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Indoor Residual Spraying (IRS 2) Task Order Six

**AIRS MALI  
ENTOMOLOGICAL  
MONITORING**

**FINAL REPORT**

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# ACRONYMS

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<b>f(Ace-IR)</b>	Frequency of the Acetylcholinesterase-I Resistant Gene
<b>AIRS</b>	Africa Indoor Residual Spraying
<b>CDC</b>	Centers for Disease Control and Prevention
<b>CREC</b>	Entomological Research Center of Cotonou
<b>CSP</b>	Circumsporozoite Protein
<b>ELISA</b>	Enzyme-Linked Immunosorbent Assay
<b>HBR</b>	Human Biting Rate
<b>HLC</b>	Human Landing Catch
<b>IRS</b>	Indoor Residual Spraying
<b>KDR</b>	Knock Down Resistance
<b>LBMA</b>	<i>Laboratoire de Biologie Moléculaire Appliquée</i>
<b>NMCP</b>	National Malaria Control Program
<b>PCR</b>	Polymerase Chain Reaction
<b>PMI</b>	President's Malaria Initiative
<b>PSC</b>	Pyrethrum Spray Catch
<b>RR</b>	Homozygous Resistant
<b>RS</b>	Heterozygous Resistant
<b>SI</b>	Sporozoite Index
<b>SS</b>	Homozygous susceptible
<b>SSRBP</b>	Site with Sign of Resistance to Bendiocarb and/or Pirimiphos-methyl
<b>TO</b>	Task Order
<b>USAID</b>	United States Agency for International Development
<b>WHO</b>	World Health Organization





# EXECUTIVE SUMMARY

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This report is the summary of all entomological data collected in 2017 by AIRS Mali.

Lab training to improve the quality of molecular results was conducted for staff from Laboratoire de Biologie Moléculaire Appliquée (LBMA) from June 11-23, 2017. Later, a regional lab training was carried out for two weeks in Cotonou with two participants from LBMA. A follow up of these two trainings was performed in Mali by the University of Notre Dame from December 4-8, 2017 and indicated that good progress had been made in terms of quality of the data provided by molecular analyses, particularly for molecular species identification and sporozoite Enzyme Linked Immuno Sorbent Assay (ELISA).

World Health Organization (WHO) tube tests performed under the framework of the national insecticide resistance monitoring plan revealed full susceptibility to pirimiphos-methyl 0.25% in all sites. Several sites showed resistance or possible resistance to bendiocarb. Resistance to permethrin 0.75%, alphacypermethrin 0.05%, and deltamethrin 0.05% was recorded in all 15 surveyed sites. The intensity of resistance was generally higher to permethrin than deltamethrin. The intensity of resistance to these two pyrethroids is strong—there were survivors in all sites at 10 times the diagnostic dosage. Data showed mortality rates in the four IRS sites with clothianidin 13.2 mg/paper of less than or equal to 90 percent after four days and greater than or equal to 98 percent after 6 days, but further optimization of the protocol may be needed. Since the protocol used to test clothianidin is not yet standardized and validated by the WHO, the mortality rates of less than 98 percent that we obtained cannot be interpreted as signs of resistance.

In September (one month after spraying), mortality rates with WHO cone bioassays, using *An. gambiae* Kisumu, were still at 100 percent, regardless of the type of wall. The fumigant air-borne effect of the sprayed insecticide was between 77 and 100 percent depending on the wall substrate. At the end of bioassays, residual duration was three months for mud, and longer for painted substrates such as painted cement, painted mud, and a combination of painted mud and cement. The fumigant effect lasted longer than expected with particularly high mortality rates occurring two months after spraying.

Morphological identification of mosquitoes collected from Human Landing Catch (HLC) and Pyrethrum Spray Catch (PSC) indicated that *An. gambiae* s.l. was the predominant malaria vector (96 percent of the collected vectors) followed by *An. pharoensis*, *An. rufipes*, *An. ziemanni* and *An. funestus*. *An. coluzzii* was the most common species of the *An. gambiae* s.l. complex. Overall, from PSC conducted before IRS (June-July), there was a mean of 6.2 *An. gambiae* s.l. per house in Mopti Region against 1.8 *An. gambiae* s.l. per house after IRS (August-December). In unsprayed former IRS sites, Koulikoro and Barouéli, the mean indoor vector density was 2.1 *An. gambiae* s.l. per house/day in June-July and 5.9 in August-December.

In the Mopti Region, before IRS, the average human biting rate (HBR) was 1.4 b/h/n<sup>1</sup> in Djénné, 1.1 b/h/n in Mopti District, 2.9 b/h/n in Bandiagara and 0.5 b/h/n in Bankass. Post-IRS, the mean HBR ranged from 0.5 b/h/n in Bankass to 7.5 b/h/n in Mopti District. Biting behavior was more pronounced late at night and was similar outdoors (2.9 b/h/n) and indoors (2.4 b/h/n) after IRS. In Koulikoro and Barouéli (former IRS sites), mean biting rates for August to September 2017 were particularly high at 106.3 b/h/n and 13.4 b/h/n, respectively. In Kati, a control site that was not sprayed in either 2016 or 2017, the mean biting rate was 80.5 b/h/n from August to September 2017. Between August and September 2016 and the same period in 2017, there was a 4.9 fold increase in biting rate in Koulikoro (former IRS site) compared to a 1.7 fold increase in Kati (the paired control). Furthermore, after combining the post-IRS data, the mean HBR in August to December was 2.7 b/h/n

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<sup>1</sup>b/h/n = bites per human per night

in IRS sites, 28.7 b/h/n in former IRS sites and 36.5 b/h/n in Kati, a control site. In former IRS sites, from August to December, infection of vectors was 0.8 percent (95% CI: 0.2-1.5) in 2017 as opposed to 1.3 percent in 2016 (95% CI: 0.2-2.5) in 2016;  $p=0.42$ . The entomological inoculation rate was 7.05 ib/h/5 months<sup>2</sup> for these sites in 2016 while it was 15.37 ib/h/5months in 2017, which equates to a two-fold increase after IRS withdrawal. In Kati, the control site, the EIR was 18.6 ib/h/5 months in 2016 while it was 6.21 ib/h/5months in 2017. Furthermore, in IRS sites, the EIR ranged from 1.7 ib/h/5 months in Bankass to 12.2 ib/h/5 months in Mopti. Overall, relocation of IRS may have contributed to an increase in the key indicators of malaria transmission, particularly human biting rates.

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<sup>2</sup> ib/h/5 months = infective bites per human over 5 months

# I. INTRODUCTION

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In 2008, malaria vector control by indoor residual spraying started in Mali with financial support from the President's Malaria Initiative (PMI). At that time, a pyrethroid insecticide (lambdacyhalothrin) was used in Bla and Koulikoro districts, with the addition of Baroueli district in 2011. Given the large expansion of vector resistance to pyrethroids, lambdacyhalothrin was replaced by bendiocarb (carbamate) for IRS in 2012. However, the short residual duration of bendiocarb on sprayed walls led the National Malaria Control Program (NMCP) and its partners to spray Actellic 300CS (organophosphate) starting in 2014. With the support of the UNITAID-funded project NGenIRS, the 2016 campaign was carried out in Fana, in addition to the two districts (Koulikoro and Baroueli) covered by the PMI funding.

The Malaria Indicator Survey conducted in 2015 revealed that the incidence of malaria in the Mopti region was twice that of the national average. On the basis of these data, the NMCP of Mali in collaboration with PMI, decided to relocate IRS operations to Mopti region in 2017. IRS started at the end of July 2017, being timed to provide maximum impact during the peak malaria transmission period.

The 2017 campaign started on July 24, and was implemented in four districts, namely Djenne, Mopti, Bandiagara, and Bankass. Entomological monitoring began in June in seven sites—four IRS sites in Mopti region, two sites where IRS was withdrawn in Koulikoro and Ségou regions, and one unsprayed control site in Koulikoro region. In this report, we present data collected during IRS entomological monitoring carried out from June to December 2017 and nationwide vector resistance surveys.

Specific aims (covered in this final report) were to determine:

- Vector species composition
- Vector indoor resting densities
- Vector biting rates, times and location (indoor or outdoor)
- Sporozoite index (SI) and Entomological Inoculation Rate (EIR)
- Vector blood meal origin
- Quality of spraying and insecticide decay rate
- Susceptibility levels of *An. gambiae* s.l. to four classes of insecticides used in public health and to new vector control products (clothianidin and chlorfenapyr), resistance intensity and frequency of resistance mechanisms.



## 2. METHODOLOGY

### 2.1 STUDY AREA

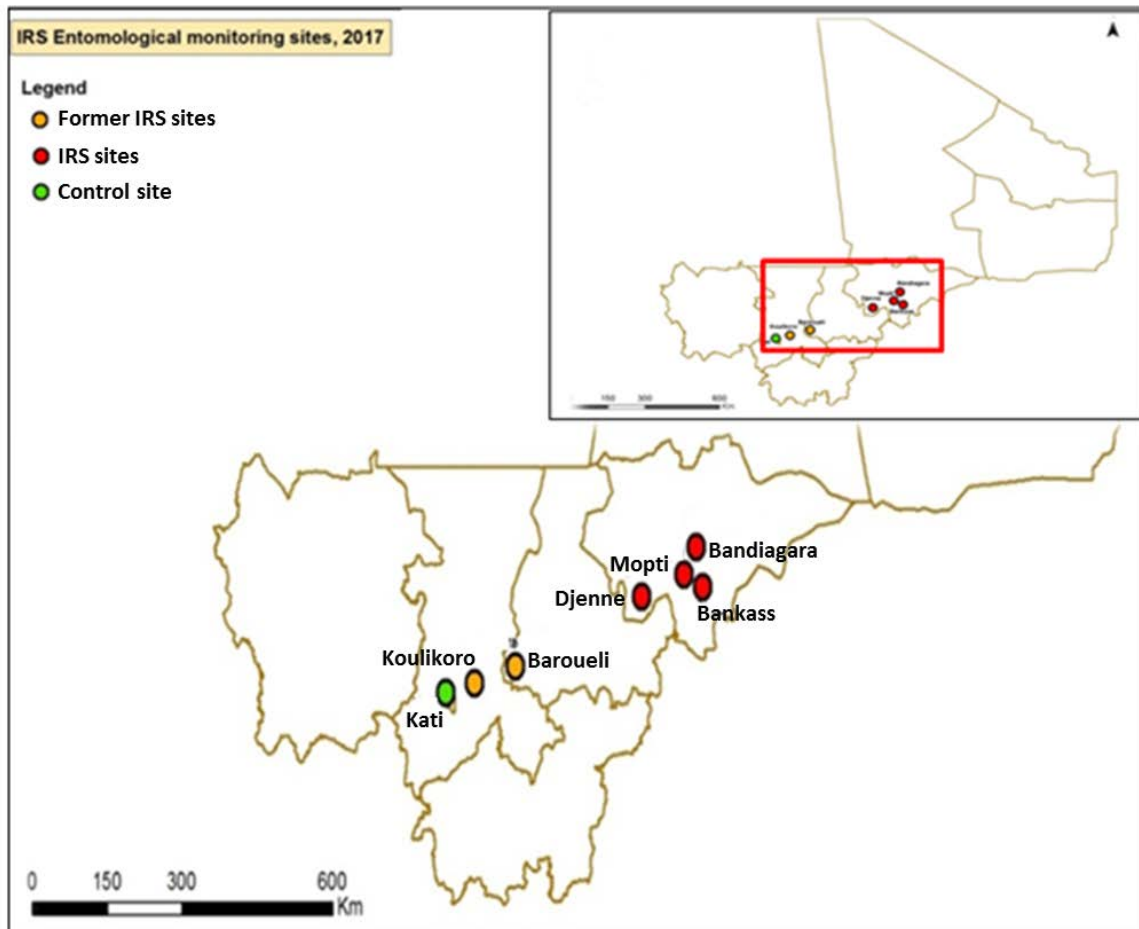
#### 2.1.1 IRS ENTOMOLOGICAL SURVEILLANCE SITES

In 2017, AIRS Mali collected data on key entomological indicators (all PMI basic and most advanced entomological indicators) from surveillance sites in the Mopti region as well as some sites in Koulikoro and Segou from which IRS was withdrawn (Table 1, Figure 1). Monthly surveillance was conducted in seven sentinel sites, four in Mopti and three in Koulikoro/Segou. All four sites in Mopti region are located in the IRS-targeted districts. There were no unsprayed control sites monitored in this area as there were no suitable, neighboring areas due to security concerns (i.e. all 'secure' sites were sprayed). Entomological surveillance was also conducted in Tienfala (Koulikoro) and Diaka Were (Baroueli), where IRS was conducted annually from 2013 to 2016, to monitor for signs of increased sporozoite rates, indoor resting densities, and biting rates.

**Table 1: IRS Entomological Surveillance Sites for 2017**

Region	District	Health Area	Site (village)	Spray Status	Geographic Zone	IRS History
Mopti	Mopti	Tongorongo	Tongorongo	Sprayed	Sahelian	2017 first year of OP IRS
	Badiangara	Badiangara Central	Dandoly	Sprayed		2017 first year of OP IRS
	Bankass	Bankass Central	Bankass	Sprayed		2017 first year of OP IRS
	Djenné	Madiama	Madiama	Sprayed	Sahelian Flooded	2017 first year of OP IRS
Koulikoro	Kati	N'gabakoro-droit	Sala	Unsprayed	Northern Sudanese	Unsprayed 2013-17
	Koulikoro	Tienfala	Tienfala	Sprayed		Carbamate 2013-14 OP 2015-16
Segou	Baroueli	Tigui	Diaka were	Sprayed	Sahelian	Carbamate 2013-14 OP 2015-16

**Figure 1: IRS Entomological Surveillance Sites and Boundaries of the Eight Regions of Mali**



### 2.1.2 NATIONWIDE SITES FOR INSECTICIDE RESISTANCE MAPPING

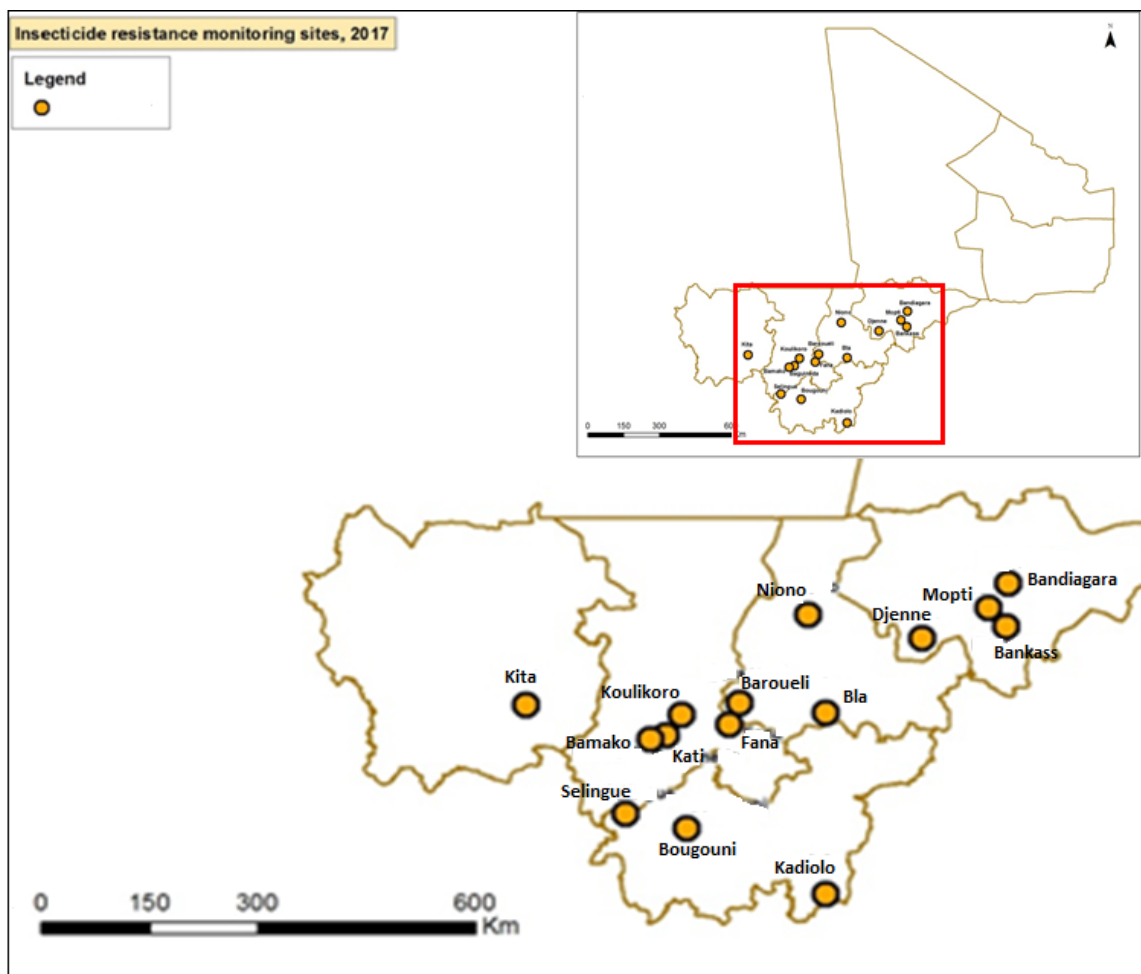
There were 15 resistance monitoring sites nationwide, the majority of which have been used for several years. The 15 sites include the 4 IRS target sites mentioned above and 11 other sentinel sites selected for various reasons shown in Table 2. The nationwide insecticide resistance survey was previously implemented in 2012, 2014, 2015 and 2016. Figure 2 shows the geographical position of each of the 15 sites.

**Table 2: Surveillance Sites Used For Insecticide Resistance Mapping**

Region	Site (District)	Village	Reason for Selection	Geographic Zone
Kayes	Kita	Fourgna Berda/ Banfara	Intense use of insecticides for agriculture	Northern Sudanese
Koulikoro	Koulikoro	Tienfala	Former IRS campaign area	
	Kati	Baguineda	Areas where long-lasting mosquito nets have been distributed and are used. Significant use of irrigation.	Northern Sudanese
	Fana	Gouana	Former IRS campaign area from 2016	Northern Sudanese

Region	Site (District)	Village	Reason for Selection	Geographic Zone
Segou	Niono	Sokourani/ Toumakoro	Significant use of irrigation	Sahelian flooded
	Bla	Tia, Touna	Former IRS campaign area	Sahelian
	Baroueli	Bouadie/Tigui	Former IRS campaign area	
Sikasso	Bougouni	Massabla/Dalabani	Intense use of insecticides for agriculture	South Sudanese
	Yanfolila	Selingue	Significant use of irrigation	South Sudanese Flooded
	Kadiolo	Kadiolo	Intense use of insecticides for agriculture	South Sudanese
Mopti	Mopti	Tongorongou	New IRS campaign area for 2017	Sahelian
	Bandiagara	Dandoly		
	Bankass	Bankass		
	Djenné	Madiama		Sahelian Flooded
Bamako	Commune IV	Djicoroni Para	Areas where long-lasting mosquito nets have been distributed and are used.	Northern Sudanese Suburban

**Figure 2: Insecticide Resistance Surveillance Sites and Boundaries of the Eight Regions of Mali**



## 2.2 HUMAN LANDING CATCH

To determine human biting rates for the main malaria vector (*An. gambiae* s.l.), human landing catches (HLC) were conducted for two consecutive nights each month from 06:00 pm to 06:00 am in four randomly selected houses of each site. The same houses were used each month throughout the collection period (June-December 2017). During each night of HLC, two collectors each equipped with a mouth aspirator and a torch (Figure 3) sat in each house, one indoors and the second outdoors. The indoor collector was positioned in the living room of the house while the outdoor collector was located within 2m of the house. The indoor/outdoor collectors were rotated every hour to compensate for variation in their attractiveness. The following morning, mosquito identification was carried out using the Gillies & de Meillon (1968) key.

Figure 3: Indoor Landing Catch



## 2.3 PYRETHRUM SPRAY CATCH

Spritex aerosol containing tetramethrin 0.3% and permethrin 0.03% was used to perform pyrethrum spray catch (PSC) in 20 houses per site to sample indoor resting mosquitoes. Ten houses per day

Figure 4: House Spraying with Aerosol



processing in a molecular lab.

were visited during two consecutive days per month from 7:00 a.m. to 12:00 p.m. The surveyed houses were the same from June to December 2017. Before the start of PSC, all people, animals and food were removed from the houses. All the openings present in each house were blocked. White sheets were laid on the entire floor and the remaining furniture. Thereafter, a collector sprayed the houses (Figure 4). After spraying, the door was closed for 10-15 minutes. The collectors removed the canvas from the houses and then collected mosquitoes that were fallen. Mosquitoes were put in labeled petri dishes. They were morphologically identified as *Anopheles* and culicines and separated by sex. Abdominal status (unfed, fed, half gravid and gravid) of all female *Anopheles* was determined. Females of *Anopheles gambiae* s.l. were preserved in 1.5 ml labeled Eppendorf tubes containing 70% ethanol for further

## 2.4 WALL CONE BIOASSAY AND FUMIGANT TESTS

To determine the quality of spraying on walls and the decay rate of the insecticide, bioassays were conducted one to two days after IRS followed by a monthly monitoring, using a susceptible colony of *An. gambiae* s.s. Kisumu strain that is reared in Bamako at the AIRS insectary. Mosquitoes were transported monthly by road in cooler boxes for bioassay testing. Cone bioassays were conducted in the four IRS districts (Mopti, Bandiagara, Bankass, and Djenne) according to WHO protocols.

In total, cone bioassays were conducted in 20 structures, which is equal to 5 houses per district. In each sprayed house, Kisumu strain were exposed on a wall at varying heights 0.5 m, 1.0 m, 1.5 m and 2.0m from the floor. A control cone was set on a plywood board outside of each sprayed house in a shaded area near to the house.



Fumigant bioassays were also carried out in each tested house to determine the contribution of airborne effects to overall mortality in cone bioassays. A small wire cage measuring 15cm by 10cm covered with untreated polyester netting material was placed approximately 10cm from a sprayed wall and about 1 meter above the floor. Exposure time was 30 minutes, with mortality subsequently recorded 24 hours later. A parallel control test was performed by exposing the same number of mosquitoes in a wire cage in a neighboring untreated house.

## 2.5 MOSQUITO LARVAE COLLECTIONS AND REARING

From June to September 2017, all 15 insecticide resistance monitoring sites were surveyed by the AIRS Mali team using larval collections. Larvae and pupae of *An. gambiae* s.l. were collected from sites using dippers (Figure 5). Larvae and pupae were sorted by genus and brought back to the field rearing room until the adult stage.

**Figure 5: Mosquito Larvae Collection on the Field**



## 2.6 WHO SUSCEPTIBILITY TUBE TESTS

Four batches of 20 to 25 non-blood-fed females *Anopheles*, aged two to five days were used for the susceptibility tube tests (Figure 6) according to the World Health Organization protocol (WHO, 2016). These tests were performed with permethrin 0.75%, alphacypermethrin 0.05%, deltamethrin 0.05%, bendiocarb 0.1% and pirimiphos-methyl 0.25%. Knock-down was recorded in time intervals (10, 15, 20, 30, 40, 50 and 60 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 the 24-hour mark.

**Figure 6: WHO Susceptibility Tests**



Furthermore, susceptibility tube tests were performed in the four IRS sites using filter papers impregnated with clothianidin, 13.2 mg ai per paper. Papers treated with distilled water were used as negative control. Mortality rates were recorded each day for seven days after the tests. Sumitomo recommends using seven days as the time for recording total mortality.

Dead and live mosquitoes were stored in 1.5 ml Eppendorf tubes containing silica gel for future molecular analyses (polymerase chain reaction (PCR) species, *kdr* 1014F, *kdr* 1014S and *Ace1<sup>R</sup>*).

## 2.7 BOTTLE BIOASSAYS

Insecticide resistance intensity was assessed by the United States' Center for Disease Control and Prevention (CDC) bottle assay which involved coating 250ml bottles with varying concentrations of permethrin or deltamethrin (1x, 2x, 5x, and 10x of the diagnostic concentration).

Chlorfenapyr Technical Grade of the Active Ingredient was received in October and bottle bioassays were conducted at different dosages: 12.5 µg/ml, 25 µg/ml, 50 µg/ml, 100 µg/ml, 200 µg/ml.

The bottles were air-dried overnight and two- to five-day-old mosquitoes were exposed in the treated bottles with mortality monitored every 15 minutes for one hour (Figure 7). The diagnostic time was 30 minutes. A bottle coated with acetone was used as a control.

**Figure 7: Pyrethroid Resistance Intensity Assay with CDC Bottle Tests**



## 2.8 MOLECULAR CHARACTERIZATION OF MOSQUITOES

All molecular analysis was conducted by LBMA, Bamako. Female *Anopheles* were analyzed by PCR for species identification according to the protocol described by Santolamazza et al., 2008. This method allows both identification of *Anopheles* species and molecular forms discrimination. The L1014F and L1014S *kdr* mutations were respectively identified according to the protocol of Huynh et al., (2007). The detection of the *Ace1<sup>R</sup>* mutation was done following the protocol described by Weill et al. (2004).

Adult females collected by HLC were used for circumsporozoite protein (CSP) enzyme-linked immunosorbent assays (ELISA) using the protocol of Wirtz et al. (1987). Blood meal origin was performed according to the PCR protocol of Kent et al. (2005).

## 2.9 DATA ANALYSIS

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

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

The HBR was determined from HLC using the formula:

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

A Chi-square test was used to compare the indoor and the outdoor biting rates.

For the susceptibility tests, the resistance status of each site was determined according to the WHO criteria (2013). When the mortality rate is below 90 percent, the mosquito population is considered resistant. When the mortality rate is between 90 and 97 percent, there is a suspicion of resistance. If the mortality rate is above 98 percent, the mosquito population is considered susceptible.

The sporozoite index was calculated by dividing the total number of infected vectors by the total number of vectors tested with ELISA and multiplying by 100.

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

# 3. RESULTS

## 3.1 VECTOR SPECIES COMPOSITION

Five different *Anopheles* species were encountered: *An. gambiae* s.l., *An. pharoensis*, *An. rufipes*, *An. funestus* s.l., and *An. ziemanni*. The most predominant vector was *An. gambiae* s.l. which accounted for more than 96 percent of the collected *Anopheles* mosquitoes irrespective of the sampling method (Table 3). After combining data from the two collection methods (HLC and PSC), only 371 *An. pharoensis*, nine *An. rufipes*, four *An. ziemanni* and three *An. funestus* were captured out of 13,935 *Anopheles*. The total number of each mosquito species collected is presented in Table 3.

Mosquitoes collected from the two sampling methods (HLC and PSC) were used for molecular species identification. A maximum of 35 specimens of *An. gambiae* s.l. captured from each site were then analyzed by PCR on a monthly basis. The results revealed, out of the 686 specimens of *An. gambiae* s.l. that were tested, 92.7 percent were *An. coluzzii* (n=636), 5 percent were *An. gambiae* (n=34), 0.9 percent were *An. arabiensis* (n=6), 0.1 percent were hybrid *coluzzii* x *gambiae* (n=1), and 1.3 percent (n=9) did not amplify (Figure 8). More details are in Annex A, Table A1. The proportion of each species for each site is presented in Figures 9 through 12.

Figure 8: *An. gambiae* s.l. Sampling Species PCR for All Sites

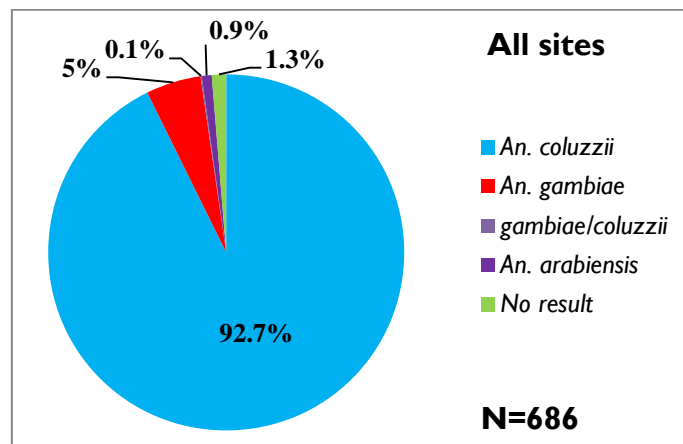
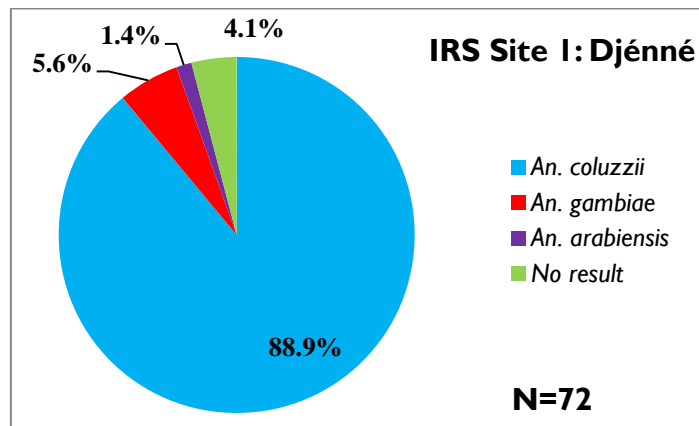


Figure 9: *An. gambiae* s.l. Sampling Species PCR for Djénné (IRS Site I)





**Table 3: Vector Species Composition in Entomological Monitoring Sites, June-December 2017**

Sampling methods	Mosquito species	Djénné (IRS Site 1)	Mopti (IRS Site 2)	Bandiagara (IRS Site 3)	Bankass (IRS Site 4)	Koulikoro (Former IRS Site 1)	Barouéli (Former IRS Site 2)	Kati (Control site)	Total
<b>HLC</b>	<i>An. gambiae</i>	192 (97.5%)	638 (75.2%)	154 (98.1%)	57 (100%)	4,651 (99.1%)	430 (100%)	3566 (96.7%)	<b>9,688 (96.2%)</b>
	<i>An. funestus</i>	1 (<1%)	0	0	0	0	0	0	<b>1 (&lt;1%)</b>
	<i>An. pharoensis</i>	2 (<1%)	206 (24.3%)	1 (<1%)	0	41 (<1%)	0	120 (3.2%)	<b>370 (3.7%)</b>
	<i>An. ziemanni</i>	1 (<1%)	3 (<1%)	0	0	0	0	0	<b>4 (&lt;1%)</b>
	<i>An. rufipes</i>	1 (<1%)	1 (<1%)	2 (<1%)	0	0	0	1 (<1%)	<b>5 (&lt;1%)</b>
	<b>Total/Site</b>	<b>197</b>	<b>848</b>	<b>157</b>	<b>57</b>	<b>4,692</b>	<b>430</b>	<b>3,687</b>	<b>10,068</b>
<b>PSC</b>	<i>An. gambiae</i>	493 (99.8%)	612 (99.8%)	431 (99.5%)	178 (98.8%)	694 (100%)	658 (99.8%)	794 (100%)	<b>3,860 (99.8%)</b>
	<i>An. funestus</i>	0	0	0	1 (<1%)	0	1 (<1%)	0	<b>2 (&lt;1%)</b>
	<i>An. pharoensis</i>	0	1 (<1%)	0	0	0	0	0	<b>1 (&lt;1%)</b>
	<i>An. rufipes</i>	1 (<1%)	0	2 (<1%)	1 (<1%)	0	0	0	<b>4 (&lt;1%)</b>
	<b>Total/Site</b>	<b>494</b>	<b>613</b>	<b>433</b>	<b>180</b>	<b>694</b>	<b>659</b>	<b>794</b>	<b>3,867</b>

Figure 10: *An. gambiae* s.l. Sampling Species PCR for Mopti and Bandiagara

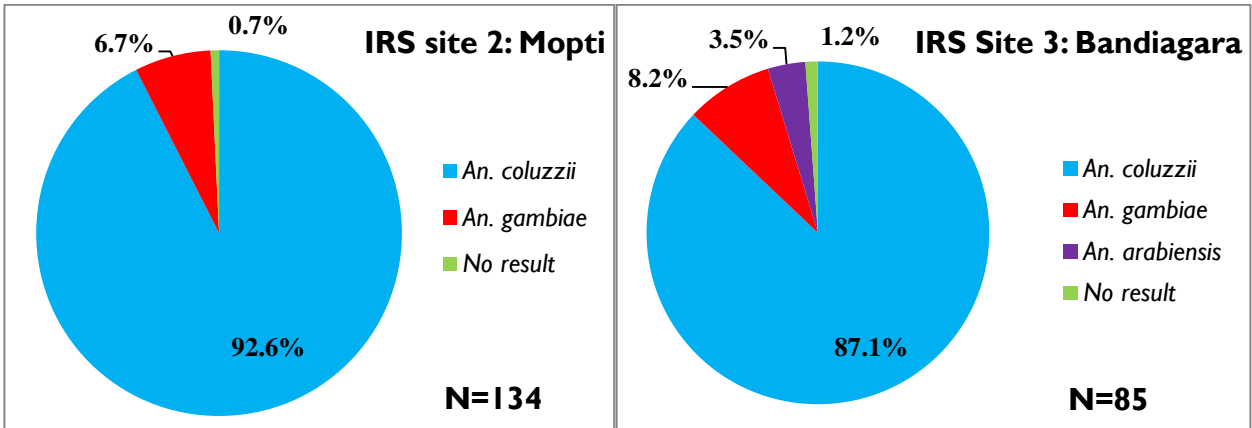


Figure 11: *An. gambiae* s.l. Sampling Species PCR for Bankass and Koulikoro

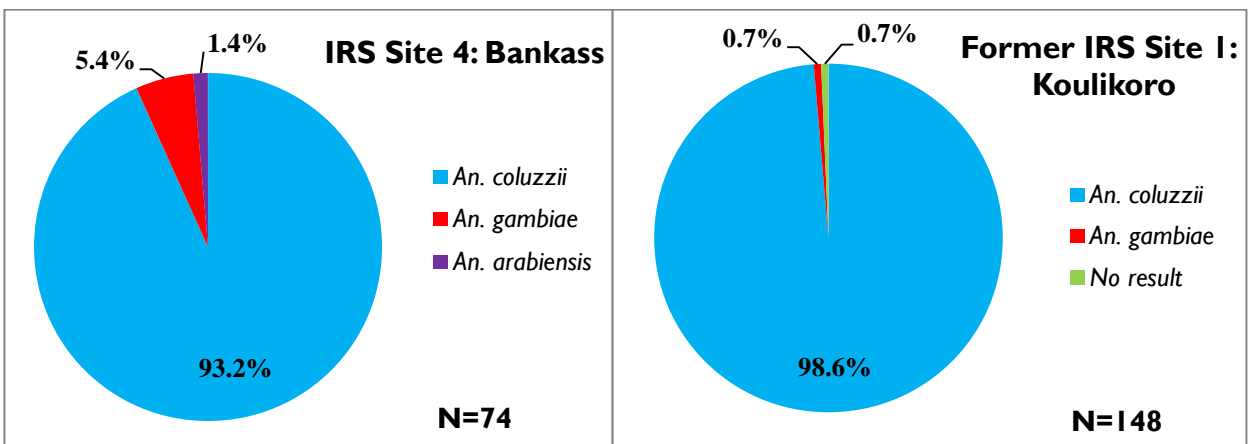
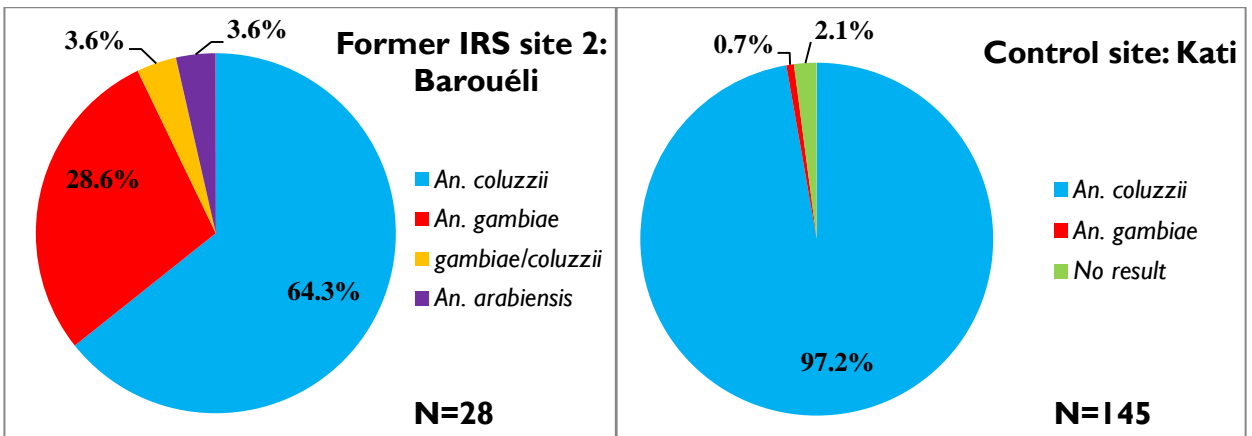


Figure 12: *An. gambiae* s.l. Sampling Species PCR for Barouéli and Kati



### 3.2 INDOOR VECTOR RESTING DENSITY

Over the study period, the indoor vector density in Djénné, Mopti, Bandiagara, and Bankass (IRS sites) was between 0.1-19.3, 1.5-11, 0.2-10.7 and 0-3.6 *An. gambiae* s.l. per house per day, respectively. The cumulative data in IRS sites revealed a post-IRS vector density of 1.8 *An. gambiae* s.l. per house per day, while the density was 6.2 *An. gambiae* s.l. per house per day before IRS. Overall, indoor resting densities decreased as expected after the spraying except in Mopti in October and Bankass in September. In IRS sites, the month during which the highest indoor vector density was recorded varied by district—July in Djénné and Bandiagara, August in Bankass, and October in Mopti (Table 4).

In unsprayed former IRS sites, the mean indoor vector density was between 0.6-12.3 and 0.1-13.5 *An. gambiae* s.l. per house per day in Koulikoro and Barouéli, respectively. In Kati, where spraying has never occurred, the indoor vector density ranged from 0.3 to 13.9 *An. gambiae* s.l. per house per day. In unsprayed former IRS sites and in the control site, the peak in indoor vector density was obtained in August/September (Table 4).





**Table 4: Indoor Vector Density in Entomological Monitoring Sites**

Period	Parameters	Djenné (IRS Site 1)	Mopti (IRS Site 2)	Bandiagara (IRS Site 3)	Bankass (IRS Site 4)	Mean	Koulikoro (Former IRS Site 1)	Barouéli (Former IRS Site 2)	Mean	Kati (Control Site)
<b>Pre-IRS</b>										
June	N collected	67	29	79	42	217	59	11	70	45
	N houses	20	20	20	20	80	20	20	40	20
	<b>Density (An/h/d)</b>	<b>3.4</b>	<b>1.5</b>	<b>4</b>	<b>2.1</b>	<b>2.7</b>	<b>3</b>	<b>0.6</b>	<b>1.8</b>	<b>2.3</b>
July	N collected	386	138	214	29	767	40	59	99	207
	N houses	20	20	20	20	80	20	20	40	20
	<b>Density (An/h/d)</b>	<b>19.3</b>	<b>6.9</b>	<b>10.7</b>	<b>1.5</b>	<b>9.6</b>	<b>2</b>	<b>3</b>	<b>2.5</b>	<b>10.4</b>
Total (Pre-IRS)	N collected	453	167	293	71	984	99	70	169	252
	N houses	40	40	40	40	160	40	40	80	40
	<b>Density (An/h/d)</b>	<b>11.3</b>	<b>4.2</b>	<b>7.3</b>	<b>1.8</b>	<b>6.2</b>	<b>2.5</b>	<b>1.75</b>	<b>2.1</b>	<b>6.3</b>
<b>Post-IRS</b>										
August (IRS intervention)	N collected	17	39	27	71	154	246	208	454	159
	N houses	20	20	20	20	80	20	20	40	20
	<b>Density (An/h/d)</b>	<b>0.9</b>	<b>2</b>	<b>1.4</b>	<b>3.6</b>	<b>1.9</b>	<b>12.3</b>	<b>10.4</b>	<b>11.4</b>	<b>8</b>
September	N collected	7	90	49	22	168	102	269	371	277
	N houses	20	20	20	20	80	20	20	40	20
	<b>Density (An/h/d)</b>	<b>0.4</b>	<b>4.5</b>	<b>2.5</b>	<b>1.1</b>	<b>2.1</b>	<b>5.1</b>	<b>13.5</b>	<b>9.3</b>	<b>13.9</b>
October	N collected	6	219	49	7	281	178	82	260	94
	N houses	20	20	20	20	80	20	20	40	20
	<b>Density (An/h/d)</b>	<b>0.3</b>	<b>11</b>	<b>2.5</b>	<b>0.4</b>	<b>3.5</b>	<b>8.9</b>	<b>4.1</b>	<b>6.5</b>	<b>4.7</b>
November	N collected	8	59	9	7	83	57	28	85	7
	N houses	20	20	20	20	80	20	20	40	20
	<b>Density (An/h/d)</b>	<b>0.4</b>	<b>3</b>	<b>0.5</b>	<b>0.4</b>	<b>1</b>	<b>2.9</b>	<b>1.4</b>	<b>2.1</b>	<b>0.4</b>
December	N collected	2	38	4	0	44	12	1	13	5
	N houses	20	20	20	20	80	20	20	40	20
	<b>Density (An/h/d)</b>	<b>0.1</b>	<b>1.9</b>	<b>0.2</b>	<b>0</b>	<b>0.6</b>	<b>0.6</b>	<b>0.1</b>	<b>0.3</b>	<b>0.3</b>
Total (Post-IRS)	N collected	40	445	138	107	730	595	588	1183	542
	N houses	100	100	100	100	400	100	100	200	100
	<b>Density (An/h/d)</b>	<b>0.4</b>	<b>4.5</b>	<b>1.4</b>	<b>1.1</b>	<b>1.8</b>	<b>6</b>	<b>5.9</b>	<b>5.9</b>	<b>5.4</b>

### 3.3 HUMAN BITING RATE IN *AN. GAMBIAE* S.L., SEASONALITY

Overall, the peak in *An. gambiae* s.l. biting rate was obtained in August and September at all sites.

In the Mopti Region, before IRS, the average *An. gambiae* s.l. HBR was 1.4 b/h/n in Djénné, 1.1 b/h/n in Mopti district, 2.9 b/h/n in Bandiagara, and 0.5 b/h/n in Bankass (Table 5). In the former IRS sites, the mean *An. gambiae* s.l. HBR during the same period was zero in Barouéli while it was 15.2 b/h/n in Koulikoro. The highest mean HBR (20.2 b/h/n) was obtained in Kati, a control site that has never been sprayed.

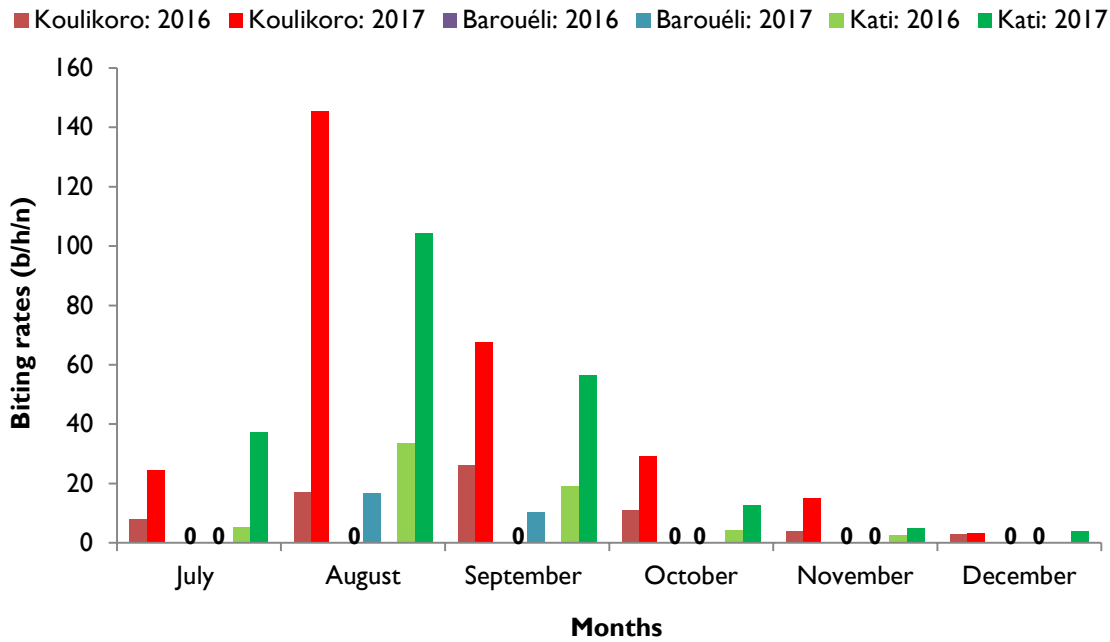
Post-IRS, in the Mopti Region (sprayed sites), the mean *An. gambiae* s.l. HBR ranged from 0.5 b/h/n in Bankass to 7.5 b/h/n in Mopti District. The mean *An. gambiae* s.l. HBRs for August and September 2017 in Koulikoro and Barouéli (former IRS sites) were particularly high at 106.3b/h/n and 13.4 b/h/n, respectively, as compared to the same period of 2016 when it was 21.5 b/h/n and 0 b/h/n, respectively (Figure 13). The tendency was the same in Kati, a control site that wasn't sprayed in either 2016 or 2017, with a mean HBR of 80.5 b/h/n in August through September 2017 compared with 46.2 b/h/n in 2016. Between August-September 2017, there was a 4.9 fold increase in HBR in Koulikoro compared with the same period in 2016, while the increase in Kati (the paired control) was to a lower magnitude (1.7 fold). Overall, after combining the 2017 data, the mean HBR was 2.7 b/h/n in IRS sites, 28.7 b/h/n in former IRS sites, and 36.5 b/h/n in Kati, a control site. More details are given in Annex B, Table BI.

**Table 5: *An. gambiae* s.l., Mean Human Biting Rate in Entomological Monitoring Sites, June-December 2017**

SITES	Pre-IRS			Post-IRS					
	June	July	Mean	August	September	October	November	December	Mean
IRS site 1: Djénné	0.2	2.7	1.4	7.2	1.5	0.2	0.1	0.1	1.8
IRS site 2: Mopti	0.4	1.8	1.1	8.6	20.3	6.4	2.2	0.3	7.5
IRS site 3: Bandiagara	2.4	3.4	2.9	1.6	1.4	0.8	0.1	0.0	0.8
IRS site 4: Bankass	0.5	0.6	0.5	1.4	0.5	0.6	0.0	0.0	0.5
<b>Grand Total: IRS sites</b>	<b>0.9</b>	<b>2.1</b>	<b>1.5</b>	<b>4.7</b>	<b>5.9</b>	<b>2.0</b>	<b>0.6</b>	<b>0.1</b>	<b>2.7</b>
Former IRS site 1: Koulikoro	6.1	24.3	15.2	145.3	67.4	29.0	15.1	3.4	52.1
Former IRS site 2: Barouéli	0.0	0.0	0	16.6	10.3	0.0	0.0	0.0	5.4
<b>Grand Total: Former IRS sites</b>	<b>3.1</b>	<b>12.2</b>	<b>7.6</b>	<b>81.0</b>	<b>38.8</b>	<b>14.5</b>	<b>7.6</b>	<b>1.7</b>	<b>28.7</b>
<b>Control site: Kati</b>	<b>3.0</b>	<b>37.3</b>	<b>20.2</b>	<b>104.4</b>	<b>56.6</b>	<b>12.7</b>	<b>4.9</b>	<b>3.9</b>	<b>36.5</b>

b/h/n: bite per human per night

**Figure 13: Variation of Mean Biting Rates in Koulikoro and Barouéli (Former IRS) and Kati (Control Site Between 2016 and 2017)**



### 3.4 BITING TIMES OF *AN. GAMBIAE* S.L.

Figures 14 through 18 display the same tendency in terms of biting times for *An. gambiae* s.l. in IRS, former IRS, and control sites. The biting behavior was more pronounced late at night indoors and outdoors, with little biting before 10 at night. The biting times are not presented for Bandiagara and Bankass given fewer than 160 *An. gambiae* s.l. were collected. More details are available in Annex C, Tables C1 and C2.

**Figure 14: *An. gambiae* s.l. Hourly Biting Rates in Djénné (IRS Site 1), June-December 2017**

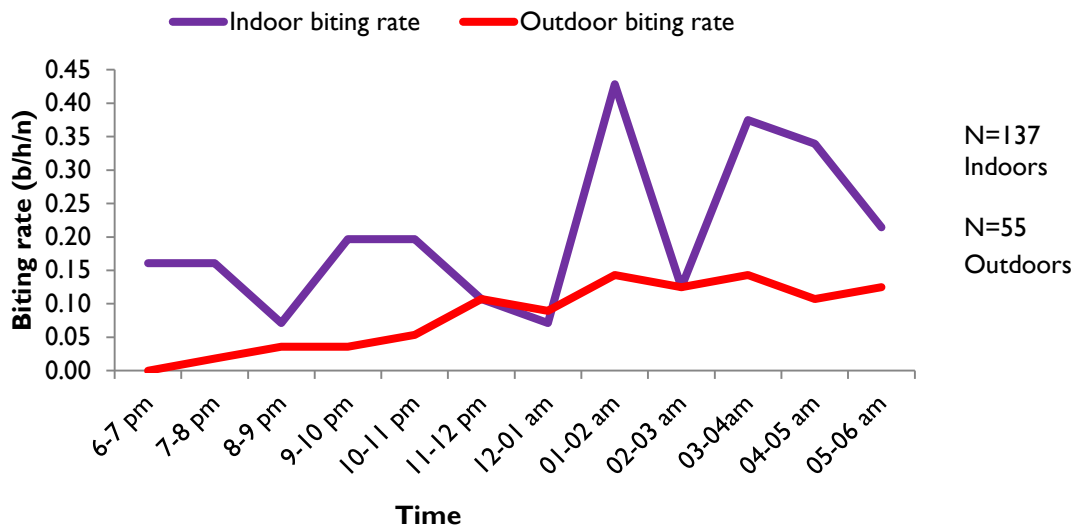


Figure 15: *An. gambiae* s.l. Hourly Biting Rates in Mopti (IRS Site 2), June-December 2017

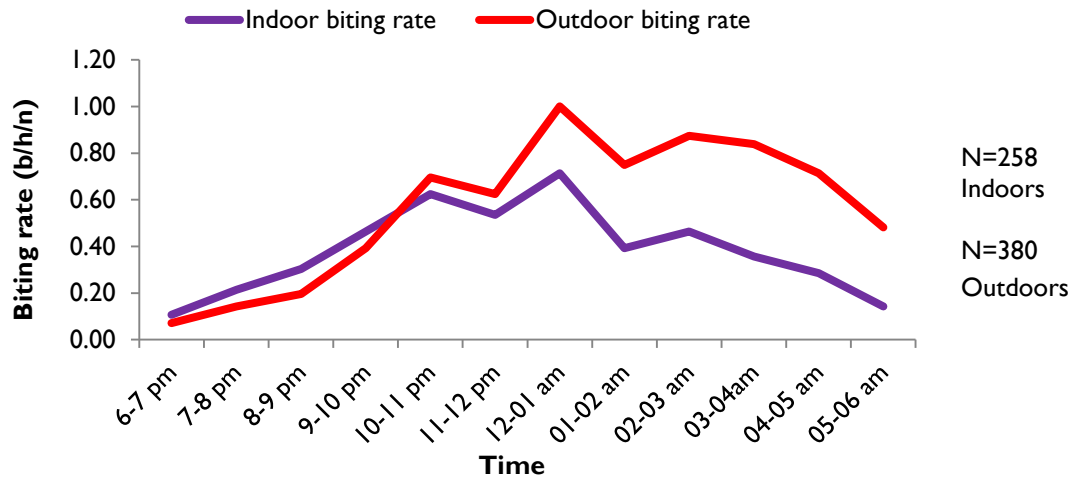


Figure 16: *An. gambiae* s.l. Hourly Biting Rates in Koulikoro (Former IRS Site 1), June-December 2017

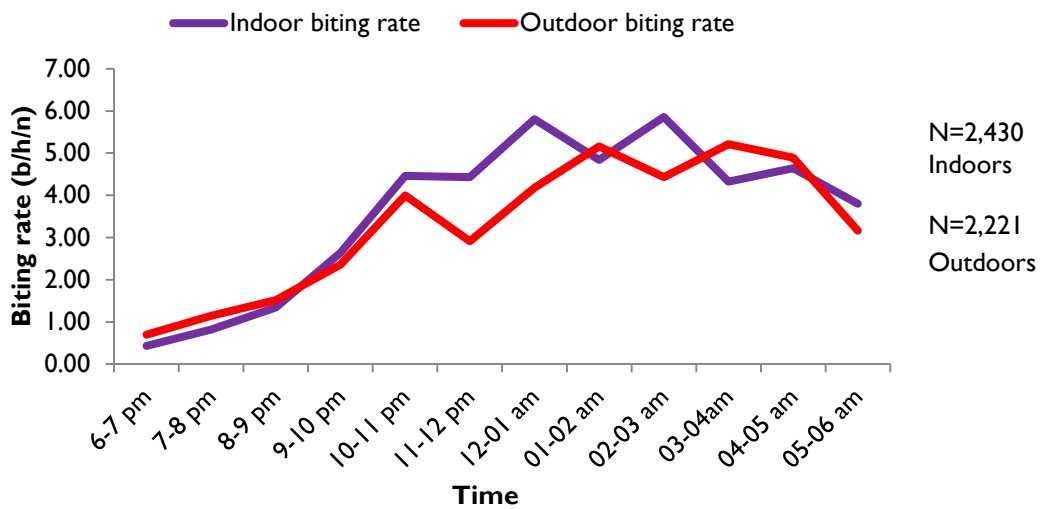


Figure 17: *An. gambiae* s.l. Hourly Biting Rates in Baroueli (Former IRS Site 2), June-December 2017

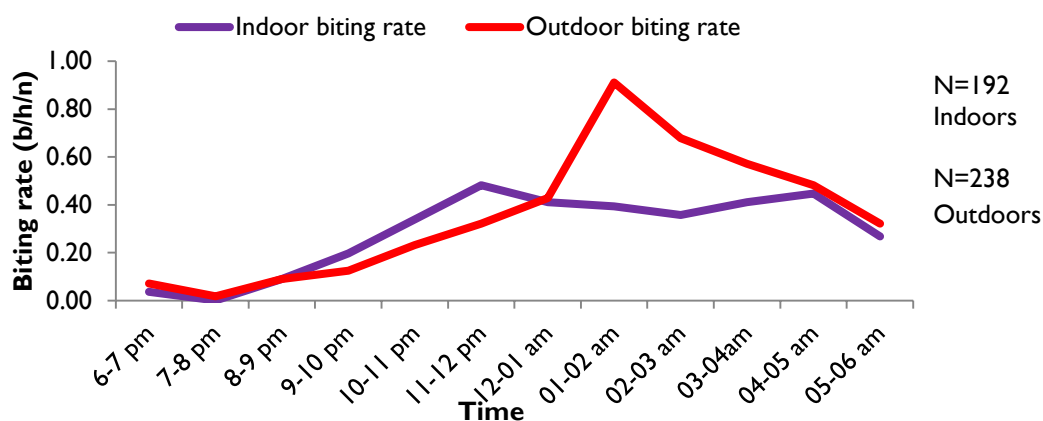
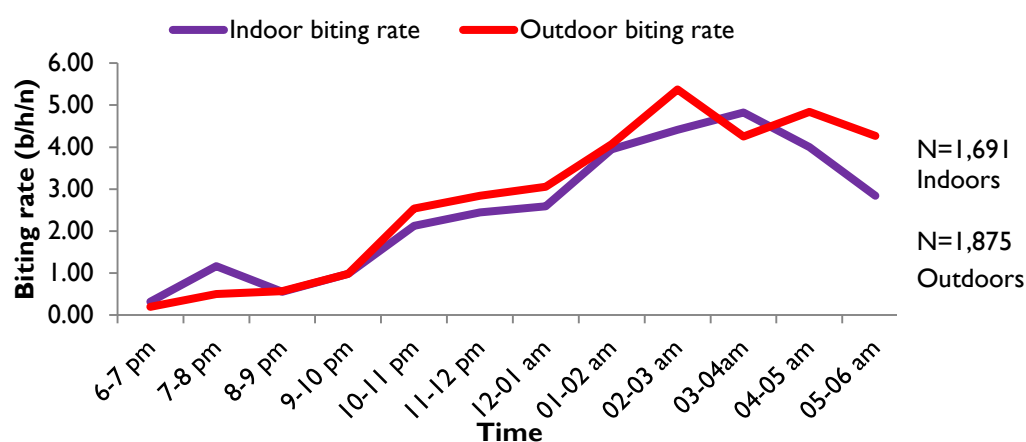


Figure 18: *An. gambiae* s.l. Hourly Biting Rates in Kati (Control Site), June-December 2017



### 3.5 BITING LOCATION OF *AN. GAMBIAE* S.L.

The frequency of indoor and outdoor biting rates of *An. gambiae* s.l. in each site is shown in Table 6 (more details are provided in Annex D, Tables D1 and D2). Prior to IRS, *An. gambiae* s.l. biting rates were similar indoors and outdoors at all sites except for Djénné (IRS site 1) and Bandiagara (IRS site 3) where there was more biting indoors. As pre-IRS sampling was only done for two months, no valid conclusion can be drawn regarding the differences observed. Post-IRS, biting behavior in *An. gambiae* s.l. was variable according to each IRS site (Djénné, Mopti, Bandiagara, and Bankass), but after combining the data, biting rates were similar indoors and outdoors in IRS sites. There were also similar indoor and outdoor biting rates in former IRS sites and the control site (Table 6).

Table 6: *An. gambiae* s.l. Mean Human Biting Rate per Night, Indoors and Outdoors, June-December 2017

Period/site	Indoor	Outdoor	In:Out Ratio
<b>Pre IRS</b>			
Djénné: IRS site 1	2 <sup>a</sup>	0.9 <sup>b</sup>	0.7:0.3
Mopti: IRS site 2	1.3 <sup>a</sup>	0.9 <sup>a</sup>	0.6:0.4
Bandiagara: IRS site 3	3.8 <sup>a</sup>	2 <sup>b</sup>	0.65:0.35
Bankass: IRS site 4	0.5 <sup>a</sup>	0.6 <sup>a</sup>	0.47:0.53
<b>MEAN: IRS sites</b>	<b>1.9<sup>a</sup></b>	<b>1.1<sup>b</sup></b>	<b>0.64:0.36</b>
Koulikoro: Former IRS site 1	14.4 <sup>a</sup>	16.1 <sup>a</sup>	0.47:0.53
Barouéli: Former IRS site 2	0	0	-
<b>MEAN: Former IRS sites</b>	<b>7.2<sup>a</sup></b>	<b>8<sup>a</sup></b>	<b>0.47:0.53</b>
<b>Kati: Control site</b>	<b>19.7<sup>a</sup></b>	<b>20.6<sup>a</sup></b>	<b>0.49:0.51</b>

Post IRS			
Djénné: IRS site 1	2.6 <sup>a</sup>	1 <sup>b</sup>	0.72:0.28
Mopti: IRS site 2	5.9 <sup>a</sup>	9.2 <sup>b</sup>	0.39:0.61
Bandiagara: IRS site 3	0.8 <sup>a</sup>	0.7 <sup>a</sup>	0.53:0.47
Bankass: IRS site 4	0.4 <sup>a</sup>	0.6 <sup>a</sup>	0.38:0.62
<b>MEAN: IRS sites</b>	<b>2.4<sup>a</sup></b>	<b>2.9<sup>a</sup></b>	<b>0.46:0.54</b>
Koulikoro: Former IRS site 1	55 <sup>a</sup>	49.1 <sup>b</sup>	0.52:0.48
Barouéli: Former IRS site 2	4.8 <sup>a</sup>	6 <sup>b</sup>	0.45:0.55
<b>MEAN: Former IRS sites</b>	<b>29.9<sup>a</sup></b>	<b>27.5<sup>a</sup></b>	<b>0.52:0.48</b>
<b>Kati: Control site</b>	<b>34.4<sup>a</sup></b>	<b>38.6<sup>a</sup></b>	<b>0.47:0.53</b>

Note: HBR indoor and outdoor with different superscript are statistically different.

### 3.6 SPOROZOITE INDEX

In each assessment site, the proportion of infected mosquitoes was evaluated on a monthly basis (Table 7). The details on the number of mosquitoes tested by ELISA for CSP and the number found positive for the presence of *Plasmodium falciparum* antigen are in Annex E, Table E1.

Overall, for the pre-IRS period, the mean sporozoite index (SI) was 3 percent for IRS sites (95% CI: 1.3-4.8), 0.7 percent for former IRS sites (95% CI: 0.0-2.0) and 0.8 percent for the control site (95% CI: 0.0-2.3). Post-IRS, it was 1.5 percent for IRS sites (95% CI: 0.5-2.4), and 0.8 percent for the former IRS sites and the control site (95% CI: 0.0-1.7). Although not significant ( $p=0.09$ ), a reduction in mosquito infection was observed in the combined four IRS sites after the implementation of the control strategy. In former IRS sites, the sporozoite index was similar for both years at 0.8% (95%CI: 0.2-1.5) in 2017 versus 1.3% (95%CI: 0.2-2.5) in 2016;  $p=0.42$ .

**Table 7: Monthly Sporozoite Index from HLC at All Seven Sentinel Sites**

SITES	Pre-IRS				Post-IRS						
	SI : % (N)				SI : % (N)						
	June	July	Mean	CI	August	September	October	November	December	Mean	CI
<b>IRS Site 1: Djénné</b>	5.5% (55)	1.7% (60)	<b>3.5%</b> <b>(115)</b>	[0.1-6.8]	0% (50)	0% (35)	33.3% (3)	50% (2)	0% (4)	<b>2.1%</b> <b>(94)</b>	[0.0– 5.0]
<b>IRS Site 2: Mopti</b>	0% (8)	0% (60)	<b>0%</b> <b>(68)</b>	-	0% (50)	1.3% (75)	0% (152)	6.3% (32)	0% (40)	<b>0.9%</b> <b>(349)</b>	[0.0 - 1.8]
<b>IRS Site 3: Bandiagara</b>	2.9% (70)	3.3% (60)	<b>3.1%</b> <b>(130)</b>	[0.1- 6.0]	0% (33)	5% (40)	2.9% (34)	0% (2)	-	<b>2.8%</b> <b>(109)</b>	[0.0 - 5.8]
<b>IRS Site 4: Bankass</b>	4% (25)	8.7% (23)	<b>6.3%</b> <b>(48)</b>	[0.0-13.1]	0% (22)	0% (32)	9.1% (11)	-	-	<b>1.5%</b> <b>(65)</b>	[0.0 - 4.5]
<b>Former IRS Site 1: Koulikoro</b>	0% (90)	1.7% (60)	<b>0.7%</b> <b>(150)</b>	[0.0- 2.0]	0% (205)	0% (124)	1.3% (150)	0% (160)	2.9% (69)	<b>0.6%</b> <b>(708)</b>	[0.0 - 1.1]
<b>Former IRS Site 2: Barouéli</b>	-	-	-	-	0% (60)	5.3% (57)	-	-	-	<b>2.6%</b> <b>(117)</b>	[0.0 - 5.4]
<b>Control Site: Kati</b>	1.4% (72)	0% (59)	<b>0.8%</b> <b>(131)</b>	[0.0-2.3]	0% (93)	0% (64)	0% (75)	1.3% (79)	3.7% (54)	<b>0.8%</b> <b>(365)</b>	[0.0 - 1.7]
<b>Grand Total: IRS sites</b>	3.8% (158)	2.5% (203)	<b>3%</b> <b>(361)</b>	[1.3-4.8]	0% (155)	1.6% (182)	1.5% (200)	8.3% (36)	0% (44)	<b>1.5%</b> <b>(617)</b>	[0.5 - 2.4]
<b>Grand Total: Former IRS sites</b>	0% (90)	1.7% (60)	<b>0.7%</b> <b>(150)</b>	[0.0-2.0]	0% (265)	1.7% (181)	1.3% (150)	0% (160)	2.9% (69)	<b>0.8%</b> <b>(825)</b>	[0.2 - 1.5]

N: number of *An. gambiae* s.l. for ELISA CSP

### 3.7 ENTOMOLOGICAL INOCULATION RATE (EIR)

The details on monthly variation of the EIR are provided by Annex F, Table F1.

Post-IRS, the peak of transmission was reached in all sites in September or October, except for Kati where the peak was observed in December because of the null SI recorded during the period of peak biting rate. In IRS sites, the monthly peak was 1.88, 8.1, 2.16 and 1.70 infectious bites per human per month (ib/h/month) respectively in Djénné, Mopti, Bandiagara and Bankass (Table 8). In the former IRS sites the monthly peak in September/October was 11.6 ib/h/month in Koulikoro and 16.18 ib/h/month in Barouéli. Overall, following IRS relocation, EIR in former IRS sites increased two fold to 15.37 infectious bites/human/5months in 2017 versus 7.05 ib/h/5months in 2016.

In IRS sites, the highest total EIR over the entire five month post-IRS period occurred in Mopti at 12.2 ib/h/5 months; the lowest was in Bankass (1.7 ib/h/5 months). The mean for all IRS sites post-IRS was 5.12 ib/h/5 months compared with 15.37 ib/h/5 months for the former IRS sites and 6.21 ib/h/5 months for Kati, the control site.

**Table 8: Monthly EIR For All Seven Sites Before and After IRS**

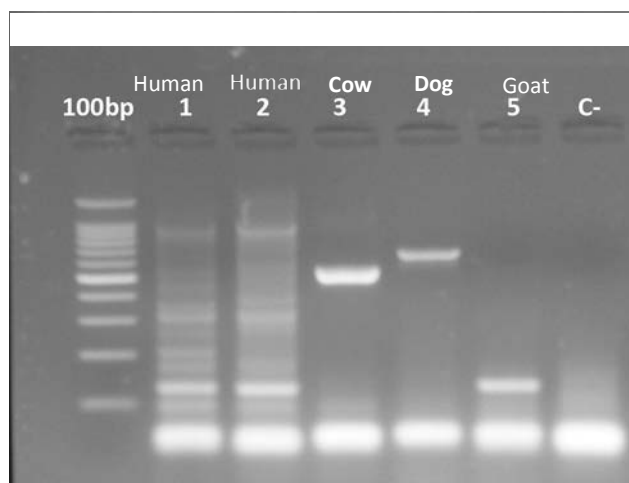
SITES	Pre-IRS			Post-IRS					
	Monthly EIR			Monthly EIR					
	June	July	Total (ib/h/2m)	August	September	October	November	December	Total (ib/h/5m)
<b>IRS Site 1: Djénné</b>	0.31	1.34	<b>1.65</b>	0	0	1.88	1.88	0	<b>3.75</b>
<b>IRS Site 2: Mopti</b>	0	0	<b>0</b>	0	8.10	0	4.10	0	<b>12.20</b>
<b>IRS Site 3: Bandiagara</b>	2.04	3.38	<b>5.41</b>	0	2.16	0.66	0	0	<b>2.82</b>
<b>IRS Site 4: Bankass</b>	0.60	1.47	<b>2.07</b>	0	0	1.70	0	0	<b>1.70</b>
<b>Former IRS Site 1: Koulikoro</b>	0	12.16	<b>12.16</b>	0	0	11.60	0	2.96	<b>14.56</b>
<b>Former IRS Site 2: Barouéli</b>	0	0	<b>0</b>	0	16.18	0	0	0	<b>16.18</b>
<b>Control site: Kati</b>	1.25	0	<b>1.25</b>	0	0	0	1.88	4.33	<b>6.21</b>
<b>Grand Total: IRS sites</b>	<b>0.7</b>	<b>1.5</b>	<b>2.28</b>	<b>0</b>	<b>2.56</b>	<b>1.06</b>	<b>1.49</b>	<b>0</b>	<b>5.12</b>
<b>Grand Total: Former IRS sites</b>	<b>0</b>	<b>6.1</b>	<b>6.08</b>	<b>0</b>	<b>8.09</b>	<b>5.80</b>	<b>0</b>	<b>1.48</b>	<b>15.37</b>

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

### 3.8 BLOOD MEAL ORIGIN

In 2017 we changed from blood-meal ELISA to PCR (MR4) due to the large proportion of unidentified samples through ELISA and the greater specificity of PCR. However, the protocol of Kent et al (2005) did not prove specific to determine human and goat bands separately during optimization (Figure 19). Indeed, the amplification of DNA extracted directly from mosquitoes that

were fed on humans presented bands for goat. A different protocol will be used for the 2018 activities.



### 3.9 QUALITY OF SPRAYING AND INSECTICIDE DECAY RATE

The results of WHO cone bioassays performed 1-2 days after spraying showed mean mortality rates of 100 percent at the four different heights (Table 9). Tests during the same period for the fumigant air-borne effect of Actellic 300 CS produced mortality ranging from 96.8 percent to 100 percent according to the different type of wall encountered (Figure 20). These results indicate that IRS was conducted satisfactorily in the tested houses.

**Table 9: Results of Quality Assurance Cone Bioassay Testing Following IRS (August 2017)**

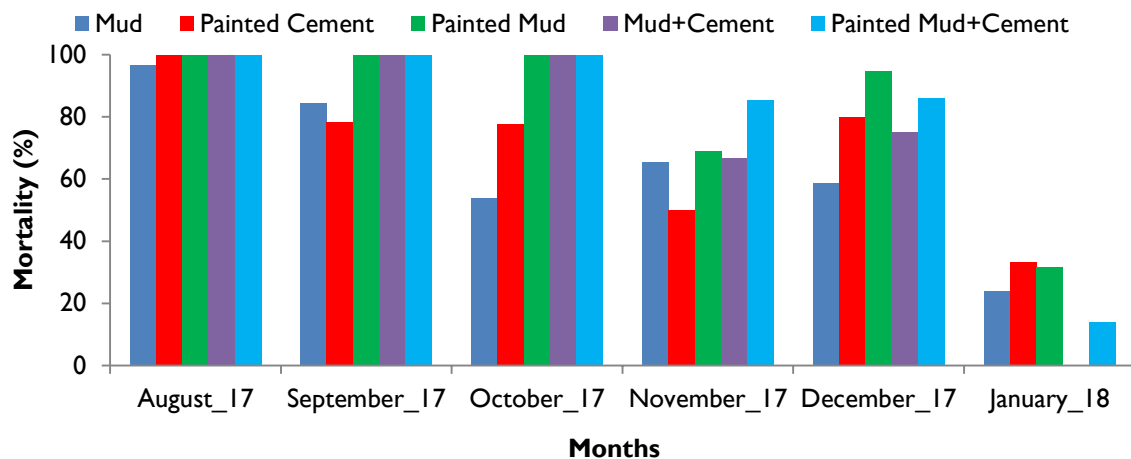
Cone Position	No. Structures	No. Mosquitoes	No. Mosquitoes Knocked Down 30 Min	% Knock Down 30 Min	No. Mosquitoes Dead after 24 Hrs.	% Observed Mortality
0.5 m	20	236	82	34.7%	236	100%
1 m	20	236	53	22.5%	236	100%
1.5 m	20	237	60	25.3%	237	100%
2 m	20	232	52	22.4%	232	100%
<b>Total test</b>	<b>80</b>	<b>941</b>	<b>247</b>	<b>26.2%</b>	<b>941</b>	<b>100%</b>

The monthly mortality rates obtained after cone testing per type of wall are shown in Figure 21. Mortality remained more than 80 percent for five months on painted mud and the combination of painted mud and cement, while mortality remained above 80 percent for four months on painted cement and for two months on the combination of mud (unpainted) and cement. The most frequently encountered material, mud, had a residual efficacy of 3 months. More details can be found in Annex G, Table G1.

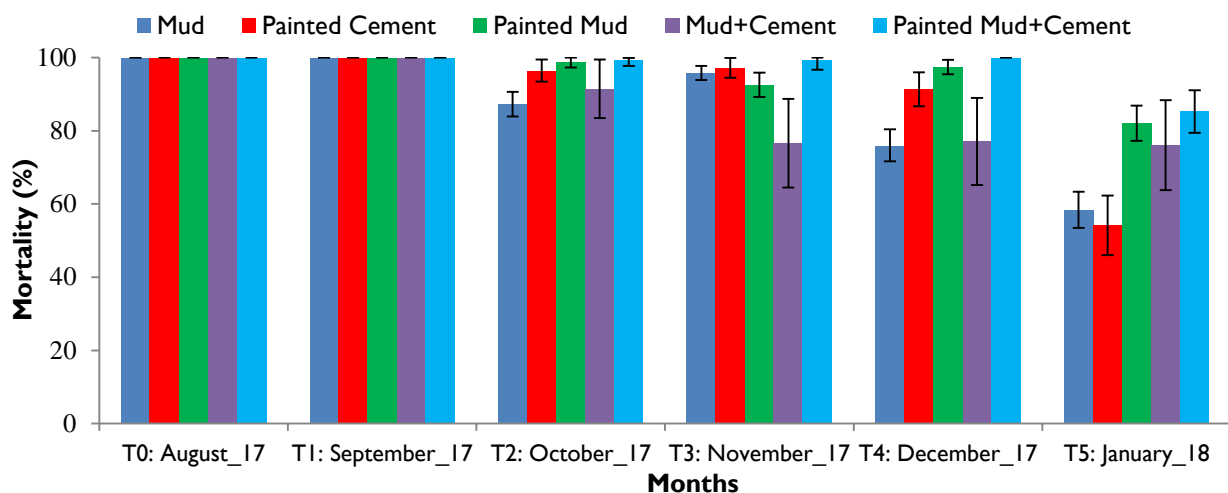
With 30 minutes of exposure, the fumigant effect produced particularly high mortality rates up to two months after spraying, while mortality remained above 60 percent on all wall surfaces for four months after spraying, followed by a large decrease after five months (Figure 20).



**Figure 20: Monthly % Mortality of *An. Gambiae* s.l. Kisumu After 30 Minutes of Exposure in Wire Cages to the Airborne Fumigant Effect of Actellic 300 CS in Djenne, Mopti, Bandiagara, and Bankass**



**Figure 21: Monthly % Mortality of *An. gambiae* s.l. Kisumu After 30 Minutes Exposure in WHO Cone Tests on Walls Sprayed with Actellic 300CS in Djenne, Mopti, Bandiagara, and Bankass**



### 3.10 VECTOR SUSCEPTIBILITY TO INSECTICIDES

Figure 22 is a series of nationwide maps showing the resistance status of *Anopheles gambiae* s.l. to five insecticides, based on modified WHO categories of 98 to 100 percent mortality indicating susceptibility, 91 to 97 percent mortality indicating possible resistance, 66 to 90 percent indicating resistance, 41 to 65 percent indicating high frequency resistance, and 0 to 40 indicating very high frequency resistance.

#### SUSCEPTIBILITY TO PRIMIPHOS-METHYL 0.25%

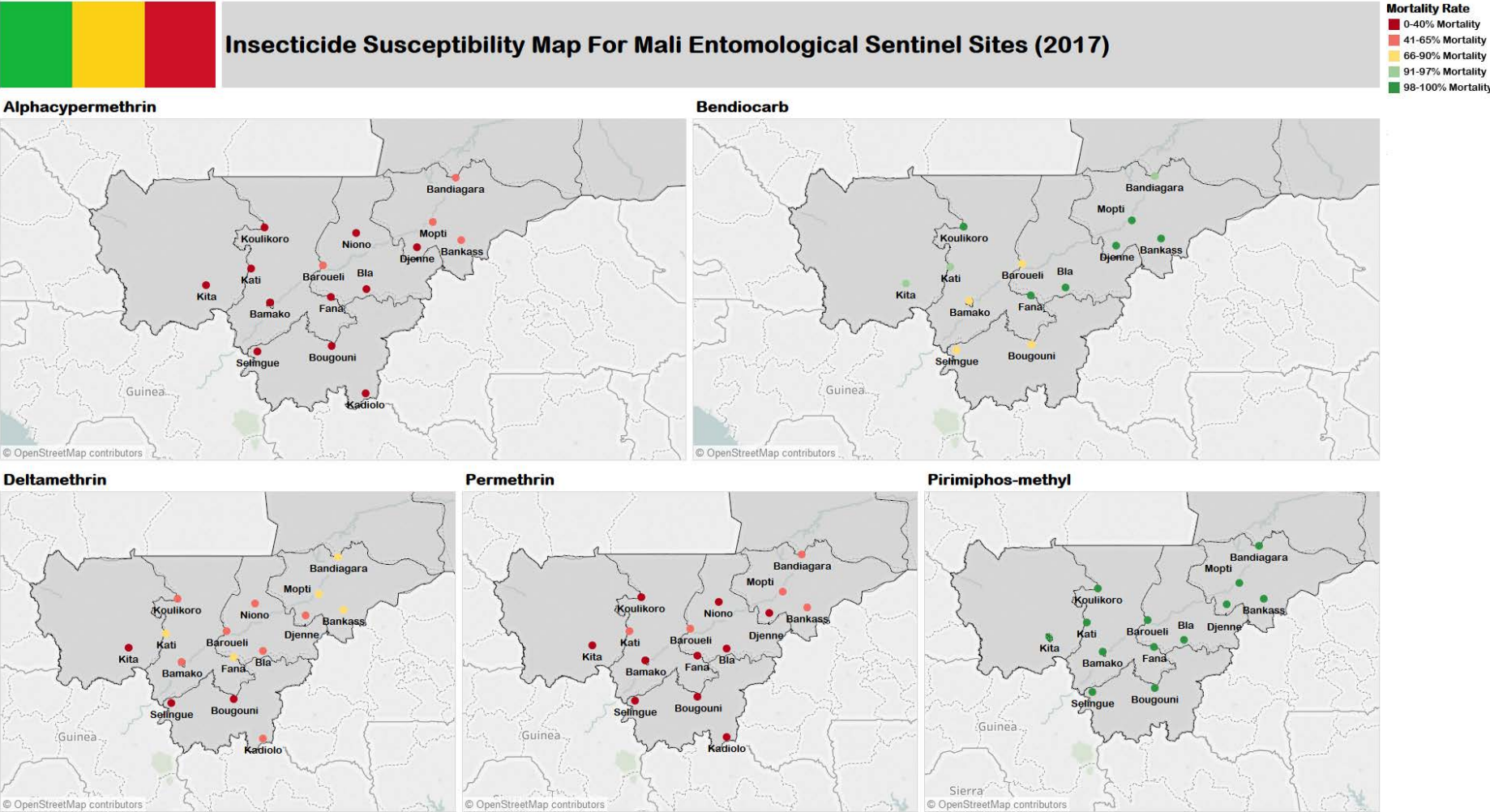
In 2017, full susceptibility was observed in all sites (Table 10). More details are in Annex H, Table H1.

**Table 10: Mortality of *An. gambiae* s.l. Tested with 0.25% Pirimiphos-Methyl in WHO Susceptibility Tests in 2017 (Compared with 2015-16 Data)**

District (Site)	2015% Mortality	2016% Mortality	2017% Mortality
Koulikoro	100%	100%	100%
Kati	100%	100%	100%
Bamako	100%	100%	100%
Bla	100%	99%	100%
Baroueli	100%	100%	100%
Selingue	100%	100%	100%
Bougouni	100%	100%	100%
Djenne	100%	100%	100%
Mopti	n/a	n/a	100%
Bandigara	100%	100%	100%
Bankass	100%	100%	100%
Kita	n/a	100%	100%
Fana	n/a	100%	100%
Mean	100%	>99%	100%

n/a: no data available

**Figure 22: Map of *An. Gambiae* s.l. Susceptibility to 0.05% Alphacypermethrin, 0.1% Bendiocarb, 0.05% Deltamethrin, 0.75% Permethrin, And 0.25% Pirimiphos-Methyl, 2017**



## SUSCEPTIBILITY TO PYRETHROID INSECTICIDES (PERMETHRIN 0.75%, DELTAMETHRIN 0.05%, AND ALPHACYPERMETHRIN 0.05%)

Based on WHO 2013 criteria, populations of *An. gambiae* s.l. collected from all sites were resistant to all pyrethroid insecticides tested in 2017. The mean mortality rates across all sites revealed that vectors were more resistant to permethrin (0.75%) than to deltamethrin (0.05%) in 2017, as was also observed in 2015 and 2016 (Table 11). In 2017, a similar mean mortality rate was observed with permethrin (0.75%) (26%; 95%CI: 24.2-28.7) and alphacypermethrin (0.05%) (31%; 95%CI: 28.2-32.9). Overall, for permethrin 0.75%, mortality rates varied from 11% (Kadiolo) to 55% (Bankass) (Table 11) in 2017. For alphacypermethrin 0.05% and deltamethrin 0.05%, they ranged respectively from 6% in Kati to 51% in Mopti and 39% in Kita to 86% in Bandiagara (See Annex H, Table H1).

**Table 11: Mortality of *An. gambiae* s.l. Tested with 0.75% Permethrin, 0.05% Deltamethrin, and 0.05% Alphacypermethrin in WHO Susceptibility tests in 2017 (Compared with 2015-16 Data)**

District (site)	Permethrin 0.75%			Deltamethrin 0.05%			Alphacypermethrin 0.05%
	2015	2016	2017	2015	2016	2017	2017
	% Mortality	% Mortality	% Mortality	% Mortality	% Mortality	% Mortality	% Mortality
Kita	65%	74%	15%	98%	95%	39%	35%
Koulikoro	14%	19%	22%	49%	85%	63%	36%
Kati	7%	6%	51%	38%	65%	78%	6%
Bamako	63%	50%	12%	79%	50%	49%	14%
Bla	83%	41%	18%	64%	64%	43%	20%
Baroueli	21%	11%	41%	66%	81%	62%	44%
Niono	49%	n/a	26%	58%	20%	51%	29%
Selingue	57%	33%	9%	56%	40%	24%	17%
Bougouni	85%	67%	13%	79%	76%	26%	16%
Kadiolo	54%	21%	11%	63%	47%	54%	24%
Djenne	19%	14%	19%	41%	28%	45%	25%
Bandiagara	22%	58%	45%	45%	89%	86%	55%
Bankass	87%	64%	55%	90%	93%	77%	64%
Fana	n/a	41%	16%	n/a	52%	81%	24%
Mopti	n/a	n/a	42%	n/a	n/a	75%	51%
<b>Mean</b>	<b>48%</b>	<b>38%</b>	<b>26%</b>	<b>64%</b>	<b>64%</b>	<b>57%</b>	<b>31%</b>

n/a: no data available

## SUSCEPTIBILITY TO BENDIOCARB 0.1%

In 2017, out of the 13 sites where testing was performed, full susceptibility was noted in six sites, possible resistance in four sites and resistance in three sites—Barouéli, Sélingué and Bougouni, as shown in Table 12. More details are provided Annex H, Table H1.

**Table 12: Mortality of *An. gambiae* s.l. Tested with 0.1% Bendiocarb in WHO Susceptibility Tests in 2017 (Compared With 2015-16 Data)**

Districts (Site)	2015 % Mortality	2016 % Mortality	2017 % Mortality
Koulikoro	100%	99%	99%
Kati	100%	98%	97%
Bamako	98%	98%	90%
Bla	100%	100%	98%
Baroueli	99%	98%	89%
Niono	100%	94%	n/a
Selingue	100%	100%	86%
Bougouni	100%	92%	76%
Kadiolo	94%	100%	n/a
Djenne	100%	100%	98%
Bandiagara	100%	100%	97%
Bankass	100%	100%	99%
Kita	n/a	100%	95%
Fana	n/a	100%	98%
Mopti	n/a	n/a	100%
Mean	99%	99%	94%

#: Mortality percentage; n/a – no data available

### SUSCEPTIBILITY TO CLOTHIANIDIN 2.0% (13.2MG/PAPER)

For clothianidin, mortality rates using first filial generation (F1) wild *An. gambiae* s.l. from the four IRS districts were at least 98 percent after six days (the diagnostic time recommended by Sumitomo being seven days) (see Table 13). For the negative controls, mortality rates were 6 percent or less after five days (except for Koulikoro). For the susceptible strain (Kisumu), 100 percent mortality was recorded after two days (Table 13). In Bankass and Bandiagara, mortality of wild *An. gambiae* s.l. was 96 percent five days after testing, which indicates possible resistance. However, as this is a new testing protocol, the finding of less than 100 percent mortality may also be due to test variation.

**Table 13: Mortality of *An. gambiae* s.s. Tested with Clothianidin 2% (13.2 mg ai/Paper)**

Sites	Strains	Total tested	Mortality Rate (%)						
			Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
<b>Djénné</b>	Control	48	0	0	0	0	0	0	0
	Field Strain	99	86	99	100	100	100	100	100
<b>Mopti</b>	Control	50	0	0	4	6	6	6	6
	Field Strain	100	85	94	97	98	99	99	99
<b>Bandiagara</b>	Control	50	0	0	0	0	0	0	0
	Field Strain	95	88	95	96	96	96	98	100
<b>Bankass</b>	Control	50	0	0	0	0	0	0	0
	Field Strain	100	39	54	66	90	96	100	100
<b>Koulikoro</b>	Control	20	0	0	0	0	50	50	50
	Kisumu strain	80	98.75	100	100	100	100	100	100
	Field Strain	80	88.75	100	100	100	100	100	100

### 3.11 INSECTICIDE RESISTANCE INTENSITY ASSAYS WITH PERMETHRIN AND DELTAMETHRIN

Table 14 shows results obtained after exposing *An. gambiae* s.l. from the 15 surveyed sites to permethrin and deltamethrin at one, two, five and ten times the diagnostic dosages to assess the intensity of insecticide resistance.

Mortality rates of populations of *An. gambiae* s.l. exposed to 10 times the diagnostic dosage of permethrin ranged from 35 percent to 85 percent in all sites. After exposure to 10 times the diagnostic dosage of deltamethrin, *An. gambiae* s.l. showed a mortality rate varying from 72 percent to 97 percent. 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, increased mortalities are observed as the insecticide concentration increases.

**Table 14: Insecticide Resistance Intensity Results of *An. gambiae* s.l. Tested Against Permethrin and Deltamethrin**

SITES	Perm 1X		Perm 2X		Perm 5X		Perm 10X		Delta 1X		Delta 2X		Delta 5X		Delta 10X	
	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N
Djénné	5	100	8	100	38	100	66	100	11	100	14	100	71	100	75	100
Mopti	6	100	9	100	36	100	54	100	29	100	59	100	83	96	91	95
Bandiagara	26	100	39	100	58	100	72	100	41	100	45	100	69	100	94	97
Bankass	2	100	21	98	40	99	60	100	22	101	39	99	72	87	81	100
Koulikoro	6	100	10	100	59	100	80	100	26	100	48	100	55	100	72	100
Fana	12	100	29	100	55	99	66	100	36	100	45	100	70	100	74	100
Baroueli	10	99	12	100	17	100	55	100	12	100	31	100	65	99	82	100
Bamako	0	100	2	100	47	100	63	100	7	97	28	98	54	100	86	100
Kati	6	100	30	100	40	100	68	99	26	100	48	100	61	94	83	93
Bla	12	100	18	94	68	100	74	100	18	100	30	99	76	100	87	100
Selingue	5	96	13	96	24	96	64	100	11	96	23	96	43	93	86	100
Bougouni	16	100	21	100	28	99	35	97	14	98	23	95	35	98	80	97
Niono	5	100	10	99	43	100R	63	100	26	100	45	100	69	100	81	100
Kita	4	100	17	100	35	100	60	100	11	100	33	100	58	99	78	100
Kadiolo	6	100	14	100	71	100	85	100	74	100	75	100	87	99	97	100

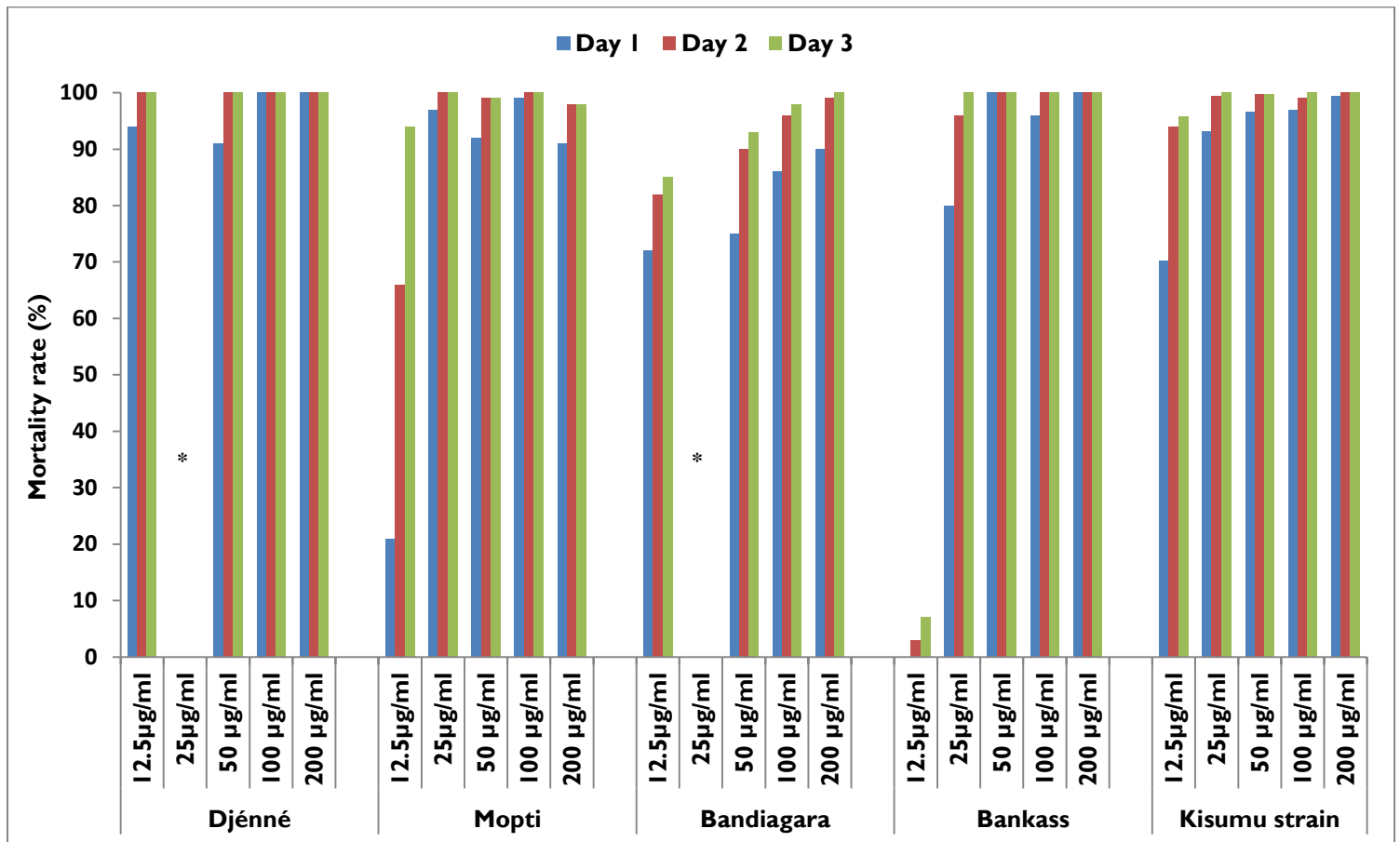
%; Mortality percentage; N: number tested; n/a - results not available

■ = resistant    ■ = possible resistance

### 3.12 DETERMINING THE DIAGNOSTIC CONCENTRATION OF CHLORFENAPYR IN CDC BOTTLE BIOASSAYS

Figure 23 displays mortality rates obtained after exposing populations of *An. gambiae* s.l. from four IRS sites and Kisumu strain to chlorfenapyr at concentrations of 12.5µg, 25µg, 50µg, 100µg and 200µg/bottle. At Day 3, only chlorfenapyr 100 and 200 µg/ml gave full susceptibility (defined as a mortality rate of more than 98 percent) with both field and Kisumu strains. The 100 µg/ml could be considered as the diagnostic concentration, as has been recommended by the CDC. More details are provided in Annex I, Table II.

**Figure 23: Results of *An. gambiae* s.l. (Field Strain and Kisumu) Tested Against Chlorphenapyr 12.5 µg/MI, 25 µg/MI, 50 µg/MI, 100 µg/ml, 200 µg/ml in Djenne, Mopti, Bandiagara, and Bankass**



\*Not tested



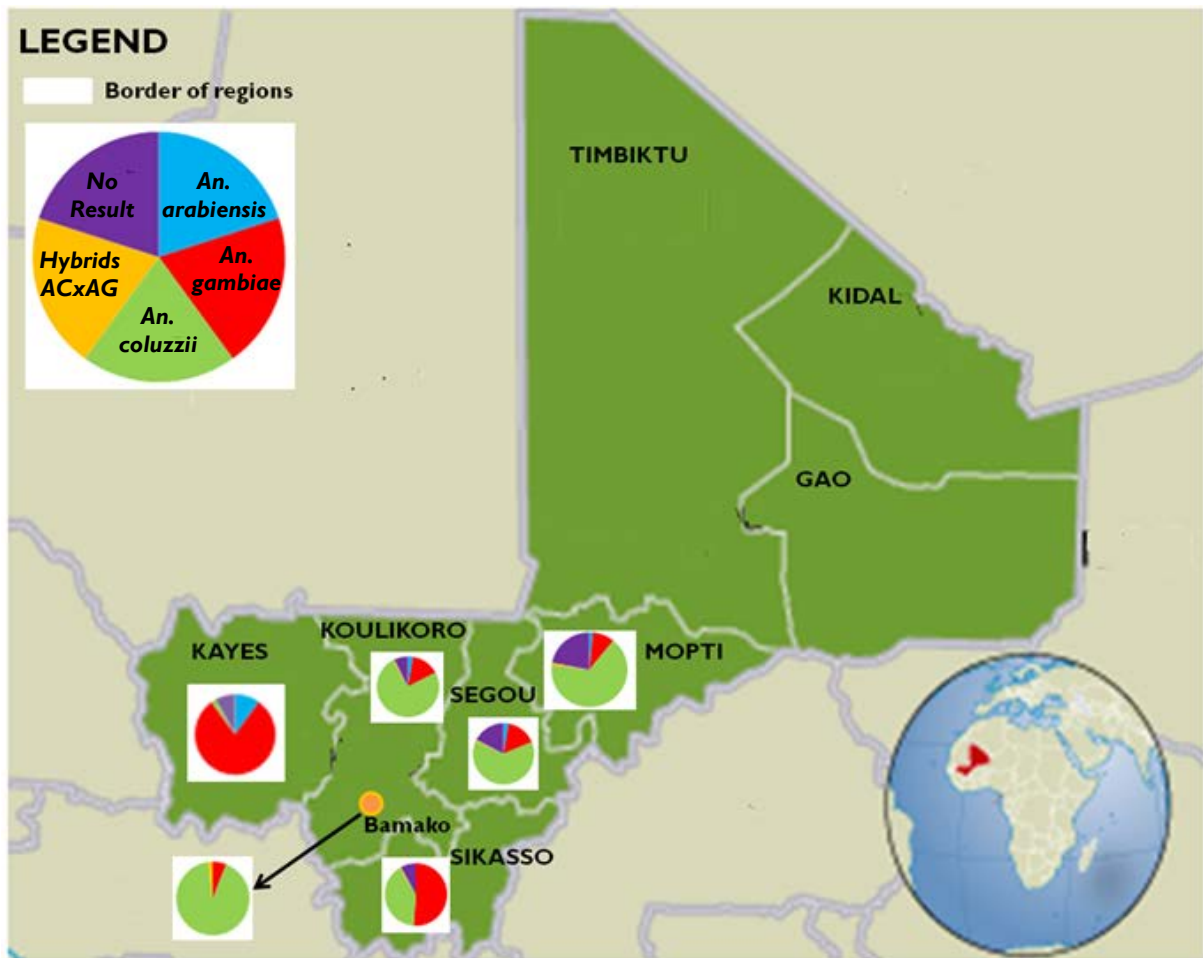
### 3.13 VECTOR SPECIES IDENTIFICATION FROM NATIONWIDE RESISTANCE SURVEYS

In total, 750 specimens of *An. gambiae* s.l. from the 15 selected sentinel sites (50 mosquitoes/site) were tested by PCR for species identification. *An. coluzzii* was the primary vector in eleven sites, followed by *An. gambiae* in two sites (Kita and Bougouni). Both species were in similar proportion in Barouéli and Sélingué ( $p>0.05$ ). Some rare specimens of *An. arabiensis* and hybrids (*coluzzii* x *gambiae*) were also detected (Table 15). Overall, the frequency of each species was as follows: 59.5% *An. coluzzii*, 24.7% *An. gambiae*, 2.1% *An. arabiensis* and 0.8% *An. coluzzii* x *gambiae* hybrids (Table 15), while 12.9% of samples did not amplify. Figure 24 displays molecular species distribution by region.

**Table 15: *An. gambiae* s.l. Sibling Species Composition from Insecticide Resistance Monitoring Tests in 15 Surveillance Sites**

Regions	Sites	Total tested	<i>An. arabiensis</i>		<i>An. gambiae</i>		<i>An. coluzzii</i>		Hybrid <i>coluzzii</i> & <i>gambiae</i>		Total Non Amplified	
			N	%	N	%	N	%	N	%	N	%
Kayes	Kita	50	5	10	40	80	1	2	0	0	4	8
Koulikoro	Koulikoro	50	2	4	9	18	35	70	1	2	3	6
	Fana	50	2	4	11	22	33	66	0	0	4	8
	Kati	50	0	0	2	4	44	88	0	0	4	8
	<b>Total</b>	<b>150</b>	<b>4</b>	<b>2.7</b>	<b>22</b>	<b>14.7</b>	<b>112</b>	<b>74.7</b>	<b>1</b>	<b>0.7</b>	<b>11</b>	<b>7.3</b>
Segou	Niono	50	0	0	0	0	45	90	0	0	5	10
	Bla	50	0	0	4	8	34	68	0	0	12	24
	Baroueli	50	4	8	20	40	16	32	1	2	9	18
	<b>Total</b>	<b>150</b>	<b>4</b>	<b>2.7</b>	<b>24</b>	<b>16</b>	<b>95</b>	<b>63.3</b>	<b>1</b>	<b>0.7</b>	<b>26</b>	<b>17.3</b>
Sikasso	Bougouni	50	0	0	44	88	3	6	1	2	2	4
	Selingue	50	0	0	24	48	18	36	0	0	8	16
	Kadiolo	50	0	0	9	18	39	78	0	0	2	4
	<b>Total</b>	<b>150</b>	<b>0</b>	<b>0</b>	<b>77</b>	<b>51.3</b>	<b>60</b>	<b>40</b>	<b>1</b>	<b>0.7</b>	<b>12</b>	<b>8</b>
Mopti	Djenne	50	1	2	5	10	38	76	1	2	5	10
	Mopti	50	0	0	1	2	30	60	0	0	19	38
	Bandiagara	50	2	4	9	18	29	58	1	2	9	18
	Bankass	50	0	0	4	8	35	70	0	0	11	22
	<b>Total</b>	<b>200</b>	<b>3</b>	<b>1.5</b>	<b>19</b>	<b>9.5</b>	<b>132</b>	<b>66</b>	<b>2</b>	<b>1</b>	<b>44</b>	<b>22</b>
Bamako	Bamako IV	50	0	0	3	6	46	92	1	2	0	0
<b>Grand Total</b>		<b>750</b>	<b>16</b>	<b>2.1</b>	<b>185</b>	<b>24.7</b>	<b>446</b>	<b>59.5</b>	<b>6</b>	<b>0.8</b>	<b>97</b>	<b>12.9</b>

Figure 24: *An. gambiae* s.l. Sibling Species by Region



### 3.14 VECTOR RESISTANCE MECHANISMS (*KDR* 1014 F, 1014S, AND *ACE1<sup>R</sup>*)

Molecular results of the 15 investigated sites are presented here. Only *An. gambiae* s.l. mosquitoes from IRS sites, former IRS sites, and sites with signs of resistance to bendiocarb and/or pirimiphos-methyl (only alive mosquitoes) were screened for the presence of *Ace1<sup>R</sup>* mutation (Table 16). Results obtained showed frequencies ranging from 0 percent in Sélingué to 53 percent in Bougouni, a site where resistance to bendiocarb was observed (76 percent mortality rate). In Bougouni, agricultural use of insecticides is practiced in high volumes. In current IRS sites of Djenne and Bandiagara, low frequency *Ace-1<sup>R</sup>* resistance was detected and should be closely monitored.

For the 1014F *kdr* mutation, 588 out of 750 specimens of *An. gambiae* s.l. were amplified (details are in Table 17 & Annex J, Tables J1- J5). Combined data of the seven sites showed the mean frequency was 65 percent for *An. coluzzii* and 89 percent for *An. gambiae* (Table 17). The *kdr* 1014S mutation was absent in most sites and at very low frequency where present (Table 18).

**Table 16: *AceI<sup>R</sup>* Mutation Allelic Frequencies in *An. gambiae* s.l. from 10 Insecticide Resistance Monitoring Sites**

Sites	Type of site	Status	Total amplified	Total Non Amplified	RR	SS	RS	f( <i>AceI<sup>R</sup></i> )
Bamako	SSRBP	Alive	9	0	0	7	2	0.11
Selingue	SSRBP	Alive	3	0	0	3	0	0
Kita	SSRBP	Alive	5	0	0	0	5	0.50
Bougouni	SSRBP	Alive	29	2	2	0	27	0.53
Kati	—	—	45	3	0	44	1	0.01
Koulikoro	Former IRS site	Dead and Alive	49	1	1	42	6	0.08
Fana	Former IRS site	Dead and Alive	46	2	0	39	7	0.08
Bla	Former IRS site	Dead and Alive	48	1	0	46	2	0.02
Baroueli	Former IRS site	Dead and Alive	31	19	1	19	11	0.2
Djénné	IRS site	Dead and Alive	44	4	1	41	2	0.05
Bandiagara	IRS site	Dead and Alive	43	4	1	42	0	0.02
Mopti	IRS site	Dead and Alive	22	28	0	21	1	0.02
Bankass	IRS site	Dead and Alive	36	13	0	34	2	0.03

SSRBP: Site with sign of resistance to bendiocarb and/or pirimiphos-methyl, RR: homozygous resistant, SS: homozygous susceptible, RS: Heterozygous resistant, f(*AceI<sup>R</sup>*): frequency of the acetylcholinesterase-I resistant gene

**Table 17: *An. gambiae* s.l. *Kdr* L1014F Mutation Allelic Frequencies from Insecticide Resistance Monitoring Sites**

Sites	Total amplified	Total Non Amplified	<i>An. arabiensis</i>				<i>An. gambiae</i>				<i>An. coluzzii</i>			
			RR	SS	RS	f( <i>kdr</i> )	RR	SS	RS	f( <i>kdr</i> )	RR	SS	RS	f( <i>kdr</i> )
Koulikoro	47	3	0	0	2	0.5	9	0	0	1	14	6	14	0.62
Bla	37	13	0	0	0	-	4	0	0	1	15	5	13	0.65
Selingue	42	8	0	0	0	-	22	1	1	0.94	5	3	10	0.56
Bamako	48	2	0	0	0	-	3	0	0	1	23	5	17	0.7
Niono	45	5	0	0	0	-	0	0	0	-	24	3	18	0.73
Kita	46	4	0	5	0	0	38	2	0	0.95	1	0	0	1
Kadiolo	48	2	0	0	0	-	8	0	2	0.9	12	5	21	0.59
Bougouni	43	7	0	0	0	-	36	1	3	0.93	1	0	2	0.66
Djenne	42	8	0	1	0	0	2	1	2	0.6	6	9	20	0.46
Fana	43	7	0	2	0	0	8	0	2	0.9	14	4	13	0.66
Mopti	7	43	0	0	0	-	0	0	0	-	0	0	7	0.5
Baroueli	26	24	0	3	0	0	9	1	2	0.83	5	1	5	0.68
Bandiagara	36	14	1	1	0	0.5	1	4	3	0.31	10	5	10	0.6
Bankass	33	17	0	0	0	-	3	0	1	0.87	18	3	8	0.75
Kati	45	5	0	0	0	-	2	0	0	1	23	4	16	0.72
<b>Grand Total</b>	<b>588</b>	<b>162</b>	<b>1</b>	<b>12</b>	<b>2</b>	<b>0.13</b>	<b>145</b>	<b>10</b>	<b>16</b>	<b>0.89</b>	<b>171</b>	<b>53</b>	<b>174</b>	<b>0.65</b>

RR: homozygous resistant, SS: homozygous susceptible, RS: Heterozygous resistant, f(*kdr*): frequency of the *kdr* resistant gene

**Table 18: *An. gambiae* s.l. *Kdr* L1014S Mutation Allelic Frequencies in Insecticide Resistance Monitoring Sites**

Sites	Total amplified	Total Non Amplified	<i>An. arabiensis</i>				<i>An. gambiae</i>				<i>An. coluzzii</i>			
			RR	SS	RS	f(kdr)	RR	SS	RS	f(kdr)	RR	SS	RS	f(kdr)
Koulikoro	47	3	0	2	0	0	0	8	0	0	0	34	2	0.03
Bla	37	13	0	0	0	-	0	4	0	0	0	34	0	0
Selingue	42	8	0	0	0	-	0	24	0	0	0	18	0	0
Bamako	49	1	0	0	0	-	0	3	0	0	0	46	0	0
Niono	45	5	0	0	0	-	0	0	0	-	0	45	0	0
Kita	46	4	0	4	1	0.1	0	40	0	0	0	1	0	0
Kadiolo	49	1	0	0	0	-	0	9	0	0	0	40	0	0
Bougouni	47	3	0	0	0	-	0	43	0	0	0	3	0	0
Djenne	39	11	0	1	0	0	1	3	0	0.25	0	33	0	0
Fana	44	6	0	1	1	0.25	0	11	0	0	0	31	0	0
Mopti	41	9	0	0	0	-	0	0	0	-	0	9	0	0
Baroueli	23	27	0	3	0	0	0	12	0	0	0	8	0	0
Bandiagara	36	14	0	1	1	0.25	0	5	2	0.14	0	27	0	0
Bankass	32	18	0	0	0	-	0	3	1	0.12	0	28	0	0
Kati	30	20	0	0	0	-	0	1	0	0	0	29	0	0
<b>Grand Total</b>	<b>607</b>	<b>143</b>	<b>0</b>	<b>12</b>	<b>3</b>	<b>0.10</b>	<b>1</b>	<b>166</b>	<b>3</b>	<b>0.01</b>	<b>0</b>	<b>386</b>	<b>2</b>	<b>0.00</b>

RR: homozygous resistant, SS: homozygous susceptible, RS: Heterozygous resistant, f(kdr): frequency of the kdr resistant gene

### 3.15 MALI LAB TRAINING

In 2016, a review of raw lab data from LBMA highlighted weaknesses with results from several protocols, in particular for sporozoite ELISA and species identification PCR. To address this several training sessions were conducted.

- 1) Laboratory training was held at LBMA from June 12-23, 2017. In total, 13 people participated in the training, which was led by consultants from Entomological Research Center of Cotonou (CREC), Benin.
- 2) Regional laboratory training was held at CREC, Benin from November 13-24, 2017 and covered all commonly used AIRS laboratory protocols. There were two participants from LBMA, Mali.
- 3) Follow-up practical training conducted by University of Notre Dame was held at LBMA on December 4-8, 2017. Six persons from LBMA attended this training. The focus was on specific protocols for species identification and sporozoite ELISA where specific issues were observed.

Progress made:

- LBMA now conduct laboratory testing on a monthly basis as soon as samples are received from the field. A monthly report, including the raw data (i.e., gel images for PCR and optical density values for sporozoite ELISA) is shared with Abt, University of Notre Dame, and CREC for review.
- Based on this system we have been able to quickly identify testing issues and implement solutions.
- We have high confidence in the sporozoite ELISA data for 2017. Changes in procedure include adjusting the wavelength of the plate reader, ensuring that all positive samples are repeat tested for confirmation, ensuring that the positive controls produce a value that is several times greater than the negative cut-off, and testing of 8 negative controls and 3 positive controls per plate.
- The protocol for species identification was changed and LBMA are now using the Santolamazza SINE 200 PCR method for detection of *An. arabiensis*, *An. gambiae*, and *An. coluzzii*. Previously, the proportion of hybrids detected was very high—several steps have been taken to reduce any risk of sample contamination, and hybrid rate detection in 2017 is within expected levels (less than 2%) compared with more than 30% in 2016. Any hybrids detected are repeat tested. Positive controls for all three species are now used.
- Some issues remain with blood-meal PCR which are being addressed. Refresher lab training is highly recommended for 2018 to reinforce general measures already implemented to improve the quality of PCR and ELISA data as well as follow up on any ongoing issues with blood-meal PCR or any other unusual results received in 2018.

Overall LBMA has shown a great willingness to work with partners to improve data quality and significant progress has been made for most analyzes (ELISA CSP, DNA extraction, species identification, *kdr* West/East).

### 3.16 QUALITY ASSURANCE, VECTOR SPECIES IDENTIFICATION AND *Ace I*<sup>R</sup>

Sub-samples of mosquito samples had legs removed and testing conducted on the same samples at both LBMA, Mali and Notre Dame, USA for quality assurance testing. Results have revealed there was a 91% concordance between both labs for species ID (Table 19). Overall, great progress has been made by LBMA in 2017 compared to 2016 when only 80% concordance was obtained. For the *Ace I*<sup>R</sup> genotypes, 95% concordance was noted, which is also encouraging (Table 19).

**TABLE 19: QUALITY ASSURANCE RESULTS, COMPARISON OF MOLECULAR RESULTS FROM LBMA AND UND**

	Species ID (Ac, Ag, Aa, Acg)				<i>Ace I</i> <sup>R</sup> 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
Kadiolo	20	16	80%	20%	-	-	-	-
Niono	16	16	100%	0%	-	-	-	-
Bougouni	18	18	100%	0%	-	-	-	-
Bamako	20	19	95%	5%	-	-	-	-
Selingue	15	13	87%	13%	-	-	-	-
Kita	20	20	100%	0%	-	-	-	-
Bla	13	13	100%	0%	8	8	100%	0%
Koulikoro	19	18	95%	5%	20	20	100%	0%

Djenne	17	15	88%	12%	16	15	94%	6%
Fana	17	11	65%	35%	13	11	85%	15%
TOTAL	175	159	91%	9%	57	54	95%	5%

## 4. CONCLUSIONS

Overall, *An. coluzzii* was the most predominant species at all sites. In IRS sites, the pre-IRS data collected in June-July 2017 revealed mean indoor *An. gambiae* s.l. density of 6.2 *An. gambiae* s.l. per house per day versus 1.8 *An. gambiae* s.l. per house per day after IRS (August-December). In Mopti, up to 11 *An. gambiae* s.l. per house per day were collected only two months after IRS operations (October). This may be due to the lower efficacy of the insecticide on walls or the fact that mosquitoes found unsprayed shelters inside houses (e.g., hanging clothes). Indeed, the results we obtained after cone bioassays revealed a residual efficacy of three months with mud, the most frequently encountered substrate. A slightly longer duration was obtained on some substrates such as painted mud, painted cement, and the combination of painted mud and cement.

The peak *An. gambiae* biting rate was obtained at all sites (except Bandiagara) in August and September 2017. The peak biting times were observed between 10:00 pm and 4:00 a.m. Overall, in IRS sites, the HBR ranged from 0.5 b/h/n in Bankass to 7.5 b/h/n in Mopti. In the former IRS sites, the large increase in *An. gambiae* s.l. biting rate in August-September 2017 compared to the same period in 2016 is of concern. The greater increase in biting rate in Koulikoro (former IRS site) compared to Kati (the paired control) indicates that IRS withdrawal may be a contributing factor. In addition, no mosquitoes were caught in 2016 by HLC in Barouéli, a former IRS site where we have a mean biting rate of 5.4 b/h/n in 2017. For both sites (Koulikoro & Baroueli), over five months (August-December), mean vector sporozoite rates in 2017 were 0.8 percent (95%CI: 0.2-1.5) and 1.3 percent (95% CI: 0.2-2.5) in 2016, but there was no significant difference ( $p=0.42$ ).

The entomological inoculation rate was 7.05 ib/h/5 months for the two sites in 2016 while it was 15.37 ib/h/5months in 2017, which equates to a two-fold increase after IRS withdrawal. A similar result was obtained in Benin by Ossé et al. (2013) following IRS relocation. Moreover, in Kati, the control site, the EIR was 18.6 ib/h/5months in 2016 while it was 6.21 ib/h/5months in 2017, a three-fold reduction. This supports the hypothesis that relocation of IRS contributed to an increase in the key indicators of malaria transmission.

Resistance and possible resistance to bendiocarb 0.1% were observed in several sites while full susceptibility to pirimiphos-methyl 0.25% was observed in all sites. High frequency resistance to pyrethroids in *An. gambiae* s.l. was recorded with variable levels of resistance according to the specific insecticide. With clothianidin, mortality in all four IRS sites reached at least 98 percent after six days. In Koulikoro, a former IRS site, mortality reached 100 percent after two days. Since the protocol used to test chlothianidin is not yet standardized and validated by the WHO, the mortality rates of less than 98 percent that we obtained cannot necessarily be interpreted as signs of resistance. The intensity of resistance was higher to permethrin compared to deltamethrin but survivors were recorded at ten times the diagnostic dose in all sites to both pyrethroids. As previously reported, 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<sup>R</sup>* mutation indicates the importance of monitoring this mutation over time and raises the issue of the use of an insecticide with a mode of action different from that of carbamates and organophosphates as part of a resistance management strategy.



## 5. ANNEXES

### ANNEX A: *AN. GAMBIAE* S.L. SIBLING SPECIES PCR

Table A1: *An. gambiae* s.l. Sibling Species PCR Across Sites, June-December 2017

Sites	N tested	<i>An. coluzzi</i>	% <i>An. coluzzi</i>	<i>An. gambiae</i>	% <i>An. gambiae</i>	<i>gambiae/Coluzzi</i>	% <i>gambiae/coluzzi</i>	<i>An. arabiensis</i>	% <i>An. arabiensis</i>	NA	% NA
IRS Site 1: Djénné	72	64	88.9	4	5.6	0	0	1	1.4	3	4.2
IRS Site 2: Mopti	134	124	92.5	9	6.7	0	0	0	0	1	0.7
IRS Site 3: Bandiagara	85	74	87.1	7	8.2	0	0	3	3.5	1	1.2
IRS Site 4: Bankass	74	69	93.2	4	5.4	0	0	1	1.4	0	0
<b>Total: IRS Sites</b>	<b>365</b>	<b>331</b>	<b>90.7</b>	<b>24</b>	<b>6.6</b>	<b>0</b>	<b>0</b>	<b>5</b>	<b>1.4</b>	<b>5</b>	<b>1.4</b>
Former IRS Site 1: Koulikoro	148	146	98.6	1	0.7	0	0	0	0	1	0.7
Former IRS Site 2: Barouéli	28	18	64.3	8	28.6	1	3.6	1	3.6	0	0
<b>Total: Former IRS Sites</b>	<b>176</b>	<b>164</b>	<b>93.2</b>	<b>9</b>	<b>5.1</b>	<b>1</b>	<b>0.6</b>	<b>1</b>	<b>0.6</b>	<b>1</b>	<b>0.6</b>
<b>Control Sites: Kati</b>	145	141	97.2	1	0.7	0	0	0	0	3	2.1
<b>GRAND TOTAL</b>	<b>686</b>	<b>636</b>	<b>92.7</b>	<b>34</b>	<b>5</b>	<b>1</b>	<b>0.1</b>	<b>6</b>	<b>0.9</b>	<b>9</b>	<b>1.3</b>

NA: Non-amplified



## ANNEX B: HUMAN BITING RATE (HBR), HLC

Table B1: HBR, HLC, Seasonality in IRS sites, Former IRS Sites and Control Site, 2017

SITES	Parameters	Pre-IRS			Post-IRS					
		June	July	Mean	August	September	October	November	December	Mean
<b>Djénné : IRS site 1</b>	N of An. gamb	3	43		115	24	3	2	2	
	Human night	16	16		16	16	16	16	16	
	HBR (b/h/n)	0.2	2.7	1.4	7.2	1.5	0.2	0.1	0.1	1.8
	HBR period	5.6	80.6	86.3	215.6	45	5.6	3.8	3.8	273.8
<b>Mopti : IRS site 2</b>	N of An. gamb	6	29		137	324	103	35	4	
	Human night	16	16		16	16	16	16	16	
	HBR (b/h/n)	0.4	1.8	1.1	8.6	20.3	6.4	2.2	0.3	7.5
	HBR period	11.3	54.4	65.6	256.9	607.5	193.1	65.6	7.5	1130.6
<b>Bandiagara : IRS site 3</b>	N of An. gamb	38	54		25	23	12	2	0	
	Human night	16	16		16	16	16	16	16	
	HBR (b/h/n)	2.4	3.4	2.9	1.6	1.4	0.8	0.1	0	0.8
	HBR period	71.3	101.3	172.5	46.9	43.1	22.5	3.8	0	116.3
<b>Bankass : IRS site 4</b>	N of An. gamb	8	9		22	8	10	0	0	
	Human night	16	16		16	16	16	16	16	
	HBR (b/h/n)	0.5	0.6	0.5	1.4	0.5	0.6	0	0	0.5
	HBR period	15	16.9	31.9	41.3	15	18.8	0	0	75
<b>Koulikoro : Former IRS site 1</b>	N of An. gamb	98	389		2325	1078	464	242	55	
	Human night	16	16		16	16	16	16	16	
	HBR (b/h/n)	6.1	24.3	15.2	145.3	67.4	29	15.1	3.4	52.1
	HBR period	183.8	729.4	913.1	4359.4	2021.3	870	453.8	103.1	7807.5
<b>Barouéli: Former IRS site 2</b>	N of An. gamb	0	0		266	164	0	0	0	
	Human night	16	16		16	16	16	16	16	
	HBR (b/h/n)	0	0	0	16.6	10.3	0	0	0	5.4
	HBR period	0	0	0	498.8	307.5	0	0	0	806.3

SITES	Parameters	Pre-IRS			Post-IRS					
		June	July	Mean	August	September	October	November	December	Mean
<b>Kati: Control site</b>	N of <i>An. gamb</i>	48	597		1671	905	203	79	63	
	Human night	16	16		16	16	16	16	16	
	HBR (b/h/n)	3	37.3	20.2	104.4	56.6	12.7	4.9	3.9	36.5
	HBR period	90	1119.4	1209.4	3133.1	1696.9	380.6	148.1	118.1	5476.9
<b>Grand Total: IRS sites</b>	<b>HBR (b/h/n)</b>	<b>0.9</b>	<b>2.1</b>	<b>1.5</b>	<b>4.7</b>	<b>5.9</b>	<b>2</b>	<b>0.6</b>	<b>0.1</b>	<b>2.7</b>
	<b>HBR period</b>	<b>25.8</b>	<b>63.3</b>	<b>89.1</b>	<b>140.2</b>	<b>177.7</b>	<b>60</b>	<b>18.3</b>	<b>2.8</b>	<b>398.9</b>
<b>Grand Total: Former IRS sites</b>	<b>HBR (b/h/n)</b>	<b>3.1</b>	<b>12.2</b>	<b>7.6</b>	<b>81</b>	<b>38.8</b>	<b>14.5</b>	<b>7.6</b>	<b>1.7</b>	<b>28.7</b>
	<b>HBR period</b>	<b>91.9</b>	<b>364.7</b>	<b>456.6</b>	<b>2429.1</b>	<b>1164.4</b>	<b>435</b>	<b>226.9</b>	<b>51.6</b>	<b>4306.9</b>

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

## ANNEX C: HUMAN BITING RATE (HBR), HLC, BITING TIME

TABLE CI: HBR, HLC, BITING TIME, IRS SITES, 2017

Sites	Biting location	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
<b>Djenné:</b>	Indoor	N of <i>An. gamb</i>	9	9	4	11	11	6	4	24	7	21	19	12

<b>IRS Site 1</b>		Man night	56	56	56	56	56	56	56	56	56	56	56	
		HBR (b/m/n)	0.16	0.16	0.07	0.20	0.20	0.11	0.07	0.43	0.13	0.38	0.34	0.21
	Outdoor	N of An. gamb	0	1	2	2	3	6	5	8	7	8	6	7
<b>Mopti: IRS Site 2</b>		Man night	56	56	56	56	56	56	56	56	56	56	56	
		HBR (b/m/n)	0	0.02	0.04	0.04	0.05	0.11	0.09	0.14	0.13	0.14	0.11	0.13
	Indoor	N of An. gamb	6	12	17	26	35	30	40	22	26	20	16	8
<b>Bandiagara: IRS Site 3</b>		Man night	56	56	56	56	56	56	56	56	56	56	56	
		HBR (b/m/n)	0.11	0.21	0.30	0.46	0.63	0.54	0.71	0.39	0.46	0.36	0.29	0.14
	Outdoor	N of An. gamb	4	8	11	22	39	35	56	42	49	47	40	27
<b>Bankass: IRS Site 4</b>		Man night	56	56	56	56	56	56	56	56	56	56	56	
		HBR (b/m/n)	0.07	0.14	0.20	0.39	0.70	0.63	1	0.75	0.88	0.84	0.71	0.48
	Indoor	N of An. gamb	13	4	7	4	6	6	9	9	10	9	8	8
<b>Former IRS site I</b>		Man night	56	56	56	56	56	56	56	56	56	56	56	
		HBR (b/m/n)	0.23	0.07	0.13	0.07	0.11	0.11	0.16	0.16	0.18	0.16	0.14	0.14
	Outdoor	N of An. gamb	7	2	3	0	4	8	7	11	6	2	4	7
<b>Former IRS site I</b>		Man night	56	56	56	56	56	56	56	56	56	56	56	
		HBR (b/m/n)	0.13	0.04	0.05	0	0.07	0.14	0.13	0.20	0.11	0.04	0.07	0.13
	Indoor	N of An. gamb	2	0	1	2	2	0	2	1	1	1	1	10
<b>Former IRS site I</b>		Man night	56	56	56	56	56	56	56	56	56	56	56	
		HBR (b/m/n)	0.04	0	0.02	0.04	0.04	0	0.04	0.02	0.02	0.02	0.02	0.18
	Outdoor	N of An. gamb	2	0	0	0	0	6	4	2	3	7	4	6
<b>Former IRS site I</b>		Man night	56	56	56	56	56	56	56	56	56	56	56	
		HBR (b/m/n)	0.04	0	0	0	0	0.11	0.07	0.04	0.05	0.13	0.07	0.11

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

**TABLE C2: HBR, HLC, BITING TIME, FORMER IRS SITES & CONTROL SITE, 2017**

Sites	Biting location	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
<b>Koulikoro : Former IRS site I</b>	Indoor	N of An. gamb	24	46	75	148	250	248	325	271	328	242	260	213
		Man night	56	56	56	56	56	56	56	56	56	56	56	56

		HBR (b/m/n)	0.43	0.82	1.34	2.64	4.46	4.43	5.80	4.84	5.86	4.32	4.64	3.80
	Outdoor	N of <i>An. gamb</i>	39	64	85	132	224	163	234	289	248	292	274	177
		Man night	56	56	56	56	56	56	56	56	56	56	56	56
		HBR (b/m/n)	0.70	1.14	1.52	2.36	4	2.91	4.18	5.16	4.43	5.21	4.89	3.16
Barouéli Former IRS site 2		Indoor	N of <i>An. gamb</i>	2	0	5	11	19	27	23	22	20	23	25
	Man night		56	56	56	56	56	56	56	56	56	56	56	56
	HBR (b/m/n)		0.04	0	0.09	0.20	0.34	0.48	0.41	0.39	0.36	0.41	0.45	0.27
	Outdoor	N of <i>An. gamb</i>	4	1	5	7	13	18	24	51	38	32	27	18
		Man night	56	56	56	56	56	56	56	56	56	56	56	56
		HBR (b/m/n)	0.07	0.02	0.09	0.13	0.23	0.32	0.43	0.91	0.68	0.57	0.48	0.32
Kati Control site	Indoor	N of <i>An. gamb</i>	18	65	31	55	119	137	145	221	247	270	224	159
		Man night	56	56	56	56	56	56	56	56	56	56	56	56
		HBR (b/m/n)	0.32	1.16	0.55	0.98	2.13	2.45	2.59	3.95	4.41	4.82	4	2.84
	Outdoor	N of <i>An. gamb</i>	11	28	32	55	142	159	171	228	301	238	271	239
		Man night	56	56	56	56	56	56	56	56	56	56	56	56
		HBR (b/m/n)	0.20	0.50	0.57	0.98	2.54	2.84	3.05	4.07	5.38	4.25	4.84	4.27

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

## ANNEX D: HUMAN BITING RATE (HBR), HLC, AND BITING LOCATION

Table D1: HBR, HLC, and Biting Location at IRS sites, 2017

Sites	Feeding location	Parameters	Pre-IRS			Post-IRS					
			June	July	Mean	August	September	October	November	December	Mean
<b>Djénné : IRS site 1</b>	Indoor	N of <i>An. gamb</i>	3	29		89	13	1	1	1	
		Man night	8	8		8	8	8	8	8	
		HBR (b/m/n)	0.4	3.6	2	11.1	1.6	0.1	0.1	0.1	2.6
	Outdoor	N of <i>An. gamb</i>	0	14		26	11	2	1	1	
		Man night	8	8		8	8	8	8	8	
		HBR (b/m/n)	0	1.8	0.9	3.3	1.4	0.3	0.1	0.1	1
<b>Mopti : IRS site 2</b>	Indoor	N of <i>An. gamb</i>	6	15		67	119	29	18	4	
		Man night	8	8		8	8	8	8	8	
		HBR (b/m/n)	0.8	1.9	1.3	8.4	14.9	3.6	2.3	0.5	5.9
	Outdoor	N of <i>An. gamb</i>	0	14		70	205	74	17	0	
		Man night	8	8		8	8	8	8	8	
		HBR (b/m/n)	0	1.8	0.9	8.8	25.6	9.3	2.1	0	9.2
<b>Bandiagara : IRS site 3</b>	Indoor	N of <i>An. gamb</i>	25	35		16	13	3	1	0	
		Man night	8	8		8	8	8	8	8	
		HBR (b/m/n)	3.1	4.4	3.8	2	1.6	0.4	0.1	0	0.8
	Outdoor	N of <i>An. gamb</i>	13	19		9	10	9	1	0	
		Man night	8	8		8	8	8	8	8	
		HBR (b/m/n)	1.6	2.4	2	1.1	1.3	1.1	0.1	0	0.7
<b>Bankass : IRS site 4</b>	Indoor	N of <i>An. gamb</i>	5	3		9	4	2	0	0	
		Man night	8	8		8	8	8	8	8	
		HBR (b/m/n)	0.6	0.4	0.5	1.1	0.5	0.3	0	0	0.4
	Outdoor	N of <i>An. gamb</i>	3	6		13	4	8	0	0	
		Man night	8	8		8	8	8	8	8	
		HBR (b/m/n)	0.4	0.8	0.6	1.6	0.5	1	0	0	0.6
<b>Grand total : IRS sites</b>	Indoor	N of <i>An. gamb</i>	39	82		181	149	35	20	5	
		Man night	32	32		32	32	32	32	32	
		HBR (b/m/n)	1.2	2.6	1.9	5.7	4.7	1.1	0.6	0.2	2.4
	Outdoor	N of <i>An. gamb</i>	16	53		118	230	93	19	1	
		Man night	32	32		32	32	32	32	32	
		HBR (b/m/n)	0.5	1.7	1.1	3.7	7.2	2.9	0.6	0	2.9

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

**Table D2: HBR, HLC, Biting Location, Former IRS sites, and Control site, 2017**

Sites	Feeding location	Parameters	Pre-IRS			Post-IRS					Mean
			June	July	Mean	August	September	October	November	December	
<b>Koulikoro (Former IRS site 1)</b>	Indoor	N of <i>An. gamb</i>	31	199		1285	535	217	134	29	55
		Man night	8	8		8	8	8	8	8	
		HBR (b/m/n)	3.9	24.9	14.4	160.6	66.9	27.1	16.8	3.6	
	Outdoor	N of <i>An. gamb</i>	67	190		1040	543	247	108	26	49.1
		Man night	8	8		8	8	8	8	8	
		HBR (b/m/n)	8.4	23.8	16.1	130	67.9	30.9	13.5	3.3	
<b>Barouéli Former IRS site 2</b>	Indoor	N of <i>An. gamb</i>	0	0		109	83	0	0	0	4.8
		Man night	8	8		8	8	8	8	8	
		HBR (b/m/n)	0	0	0	13.6	10.4	0	0	0	
	Outdoor	N of <i>An. gamb</i>	0	0		157	81	0	0	0	6
		Man night	8	8		8	8	8	8	8	
		HBR (b/m/n)	0	0	0	19.6	10.1	0	0	0	
<b>Kati Control site</b>	Indoor	N of <i>An. gamb</i>	28	287		778	419	117	34	28	34.4
		Man night	8	8		8	8	8	8	8	
		HBR (b/m/n)	3.5	35.9	19.7	97.3	52.4	14.6	4.3	3.5	
	Outdoor	N of <i>An. gamb</i>	20	310		893	486	86	45	35	38.6
		Man night	8	8		8	8	8	8	8	
		HBR (b/m/n)	2.5	38.8	20.6	111.6	60.8	10.8	5.6	4.4	
<b>Grand total Former IRS sites</b>	Indoor	N of <i>An. gamb</i>	31	199		1394	618	217	134	29	29.9
		Man night	16	16		16	16	16	16	16	
		HBR (b/m/n)	1.9	12.4	7.2	87.1	38.6	13.6	8.4	1.8	
	Outdoor	N of <i>An. gamb</i>	67	190		1197	624	247	108	26	27.5
		Man night	16	16		16	16	16	16	16	
		HBR (b/m/n)	4.2	11.9	8	74.8	39	15.4	6.8	1.6	

HBR: human biting rate; N of *An. gamb*: Number of *Anopheles gambiae* s.l.; b/h/n: bites/human/night

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

TABLE EI: SPOROZOITE INDEX IN *AN. GAMBIAE* S.L., MONTHLY COLLECTIONS, ALL SITES, 2017

Sites	Parameters	Pre-IRS				Post-IRS						
		June	July	Total	CI	August	September	October	November	December	Total	CI
<b>Djénne:</b> <b>IRS site 1</b>	N tested	55	60	115		50	35	3	2	4	94	
	N positive	3	1	4		0	0	1	1	0	2	
	SI (%)	5.5	1.7	3.5	[0.1 - 6.8]	0	0	33.3	50	0	2.1	[0 - 5]
<b>Mopti:</b> <b>IRS site 2</b>	N tested	8	60	68		50	75	152	32	40	349	
	N positive	0	0	0		0	1	0	2	0	3	
	SI (%)	0	0	0	-	0	1.3	0	6.3	0	0.9	[0 - 1.8]
<b>Bandiagara:</b> <b>IRS site 3</b>	N tested	70	60	130		33	40	34	2	0	109	
	N positive	2	2	4		0	2	1	0	0	3	
	SI (%)	2.9	3.3	3.1	[0.1 - 6]	0	5	2.9	0	-	2.8	[0 - 5.8]
<b>Bankass:</b> <b>IRS site 4</b>	N tested	25	23	48		22	32	11	0	0	65	
	N positive	1	2	3		0	0	1	0	0	1	
	SI (%)	4	8.7	6.3	[0 - 13.1]	0	0	9.1	-	-	1.5	[0 - 4.5]
<b>Koulikoro:</b> <b>Former IRS site 1</b>	N tested	90	60	150		205	124	150	160	69	708	
	N positive	0	1	1		0	0	2	0	2	4	
	SI (%)	0	1.7	0.7	[0 - 2]	0	0	1.3	0	2.9	0.6	[0 - 1.1]
<b>Barouéli:</b> <b>Former IRS site 2</b>	N tested	0	0	0		60	57	0	0	0	117	
	N positive	0	0	0		0	3	0	0	0	3	
	SI (%)	-	-	-	[-]	0	5.3	-	-	-	2.6	[0 - 5.4]
<b>Kati:</b> <b>Control site</b>	N tested	72	59	131		93	64	75	79	54	365	
	N positive	1	0	1		0	0	0	1	2	3	
	SI (%)	1.4	0	0.8	[0 - 2.3]	0	0	0	1.3	3.7	0.8	[0 - 1.7]
<b>Grand Total:</b> <b>IRS sites</b>	N tested	158	203	361		155	182	200	36	44	617	
	N positive	6	5	11		0	3	3	3	0	9	
	SI (%)	3.8	2.5	3	[1.3 - 4.8]	0	1.6	1.5	8.3	0	1.5	[0.5 - 2.4]
<b>Grand Total:</b> <b>Former IRS sites</b>	N tested	90	60	150		265	181	150	160	69	825	
	N positive	0	1	1		0	3	2	0	2	7	
	SI (%)	0	1.7	0.7	[0 - 2]	0	1.7	1.3	0	2.9	0.8	[0.2 - 1.5]

N: Number of *An. gambiae* s.l., SI: sporozoite index

## ANNEX F: ENTOMOLOGICAL INOCULATION RATE (EIR)

TABLE FI: EIR, MONTHLY COLLECTIONS, ALL SITES, 2017

Sites	Parameters	Pre-IRS			Post-IRS					
		June	July	Total	August	September	October	November	December	Total
<b>Djénné:</b> <b>IRS Site 1</b>	HBR (b/h/n)	0.2	2.7		7.2	1.5	0.2	0.1	0.1	
	SI (%)	5.5	1.7		0	0	33.3	50	0	
	EIR (ib/h/n)	0.01	0.04	<b>0.028</b>	0	0	0.06	0.06	0	<b>0.03</b>
	EIR (period )	0.31	1.34	<b>1.65</b>	0	0	1.88	1.88	0	<b>3.75</b>
<b>Mopti:</b> <b>IRS Site 2</b>	HBR (b/h/n)	0.4	1.8		8.6	20.3	6.4	2.2	0.3	
	SI (%)	0	0.0		0	1.3	0	6.3	0	
	EIR (ib/h/n)	0	0	<b>0</b>	0	0.27	0	0.14	0	<b>0.08</b>
	EIR (period )	0	0	<b>0</b>	0	8.10	0	4.10	0	<b>12.20</b>
<b>Bandiagara</b> <b>IRS Site 3</b>	HBR (b/h/n)	2.4	3.4		1.6	1.4	0.8	0.1	0.0	
	SI (%)	2.9	3.3		0	5	2.9	0		
	EIR (ib/h/n)	0.07	0.11	<b>0.090</b>	0	0.07	0.02	0	0	<b>0.02</b>
	EIR (period )	2.04	3.38	<b>5.41</b>	0	2.16	0.66	0	0	<b>2.82</b>
<b>Bankass</b> <b>IRS Site 4</b>	HBR (b/h/n)	0.5	0.6		1.4	0.5	0.6	0	0	
	SI (%)	4	8.7		0	0	9.1	-	-	
	EIR (ib/h/n)	0.02	0.05	<b>0.034</b>	0	0	0.06	0	0	<b>0.01</b>
	EIR (period )	0.60	1.47	<b>2.07</b>	0	0	1.70	0	0	<b>1.70</b>
<b>Koulikoro</b> <b>Former IRS Site 1</b>	HBR (b/h/n)	6.1	24.3		145.3	67.4	29.0	15.1	3.4	
	SI (%)	0	1.7		0	0	1.3	0	2.9	
	EIR (ib/h/n)	0	0.41	<b>0.203</b>	0	0	0.39	0	0.1	<b>0.10</b>
	EIR (period )	0	12.16	<b>12.16</b>	0	0	11.60	0	2.96	<b>14.56</b>
<b>Barouéli</b> <b>Former IRS Site 2</b>	HBR (b/h/n)	0	0		16.6	10.3	0	0	0	
	SI (%)	0	0		0	5.3	-	-	-	
	EIR (ib/h/n)	0	0	<b>0</b>	0	0.54	0	0	0	<b>0.11</b>
	EIR (period )	0	0	<b>0</b>	0	16.18	0	0	0	<b>16.18</b>



Sites	Parameters	Pre-IRS			Post-IRS					
		June	July	Total	August	September	October	November	December	Total
<b>Kati Control site</b>	HBR (b/h/n)	3.0	37.3		104.4	56.6	12.7	4.9	3.9	
	SI (%)	1.4	0.0		0	0	0	1.3	3.7	
	EIR (ib/h/n)	0.04	0	<b>0.021</b>	0	0	0	0.06	0.14	<b>0.04</b>
	EIR (period )	1.25	0	<b>1.25</b>	0	0	0	1.88	4.33	<b>6.21</b>
<b>Grand Total</b>	EIR (ib/h/n)	0.0	0.1	<b>0.038</b>	0	0.09	0.04	0.05	0	<b>0.03</b>
<b>IRS sites</b>	EIR (period )	0.7	1.5	<b>2.28</b>	0	2.56	1.06	1.49	0	<b>5.12</b>
<b>Grand Total</b>	EIR (ib/h/n)	0.0	0.2	<b>0.1</b>	0	0.27	0.19	0	0.05	<b>0.10</b>
<b>Former IRS sites</b>	EIR (period )	0.0	6.1	<b>6.08</b>	0	8.09	5.80	0	1.48	<b>15.37</b>

EIR: Entomological Inoculation Rate, ib/h/n: Infected bite/human/night

## ANNEX G: CONE TESTS AND FUMIGANT BIOASSAYS RESULTS

**Table G1: WHO Cone Tests Results of *An. Gambiae* Kisumu Strain - Mortality After 30 Minutes Exposure to Pirimiphos-Methyl 300CS in Djenne, Mopti, Bandiagara, and Bankass (August 2017-January 2018)**

Substrates	Number of Tested Structures	Parameters	T0 (Aug-17)	T1 (Sept-17)	T2 (Oct-17)	T3 (Nov-17)	T4 (Dec-17)	T5 (Jan-18)
Mud	8	N tested	378	402	378	387	374	380
		N Dead	378	402	330	371	284	222
		Mortality (%)	100	100	87.3	95.9	76	58.4
		Corrected Mortality	-	-	86.6	-	-	-
Painted Cement	3	N tested	141	151	140	144	139	144
		N Dead	141	151	135	140	127	78
		Mortality (%)	100	100	96.4	97.2	91.4	54.2
		Corrected Mortality	-	-	-	97.1	-	-
Painted Mud	5	N tested	233	239	237	243	235	246
		N Dead	233	239	234	225	229	202
		Mortality (%)	100	100	98.7	92.6	97.5	82.1
		Corrected Mortality	-	-	-	-	-	-
Mud+Cement	1	N tested	47	47	47	47	48	46
		N Dead	47	47	43	36	37	35
		Mortality (%)	100	100	91.5	76.6	77.1	76.1
		Corrected Mortality	-	-	-	-	-	-
Painted Mud+Cement	3	N tested	142	139	136	141	144	143
		N Dead	142	139	135	140	144	122
		Mortality (%)	100	100	99.3	99.3	100	85.3
		Corrected Mortality	-	-	-	-	-	-

**Table G2: Fumigant Bioassays Results for *An. gambiae* Kisumu Strain - Mortality After 30 Minutes Exposure To Pirimiphos-Methyl 300CS in Djenne, Mopti, Bandiagara, and Bankass (August 2017-January 2018)**

Substrates	T of Tested Structures	Parameters	T0 (Aug)	T1 (Sept)	T2 (Oct)	T3 (Nov)	T4 (Dec)	T5 (Jan)
Mud	8	N tested	93	103	93	96	92	96
		N Dead	90	87	50	63	54	23
		Mortality (%)	96.8	84.5	53.8	65.6	58.7	24
		Corrected Mortality	-	83.9	50.6	62.9	57.8	23.1
Painted Cement	3	N tested	34	37	36	36	35	39
		N Dead	34	29	28	18	28	13
		Mortality (%)	100	78.4	77.8	50	80	33.3
		Corrected Mortality	-	77.2	-	-	79.4	-
Painted Mud	5	N tested	58	60	57	58	56	60
		N Dead	58	60	57	40	53	19
		Mortality (%)	100	100	100	69	94.6	31.7
		Corrected Mortality	-	-	-	-	-	29.3
Mud+Cement	1	N tested	11	12	10	12	12	12
		N Dead	11	12	10	8	9	0
		Mortality (%)	100	100	100	66.7	75	0
		Corrected Mortality	-	-	-	63.3	-	-
Painted Mud+Cement	3	N tested	36	33	34	34	36	36
		N Dead	36	33	34	29	31	5
		Mortality (%)	100	100	100	85.3	86.1	13.9
		Corrected Mortality	-	-	-	84.8	-	-

## ANNEX H: WHO SUSCEPTIBILITY TUBE TEST RESULTS

Table HI: WHO Tube Tests Results for Permethrin 0.75%, Alphacypermethrin 0.05%, Deltamethrin 0.05%, and Pirimiphos-methyl 0.25%

SITES	Permethrin 0.75%				Alphacypermethrin 0.05%				Deltamethrin 0.05%			
	N Tested	N dead	Mortality (%)	Corrected Mortality	N Tested	N dead	Mortality (%)	Corrected Mortality	N Tested	N dead	Mortality (%)	Corrected Mortality
Djenne	100	19	19	-	100	25	25	-	100	45	45	-
Mopti	100	42	42	-	100	51	51	-	96	72	75	-
Bandiagara	100	45	45	-	100	55	55	-	100	86	86	-
Bankass	100	55	55	-	100	64	64	-	100	77	77	-
Koulikoro	96	21	22	-	95	34	36	-	100	63	63	-
Fana	98	16	16	-	100	24	24	-	100	81	81	-
Baroueli	100	41	41	-	95	42	44	-	94	58	62	-
Bamako	100	12	12	-	100	14	14	-	100	49	49	-
Kati	100	51	51	-	100	6	6	-	98	76	78	-
Bla	96	17	18	-	100	20	20	-	100	43	43	-
Selingue	97	9	9	-	100	17	17	-	100	21	21	-
Bougouni	100	13	13	-	99	16	16	-	100	26	26	-
Niono	97	25	26	-	98	28	29	-	73	37	51	-
Kita	99	15	15	-	104	36	35	-	98	38	39	-
Kadiolo	99	11	11	-	100	24	24	-	100	54	54	-
<b>Mean</b>	<b>1382</b>	<b>373</b>	<b>27</b>	<b>-</b>	<b>1391</b>	<b>431</b>	<b>31</b>	<b>-</b>	<b>1359</b>	<b>781</b>	<b>57</b>	<b>-</b>

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

**Table H2: WHO Tube Tests Results for Bendiocarb 0.1% and Pirimiphos-methyl 0.25%**

SITES	Bendiocarb 0.1%				Pirimiphos methyl 0.25%			
	N Tested	N dead	Mortality (%)	Corrected Mortality	Total	N dead	Mortality (%)	Corrected Mortality
Djenne	100	98	98	-	100	100	100	-
Mopti	100	100	100	-	100	100	100	-
Bandiagara	100	97	97	-	100	100	100	-
Bankass	87	86	99	-	102	102	100	-
Koulikoro	100	99	99	-	100	100	100	-
Fana	102	100	98	-	97	97	100	-
Baroueli	100	89	89	-	100	100	100	-
Bamako	100	90	90	-	100	100	100	-
Kati	100	97	97	-	102	102	100	-
Bla	100	98	98	-	100	100	100	-
Selingue	99	85	86	-	100	100	100	-
Bougouni	100	76	76	-	100	100	100	-
Niono	-	-	-	-	-	-	-	-
Kita	100	95	95	-	97	97	100	-
Kadiolo	-	-	-	-	-	-	-	-
<b>Mean</b>	1288	1210	94	-	1298	1298	100	-

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

## ANNEX I: CDC BOTTLE TEST RESULTS WITH CHLORFENAPYR

**Table II: IR Intensity Assay Results of *An. gambiae* s.l. Tested Against Chlorphenapyr 200 µg/ml, 100 µg/ml, 50 µg/ml, 25 µg/ml, 12.5 µg/ml in Djenne, Mopti, Bandiagara, and Bankass**

Sites	Insecticide	Strains	Total tested	Mortality Rate (%)		
				Day 1	Day 2	Day 3
Djenné	Solution A (200µg/ml)	Kisumu	100	100	100	100
		Field Strain	100	100	100	100
	Solution B (100µg/ml)	Kisumu	100	100	100	100
		Field Strain	100	100	100	100
	Solution C (50 µg/ml)	Kisumu	100	95	100	100
		Field Strain	100	91	100	100
	Solution D (25 µg/ml)	Kisumu	-	-	-	-
		Field Strain	-	-	-	-
	Solution E (12.5 µg/ml)	Kisumu	100	96	100	100
		Field Strain	100	94	100	100
Mopti	Solution A (200µg/ml)	Kisumu	100	98	100	100
		Field Strain	100	91	98	98
	Solution B (100µg/ml)	Kisumu	100	98	100	100
		Field Strain	100	99	100	100
	Solution C (50 µg/ml)	Kisumu	80	100	100	100
		Field Strain	100	92	99	99
	Solution D (25 µg/ml)	Kisumu	80	95	100	100
		Field Strain	100	97	100	100
	Solution E (12.5 µg/ml)	Kisumu	100	35	94	98
		Field Strain	100	21	66	94
Bandiagara	Solution A (200µg/ml)	Kisumu	100	100	100	100
		Field Strain	100	90	99	100
	Solution B (100µg/ml)	Kisumu	100	90	96	100
		Field Strain	100	86	96	98
	Solution C (50 µg/ml)	Kisumu	100	98	100	100
		Field Strain	100	75	90	93
	Solution D (25 µg/ml)	Kisumu	-	-	-	-
		Field Strain	-	-	-	-
	Solution E (12.5 µg/ml)	Kisumu	100	98	100	100
		Field Strain	100	72	82	85
Bankass	Solution A (200µg/ml)	Kisumu	100	100	100	100
		Field Strain	100	100	100	100
	Solution B (100µg/ml)	Kisumu	100	100	100	100
		Field Strain	100	96	100	100
	Solution C (50 µg/ml)	Kisumu	100	94	99	99
		Field Strain	100	100	100	100
	Solution D (25 µg/ml)	Kisumu	80	91	99	100
		Field Strain	80	80	96	100
	Solution E (12.5 µg/ml)	Kisumu	100	78	100	100
		Field Strain	100	0	3	7

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

TABLE J1: KDRL1014F RESISTANCE GENOTYPES AND FREQUENCY, KADIOLO, BAMAKO, SELINGUE AND BLA, 2017

Site: Kadiolo	<i>An. coluzzii</i>				<i>An. gambiae</i>			
	<i>L1014F</i> genotypes			<i>f(kdr)</i>	<i>L1014F</i> genotypes			<i>f(kdr)</i>
	RR	RS	SS		RR	RS	SS	
Permethrin Dead	2	3	0	0.7	3	0	0	1
Permethrin Alive	7	9	1	0.67	2	1	0	0.83
Deltamethrin Dead	2	8	2	0.5	2	1	0	0.83
Deltamethrin Alive	1	1	2	0.37	1	0	0	1
<b>Total</b>	12	21	5	0.59	8	2	0	0.9

Site: Bamako	<i>An. coluzzii</i>				<i>An. gambiae</i>			
	<i>L1014F</i> genotypes			<i>f(kdr)</i>	<i>L1014F</i> genotypes			<i>f(kdr)</i>
	RR	RS	SS		RR	RS	SS	
Permethrin Dead	2	2	0	0.75	1	0	0	1
Permethrin Alive	11	9	3	0.67	1	0	0	1
Deltamethrin Dead	3	3	1	0.64	0	0	0	0
Deltamethrin Alive	7	3	1	0.77	1	0	0	1
<b>Total</b>	23	17	5	0.7	3	0	0	1

Site: Selingue	<i>An. coluzzii</i>				<i>An. gambiae</i>			
	<i>L1014F</i> genotypes			<i>f(kdr)</i>	<i>L1014F</i> genotypes			<i>f(kdr)</i>
	RR	RS	SS		RR	RS	SS	
Permethrin Dead	0	1	1	0.25	1	0	0	1
Permethrin Alive	3	5	1	0.61	11	1	0	0.95
Deltamethrin Dead	1	3	1	0.5	8	0	1	0.88
Deltamethrin Alive	1	1	0	0.75	2	0	0	1
<b>Total</b>	5	10	3	0.55	22	1	1	0.93

Site: Bla	<i>An. coluzzii</i>				<i>An. gambiae</i>			
	<i>L1014F</i> genotypes			<i>f(kdr)</i>	<i>L1014F</i> genotypes			<i>f(kdr)</i>
	RR	RS	SS		RR	RS	SS	
Permethrin Dead	3	2	2	0.57	2	0	0	1
Permethrin Alive	4	2	1	0.71	1	0	0	1
Deltamethrin Dead	5	8	1	0.64	1	0	0	1
Deltamethrin Alive	3	1	1	0.7	0	0	0	0
<b>Total</b>	15	13	5	0.65	4	0	0	1

**TABLE J2: KDRLI014F RESISTANCE GENOTYPES AND FREQUENCY, NIONO, KITA AND KOULIKORO, 2017**

Site: Niono	<i>An coluzzii</i>			
	<i>L1014F</i> genotypes			<i>f(kdr)</i>
	RR	RS	SS	
Permethrin Dead	8	4	1	<b>0.76</b>
Permethrin Alive	7	6	0	<b>0.76</b>
Deltamethrin Dead	6	7	2	<b>0.63</b>
Deltamethrin Alive	3	1	0	<b>0.87</b>
<b>Total</b>	<b>24</b>	<b>18</b>	<b>3</b>	<b>0.73</b>

Site: Kita	<i>An coluzzii</i>				<i>An. gambiae</i>				<i>An. arabiensis</i>			
	<i>L1014F</i> genotypes			<i>f(kdr)</i>	<i>L1014F</i> genotypes			<i>f(kdr)</i>	<i>L1014F</i> genotypes			<i>f(kdr)</i>
	RR	RS	SS		RR	RS	SS		RR	RS	SS	
Permethrin Dead	1	0	0	1	7	0	0	1	0	0	0	0
Permethrin Alive	0	0	0	0	19	0	1	<b>0.95</b>	0	0	1	0
Deltamethrin Dead	0	0	0	0	2	0	1	<b>0.66</b>	0	0	1	0
Deltamethrin Alive	0	0	0	0	10	0	0	1	0	0	3	0
<b>Total</b>	<b>1</b>	<b>0</b>	<b>0</b>	<b>1</b>	<b>38</b>	<b>0</b>	<b>2</b>	<b>0.95</b>	<b>0</b>	<b>0</b>	<b>5</b>	<b>0</b>

Site: Koulikoro	<i>An coluzzii</i>				<i>An. gambiae</i>				<i>An. arabiensis</i>				<i>Hybrid An. gambiae/coluzzii</i>			
	<i>L1014F</i> genotypes			<i>f(kdr)</i>	<i>L1014F</i> genotypes			<i>f(kdr)</i>	<i>L1014F</i> genotypes			<i>f(kdr)</i>	<i>L1014F</i> genotypes			<i>f(kdr)</i>
	RR	RS	SS		RR	RS	SS		RR	RS	SS		RR	RS	SS	
Permethrin Dead	1	0	0	1	3	0	0	1	0	2	0	<b>0.5</b>	0	0	0	0
Permethrin Alive	9	7	3	<b>0.65</b>	4	0	0	1	0	0	0	0	0	0	0	0
Deltamethrin Dead	3	4	3	<b>0.5</b>	2	0	0	1	0	0	0	0	0	0	0	0
Deltamethrin Alive	1	3	0	<b>0.62</b>	0	0	0	0	0	0	0	0	1	0	0	1
<b>Total</b>	<b>14</b>	<b>14</b>	<b>6</b>	<b>0.61</b>	<b>9</b>	<b>0</b>	<b>0</b>	<b>1</b>	<b>0</b>	<b>2</b>	<b>0</b>	<b>0.5</b>	<b>1</b>	<b>0</b>	<b>0</b>	<b>1</b>



TABLE J3: KDRL1014F RESISTANCE GENOTYPES AND FREQUENCY, BOUGOUNI, MOPTI AND FANA, 2017

Bougouni	<i>An. coluzzii</i>				<i>An. gambiae</i>			
	<i>L1014F</i> genotypes			<i>f(kdr)</i>	<i>L1014F</i> genotypes			<i>f(kdr)</i>
	RR	RS	SS		RR	RS	SS	
Permethrin Dead	0	0	0	0	4	0	0	1
Permethrin Alive	0	1	0	0.5	23	0	0	1
Deltamethrin Dead	0	0	0	0	4	0	1	0.8
Deltamethrin Alive	1	1	0	0.75	5	3	0	0.81
<b>Total</b>	1	2	0	<b>0.66</b>	36	3	1	<b>0.93</b>

Mopti	<i>An. coluzzii</i>				<i>An. gambiae</i>			
	<i>L1014F</i> genotypes			<i>f(kdr)</i>	<i>L1014F</i> genotypes			<i>f(kdr)</i>
	RR	RS	SS		RR	RS	SS	
Permethrin Dead	0	0	0	0	0	0	0	0
Permethrin Alive	0	1	0	0.5	0	0	0	0
Deltamethrin Dead	0	4	0	0.5	0	0	0	0
Deltamethrin Alive	0	2	0	0.5	0	0	0	0
<b>Total</b>	0	7	0	<b>0.5</b>	0	0	0	0

Fana	<i>An. coluzzii</i>				<i>An. gambiae</i>				<i>An. arabiensis</i>			
	<i>L1014F</i> genotypes			<i>f(kdr)</i>	<i>L1014F</i> genotypes			<i>f(kdr)</i>	<i>L1014F</i> genotypes			<i>f(kdr)</i>
	RR	RS	SS		RR	RS	SS		RR	RS	SS	
Permethrin Dead	0	6	1	0.42	0	1	0	0.5	0	0	1	0
Permethrin Alive	8	1	2	0.77	2	1	0	0.83	0	0	0	0
Deltamethrin Dead	5	2	0	0.85	4	0	0	1	0	0	1	0
Deltamethrin Alive	1	4	1	0.5	2	0	0	1	0	0	0	0
<b>Total</b>	14	13	4	<b>0.66</b>	8	2	0	<b>0.9</b>	0	0	2	0

**TABLE J4: KDRL1014F RESISTANCE GENOTYPES AND FREQUENCY, BANKASS, KATI AND DJENNE, 2017**

Bankass	<i>An. coluzzii</i>				<i>An. gambiae</i>			
	<i>L1014S</i> genotypes			<i>f(kdr)</i>	<i>L1014S</i> genotypes			<i>f(kdr)</i>
	RR	RS	SS		RR	RS	SS	
Permethrin Dead	0	0	8	0	0	0	1	0
Permethrin Alive	0	0	8	0	0	0	1	0
Deltamethrin Dead	0	0	6	0	0	1	0	0.5
Deltamethrin Alive	0	0	6	0	0	0	1	0
<b>Total</b>	0	0	28	0	0	1	3	0.12

Kati	<i>An. coluzzii</i>				<i>An. gambiae</i>			
	<i>L1014S</i> genotypes			<i>f(kdr)</i>	<i>L1014S</i> genotypes			<i>f(kdr)</i>
	RR	RS	SS		RR	RS	SS	
Permethrin Dead	0	0	8	0	0	0	0	0
Permethrin Alive	0	0	2	0	0	0	1	0
Deltamethrin Dead	0	0	10	0	0	0	0	0
Deltamethrin Alive	0	0	9	0	0	0	0	0
<b>Total</b>	0	0	29	0	0	0	1	0

Djenne	<i>An. coluzzii</i>				<i>An. gambiae</i>				Hybrid <i>An. gambiae/coluzzii</i>			<i>An. arabiensis</i>				
	<i>L1014F</i> genotypes			<i>f(kdr)</i>	<i>L1014F</i> genotypes			<i>f(kdr)</i>	<i>L1014F</i> genotypes			<i>f(kdr)</i>	<i>L1014F</i> genotypes			<i>f(kdr)</i>
	RR	RS	SS		RR	RS	SS		RR	RS	SS		RR	RS	SS	
Permethrin Dead	1	8	0	0.55	0	1	1	0.25	0	1	0	0.5	0	0	0	0
Permethrin Alive	3	3	3	0.5	0	1	0	0.5	0	0	0	0	0	0	0	0
Deltamethrin Dead	1	7	3	0.4	1	0	0	1	0	0	0	0	0	0	1	0
Deltamethrin Alive	1	2	3	0.33	1	0	0	1	0	0	0	0	0	0	0	0
<b>Total</b>	6	20	9	0.45	2	2	1	0.6	0	1	0	0.5	0	0	1	0

**TABLE J5: KDRL1014F RESISTANCE GENOTYPES AND FREQUENCY, BAROUELI AND BANDIAGARA, 2017**

Baroueli	<i>An coluzzii</i>			<i>f(kdr)</i>	<i>An. gambiae</i>			<i>f(kdr)</i>	<i>Hybrid An. gambiae/coluzzii</i>			<i>f(kdr)</i>	<i>An. arabiensis</i>			<i>f(kdr)</i>
	<i>L1014S</i> genotypes				<i>L1014S</i> genotypes				<i>L1014S</i> genotypes				<i>L1014S</i> genotypes			
	RR	RS	SS	RR	RS	SS	RR	RS	SS	RR	RS	SS	RR	RS	SS	
Permethrin Dead	0	0	5	0	0	0	2	0	0	0	0	0	0	0	1	0
Permethrin Alive	0	0	1	0	0	0	5	0	0	0	0	0	0	0	0	0
Deltamethrin Dead	0	0	0	0	0	0	4	0	0	0	0	0	0	0	2	0
Deltamethrin Alive	0	0	2	0	0	0	1	0	0	0	0	0	0	0	0	0
<b>Total</b>	0	0	8	0	0	0	12	0	0	0	0	0	0	0	3	0

Bandiagara	<i>An coluzzii</i>			<i>f(kdr)</i>	<i>An. gambiae</i>			<i>f(kdr)</i>	<i>Hybrid An. gambiae/coluzzii</i>			<i>f(kdr)</i>	<i>An. arabiensis</i>			<i>f(kdr)</i>
	<i>L1014S</i> genotypes				<i>L1014S</i> genotypes				<i>L1014S</i> genotypes				<i>L1014S</i> genotypes			
	RR	RS	SS	RR	RS	SS	RR	RS	SS	RR	RS	SS	RR	RS	SS	
Permethrin Dead	0	0	9	0	0	0	1	0	0	0	0	0	0	0	1	0
Permethrin Alive	0	0	7	0	0	0	0	0	0	0	0	0	0	1	0	0.5
Deltamethrin Dead	0	0	8	0	0	1	1	0.25	0	0	0	0	0	0	0	0
Deltamethrin Alive	0	0	3	0	0	1	3	0.12	0	0	0	0	0	0	0	0
<b>Total</b>	0	0	27	0	0	2	5	0.14	0	0	0	0	0	1	1	0.25

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