS	f(*kdr*)	RR	SS	RS	f(*kdr*)	RR	SS	RS	f(*kdr*)
Koulikoro	46	4	0	0	0	n/a	4	1	0	0.8	39	0	1	0.98	1	0	0	1
Kati	47	3	0	0	0	n/a	0	4	0	0	32	1	9	0.86	1	0	0	1
Bougouni	48	2	0	0	0	n/a	35	0	1	0.98	9	0	0	1	3	0	0	1
Bankass	46	4	2	9	0	0.18	7	2	0	0.77	15	2	3	0.82	3	2	1	0.58
Bandiagara	50	0	0	0	0	n/a	12	3	0	0.8	25	1	7	0.86	2	0	0	1
Bla	47	3	0	0	0	n/a	4	3	0	0.57	14	3	22	0.64	0	1	0	0
Selingue	37	13	0	0	0	n/a	0	0	0	n/a	23	0	0	1	14	0	0	1
Bamako	48	2	0	0	0	n/a	2	0	0	1	37	0	0	1	9	0	0	1
Niono	39	11	0	0	0	n/a	0	0	0	n/a	24	1	6	0.87	6	0	2	0.87
Kita	27	23	1	5	0	0.16	6	5	1	0.54	4	1	0	0.8	2	2	0	0.5
Kadiolo	50	0	0	0	0	n/a	15	0	0	1	23	2	8	0.81	1	0	1	0.75
Djenne	49	1	0	0	0	n/a	1	0	0	1	38	0	0	1	10	0	0	1
Baroueli	18	32	1	0	0	1	14	0	0	1	3	0	0	1	0	0	0	n/a
Fana	50	0	1	6	0	0.14	22	2	0	0.91	11	2	3	0.78	3	0	0	1
Grand Total	602	98	5	20	0	0.2	122	20	2	0.85	297	13	59	0.88	55	5	4	0.89

n/a: Not Applicable

TABLE 21: *AN. GAMBIAE* S.L. *KDR* 1014S AFRICA MUTATION ALLELIC FREQUENCIES IN INSECTICIDE RESISTANCE MONITORING SITES

			An. arabiensis				*An. gambiae s.s.*				*An coluzzii*				*Hybrids coluzzii and gambiae*			
Sites	Total Amplified	Total Non Amplified	RR	SS	RS	f(*kdr*)	RR	SS	RS	f(*kdr*)	RR	SS	RS	f(*kdr*)	RR	SS	RS	f(*kdr*)
Koulikoro	23	27	0	0	0	n/a	0	0	0	n/a	0	23	0	0	0	0	0	n/a
Kati	24	26	0	0	0	n/a	0	4	0	0	0	20	0	0	0	0	0	n/a
Bougouni	8	42	0	0	0	n/a	0	2	0	0	0	6	0	0	0	0	0	n/a
Bankass	24	26	0	10	0	0	0	2	0	0	0	9	0	0	0	3	0	0
Bandiagara	33	17	0	0	0	n/a	1	13	1	0.1	0	18	0	0	0	0	0	n/a
Bla	30	20	0	0	0	n/a	1	3	0	0.25	0	25	0	0	0	1	0	0
Selingue	18	32	0	0	0	n/a	0	0	0	n/a	0	10	0	0	0	8	0	0
Bamako	23	27	0	0	0	n/a	0	0	0	n/a	0	19	0	0	0	4	0	0
Niono	12	38	0	0	0	n/a	0	0	0	n/a	0	11	0	0	0	1	0	0
Kita	30	20	0	13	0	0	0	8	0	0	0	4	0	0	0	5	0	0
Kadiolo	21	29	0	0	0	n/a	0	1	0	0	0	18	1	0.02	0	1	0	0
Djenne	29	21	0	0	0	n/a	0	1	0	0	0	22	0	0	0	6	0	0
Baroueli	9	41	0	5	0	0	0	3	0	0	0	1	0	0	0	0	0	0
Fana	20	30	0	4	2	0.16	0	3	0	0	0	9	0	0	0	2	0	0
Grand Total	304	396	0	32	2	0.03	2	40	1	0.06	0	195	1	0	0	31	0	0

n/a: Not Applicable

3.17 QUALITY ASSURANCE, VECTOR SPECIES IDENTIFICATION AND RESISTANCE MECHANISMS

Results from CREC revealed *An. arabiensis* only at Bankass but results of LBMA showed in addition to Bankass, the presence of this species at Kita and Fana. No hybrids (coluzzii/gambiae) were detected by CREC but LBMA found them in several sites. Overall, for all sites, the proportion of the different sibling species of *An. gambiae* s.l. identified by both labs was similar for Kati, Bla, Kadiolo and Djenne, but different for all other sites (Table 22). There was a 20% discordance between the two labs for species identification (Table 22).

For the *kdr* 1014F mutation, the proportion of the three genotypes (RR, RS and SS) was different between both labs in all sites except for Baroueli and Fana where similar results were obtained (Table 22). There was a 31% discordance rate for *kdr* 1014F after cumulating data of all sites (Table 22). This raises the issue of the quality of molecular results. A regional molecular laboratory training course will be conducted in partnership with CREC and the University of Notre Dame later in 2017. For the *kdr* 1014S Genotypes, no great discordance was observed between both labs (Table 22) but LBMA have met difficulties amplifying this mutation in many mosquitoes.

TABLE 22: QUALITY ASSURANCE RESULTS, COMPARISON OF MOLECULAR RESULTS FROM LBMA AND CREC

	Species ID (Ac, Ag, Aa, Acg)				*kdr* 1014F Genotypes (RR, RS, SS)				*kdr* 1014S Genotypes (RR, RS, SS)			
	N identified by both labs	N of Concordant species between both labs	% of concordance	% of discordance	N identified by both labs	N of Concordant genotypes between both labs	%of concordance	%of discordance	N identified by both labs	N of Concordant genotypes between both labs	% of concordance	% of discordance
Koulikoro	20	17	85%	15%	18	6	33%	67%	12	12	100%	0%
Kati	18	18	100%	0%	19	9	47%	53%	11	11	100%	0%
Bougouni	19	10	53%	47%	19	15	79%	21%	2	2	100%	0%
Bankass	20	9	45%	55%	18	4	22%	78%	12	12	100%	0%
Bandiagara	20	18	90%	10%	20	15	75%	25%	11	11	100%	0%
Bla	19	19	100%	0%	18	10	56%	44%	9	9	100%	0%
Selingue	20	16	80%	20%	17	14	82%	18%	4	4	100%	0%
Bamako	20	14	70%	30%	19	10	53%	47%	10	10	100%	0%
Niono	18	15	83%	17%	14	11	79%	21%	6	6	100%	0%
Kita	20	10	50%	50%	12	9	75%	25%	10	10	100%	0%
Kadiolo	20	20	100%	0%	20	16	80%	20%	6	5	83%	17%
Djenne	20	20	100%	0%	20	18	90%	10%	11	11	100%	0%
Baroueli	14	14	100%	0%	11	11	100%	0%	2	2	100%	0%
Fana	20	15	75%	25%	20	20	100%	0%	6	6	100%	0%
TOTAL	268	215	80%	20%	245	168	69%	31%	112	111	99%	1%

Ac: *Anopheles coluzzii*, Ag: *Anopheles gambiaes.s.*, Aa: *Anopheles arabiensis*, Acg: Hybrids *Anopheles coluzziixgambiae*

4. CONCLUSIONS

In 2016, a concern after the implementation of IRS in Mali was the short residual activity of pirimiphos-methyl 300 CS, which was generally two months (although mortality was >60% after 5 months), while it was around 3 months in 2015. The residual life of two months is shorter than what was observed in other AIRS countries but is generally in keeping with neighboring Senegal which had a duration of 3 months in 2016. The houses in Koulikoro and Baroueli typically have mud walls with some plastered on the surface with kaolin. It was important to gain a better understanding of the factors that are critical to insecticide residual performance and 20kg of dried mud bricks were taken from Koulikoro to the ACS Soil Testing Facility in the UK to determine the characteristics (physical and chemical composition) of the mud. Results are expected by April 2017.

The rainy season and peak vector season in IRS areas of Mali are very short, with the highest vector densities and biting rates occurring in August-September. *Anopheles gambiae* s.l. was the predominant vector collected by PSC and human landing catches. Subsequent laboratory analysis showed that *An. coluzzii* was the most common species followed respectively by *An. gambiae, gambiae/coluzzii* (hybrids*)* and *An. arabiensis*. The three IRS sites displayed lower indoor resting densities of malaria vectors, for a duration of 2 months, when compared to control areas. While overall indoor resting densities were lower in IRS sites, in Koulikoro (IRS site 1) up to 10 *An. gambiae* s.l. per house per day were collected just 2 months after spraying. This may indicate that some mosquitoes are finding unsprayed refuges indoors such as on the ceiling, roof, furniture and clothing. Similarly, the human biting rate was significantly lower in sprayed areas but the nightly human biting rates were still very high in August and September both indoors and outdoors. The peak in biting was observed late at night between 10 p.m. and 4:00 a.m. Therefore, people who frequently use LLINs should be more protected.

The sporozoite rate was not significantly lower in IRS sites. The entomological inoculation rate was 84.5% lower in sprayed sites compared to control sites over the five months of monitoring. This reduction is somewhat less than the 99.2% reduction observed by Aïkpon *et al.*, (2014) in Atacora-Donga region (Benin) after IRS implementation with pirimiphos-methyl 50 EC. Despite a 84.5% lower EIR in IRS areas compared to unsprayed controls, the EIR in IRS areas was not negligible at 7.03 infectious bites per person over 5 months. Due to design limitations including a lack of sufficient baseline data before IRS implementation, we cannot be certain that any differences in entomological indices between control and IRS areas were due to IRS. The short 1 month baseline is due to the short rainy season in Mali. The relatively high EIR despite use of LLINs and IRS supports the need for complimentary control strategies.

In 2016, nationwide WHO tube tests confirmed widespread resistance to DDT, permethrin and deltamethrin. The intensity of resistance is generally greater to permethrin than deltamethrin in most sites. There were survivors at all sites when permethrin and deltamethrin were tested at 10X the diagnostic dosage, indicating a high intensity of resistance. MFOs were only partially implicated in the resistance of *An. gambiae* s.l. to permethrin and deltamethrin, indicating that other mechanisms are likely to be important. The intensity of resistance has remained stable between 2014 and 2016. Full susceptibility to bendiocarb was observed in all sites except Niono and Bougouni where possible resistance was recorded. Data also indicated full susceptibility to pirimiphos methyl in all sites except Niono (a non-IRS site) where repeat testing is needed to confirm this result. Spraying in 2017 is due to take place in the Mopti Region where full susceptibility was recorded for pirimiphos-methyl. As in previous years, the 1014F *kdr* mutation was identified at high frequencies in all sites. The detection of a few homozygous resistant (RR) and heterozygous genotypes (RS) for *Ace1*^{R} mutation indicates the importance of monitoring this mutation over time.

5. ANNEX

ANNEX A: AN. GAMBIAE S.L. SIBLING SPECIES PCR, 2016

TABLE A1: *AN. GAMBIAE* S.L. SIBLING SPECIES PCR, KOULIKORO, FANA, BAROUÉLI, KATI, DIOILA, SÉGOU AND BLA, JULY-DECEMBER 2016

	N tested	*An. gambiae*	%	*An. coluzzii*	%	Hybrid *gambiae/coluzzii*	%	*An. arabiensis*	%	Non Amplified	%
Koulikoro: IRS Site 1	176	4	2.3	151	85.8	12	6.8	0	0	9	5.1
Fana: IRS site 2	114	39	34.2	46	40.4	24	21.1	0	0	5	4.4
Barouéli: IRS site 3	50	7	14	37	74	3	6	0	0	3	6
Kati: Control site 1	141	2	1.4	118	83.7	13	9.2	0	0	8	5.7
Dioila: Control site 2	137	62	45.3	55	40.1	6	4.4	2	1.5	12	8.8
Ségou: Control site 3	50	5	10	30	60	4	8	0	0	11	22
Bla: Former IRS site	136	10	7.4	99	72.8	18	13.2	1	0.7	8	5.9
Total: IRS sites	340	50	14.7	234	68.8	39	11.5	0	0	17	5
Total:Control sites	328	69	21	203	61.9	23	7	2	0.6	31	9.5
Grand Total	804	129	16	536	66.7	80	10	3	0.4	56	7

ANNEX B: VECTOR DENSITY

TABLE B1: MEAN INDOOR RESTING DENSITY (PSC) OF *AN. GAMBIAE* S.L. BY MONTH IN ALL SITES, JULY-DECEMBER 2016

Period	Parameters	Koulikoro IRS site 1	Fana IRS site 2	Barouéli IRS site 3	Mean IRS sites	Kati Contol site 1	Dioila Contol site 2	Ségou Contol site 3	Mean Contol sites	Bla Former IRS site
Pre-IRS										
July	N collected	24	7	6	37	17	45	7	69	147
	N houses	20	20	20	60	20	20	20	60	20
	Density (*An*/h/d)	1.2	0.4	0.3	0.6	0.9	2.3	0.4	1.2	7.4
Post-IRS										
August	N collected	113	127	21	261	403	147	335	885	673
	N houses	20	20	20	60	20	20	20	60	20
	Density (*An*/h/d)	5.7	6.4	1.1	4.4	20.2	7.4	16.8	14.8	33.7
September	N collected	220	55	41	316	655	158	158	971	342
	N houses	20	20	20	60	20	20	20	60	20
	Density (*An*/h/d)	11.0	2.8	2.1	5.3	32.8	7.9	7.9	16.2	17.1
October	N collected	46	32	31	109	114	126	16	256	168
	N houses	20	20	20	60	20	20	20	60	20
	Density (*An*/h/d)	2.3	1.6	1.6	1.8	5.7	6.3	0.8	4.3	8.4
November	N collected	66	23	10	99	28	50	12	90	73
	N houses	20	20	20	60	20	20	20	60	20
	Density (*An*/h/d)	3.3	1.2	0.5	1.7	1.4	2.5	0.6	1.5	3.7
December	N collected	36	6	1	43	8	5	0	13	23
	N houses	20	20	20	60	20	20	20	60	20
	Density (*An*/h/d)	1.8	0.3	0.1	0.7	0.4	0.3	0.0	0.2	1.2
Total (Post-IRS)										
	N collected	481	243	104	828	1208	486	521	2215	1279
	N houses	100	100	100	300	100	100	100	300	100
	Density (*An*/h/d)	4.8	2.4	1.0	2.8	12.1	4.9	5.2	7.4	12.8

N of *An. gamb:* Number of *An. gambiae* s.l., N houses: Number of houses, *An*/h/d: *An. gambiae* s.l./house/day

ANNEX C: HUMAN BITING RATE (HBR), HLC, SEASONALITY AND LOCATION

TABLE C1: HBR, HLC, SEASONALITY AND LOCATION, IRS SITES

			Pre IRS	Post IRS						
Sites	Feeding location	Parameters	July	August	September	October	November	December	Total (Post-IRS)	Total (Pre+Post-IRS)
Koulikoro (IRS site 1)	Indoor	N of *An. gamb*	37	36	95	31	22	4	188	225
		Human night	4	4	4	4	4	4	20	24
		HBR (b/h/n)	9.3	9.0	23.8	7.8	5.5	1	9.4	9.4
	Outdoor	N of *An. gamb*	27	100	113	55	9	21	298	325
		Human night	4	4	4	4	4	4	20	24
		HBR (b/h/n)	6.8	25	28.3	13.8	2.3	5.3	14.9	13.5
	Total	N of *An. gamb*	64	136	208	86	31	25	486	550
		Human night	8	8	8	8	8	8	40	48
		HBR (b/h/n)	8	17	26	10.8	3.9	3.1	12.2	11.5
Fana (IRS site 2)	Indoor	N of *An. gamb*	19	145	42	2	2	0	191	210
		Human night	4	4	4	4	4	4	20	24
		HBR (b/h/n)	4.8	36.3	10.5	0.5	0.5	0	9.6	8.8
	Outdoor	N of *An. gamb*	2	54	3	2	1	0	60	62
		Human night	4	4	4	4	4	4	20	24
		HBR (b/h/n)	0.5	13.5	0.8	0.5	0.3	0	3	2.6
	Total	N of *An. gamb*	21	199	45	4	3	0	251	272
		Human night	8	8	8	8	8	8	40	48
		HBR (b/h/n)	2.6	24.9	5.6	0.5	0.4	0	6.3	5.7
Barouéli (IRS site 3)	Indoor	N of *An. gamb*	0	0	0	0	0	0	0	0
		Human night	4	4	4	4	4	4	20	24
		HBR (b/h/n)	0	0	0	0	0	0	0	0
	Outdoor	N of *An. gamb*	0	0	0	0	0	0	0	0
		Human night	4	4	4	4	4	4	20	24
		HBR (b/h/n)	0	0	0	0	0	0	0	0
	Total	N of *An. gamb*	0	0	0	0	0	0	0	0
		Human night	8	8	8	8	8	8	40	48
		HBR (b/h/n)	0	0	0	0	0	0	0	0
Grand total (IRS sites)	Indoor	N of *An. gamb*	56	181	137	33	24	4	379	435
		Human night	12	12	12	12	12	12	60	72
		HBR (b/h/n)	4.7	15.1	11.4	2.8	2	0.3	6.3	6
	Outdoor	N of *An. gamb*	29	154	116	57	10	21	358	387
		Human night	12	12	12	12	12	12	60	72
		HBR (b/h/n)	2.4	12.8	9.7	4.8	0.8	1.8	6	5.4
	Total	N of *An. gamb*	85	335	253	90	34	25	737	822
		Human night	24	24	24	24	24	24	120	144
		HBR (b/h/n)	3,5	14	10.5	3.8	1.4	1	6.1	5.7

N of *An. gamb*: Number of *Anopheles gambiae* s.l., b/h/n: bites/human/night

TABLE C2: HBR, HLC, SEASONALITY AND LOCATION, CONTROL SITES & FORMER IRS SITE

			Pre-IRS	Post-IRS						
Sites	Feeding location	Parameters	July	August	September	October	November	December	Total (Post-IRS)	Total (Pre+Post-IRS)
Kati (Control site 1)	Indoor	N of *An. gamb*	45	238	144	43	31	6	462	507
		Human night	4	4	4	4	4	4	20	24
		HBR (b/h/n)	11.3	59.5	36	10.8	7.8	1.5	23.1	21.1
	Outdoor	N of *An. gamb*	42	209	148	40	20	4	421	463
		Human night	4	4	4	4	4	4	20	24
		HBR (b/h/n)	10.5	52.3	37	10	5	1	21.1	19.3
	Total	N of *An. gamb*	87	447	292	83	51	10	883	970
		Human night	8	8	8	8	8	8	40	48
		HBR (b/h/n)	10.9	55.9	36.5	10.4	6.4	1.3	22.1	20.2
Dioila (Control site 2)	Indoor	N of *An. gamb*	23	136	81	6	4	0	227	250
		Human night	4	4	4	4	4	4	20	24
		HBR (b/h/n)	5.8	34	20.3	1.5	1	0	11.4	10.4
	Outdoor	N of *An. gamb*	13	139	75	13	2	0	229	242
		Human night	4	4	4	4	4	4	20	24
		HBR (b/h/n)	3.3	34.8	18.8	3.3	0.5	0	11.5	10.1
	Total	N of *An. gamb*	36	275	156	19	6	0	456	492
		Human night	8	8	8	8	8	8	40	48
		HBR (b/h/n)	4.5	34.4	19.5	2.4	0.8	0	11.4	10.3
Ségou (Control site 3)	Indoor	N of *An. gamb*	1	47	5	0	0	0	52	53
		Human night	4	4	4	4	4	4	20	24
		HBR (b/h/n)	0.3	11.8	1.3	0	0	0	2.6	2.2
	Outdoor	N of *An. gamb*	0	35	4	0	0	0	39	39
		Human night	4	4	4	4	4	4	20	24
		HBR (b/h/n)	0	8.8	1	0	0	0	2	1.6
	Total	N of *An. gamb*	1	82	9	0	0	0	91	92
		Human night	8	8	8	8	8	8	40	48
		HBR (b/h/n)	0.1	10.3	1.1	0	0	0	2.3	1.9
Bla (Former IRS site)	Indoor	N of *An. gamb*	5	36	7	1	1	2	47	52
		Human night	4	4	4	4	4	4	20	24
		HBR (b/h/n)	1.3	9	1.8	0.3	0.3	0.5	2.4	2.2
	Outdoor	N of *An. gamb*	1	39	7	2	4	0	52	53
		Human night	4	4	4	4	4	4	20	24
		HBR (b/h/n)	0.3	9.8	1.8	0.5	1	0	2.6	2.2
	Total	N of *An. gamb*	6	75	14	3	5	2	99	105
		Human night	8	8	8	8	8	8	40	48
		HBR (b/h/n)	0.8	9.4	1.8	0.4	0.6	0.3	2.5	2.2
Grand total (Control sites)	Indoor	N of *An. gamb*	69	421	230	49	35	6	741	810
		Human night	12	12	12	12	12	12	60	72
		HBR (b/h/n)	5.8	35.1	19.2	4.1	2.9	0.5	12.4	11.3
	Outdoor	N of *An. gamb*	55	383	227	53	22	4	689	744
		Human night	12	12	12	12	12	12	60	72
		HBR (b/h/n)	4.6	31.9	18.9	4.4	1.8	0.3	11.5	10.3
	Total	N of *An. gamb*	124	804	457	102	57	10	1430	1554
		Human night	24	24	24	24	24	24	120	144
		HBR (b/h/n)	5.2	33.5	19	4.3	2.4	0.4	11.9	10.8

N of *An. gamb*: Number of *Anopheles gambiae* s.l., b/h/n: bites/human/night

ANNEX D: HUMAN BITING RATE (HBR), HLC, BITING TIME

TABLE D1: HBR, HLC, BITING TIME, IRS SITES

			TIMES											
Sites	Locations	Parameters	6-7 pm	7-8 pm	8-9 pm	9-10 pm	10-11 pm	11-12 pm	12-01 am	01-02 am	02-03 am	03-04am	04-05 am	05-06 am
	Indoor	N of *An. gamb*	3	5	7	16	31	30	26	21	26	21	25	14
		Human night	24	24	24	24	24	24	24	24	24	24	24	24
Koulikoro		HBR (b/h/n)	0.1	0.1	0.3	0.7	1.3	1.3	1.08	0.9	1.1	0.9	1	0.6
IRS site 1	Outdoor	N of *An. gamb*	7	8	12	25	37	33	36	46	40	31	36	14
		Human night	24	24	24	24	24	24	24	24	24	24	24	24
		HBR (b/h/n)	0.3	0.3	0.5	1.	1.5	1.4	1.5	1.9	1.7	1.3	1.5	0.6
	Indoor	N of *An. gamb*	1	0	1	4	7	6	20	44	43	32	31	21
		Human night	24	24	24	24	24	24	24	24	24	24	24	24
Fana		HBR (b/h/n)	0.04	0	0.04	0.2	0.3	0.3	0.8	1.8	1.8	1.33	1.3	0.9
IRS site 2	Outdoor	N of *An. gamb*	3	2	1	2	6	2	7	13	3	14	0	9
		Human night	24	24	24	24	24	24	24	24	24	24	24	24
		HBR (b/h/n)	0.1	0.1	0.04	0.1	0.25	0.1	0.3	0.5	0.1	0.6	0	0.4
	Indoor	N of *An. gamb*	0	0	0	0	0	0	0	0	0	0	0	0
		Human night	24	24	24	24	24	24	24	24	24	24	24	24
Baroueli		HBR (b/h/n)	0	0	0	0	0	0	0	0	0	0	0	0
IRS site 3	Outdoor	N of *An. gamb*	0	0	0	0	0	0	0	0	0	0	0	0
		Human night	24	24	24	24	24	24	24	24	24	24	24	24
		HBR (b/h/n)	0	0	0	0	0	0	0	0	0	0	0	0

N of *An. gamb*: Number of *Anopheles gambiae* s.l., b/h/n: bite/human/night

TABLE D2: HBR, HLC, BITING TIME, CONTROL SITES, FORMER IRS SITE

			TIMES											
Sites	Locations	Parameters	6-7 pm	7-8 pm	8-9 pm	9-10 pm	10-11 pm	11-12 pm	12-01 am	01-02 am	02-03 am	03-04am	04-05 am	05-06 am
	Indoor	N of *An. gamb*	4	10	16	9	26	35	72	70	70	78	58	59
		Human night	24	24	24	24	24	24	24	24	24	24	24	24
Kati		HBR (b/h/n)	0.2	0.4	0.7	0.4	1.1	1.5	3	2.9	2.9	3.3	2.4	2.5
Control site 1	Outdoor	N of *An. gamb*	3	13	12	28	30	51	59	63	50	54	54	46
		Human night	24	24	24	24	24	24	24	24	24	24	24	24
		HBR (b/h/n)	0.1	0.5	0.5	1.2	1.3	2.1	2.5	2.6	2.1	2.3	2.3	1.9
	Indoor	N of *An. gamb*	13	10	9	13	9	16	23	30	37	37	32	21
		Human night	24	24	24	24	24	24	24	24	24	24	24	24
Dioila		HBR (b/h/n)	0.5	0.4	0.4	0.5	0.4	0.7	1	1.3	1.5	1.5	1.3	0.9
Control site 2	Outdoor	N of *An. gamb*	7	4	11	3	11	13	26	33	39	40	28	27
		Human night	24	24	24	24	24	24	24	24	24	24	24	24
		HBR (b/h/n)	0.3	0.2	0.5	0.1	0.5	0.5	1.1	1.4	1.6	1.7	1.2	1.1
	Indoor	N of *An. gamb*	2	4	7	6	5	4	3	8	8	0	6	0
		Human night	24	24	24	24	24	24	24	24	24	24	24	24
Segou		HBR (b/h/n)	0.1	0.2	0.3	0.3	0.2	0.2	0.1	0.3	0.3	0	0.3	0
Control site 3	Outdoor	N of *An. gamb*	2	0	8	3	5	2	3	8	8	0	0	0
		Human night	24	24	24	24	24	24	24	24	24	24	24	24
		HBR (b/h/n)	0.1	0	0.3	0.1	0.2	0.1	0.1	0.3	0.3	0	0	0
	Indoor	N of *An. gamb*	2	2	1	2	3	1	8	7	11	4	7	4
		Human night	24	24	24	24	24	24	24	24	24	24	24	24
Bla		HBR (b/h/n)	0.1	0.1	0	0.1	0.1	0	0.3	0.3	0.5	0.2	0.3	0.2
Former IRS site	Outdoor	N of *An. gamb*	0	0	0	1	3	3	2	4	9	10	11	10
		Human night	24	24	24	24	24	24	24	24	24	24	24	24
		HBR (b/h/n)	0	0	0	0.04	0.1	0.1	0.1	0.2	0.4	0.4	0.5	0.4

N of *An. gamb*: Number of *Anopheles gambiae* s.l., b/h/n: bite/human/night

ANNEX E: SPOROZOITE INDEX IN *AN. GAMBIAE* S.L.

TABLE E1: SPOROZOITE INDEX IN *AN. GAMBIAE* S.L., MONTHLY COLLECTIONS, ALL SITES, 2016

		Pre IRS	Post IRS						Pre+ Post IRS	
SITES	**Parameters**	**July**	**August**	**September**	**October**	**November**	**December**	**Total Post-IRS**	**Grand Total**	**CI**
Koulikoro	N tested	68	60	60	80	60	44	304	372	
IRS site 1	N positive	0	0	0	2	2	1	5	5	
	SI (%)	0	0	0	2.5	3.3	2.3	1.6	1.3	[0.2-2.5]
Fana	N tested	26	60	59	20	22	5	166	192	
IRS site 2	N positive	0	0	2	1	1	1	5	5	
	SI (%)	0	0	3.4	5	4.5	20	3	2.6	[0.4-4.9]
Baroueli	N tested	0	0	0	0	0	0	0	0	
IRS site 3	N positive	0	0	0	0	0	0	0	0	
	SI (%)	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Kati	N tested	123	50	58	70	60	13	251	374	
Control site 1	N positive	0	0	1	0	0	0	1	1	
	SI (%)	0	0	1.7	0	0	0	0.4	0.3	[0-0.8]
Dioila	N tested	33	60	60	70	30	3	223	256	
Control site 2	N positive	1	2	7	4	0	0	13	14	
	SI (%)	3	3.3	11.7	5.7	0	0	5.8	5.5	[2.7-8.3]
Ségou	N tested	6	60	50	0	0	0	110	116	
Control site 3	N positive	0	2	1	n/a	n/a	n/a	3	3	
	SI (%)	0	3.3	2	n/a	n/a	n/a	2.7	2.6	[0-5.5]
Bla	N tested	94	60	60	69	60	13	262	356	
Former IRS site	N positive	5	0	2	0	0	0	2	7	
	SI (%)	5.3	0	3.3	0	0	0	0.8	2	[0.5-5.4]
Grand Total	N tested	94	120	119	100	82	49	470	564	
IRS sites	N positive	0	0	2	3	3	2	10	10	
	SI (%)	0	0	1.7	3	3.7	4.1	2.1	1.8	[0.7-2.9]
Grand Total	N tested	162	170	168	140	90	16	584	746	
Control sites	N positive	1	4	9	4	0	0	17	18	
	SI (%)	0.6	2.4	5.4	2.9	0	0	2.9	2.4	[1.3-3.5]

N: Number of *An. gambiae* s.l., SI: sporozoite index, n/a: result not available

ANNEX F: ENTOMOLOGICAL INOCULATION RATE (EIR)

TABLE F1: EIR, MONTHLY COLLECTIONS, ALL SITES, 2016

		Pre IRS	Post IRS					Pre+ Post IRS
Sites	Parameters	July	August	September	October	November	December	Total Post-IRS	Grand Total
Koulikoro IRS site 1	HBR (b/h/n)	8	17	26	10.8	3.9	3.1		
	SI (%)	0	0	0	2.5	3.3	2.3		
	EIR (ib/h/n)	0	0	0	0.3	0.1	0.1		
	EIR (period)	0	0	0	8.1	3.9	2.1	14.1	14.1
Fana IRS site 2	HBR (b/h/n)	2.6	26	5.6	0.5	0.4	0		
	SI (%)	0	0	3.4	5	4.5	20		
	EIR (ib/h/n)	0	0	0.2	0.02	0	0		
	EIR (period)	0	0	5.7	0.8	0.5	0	7	7
Baroueli IRS site 3	HBR (b/h/n)	0	0	0	0	0	0		
	SI (%)	n/a	n/a	n/a	n/a	n/a	n/a		
	EIR (ib/h/n)	0	0	0	0	0	0		
	EIR (ib/h/n)	0	0	0	0	0	0	0	0
Kati Control site 1	HBR (b/h/n)	10.9	55.9	36.5	10.4	6.4	1.3		
	SI (%)	0	0	1.7	0	0	0		
	EIR (ib/h/n)	0	0	0.6	0	0	0		
	EIR (period)	0	0	18.6	0	0	0	18.6	18.6
Dioila Control site 2	HBR (b/h/n)	4.5	34.4	19.5	2.4	0.8	0		
	SI (%)	3	3.3	11.7	5.7	0	0		
	EIR (ib/h/n)	0.1	1.1	2.3	0.1	0	0		
	EIR (period)	4.1	34.1	68.3	4.1	0	0	106.5	110.6
Ségou Control site 3	HBR (b/h/n)	0.1	10.3	1.1	0	0	0		
	SI (%)	0	3.3	2	n/a	n/a	n/a		
	EIR (ib/h/n)	0	0.3	0	0	0	0		
	EIR (period)	0	10.2	0.7	0	0	0	10.9	10.9
Bla Former IRS site	HBR (b/h/n)	0.8	9.4	1.8	0.4	0.6	0.3		
	SI (%)	5.3	0	3.3	0	0	0		
	EIR (ib/h/n)	0.04	0	0.06	0	0	0		
	EIR (period)	1.2	0	1.8	0	0	0	1.8	3
Grand Total IRS sites	EIR (ib/h/n)	0	0	0.07	0.11	0.03	0.03		
	EIR (period)	0	0	1.9	2.97	1.47	0.7	7.04	7.04
Grand Total Control sites	EIR (ib/h/n)	0.03	0.47	0.97	0.03	0	0		
	EIR (period)	1.37	14.77	29.2	1.37	0	0	45.34	46.71

HBR: human biting rate, SI: sporozoite index, EIR: entomological inoculation rate, ib/h/n: infective bite/h/night

ANNEX G: PARITY RATE IN *AN. GAMBIAE* S.L.

TABLE G1: PARITY RATE, MONTHLY COLLECTIONS, ALL SITES, 2016

		Pre IRS	Post IRS						
Sites	**Parameters**	**July**	**August**	**September**	**October**	**November**	**December**	**Total Post-IRS**	**(Pre+ Post -IRS)**
Koulikoro IRS site 1	N tested	38	103	144	56	29	21	353	391
	N parous	21	76	123	49	23	17	288	309
	Parity rate (%)	55.3	73.8	85.4	87.5	79.3	81	81.6	79
	CI	[39.5-71.1]	[65.3-82.3]	[79.7-91.2]	[78.8-96.2]	[64.6-94.1]	[64.2-97.7]	[77.5-85.6]	[75-83.1]
Fana IRS site 2	N tested	7	94	63	9	12	2	180	187
	N parous	6	4	62	9	8	2	85	91
	Parity rate (%)	85.7	4.3	98.4	100	66.7	100	47.2	48.7
	CI	[59.8-111.6]	[0.2-8.3]	[95.3-100]	n/a	[40-93.3]	n/a	[39.9-54.5]	[41.5-55.8]
Baroueli IRS site 3	N tested	1	8	6	1	3	0	18	19
	N parous	1	7	5	1	3	0	16	17
	Parity rate (%)	100	87.5	83.3	100	100	n/a	88.9	89.5
	CI	n/a	[64.6-100]	[53.5-100]	n/a	n/a	n/a	[74.4-100]	[75.7-100]
Kati Control site 1	N tested	64	298	237	66	32	5	638	702
	N parous	28	176	45	40	28	3	292	320
	Parity rate (%)	43.8	59.1	19	60.6	87.5	60	45.8	45.6
	CI	[31.6-55.9]	[53.5-64.6]	[14-24]	[48.8-72.4]	[76-99]	[17.1-100]	[41.9-49.6]	[41.9-49.3]
Dioila Control site 2	N tested	33	180	105	39	14	0	338	371
	N parous	28	45	98	38	11	0	192	220
	Parity rate (%)	84.8	25	93.3	97.4	78.6	n/a	56.8	59.3
	CI	[72.6-97.1]	[18.7-31.3]	[88.6-98.1]	[92.5-100]	[57.1-100]	n/a	[51.5-62.1]	[54.3-64.3]
Ségou Control site 3	N tested	5	136	28	0	0	0	164	169
	N parous	4	80	27	0	0	0	107	111
	Parity rate (%)	80	58.8	96.4	n/a	n/a	n/a	65.2	65.7
	CI	[44.9-100]	[50.6-67.1]	[89.6-100]	n/a	n/a	n/a	[58-72.5]	[58.5-72.8]
Bla Former IRS site	N tested	59	95	51	12	13	8	179	238
	N parous	55	23	43	12	11	5	94	149
	Parity rate (%)	93.2	24.2	84.3	100	84.6	62.5	52.5	62.6
	CI	[86.8-99.6]	[15.6-32.8]	[74.3-94.3]	n/a	[65-100]	[29-96]	[45.2-59.8]	[56.5-68.8]
Grand Total IRS sites	N tested	46	205	213	66	44	23	551	597
	N parous	28	87	190	59	34	19	389	417
	Parity rate (%)	60.9	42.4	89.2	89.4	77.3	82.6	70.6	69.8
	CI	[46.8-75]	[35.7-49.2]	[85-93.4]	[82-96.8]	[64.9-89.7]	[67.1-98.1]	[66.8-74.4]	[66.2-73.5]
Grand Total Control sites	N tested	102	614	370	105	46	5	1140	1242
	N parous	60	301	170	78	39	3	591	651
	Parity rate (%)	58.8	49	45.9	74.3	84.8	60	51.8	52.4
	CI	[49.3-68.4]	[45.1-53]	[40.98-51]	[65.9-82.6]	[74.4-95.2]	[17.1-100]	[48.9-54.7]	[49.6-55.2]

N: number of *An. gambiae* s.l., CI: confidence interval, n/a: result not available

ANNEX H: BLOOD MEAL ORIGIN

TABLE H1: BLOOD MEAL ORIGIN, MONTHLY COLLECTIONS, ALL SITES, 2016

			Human +		Bovine +		Human/Bovine +		Others	
Sites	Sampling months	N Tested	N	%	N	%	N	%	N	%
Koulikoro IRS site 1	July	12	0	0	0	0	0	0	12	100
	August	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
	September	14	0	0	0	0	0	0	14	100
	October	24	0	0	0	0	0	0	24	100
	Total	50	0	0	0	0	0	0	50	100
Fana IRS site 2	July	4	0	0	0	0	0	0	4	100
	August	21	1	4.8	0	0	0	0	20	95.2
	September	22	2	9.1	0	0	0	0	20	90.9
	October	3	0	0	0	0	0	0	3	100
	Total	50	3	6	0	0	0	0	47	94
Baroueli IRS site 3	July	5	0	0	0	0	0	0	5	100
	August	11	0	0	0	0	0	0	11	100
	September	18	0	0	0	0	0	0	18	100
	October	16	0	0	0	0	0	0	16	100
	Total	50	0	0	0	0	0	0	50	100
Kati Control site 1	July	15	1	6.7	0	0	0	0	14	93.3
	August	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
	September	20	0	0	0	0	0	0	20	100
	October	15	1	6.7	0	0	0	0	14	93.3
	Total	50	2	4.0	0	0	0	0	48	96
Dioila Control site 2	July	15	0	0	0	0	1	6.7	14	93.3
	August	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
	September	20	0	0	3	15.0	0	0	17	85.0
	October	15	9	60	0	0	0	0	6	40
	Total	50	9	18	3	6	1	2	37	74
Ségou Control site 3	July	3	3	100	0	0	0	0	0	0
	August	22	3	13.6	2	9.1	2	9.1	15	68.2
	September	16	0	0	0	0	0	0	16	100
	October	9	0	0	0	0	0	0	9	100
	Total	50	6	12	2	4	2	4	40	80
Bla Control site 4	July	18	1	5.6	0	0	0	0	17	94.4
	August	17	0	0	10	58.8	0	0	7	41.2
	September	9	1	11.1	1	11.1	0	0	7	77.8
	October	6	2	33.3	2	33.3	1	16.7	1	16.7
	Total	50	4	8	13	26	1	2	32	64
Grand Total IRS sites	July	21	0	0	0	0	0	0	21	100
	August	32	1	3.1	0	0	0	0	31	96.9
	September	54	2	3.7	0	0	0	0	52	96.3
	October	43	0	0	0	0	0	0	43	100
	Total	150	3	2	0	0	0	0	147	98
Grand Total Control sites	July	51	5	9.8	0	0	1	2.0	45	88.2
	August	39	3	7.7	12	30.8	2	5.1	22	56.4
	September	65	1	1.5	4	6.2	0	0	60	92.3
	October	45	12	26.7	2	4.4	1	2.2	30	66.7
	Total	200	21	10.5	18	9	4	2	157	78.5

N: Number of *An. gambiae* s.l. tested, July: Pre-IRS, August-September-October: Post-IRS

ANNEX I: CONE TESTS RESULTS

TABLE II: WHO CONE TESTS RESULTS, *AN. GAMBIAE* KISUMU STRAIN MORTALITY AFTER 30 MINUTES EXPOSURE TO PIRIMIPHOS-METHYL 300 CS; KOULIKORO, FANA AND BAROUÉLI (JULY-DECEMBER 2016)

Substrates	Parameters	T0 (Jul)	T1 (Aug)	T2 (Sept)	T3 (Oct)	T4 (Nov)	T5 (Dec)
Mud	N tested	285	272	283	287	285	287
	N Dead	279	267	279	205	224	179
	Mortality (%)	97.9	98.2	98.6	71.4	78.6	62.4
Mud+kaolin	N tested	171	179	173	168	180	172
	N Dead	171	158	164	141	137	135
	Mortality (%)	100	88.3	94.8	83.9	76.1	78.5
Cement	N tested	144	141	141	141	144	148
	N Dead	144	141	135	100	95	106
	Mortality (%)	100	100	95.7	70.9	66	71.6
Painted cement	N tested	72	71	71	65	74	74
	N Dead	72	71	67	47	53	51
	Mortality (%)	100	100	94.4	72.3	71.6	68.9

N: Number of *An. gambiae* s.l., Jul: July, Aug: august, Sept: September, Oct: October, Nov: November, Dec: December

ANNEX J: WHO SUSCEPTIBILITY TUBE TESTS RESULTS

TABLE J1: PIRIMIPHOSMETHYL IR TESTS RESULTS AFTER 2016 IRS

District	Insecticide Tested	Number of Mosquitoes Exposed	Number of Dead	Test Mortality (%)	Corrected Mortality(%)
Kita	Pirimiphos-methyl	100	100	100	-
Fana	Pirimiphos-methyl	100	100	100	-
Koulikoro	Pirimiphos-methyl	103	103	100	-
Kati	Pirimiphos-methyl	100	100	100	-
Bamako	Pirimiphos-methyl	100	100	100	-
Bla	Pirimiphos-methyl	100	99	99	-
Baroueli	Pirimiphos-methyl	100	100	100	-
Niono	Pirimiphos-methyl	75	37	49.3	-
Selingue	Pirimiphos-methyl	100	100	100	-
Bougouni	Pirimiphos-methyl	81	81	100	-
Kadiolo	Pirimiphos-methyl	100	100	100	-
Djenne	Pirimiphos-methyl	94	94	100	-
Bandigara	Pirimiphos-methyl	104	104	100	-
Bankass	Pirimiphos-methyl	104	104	100	-

TABLE J2: PERMETHRIN IR TESTS RESULTS AFTER 2016 IRS

District	Insecticide Tested	Number of Mosquitoes Exposed	Number of Dead	Test Mortality (%)	Corrected Mortality(%)
Kita	Permethrin	100	74	74	-
Fana	Permethrin	100	41	41	-
Koulikoro	Permethrin	100	19	19	-
Kati	Permethrin	100	6	6	-
Bamako	Permethrin	100	50	50	-
Bla	Permethrin	95	39	41.1	-
Baroueli	Permethrin	100	11	11	-
Niono	Permethrin	n/a	n/a	n/a	-
Selingue	Permethrin	99	33	33.3	-
Bougouni	Permethrin	100	67	67	-
Kadiolo	Permethrin	100	21	21	-
Djenne	Permethrin	100	14	14	-
Bandigara	Permethrin	100	58	58	-
Bankass	Permethrin	100	64	64	-

n/a: result not available

TABLE J3: DELTAMETHRIN IR TESTS RESULTS AFTER 2016 IRS

District	Insecticide Tested	Number of Mosquitoes Exposed	Number of Dead	Test Mortality (%)	Corrected Mortality(%)
Kita	Deltamethrin	100	95	95	-
Fana	Deltamethrin	100	52	52	-
Koulikoro	Deltamethrin	100	85	85	-
Kati	Deltamethrin	100	65	65	-
Bamako	Deltamethrin	100	50	50	-
Bla	Deltamethrin	100	64	64	-
Baroueli	Deltamethrin	100	81	81	-
Niono	Deltamethrin	75	15	20	-
Selingue	Deltamethrin	100	40	40	-
Bougouni	Deltamethrin	103	78	75,7	-
Kadiolo	Deltamethrin	100	47	47	-
Djenne	Deltamethrin	100	28	28	-
Bandigara	Deltamethrin	104	93	89,4	-
Bankass	Deltamethrin	98	91	92,9	-

TABLE J4: BENDIOCARB IR TESTS RESULTS AFTER 2016 IRS

District	Insecticide Tested	Number of Mosquitoes Exposed	Number of Dead	Test Mortality (%)	Corrected Mortality(%)
Kita	Bendiocarb	100	100	100	-
Fana	Bendiocarb	100	100	100	-
Koulikoro	Bendiocarb	104	103	99,0	-
Kati	Bendiocarb	100	98	98,0	-
Bamako	Bendiocarb	100	98	98,0	-
Bla	Bendiocarb	100	100	100	-
Baroueli	Bendiocarb	100	98	98,0	-
Niono	Bendiocarb	75	71	94,7	-
Selingue	Bendiocarb	98	98	100	-
Bougouni	Bendiocarb	100	92	92,0	-
Kadiolo	Bendiocarb	100	100	100	-
Djenne	Bendiocarb	100	100	100	-
Bandigara	Bendiocarb	104	104	100	-
Bankass	Bendiocarb	104	104	100	-

TABLE J5: DDT IR TESTS RESULTS AFTER 2016 IRS

District	Insecticide Tested	Number of Mosquitoes Exposed	Number of Dead	Test Mortality (%)	Corrected Mortality(%)
Kita	DDT	100	22	22	-
Fana	DDT	100	11	11	-
Koulikoro	DDT	104	3	3	-
Kati	DDT	100	4	4	-
Bamako	DDT	100	17	17	-
Bla	DDT	100	21	21	-
Baroueli	DDT	100	11	11	-
Niono	DDT	n/a	n/a	n/a	-
Selingue	DDT	100	13	13	-
Bougouni	DDT	100	12	12	-
Kadiolo	DDT	100	10	10	-
Djenne	DDT	100	11	11	-
Bandigara	DDT	100	37	37	-
Bankass	DDT	100	30	30	-

n/a: result not available

5.11 ANNEX K: *KDR* L1014F RESISTANCE GENOTYPES AND FREQUENCY IN *AN. GAMBIAE* S.L. SIBLING SPECIES

TABLE K1: *KDR* L1014F RESISTANCE GENOTYPES AND FREQUENCY, KOULIKORO, KATI AND BOUGOUNI

Site: Koulikoro	*An. coluzzii*				*coluzzii*x *gambiae*Hybrids				*An. gambiaes.s*				*An. arabiensis*			
	L1014F Genotypes			*f(kdr)*	L1014Fgenotypes			*f(kdr)*	L1014F genotypes			*f(kdr)*	L1014F genotypes			*f(kdr)*
	RR	RS	SS		RR	RS	SS		RR	RS	SS		RR	RS	SS	
Permethrin Alive	15	0	0	**1**	0	0	0	**n/a**	2	0	0	**1**	0	0	0	**n/a**
Permethrin Dead	10	1	0	**0.95**	0	0	0	**n/a**	1	0	0	**1**	0	0	0	**n/a**
Deltamethrin Alive	6	0	0	**1**	0	0	0	**n/a**	0	0	0	**n/a**	0	0	0	**n/a**
Deltamethrin Dead	8	0	0	**1**	1	0	0	**1**	1	0	1	**0.50**	0	0	0	**n/a**
Total	39	1	0	**0.99**	1	0	0	**1**	4	0	1	**0.80**	0	0	0	**n/a**
Site: Kati	***An. coluzzii***				***coluzzii*x *gambiae*Hybrids**				***An. gambiaes.s***				***An. arabiensis***			
	L1014F Genotypes			***f(kdr)***	**L1014Fgenotypes**			***f(kdr)***	**L1014F genotypes**			***f(kdr)***	**L1014F genotypes**			***f(kdr)***
Site: Kati	**RR**	**RS**	**SS**		**RR**	**RS**	**SS**		**RR**	**RS**	**SS**		**RR**	**RS**	**SS**	
Permethrin Alive	9	4	1	**0.79**	1	0	0	**1**	0	0	3	**0**	0	0	0	**n/a**
Permethrin Dead	2	0	0	**1**	0	0	0	**n/a**	0	0	1	**0**	0	0	0	**n/a**
Deltamethrin Alive	13	1	0	**0.96**	0	0	0	**n/a**	0	0	0	**n/a**	0	0	0	**n/a**
Deltamethrin Dead	8	4	0	**0.83**	0	0	0	**n/a**	0	0	0	**n/a**	0	0	0	**n/a**
Total	32	9	1	**0.87**	1	0	0	**1**	0	0	4	**0**	0	0	0	**n/a**
Site: Bougouni	***An. coluzzii***				***coluzzii*x *gambiae*Hybrids**				***An. gambiaes.s***				***An. arabiensis***			
	L1014F Genotypes			***f(kdr)***	**L1014Fgenotypes**			***f(kdr)***	**L1014F genotypes**			***f(kdr)***	**L1014F genotypes**			***f(kdr)***
	RR	**RS**	**SS**		**RR**	**RS**	**SS**		**RR**	**RS**	**SS**		**RR**	**RS**	**SS**	
Permethrin Alive	6	0	0	**1**	0	0	0	**n/a**	5	0	0	**1**	0	0	0	**n/a**
Permethrin Dead	0	0	0	**n/a**	0	0	0	**n/a**	12	0	0	**1**	0	0	0	**n/a**
Deltamethrin Alive	2	0	0	**1**	1	0	0	**1**	9	1	0	**0.95**	0	0	0	**n/a**
Deltamethrin Dead	1	0	0	**1**	2	0	0	**1**	9	0	0	**1**	0	0	0	**n/a**
Total	9	0	0	**1**	3	0	0	**1**	35	1	0	**0.99**	0	0	0	**n/a**

n/a: Not Applicable

TABLE K2: *KDR* L1014F RESISTANCE GENOTYPES AND FREQUENCY, BANKASS, BANDIAGARA AND BLA

Site: Bankass	*An. coluzzii*				*coluzzii* x *gambiae* Hybrids				*An. gambiaes.s*				*An. arabiensis*			
	L1014F Genotypes			*f(kdr)*	L1014F genotypes			*f(kdr)*	L1014F genotypes			*f(kdr)*	L1014F genotypes			*f(kdr)*
	RR	RS	SS		RR	RS	SS		RR	RS	SS		RR	RS	SS	
Permethrin Alive	4	3	1	**0.69**	0	0	0	**n/a**	1	0	0	**1**	0	0	1	**0**
Permethrin Dead	6	0	0	**1**	2	0	1	**0.67**	3	0	2	**0.60**	2	0	5	**0.29**
Deltamethrin Alive	2	0	1	**0.67**	0	0	0	**n/a**	1	0	0	**1**	0	0	0	**n/a**
Deltamethrin Dead	3	0	0	**1**	1	1	1	**0.50**	2	0	0	**1**	0	0	3	**0**
Total	15	3	2	**0.83**	3	1	2	**0.58**	7	0	2	**0.78**	2	0	9	**0.18**
Site: Bandiagara	*An. coluzzii*				*coluzzii* x *gambiae* Hybrids				*An. gambiaes.s*				*An. arabiensis*			
	L1014F Genotypes			*f(kdr)*	L1014F genotypes			*f(kdr)*	L1014F genotypes			*f(kdr)*	L1014F genotypes			*f(kdr)*
	RR	RS	SS		RR	RS	SS		RR	RS	SS		RR	RS	SS	
Permethrin Alive	7	0	0	**1**	0	0	0	**n/a**	11	0	0	**1**	0	0	0	**n/a**
Permethrin Dead	4	5	0	**0.72**	1	0	0	**1**	0	0	2	**0**	0	0	0	**n/a**
Deltamethrin Alive	6	0	0	**1**	0	0	0	**n/a**	1	0	0	**1**	0	0	0	**n/a**
Deltamethrin Dead	8	2	1	**0.82**	1	0	0	**1**	0	0	1	**0**	0	0	0	**n/a**
Total	25	7	1	**0.86**	2	0	0	**1**	12	0	3	**0.80**	0	0	0	**n/a**
Site: Bla	*An. coluzzii*				*coluzzii* x *gambiae* Hybrids				*An. gambiaes.s*				*An. arabiensis*			
	L1014F Genotypes			*f(kdr)*	L1014F genotypes			*f(kdr)*	L1014F genotypes			*f(kdr)*	L1014F genotypes			*f(kdr)*
	RR	RS	SS		RR	RS	SS		RR	RS	SS		RR	RS	SS	
Permethrin Alive	3	3	0	**0.75**	0	0	0	**n/a**	0	0	0	**n/a**	0	0	0	**n/a**
Permethrin Dead	3	7	1	**0.59**	0	0	1	**0**	0	0	3	**0**	0	0	0	**n/a**
Deltamethrin Alive	4	5	1	**0.65**	0	0	0	**n/a**	2	0	0	**1**	0	0	0	**n/a**
Deltamethrin Dead	4	7	1	**0.63**	0	0	0	**n/a**	2	0	0	**1**	0	0	0	**n/a**
Total	14	22	3	**0.64**	0	0	1	**0**	4	0	3	**0.57**	0	0	0	**n/a**

n/a: Not Applicable

TABLE K3: *KDR* L1014F RESISTANCE GENOTYPES AND FREQUENCY, SELINGUE, BAMAKO AND NIONO

Site: Selingue	*An. coluzzii*				*coluzzii*x *gambiae*Hybrids				*An. gambiaes.s*				*An. arabiensis*			
	L1014F Genotypes			*f(kdr)*	L1014Fgenotypes			*f(kdr)*	L1014F genotypes			*f(kdr)*	L1014F genotypes			*f(kdr)*
	RR	RS	SS		RR	RS	SS		RR	RS	SS		RR	RS	SS	
Permethrin Dead & Alive	14	0	0	**1**	4	0	0	**1**	0	0	0	**n/a**	0	0	0	**n/a**
DeltamethrinDead & Alive	9	0	0	**1**	10	0	0	**1**	0	0	0	**n/a**	0	0	0	**n/a**
Total	23	0	0	**1**	14	0	0	**1**	0	0	0	**n/a**	0	0	0	**n/a**
Site: Bamako	*An. coluzzii*				*coluzzii*x *gambiae*Hybrids				*An. gambiaes.s*				*An. arabiensis*			
	L1014F Genotypes			*f(kdr)*	L1014Fgenotypes			*f(kdr)*	L1014F genotypes			*f(kdr)*	L1014F genotypes			*f(kdr)*
	RR	RS	SS		RR	RS	SS		RR	RS	SS		RR	RS	SS	
Permethrin Alive	14	0	0	**1**	2	0	0	**1**	2	0	0	**1**	0	0	0	**n/a**
Permethrin Dead	0	0	0	**n/a**	0	0	0	**n/a**	0	0	0	**n/a**	0	0	0	**n/a**
Deltamethrin Alive	9	0	0	**1**	4	0	0	**1**	0	0	0	**n/a**	0	0	0	**n/a**
Deltamethrin Dead	14	0	0	**1**	3	0	0	**1**	0	0	0	**n/a**	0	0	0	**n/a**
Total	37	0	0	**1**	9	0	0	**1**	2	0	0	**1**	0	0	0	**n/a**
Site: Niono	*An. coluzzii*				*coluzzii*x *gambiae*Hybrids				*An. gambiaes.s*				*An. arabiensis*			
	L1014F Genotypes			*f(kdr)*	L1014Fgenotypes			*f(kdr)*	L1014F genotypes			*f(kdr)*	L1014F genotypes			*f(kdr)*
	RR	RS	SS		RR	RS	SS		RR	RS	SS		RR	RS	SS	
Bendiocarb Alive	1	0	0	**1**	0	0	0	**n/a**	0	0	0	**n/a**	0	0	0	**n/a**
Pyrimiphos-m Alive	3	1	0	**0.88**	2	0	0	**1**	0	0	0	**n/a**	0	0	0	**n/a**
Deltamethrin Alive	18	3	1	**0.89**	3	2	0	**0.80**	0	0	0	**n/a**	0	0	0	**n/a**
Deltamethrin Dead	2	2	0	**0.75**	1	0	0	**1**	0	0	0	**n/a**	0	0	0	**n/a**
Total	24	6	1	**0.87**	6	2	0	**0.88**	0	0	0	**n/a**	0	0	0	**n/a**

n/a: Not Applicable

TABLE K4: *KDR* L1014F RESISTANCE GENOTYPES AND FREQUENCY, KITA, KADIOLO AND DJENNE

Site: Kita	*An. coluzzii*				*coluzziix gambiae*Hybrids				*An. gambiaes.s*				*An. arabiensis*			
	L1014F Genotypes			*f(kdr)*	L1014Fgenotypes			*f(kdr)*	L1014F genotypes			*f(kdr)*	L1014F genotypes			*f(kdr)*
	RR	RS	SS		RR	RS	SS		RR	RS	SS		RR	RS	SS	
Permethrin Alive	3	0	0	**1**	1	0	0	**1**	1	0	0	**1**	1	0	1	**0.50**
Permethrin Dead	0	0	1	**0**	0	0	1	**0**	1	0	4	**0.20**	0	0	0	**n/a**
Deltamethrin Alive	1	0	0	**1**	0	0	0	**n/a**	3	1	1	**0.70**	0	0	1	**0**
Deltamethrin Dead	0	0	0	**n/a**	1	0	1	**0.50**	1	0	0	**1**	0	0	3	**0**
Total	4	0	1	**0.80**	2	0	2	**0.50**	6	1	5	**0.54**	1	0	5	**0.17**
Site: Kadiolo	***An. coluzzii***				***coluzziix gambiae*Hybrids**				***An. gambiaes.s***				***An. arabiensis***			
	L1014F Genotypes			***f(kdr)***	**L1014Fgenotypes**			***f(kdr)***	**L1014F genotypes**			***f(kdr)***	**L1014F genotypes**			***f(kdr)***
	RR	**RS**	**SS**		**RR**	**RS**	**SS**		**RR**	**RS**	**SS**		**RR**	**RS**	**SS**	
Permethrin Alive	5	3	0	**0.81**	0	0	0	**n/a**	4	0	0	**1**	0	0	0	**n/a**
Permethrin Dead	1	4	1	**0.50**	1	1	0	**0.75**	4	0	0	**1**	0	0	0	**n/a**
Deltamethrin Alive	10	0	0	**1**	0	0	0	**n/a**	3	0	0	**1**	0	0	0	**n/a**
Deltamethrin Dead	7	1	1	**0.83**	0	0	0	**n/a**	4	0	0	**1**	0	0	0	**n/a**
Total	23	8	2	**0.82**	1	1	0	**0.75**	15	0	0	**1**	0	0	0	**n/a**
Site: Djenne	***An. coluzzii***				***coluzziix gambiae*Hybrids**				***An. gambiaes.s***				***An. arabiensis***			
	L1014F Genotypes			***f(kdr)***	**L1014Fgenotypes**			***f(kdr)***	**L1014F genotypes**			***f(kdr)***	**L1014F genotypes**			***f(kdr)***
	RR	**RS**	**SS**		**RR**	**RS**	**SS**		**RR**	**RS**	**SS**		**RR**	**RS**	**SS**	
Permethrin Alive	11	0	0	**1**	3	0	0	**1**	0	0	0	**n/a**	0	0	0	**n/a**
Permethrin Dead	3	0	0	**1**	6	0	0	**1**	1	0	0	**1**	0	0	0	**n/a**
Deltamethrin Alive	13	0	0	**1**	0	0	0	**n/a**	0	0	0	**n/a**	0	0	0	**n/a**
Deltamethrin Dead	11	0	0	**1**	1	0	0	**1**	0	0	0	**n/a**	0	0	0	**n/a**
Total	38	0	0	**1**	10	0	0	**1**	1	0	0	**1**	0	0	0	**n/a**

n/a: Not Applicable

TABLE K5: *KDR* L1014F RESISTANCE GENOTYPES AND FREQUENCY, BAROUELI, FANA

Site:Baroueli	*An. coluzzii*				*coluzzii* x *gambiae* Hybrids				*An. gambiaes.s*				*An. arabiensis*			
	L1014F Genotypes			*f(kdr)*	L1014F genotypes			*f(kdr)*	L1014F genotypes			*f(kdr)*	L1014F genotypes			*f(kdr)*
	RR	RS	SS		RR	RS	SS		RR	RS	SS		RR	RS	SS	
Permethrin Alive	0	0	0	**n/a**	0	0	0	**n/a**	5	0	0	**1**	0	0	0	**n/a**
Permethrin Dead	1	0	0	**1**	0	0	0	**n/a**	3	0	0	**1**	0	0	0	**n/a**
Deltamethrin Alive	1	0	0	**1**	0	0	0	**n/a**	6	0	0	**1**	0	0	0	**n/a**
Deltamethrin Dead	1	0	0	**1**	0	0	0	**n/a**	0	0	0	**n/a**	1	0	0	**1**
Total	3	0	0	**1**	0	0	0	**n/a**	14	0	0	**1**	1	0	0	**1**
Site:Fana	*An. coluzzii*				*coluzzii* x *gambiae* Hybrids				*An. An. gambiaes.s*				*An. arabiensis*			
	L1014F Genotypes			*f(kdr)*	L1014F genotypes			*f(kdr)*	L1014F genotypes			*f(kdr)*	L1014F genotypes			*f(kdr)*
	RR	RS	SS		RR	RS	SS		RR	RS	SS		RR	RS	SS	
Permethrin Alive	5	0	0	**1**	1	0	0	**1**	7	0	0	**1**	0	0	1	**0**
Permethrin Dead	1	1	1	**0.50**	2	0	0	**1**	3	0	1	**0.75**	1	0	0	**1**
Deltamethrin Alive	3	0	0	**1**	0	0	0	**n/a**	6	0	0	**1**	0	0	2	**0**
Deltamethrin Dead	2	2	1	**0.60**	0	0	0	**n/a**	6	0	1	**0.86**	0	0	3	**0**
Total	11	3	2	**0.78**	3	0	0	**1**	22	0	2	**0.92**	1	0	6	**0.14**

n/a: Not Applicable

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