



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

**MALI:**  
**ENTOMOLOGICAL MONITORING**  
**OF 2015 IRS ACTIVITIES**

**FINAL REPORT**

**FEBRUARY 2016**

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# CONTENTS

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<b>Acronyms .....</b>	<b>iv</b>
<b>1. Introduction.....</b>	<b>1</b>
<b>2. Training and Methods.....</b>	<b>2</b>
2.1 Study Period and Area.....	2
2.2 Personnel Training.....	3
2.3 Vector Composition, Density, Resting Behavior, Longevity, Sporozoite Rate, and Blood Meal Origin .....	4
2.4 Human Landing Catches and CDC Light Traps .....	5
2.5 Quality of Spray Insecticide and Decay Rate .....	6
2.6 Insecticide Resistance Test.....	6
2.7 Pyrethroid Resistance Intensity Assay and Resistance Enzyme Oxidases.....	8
2.8 Vector Molecular Characterization.....	9
2.9 Data Analysis Methods.....	9
<b>3. Results and Discussion .....</b>	<b>11</b>
3.1 Vector Species Composition, Density, Seasonality, Resting Behavior .....	11
3.2 Vector Insecticide Resistance .....	18
3.3 Quality of Spraying and Insecticide Decay Rate.....	20
3.4 Vector Insecticide Resistance Molecular Characterization .....	22
3.5 Sporozoite Infection and Parity Rate and Blood Meal Origin .....	24
<b>4. Conclusions.....</b>	<b>30</b>
<b>Annex A: Desnsity and Rainfall .....</b>	<b>32</b>
<b>Annex B: Vector Seasonality.....</b>	<b>35</b>
<b>Annex C: Susceptibility Test Results.....</b>	<b>36</b>
<b>Annex D. Cone Test Results .....</b>	<b>37</b>
<b>Annex E. Vector Infection Rate .....</b>	<b>38</b>
<b>Annex F. Vector Parity Rate .....</b>	<b>40</b>
<b>References .....</b>	<b>41</b>

## LIST OF TABLES

Table 1. Data Collection Sites .....	2
Table 2. Staffing for Entomological Surveillance Activities.....	4
Table 3. Species Composition (PSC), All Sites, June–December .....	11
Table 4. <i>An. gambiae</i> s.l. Mean MBR Indoor and Outdoor, HLC, June–December 2015.....	17
Table 5. Number of Mosquitoes Collected (HLC), All Sites, July–December 2015 .....	18
Table 6. Number of Mosquitoes Collected (CDC light traps), All Sites, July–December 2015.....	18
Table 7. WHO Susceptibility Assays with <i>An. gambiae</i> s.l., 2015 .....	19
Table 8. Pyrethroid Resistance Intensity, Koulikoro, Baroueli, and Bla .....	19
Table 9. 2015 MFO Resistance Mechanism, Koulikoro, Baroueli, and Bla .....	20
Table 10. Summary of Quality Assurance Tests, WHO Cone Bioassay, Koulikoro and Baroueli Districts .....	21
Table 11. Cone Bioassay Test Summary Results .....	22
Table 12. L1014F Resistance Genotypes and Frequency, Koulikoro and Baroueli .....	22
Table 13. L1014F Resistance Genotypes and Frequency, Bla .....	23
Table 14. L1014S Resistance Genotypes and Frequency, Koulikoro and Baroueli .....	23
Table 15. L1014S Resistance Genotypes and Frequency, Bla, 2015 .....	24
Table 16. <i>An. gambiae</i> s.l. Plasmodium. Falciparum (Sporozoite) Infection Rates, All Sites, HLC, June–September .....	25
Table 17. <i>An. gambiae</i> s.l. Plasmodium. Falciparum (Sporozoite) Infection Rates, All Sites, HLC, October–December .....	25
Table 18. EIR, Monthly Collections, Koulikoro.....	26
Table 19. EIR, Monthly Collections, Baroueli .....	26
Table 20. EIR, Monthly Collections, Kati .....	26
Table 21. EIR, Monthly Collections, Segou.....	26
Table 22. EIR, Monthly Collections, Bla .....	27
Table 23. <i>An. gambiae</i> s.l. Parity Rate (HLC) Pre- and Post-IRS, All Sites, June–December .....	27
Table 24. Human and Bovine Blood Meal Index, Koulikoro, Monthly Collections .....	28
Table 25. Human and Bovine Blood Meal Index, Baroueli, Monthly Collections.....	28
Table 26. Human and Bovine Blood Meal Index, Kati, Monthly Collections.....	29
Table 27. Human and Bovine Blood Meal Index, Segou, Monthly Collections .....	29
Table 28. Human and Bovine Blood Meal Index, Bla, Monthly Collections.....	29
Table A-1. Density of Anopheline ( <i>An. gambiae</i> s.l.) and Culicine ( <i>Culex</i> and <i>Aedes</i> ) and Parity Rate of <i>An. gambiae</i> s.l. Captured by PSC, Koulikoro and Kati .....	32
Table A-2. Density of Anopheline ( <i>An. gambiae</i> s.l.), and Culicine ( <i>Culex</i> and <i>Aedes</i> ) and Parity Rate of <i>An. gambiae</i> s.l. Captured by PSC, Baroueli, Segou, and Bla.....	33
Table A-3. Rainfall in Koulikoro and Baroueli, April–December 2015.....	34
Table B-1. <i>An. gambiae</i> s.l. Seasonality (HLC), Koulikoro and Kati, July–December .....	35
Table B-2. <i>An. gambiae</i> s.l. Seasonality (HLC), Baroueli, Segou, and Bla, July–December .....	35
Table C-1. Susceptibility Test Results after 2015 Spraying.....	36
Table D-1. Type of Surfaces Tested with WHO Cone Test, Koulikoro and Baroueli, July–December....	37
Table D-2. WHO Cone Test Results, <i>An. gambiae</i> Kisumu Strain Mortality after 30 Minutes Exposure to Pirimiphos-methyl, Koulikoro and Baroueli, July–December .....	37
Table E-1. <i>An. gambiae</i> s.l. Plasmodium. Falciparum (Sporozoite) Infection Rates, All Sites, July–September 2014.....	38
Table E-2. <i>An. gambiae</i> s.l. Plasmodium. Falciparum (sporozoite) Infection Rates, All Sites, October–November 2014.....	38
Table E-3. EIR, Monthly Collections, Bla, 2014 .....	38

Table E-4. <i>An. gambiae</i> s.l. Plasmodium. Falciparum (Sporozoite) Infection Rates, All Sites, HLC, CDC Light Traps and PSC, June–September 2015 .....	38
Table E-5. <i>An. gambiae</i> s.l. Plasmodium. Falciparum (Sporozoite) Infection Rates, All Sites, HLC, CDC Light Traps and PSC, October–December 2015.....	39
Table F-1. <i>An. gambiae</i> s.l. Parity Rate (HLC) Pre- and Post-IRS, Bla July–December, 2014.....	40

## LIST OF FIGURES

Figure 1. Map of PMI Mali Entomological Surveillance Sites.....	3
Figure 2. Refresher Training of Technicians.....	4
Figure 3. PSC Mosquito Collection, Bla District.....	5
Figure 4. Indoor and Outdoor Mosquito Collection by HLC, Bla District .....	6
Figure 5. Larval Collection, Baroueli District.....	7
Figure 6. WHO Susceptibility Test, Koulikoro District.....	8
Figure 7. Pyrethroid Resistance Intensity Assay with CDC Bottle Test, Koulikoro District.....	9
Figure 8. <i>An. gambiae</i> s.l. Sibling Species Composition (PCR), Koulikoro, Baroueli, and Bla, June–December 2015 (n=50 per site).....	12
Figure 9. Mean Indoor Resting Density (PSC) of <i>An. gambiae</i> s.l., by Month, Koulikoro, and Kati, June–December 2015.....	13
Figure 10. Mean Indoor Resting Density (PSC) of <i>An. gambiae</i> s.l., by Month, Baroueli, Segou, and Bla, June–December 2015.....	13
Figure 11. <i>An. gambiae</i> s.l. Human Biting Rate Seasonality (HLC), Koulikoro and Kati, June–December 2015.....	13
Figure 12. <i>An. gambiae</i> s.l. Human Biting Rate Seasonality (HLC), Baroueli, Segou, and Bla, June–December 2015.....	14
Figure 13. <i>An. gambiae</i> s.l., Hourly Biting Rate (HLC), Koulikoro, June–December 2015.....	15
Figure 14. <i>An. gambiae</i> s.l., Hourly Biting Rate (HLC), Kati, June–December 2015 .....	15
Figure 15. <i>An. gambiae</i> s.l., Hourly Biting Rate (HLC), Baroueli, June–December 2015.....	16
Figure 16. <i>An. gambiae</i> s.l., Hourly Biting Rate (HLC), Segou, June–December 2015.....	16
Figure 17. <i>An. gambiae</i> s.l., Hourly Biting Rate (HLC), Bla, June–December 2015 .....	17
Figure 18. 2015 WHO Susceptibility Assays with <i>An. gambiae</i> s.l., All Sites .....	19
Figure 19. WHO Cone Test Results, <i>An. gambiae</i> Kisumu Mortality after 30 Minutes Exposure to Pirimiphos-methyl, Koulikoro and Baroueli.....	21

# ACRONYMS

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<b>AIRS</b>	Africa Indoor Residual Spraying Project
<b>DDMS</b>	Disease Data Management System
<b>EIR</b>	Entomological Inoculation Rate
<b>HBR</b>	Human Biting Rate
<b>HLC</b>	Human Landing Catches
<b>IR</b>	Insecticide Resistance
<b>IRS</b>	Indoor Residual Spraying
<i>kdr</i>	Knock down Resistance
<b>LLIN</b>	Long-lasting Insecticidal Net
<b>MFO</b>	Mixed Function Oxidase
<b>NMCP</b>	National Malaria Control Program
<b>PMI</b>	President's Malaria Initiative
<b>PSC</b>	Pyrethrum Spray Catches
<b>PBO</b>	Piperonyl Butoxide
<b>SMC</b>	Seasonal Malaria Chemoprevention
<b>WHO</b>	World Health Organization

# I. INTRODUCTION

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During 2015, The President's Malaria Initiative (PMI) Africa Indoor Residual Spraying (AIRS) project in Mali continued indoor residual spraying (IRS) in two districts, Koulikoro and Baroueli, to help reduce malaria transmission and conducted routine entomological monitoring in five districts. Objectives of the entomological monitoring were:

- Assess malaria vector density, species composition, age structure, infectivity, and blood meal origin in intervention and selected control areas,
- Understand vector feeding times and locations,
- Monitor the quality of insecticide application and insecticide decay rates, and
- Assess vector susceptibility to insecticides approved for IRS and pyrethroids' resistance intensity.

PMI used the pyrethroid insecticide lambda-cyhalothrin in the 2008 and 2009 IRS campaigns. In 2009, insecticide resistance (IR) studies funded by PMI and the Malaria Research and Training Center collected data demonstrating better performance of deltamethrin over lambda-cyhalothrin against *An. gambiae* s.l., the main malaria vector in the country. Based on these findings, the PMI program sprayed deltamethrin in 2010. The annual IR data collected in 2010, however, showed a rapid increase of vector resistance to pyrethroids.

Guided by the IR data, PMI in collaboration with the National Malaria Control Program (NMCP) and other in-country partners decided to change the insecticide used for IRS from deltamethrin to the carbamate insecticide bendiocarb. Bendiocarb was used for IRS from 2011 to 2013. However, entomological monitoring of insecticide decay rates collected by the AIRS Mali program indicated a relatively short residual life of bendiocarb (according to the WHO criteria of mortality >80% in cone bioassay): it was less than two months in Baroueli and Bla districts, which is shorter than the malaria transmission season observed in Mali. In 2014, PMI introduced a long-acting formulation organophosphate, pirimiphos-methyl 300 CS, in two of the three PMI-supported districts (Bla and Baroueli) to ensure adequate protection during the main malaria transmission season. Since the residual life was longer, one round of spraying adequately covered the entire rainy season. Bendiocarb continued to be used in the 2014 IRS campaign in Koulikoro district because the residual life there was longer than in the other two districts. In 2015, PMI in collaboration with the NMCP and other in-country partners decided to withdraw IRS from Bla district due to the high cost of pirimiphos-methyl 300 CS. Spraying was only conducted in Koulikoro and Baroueli.

This report provides information on the entomological monitoring activities carried out from June through December 2015.

## 2. TRAINING AND METHODS

### 2.1 STUDY PERIOD AND AREA

The data were collected from June to December 2015 to monitor the sites before and after the IRS campaign (which took place July-August). Data were collected in the districts listed in Table 1. Geographical locations of the sites are shown in Figure 1.

- Koulikoro (IRS site 1) was compared to unsprayed Kati (control site 1); the two sites are in the same geographical region separated by a distance of 10 km.
- Baroueli (IRS site 2) was compared to unsprayed Segou (control site 2); the two sites are in the same geographical region separated by 5 km.
- Bla (control site 3) was monitored to determine the impact of stopping IRS.

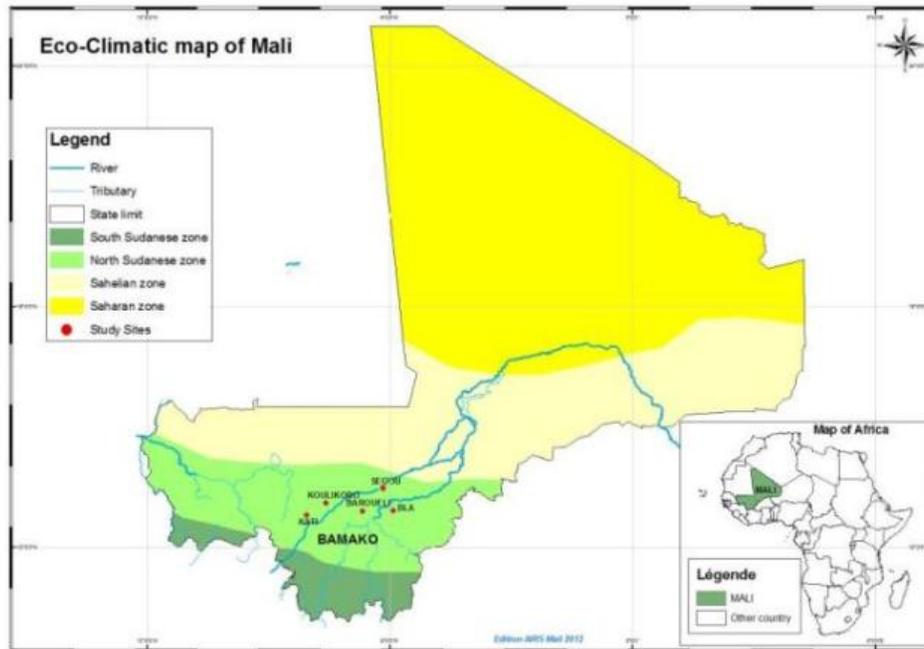
**TABLE 1. DATA COLLECTION SITES**

Region	District	Health Area	Site (village)	Status
Koulikoro	Kati	Baguineda	Baguineda	Control site 1 - Never sprayed - LLIN universal coverage in 2014 - SMC in 2015
	Koulikoro	Tienfala Kolebougou	Tienfala N'Dentila	IRS site 1 - Sprayed with bendiocarb (carbamate) in 2014 - Sprayed pirimiphos-methyl CS (organophosphate) in 2015 - LLIN universal coverage in 2014 - SMC in 2015
Segou	Baroueli	Tigui Konobougou	Diaka Konibabougou	IRS site 2 - Sprayed pirimiphos-methyl CS in 2014 and 2015 - LLIN universal coverage in 2015 - SMC in 2015
	Segou	Zambougou	Kegnebougou	Control site 2 - Never sprayed - LLIN universal coverage in 2015
	Bla	Touna	Djina	Control site 3 - Sprayed with pirimiphos-methyl CS in 2014 - Not sprayed in 2015 - LLIN universal coverage in 2015 - SMC in 2015

Note: LLIN=long-lasting insecticidal net, SMC= seasonal malaria chemoprevention

The selected sites in these districts are the best available comparable sites in terms of entomological indicators.

**FIGURE I. MAP OF PMI MALI ENTOMOLOGICAL SURVEILLANCE SITES**



## 2.2 PERSONNEL TRAINING

AIRS Mali hired entomological technicians from July through December 2015 to help complete the field work for the entomological surveillance. All of the entomological technicians had experience in mosquito sampling using pyrethrum spray catch (PSC), human landing catches (HLC), CDC light traps, and larval collection. The technicians assisted the AIRS Mali team in:

- Collection of larvae and pupae for susceptibility and bioassay tests,
- Collection of adult mosquitoes with PSC, HLC, and CDC light trap,
- Morphological identification of vector species and ovary dissections, and
- Data entry in Disease Data Management System (DDMS).

AIRS Mali conducted a four-day refresher training for the technicians in June 2015 to ensure that they all followed best practices in mosquito field collection and understood the AIRS Mali entomological monitoring standards (Table 2, Figure 2). The training covered the identification of breeding sites, larval and pupae collection, identification of anopheline larvae from culicine, how to transport larvae and pupae from the field to the rearing sites, performing PSC, HLC and CDC light trap collections, morphological identification of adult female *Anopheles* mosquitoes by species, morphological identification of *Culex* mosquitoes from *Anopheles*, dissection of mosquito ovaries, and the use of DDMS.

AIRS Mali recruited and trained one technician on insectary management. He was responsible for rearing mosquitoes and managing the insectary as well as ensuring proper temperature and humidity of the insectary. Mali's NMCP entomologist participated in the training to help build NMCP capacity in entomological monitoring.

**TABLE 2. STAFFING FOR ENTOMOLOGICAL SURVEILLANCE ACTIVITIES**

Activity	Number of Entomological Technicians Hired		Number of Local Mosquito Collectors		Number of NMCP Staff		Number of AIRS Staff	
	Male	Female	Male	Female	Male	Female	Male	Female
PSC	5	5	0	10	1	-	1	1
HLC and CDC light trap	5	5	49	1	1	-	1	1
Supervision for HLC	5	5	4	1	1	-	1	1
Mosquito morphological and physiological identification	5	5	-	-	1	-	1	1
Mosquito ovary dissection	5	5	-	-	1	-	1	1
Susceptibility test	5	5	-	-	1	-	1	1
Wall bioassay	5	5	-	-	1	-	1	1
Insectary maintenance	5	5	-	-	1	-	1	1

**FIGURE 2. REFRESHER TRAINING OF TECHNICIANS**



## 2.3 VECTOR COMPOSITION, DENSITY, RESTING BEHAVIOR, LONGEVITY, SPOROZOITE RATE, AND BLOOD MEAL ORIGIN

AIRS Mali used three entomological sampling methods, PSC, HLC, and CDC light traps, to collect adult mosquitoes to help determine key entomological indicators. The team collected data on vector composition, density, resting behavior, longevity, sporozoite rate, and blood meal origin in the following sites:

- Tienfala in Koulikoro district (IRS site 1) and Baguineda in Kati district (control site 1)
- Tigui in Baroueli district (IRS site 2), and Zambougou in Segou district and Touna in Bla district (control sites 2 and 3)

### 2.3.1 PYRETHRUM SPRAY CATCH

PSC was used to sample resting mosquitoes from 20 houses within each selected sentinel site (village) per month. For each sentinel site, the site was divided in two equal areas and 10 houses were randomly

selected in each area. The PSC was carried out in the 10 houses per day on two consecutive days between 6:00 a.m. and 11:00 a.m. Before the PSC was performed, all occupants were asked to move out of the house. Data on the number of people who slept in the house the previous night, the type of house and walls, and the numbers of treated nets present were collected.

A commercial aerosol KILIT (d-Tetramethrin 0.135% w/w, d-Allethrin 0.06% w/w, and Cypermethrin 0.46% w/w) was used to knock down mosquitoes for the PSC activity. AIRS Mali confirmed susceptibility of the local vectors to KILIT prior to the start of the field work. During the mosquito collection, the windows and other mosquito entry and escape routes around the house were sprayed first, followed by the interior of the house until the house was full of insecticide mist. The collectors then left the house with all doors and windows closed. Ten minutes later the house was opened and all mosquitoes knocked down by the chemical were collected from white sheets that had been spread on all flat surfaces of the house (Figure 3). The mosquitoes were put in precisely labeled petri dishes and taken to the dissection room in the field or to the insectary where they were sorted out morphologically by species. The abdominal status of all female *Anopheles* that were collected were sorted into four categories: unfed, blood-fed, half-gravid, and gravid females. A sub-sample of the collected mosquitoes was preserved for molecular analysis to identify the sibling species and determine infection rates. The preserved mosquitoes will be used for ELISA to identify the source of the blood meal.

**FIGURE 3. PSC MOSQUITO COLLECTION, BLA DISTRICT**



## 2.4 HUMAN LANDING CATCHES AND CDC LIGHT TRAPS

HLC and CDC light traps were used to gain information related to where most vector human contact was occurring (inside and/or outside of a structure), the vector feeding time, and changes in the feeding behavior of mosquitoes before and after IRS in two selected houses per site surveyed for two consecutive nights per month from July through December. Each sentinel site was divided into two areas of equal size and two houses were randomly selected in each area.

HLC and CDC light trapping was done from 6:00 p.m. to 6:00 a.m. For the HLC, two people were assigned to a house simultaneously, one outside and the other inside; the collectors exchanged their positions every hour (Figure 4). Volunteers involved in the collection and supervision of the work were provided with chemoprophylaxis to protect them from contracting malaria. For CDC light traps, two traps with bottles were used to sample mosquitoes from indoors and outdoors and the bottles were rotated every hour. To attract mosquitoes, volunteers slept under an untreated mosquito net indoors

and dry ice was used outdoors. During the collection, HLC and CDC light traps were rotated between the two houses every night. Data from these two sampling methods were used to assess if CDC light traps would provide comparable information with HLC.

The baseline collection was done in July to assess vector feeding behavior and parity (longevity) rates before spraying.

A sub-sample of *An. gambiae* s.l. sibling species was preserved in a 1.5 ml Eppendorf tube in silica gel for subsequent ELISA analysis.

**FIGURE 4. INDOOR AND OUTDOOR MOSQUITO COLLECTION BY HLC, BLA DISTRICT**



## 2.5 QUALITY OF SPRAY INSECTICIDE AND DECAY RATE

Cone bioassays were done in Koulikoro and Baroueli districts (10 houses/ district) based on the World Health Organization (WHO) protocol (WHO 2013). Plastic cones were placed at three different heights diagonally on sprayed wall surfaces (mud, mud with kaolin (white clay), and cement) (Annex D Table D-1). Parallel negative control tests were conducted with cones attached to unsprayed surfaces. Batches of 10–15 mosquitoes, 2–5-day-old non-blood-fed female *An. gambiae* (Kisumu strain), were introduced into each of the cones based on the number of mosquitoes available for the test. The mosquitoes were left in the cones exposed to the insecticide for 30 minutes, after which they were transferred to paper cups.

Knockdown and mortality were observed and recorded after 30 minutes exposure and after a 24-hour holding period, respectively. When mortality in the control site was between 5% and 20%, the results of the treated samples were corrected using Abbott's formula.

## 2.6 INSECTICIDE RESISTANCE TEST

After the 2015 AIRS campaign, *An. gambiae* s.l. larvae and pupae were collected from IRS target areas and reared at the AIRS Mali insectary (Figure 5). The AIRS Mali team completed vector susceptibility testing using adult mosquitoes.

**FIGURE 5. LARVAL COLLECTION, BAROUELI DISTRICT**



During the tests, female non-blood-fed adult *An. gambiae* s.l. were exposed to the standard WHO discriminating dosages of insecticide-treated papers in four replicates of 25 mosquitoes for one-hour exposure. The test tubes were kept in a vertical position (Figure 6). Exposure tests were accompanied by control tests where mosquitoes were exposed to filter papers impregnated only with oil. The numbers of mosquitoes knocked down were recorded every 10, 15, 20, 30, 40, 50, 60, and 80 minutes.

After one hour of exposure, mosquitoes were transferred to holding tubes lined with insecticide-free papers. Test and control mosquitoes were supplied with sugar solution-soaked cotton pads. During the post-exposure period, the specimens were kept in a wooden box covered with a wet towel to maintain optimal temperature and humidity. Mortality was recorded after the 24-hour holding period.

In sites where resistance to insecticide was detected, a sub-sample of the tested mosquitoes was preserved for further analysis. Molecular analysis will be done in collaboration with the Laboratory of Applied Molecular Biology of the University of Bamako to determine the mechanism of resistance. The results will be shared with PMI at the end of May, 2016.

**FIGURE 6. WHO SUSCEPTIBILITY TEST, KOULIKORO DISTRICT**



## **2.7 PYRETHROID RESISTANCE INTENSITY ASSAY AND RESISTANCE ENZYME OXIDASES**

The AIRS Mali entomological team completed IR intensity assays to pyrethroid insecticides, namely permethrin and deltamethrin, on mosquitoes from each site (Figure 7). The IR intensity assay was performed by exposing 3–5-day-old, female, non-blood-fed adult *An. gambiae* s.l. from IRS target areas in standard 250 ml glass bottles coated with insecticide.

Four replicates of 20–25 female adult mosquitoes were exposed to the following doses of permethrin: 1X, 2X, 5X, and 10X diagnostic concentration; and deltamethrin: 1X, 2X, 5X, and 10X diagnostic concentration. The test bottles were kept horizontal during exposure. In parallel, negative control tests were conducted with mosquitoes exposed to bottles coated with acetone only. The numbers of mosquitoes dead and alive were recorded at 0, 15, 30, 45, 60, 75, 105, and 120 minutes after exposure. In this report the test mortality rates recorded at the diagnostic time of 30 minutes are included.

To understand the contribution of metabolic resistance due to mixed function oxidases (MFOs), another four replicates of 20–25 female adult mosquitoes were pre-exposed to Piperonyl Butoxide (PBO) 400 µg/ml for one hour prior to being exposed to permethrin: 1X, 2X, 5X, and 10X diagnostic concentration; and deltamethrin: 1X, 2X, 5X, and 10X diagnostic concentration for 30 minutes. Exposure to PBO also was accompanied by a control that exposed mosquitoes to the bottle coated with acetone. Results from mosquitoes with and without PBO exposure were compared for each insecticide and site.

**FIGURE 7. PYRETHROID RESISTANCE INTENSITY ASSAY WITH CDC BOTTLE TEST, KOULIKORO DISTRICT**



## 2.8 VECTOR MOLECULAR CHARACTERIZATION

A sub-sample of mosquito specimens was sent to the Laboratory of Applied Molecular Biology for vector molecular characterization, specifically to conduct the following analyses:

- **Identification of sibling species and M and S molecular forms of *An. gambiae* s.s.** A total of 150 *An. gambiae* s.l. were identified by species using PCR as described by Scott et al. (1993). The molecular forms M and S of *An. gambiae* s.s. were then identified by PCR according to the protocol of Favia et al. (2001). Currently based on molecular and bionomical evidence, the *An. gambiae* molecular “M form” is named *An. coluzzii*, while the “S form” retains the nominotypical name *An. gambiae* (Coetzee et al., 2013).
- **Detection of the knock down resistance (*kdr*) and *ace-1R* mutation.** All *An. gambiae* s.l. sibling species were tested for the L1014F West Africa *kdr* mutation according to the protocol of Martinez-Torres et al. (1998) and the L1014S East Africa *kdr* mutation according to protocol described by Ranson et al. (2000). The *ace-1R* mutation was diagnosed by PCR RFLFP as described by Weill et al. (2004).

## 2.9 DATA ANALYSIS METHODS

PSC data were used to calculate the density of vectors in a room using the formula:

*Total number of vectors collected by species / Total number of rooms surveyed.*

The mean biting rate (MBR) was computed from HLC using the formula:

*Total number of mosquitoes collected by species / Total number of collectors.*

The parity rate of identified *An. gambiae* s.l. vector species collected during HLC was calculated using the formula:

*(Total number of parous vectors/total number of vectors dissected) \*100*

WHO 2013 criteria was used to interpret susceptibility test results, as it was noted that:

Susceptibility= Mortality rate of the exposed vector greater than 98%

Possible Resistance= Mortality rates that are between 90% and 97%

Resistance= Mortality rate after 24-recovery period is less than 90%.

When the control mortality was between 5% and 20%, observed mortality was corrected using Abbott's formula. An experiment was repeated when control mortality was more than 20%.

Z-test for difference in proportions was used for comparing vector infection and parity mortality rate in two populations.

## 3. RESULTS AND DISCUSSION

### 3.1 VECTOR SPECIES COMPOSITION, DENSITY, SEASONALITY, RESTING BEHAVIOR

#### 3.1.1 SPECIES COMPOSITION AND DENSITY

The primary vector group in all sites was *An. gambiae* s.l.; the secondary vector group, *An. funestus* s.l., was collected in four out of the five sites (Table 3).

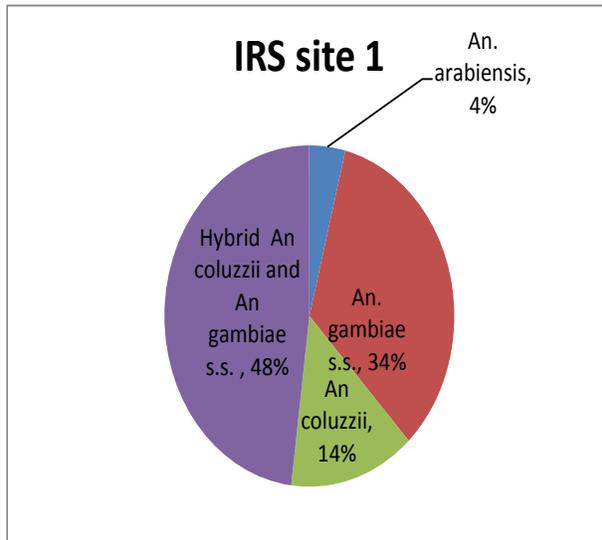
**TABLE 3. SPECIES COMPOSITION (PSC), ALL SITES, JUNE–DECEMBER**

Species	Koulikoro IRS Site 1	Kati Control Site 1	Baroueli IRS Site 2	Segou Control Site 2	Bla Control Site 3
<i>An. gambiae</i> s.l.	332 (99.70%)	1467 (99.86%)	144 (97.96%)	382 (99.22%)	1276 (91.93%)
<i>An. funestus</i> s.l.	1 (<1%)	2 (<1%)	0	1 (<1%)	1 (<1%)
<i>An. rufipes</i>	0	0	3 (2.04%)	2 (<1%)	111 (8.00%)
Total	333	1469	147	385	1388

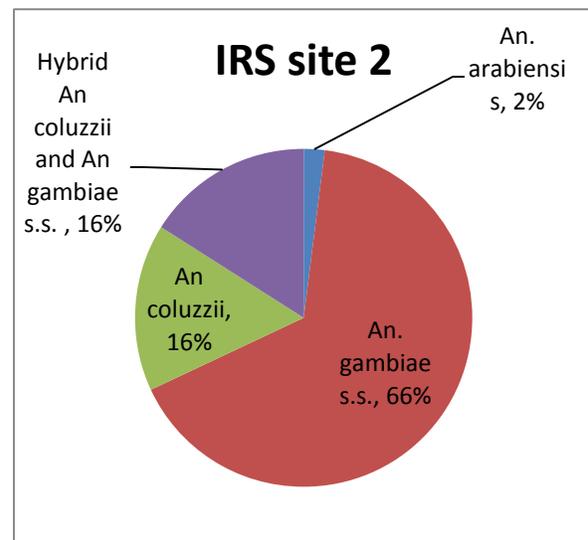
A total of 150 *An. gambiae* s.l. (50 mosquitoes per site) from Koulikoro, Baroueli, and Bla sites were identified to species using PCR. Specimens were from larval collections that were subsequently tested as adults in WHO susceptibility bioassays. The sibling species *An. gambiae* s.s., hybrid *An. coluzzii* and *An. gambiae* s.s., *An. coluzzii*, and *An. arabiensis* were identified in all sites. *An. gambiae* s.s. was predominant in Baroueli in one out of three sites, and the hybrid *An. coluzzii* and *An. gambiae* s.s. was predominant in Koulikoro and Bla in two out of three sites (Figure 8). The high frequency of hybrids was unexpected as the former M and S forms have been elevated to different species, with reproductive isolation believed to be the main barrier to hybridization. Elsewhere in Africa, the frequency of F1 hybrids was generally <2%, although there is a study from Guinea-Bissau indicating up to 20% hybrids. Previously in Mali (2012), there was a low frequency of hybrids, but this increased to nearly 20% in 2014. In 2015, the proportion of hybrids was as high as 48% in IRS site 1 (n=50), with a mean of 34% across all three villages (n=150). This result would suggest that there is no barrier to mating between *An. gambiae* s.s. and *An. coluzzi* in this area. However, we need to demonstrate that this result is quality assured, and that the high hybridization rate (never seen anywhere else at this rate) is not due to laboratory sample contamination. A sub-sample should be sent to another laboratory for quality assurance of species identification.

**FIGURE 8. AN GAMBIAE S.L. SIBLING SPECIES COMPOSITION (PCR), KOULIKORO, BAROUELI, AND BLA, JUNE–DECEMBER 2015 (N=50 PER SITE)**

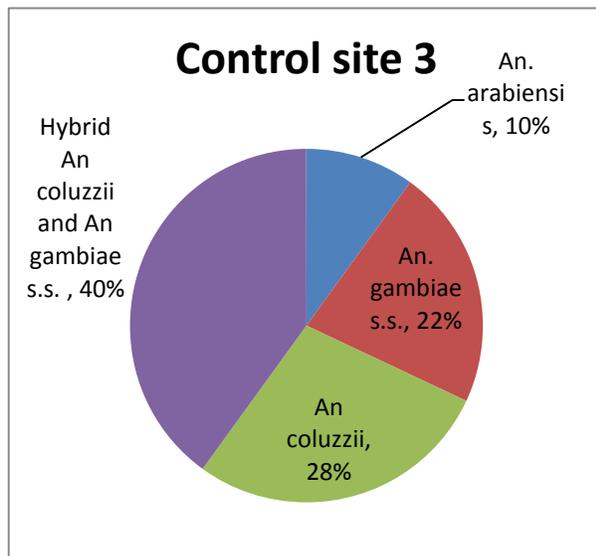
**A**



**B**



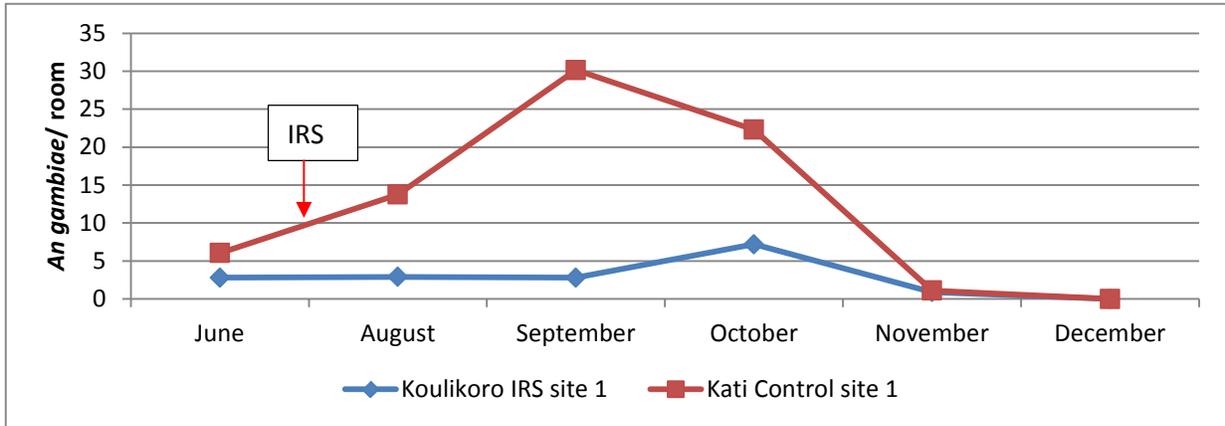
**C**



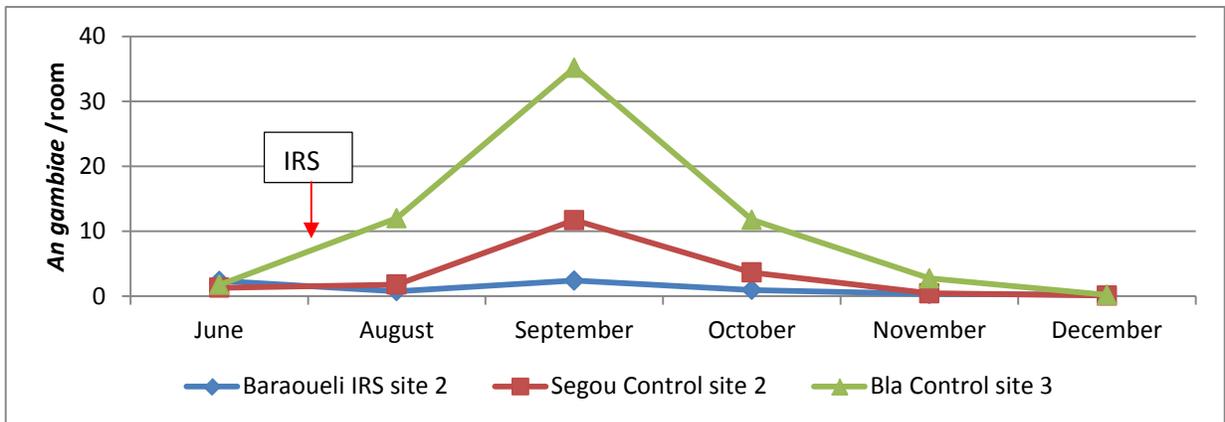
### 3.1.2 VECTOR DENSITY

Prior to IRS, indoor resting densities of malaria vectors in IRS sites were comparable to control sites during the month of June. IRS was conducted in July and post-IRS density peaked during September and October with numbers reaching a peak of 30 *An. gambiae* s.l./room in Kati (control site 1), 35 *An. gambiae* s.l./room in Bla (control site 3), and 12 *An. gambiae* s.l./room in Segou (control site 2). During the peak, both IRS sites had low densities with a peak of 7 *An. gambiae* s.l./room in October in Koulikoro (IRS site 1) and <5 mosquito/room in Baroueli (IRS site 2) (Figures 9 and 10). The low density during the peak in IRS sites as compared to control sites could be due to the impact of IRS.

**FIGURE 9. MEAN INDOOR RESTING DENSITY (PSC) OF AN. GAMBIAE S.L., BY MONTH, KOULIKORO, AND KATI, JUNE–DECEMBER 2015**



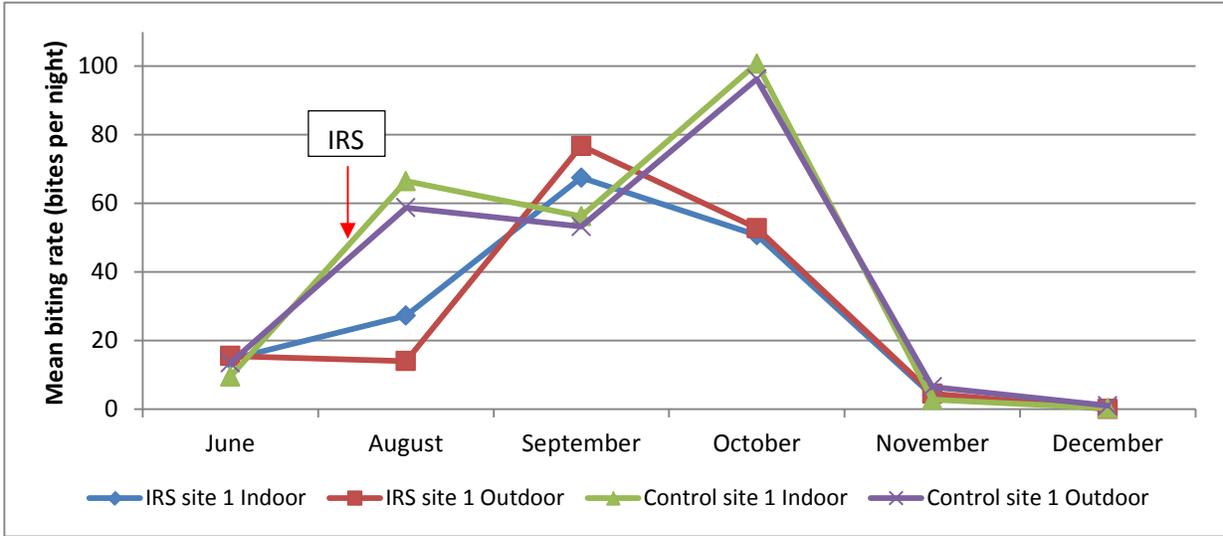
**FIGURE 10. MEAN INDOOR RESTING DENSITY (PSC) OF AN. GAMBIAE S.L., BY MONTH, BAROUELI, SEGOU, AND BLA, JUNE–DECEMBER 2015**



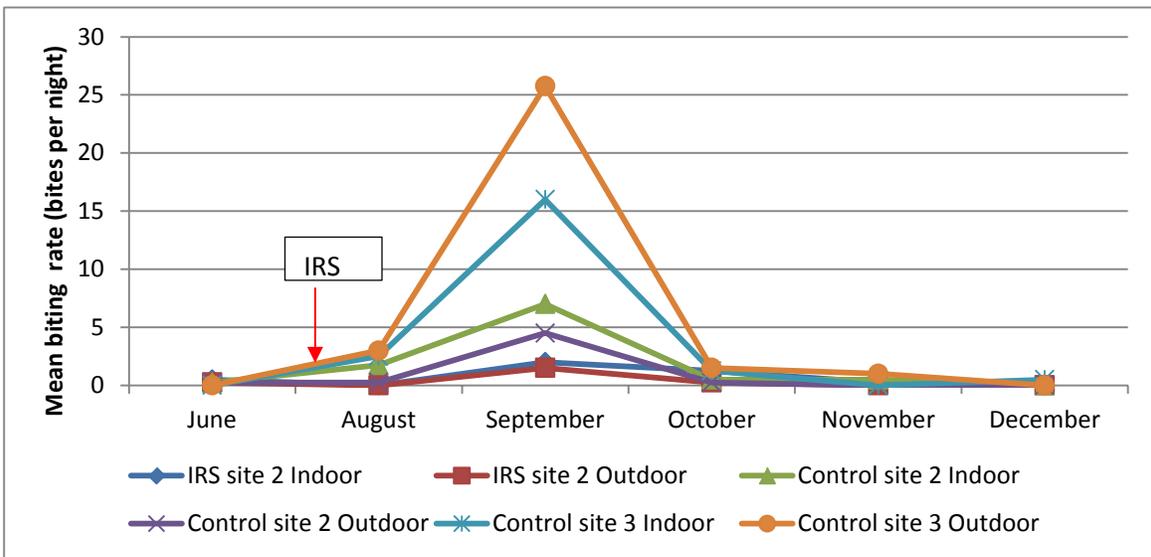
### 3.1.3 SEASONALITY, FEEDING TIME, AND LOCATION

In June (prior to IRS) malaria-vector human biting rates in IRS sites were approximately the same as control sites. After IRS, human biting rates peaked in September and October and then declined in November and December. During the peak, the IRS sites had a human biting rate of 67–78 bites per night in Koulikoro (IRS site 1) and 1–2 in Baroueli (IRS site 2) (Figures 11 and 12). The biting rates in the unsprayed villages were 96–101 in Kati (control site 1), 4-7 in Segou (control site 2), and 16–26 in Bla (control site 3). Timing and frequency of indoor and outdoor biting was approximately equal for all sites (Figure 11 and 12; Annex B Tables B-1 and B-2).

**FIGURE 11. AN. GAMBIAE S.L. HUMAN BITING RATE SEASONALITY (HLC), KOULIKORO AND KATI, JUNE–DECEMBER 2015**

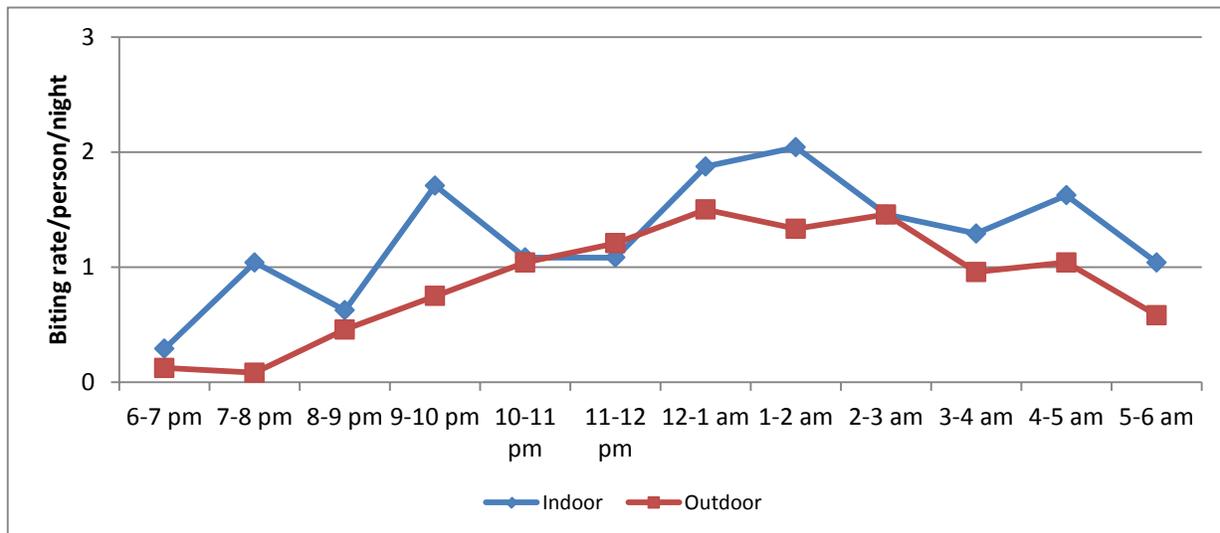


**FIGURE 12. AN. GAMBIAE S.L. HUMAN BITING RATE SEASONALITY (HLC), BAROUELI, SEGOU, AND BLA, JUNE–DECEMBER 2015**

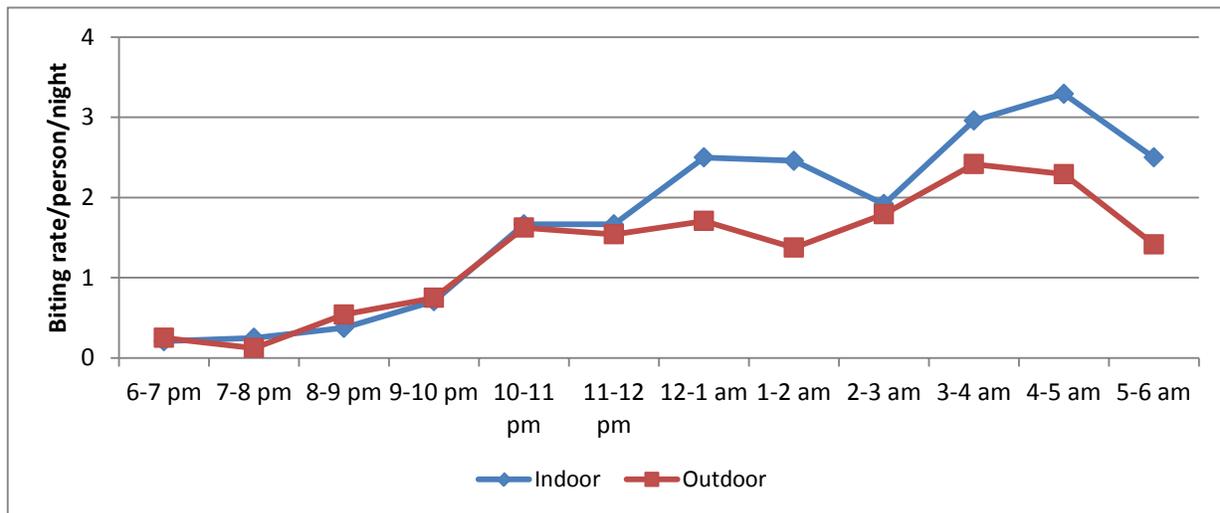


As indicated in Figures 13–17, the biting time of the main malaria vector, *An. gambiae* s.l., appears similar in the IRS and control sites. A small proportion of *An. gambiae* s.l. attempted to bite in the early evening, with the biting rate increasing progressively throughout the night. A greater human biting rate occurred in the second half of the night in both IRS and control sites. The frequency of biting indoors and outdoors fluctuated throughout the night and by location, but generally the highest biting rates were between 1 a.m. and 5 a.m.

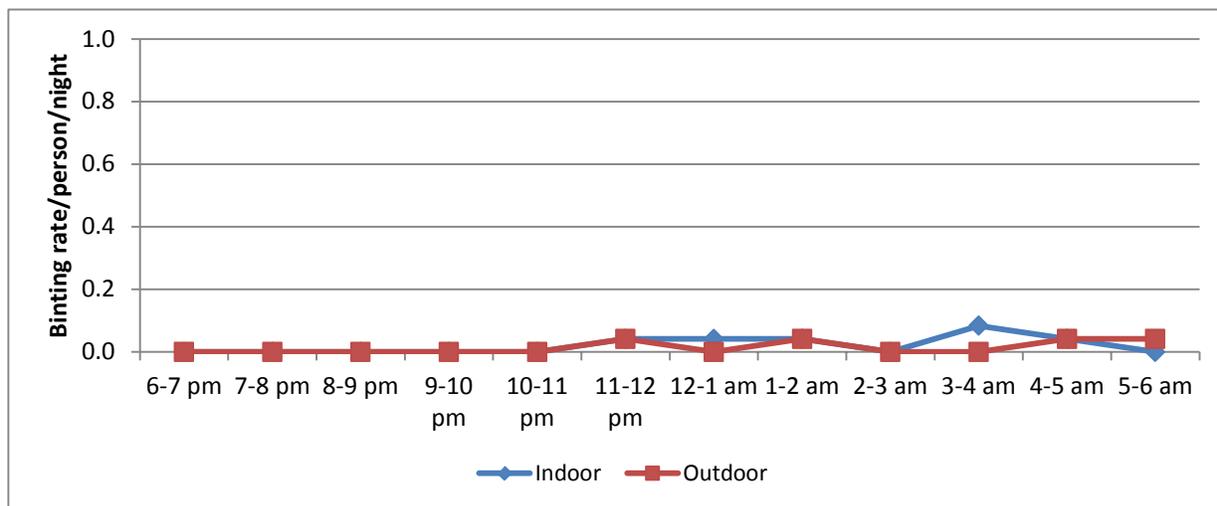
**FIGURE 13. AN. GAMBIAE S.L., HOURLY BITING RATE (HLC), KOULIKORO, JUNE–DECEMBER 2015**



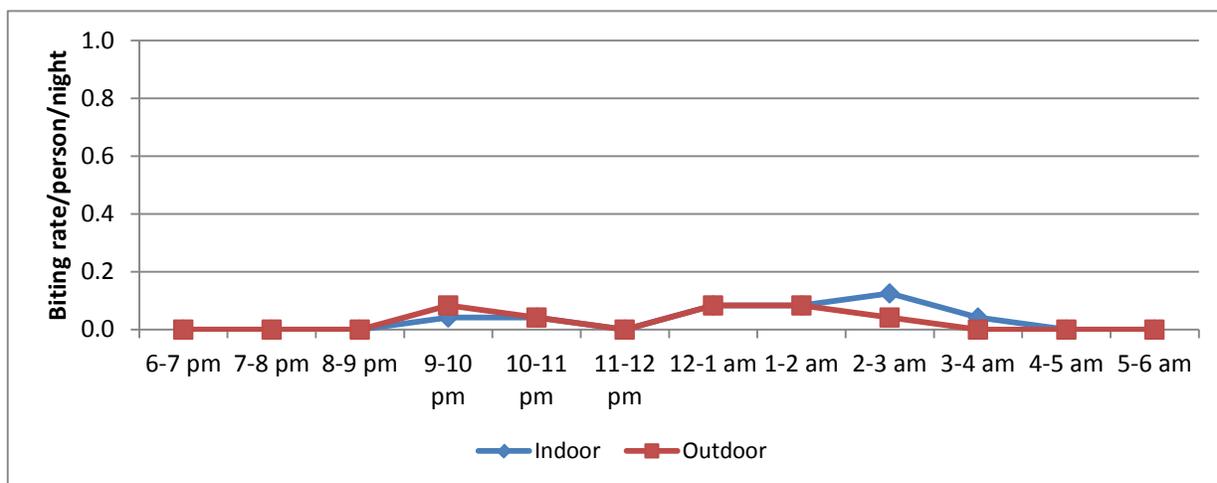
**FIGURE 14. AN. GAMBIAE S.L., HOURLY BITING RATE (HLC), KATI, JUNE–DECEMBER 2015**



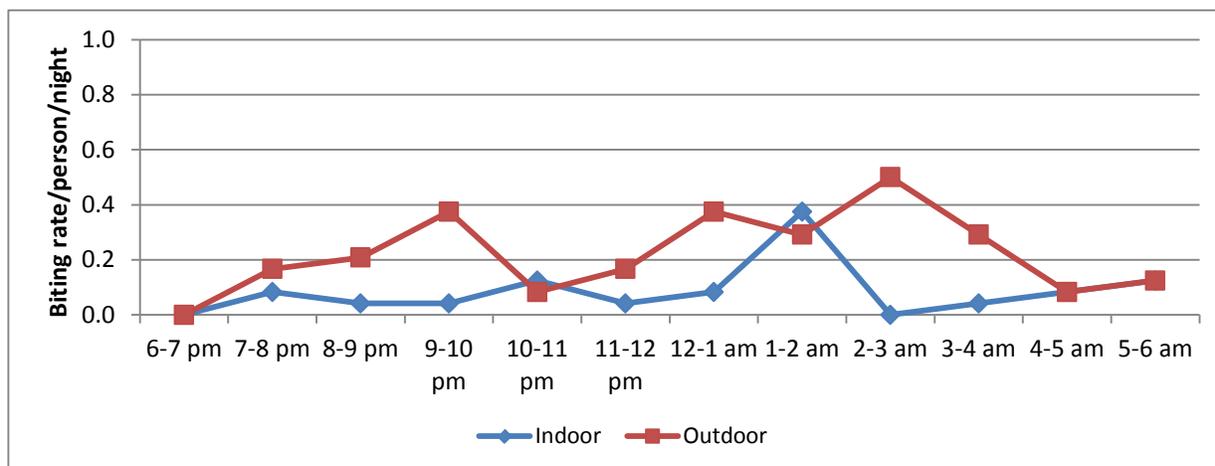
**FIGURE 15. AN. GAMBIAE S.L., HOURLY BITING RATE (HLC), BAROUELI, JUNE–DECEMBER 2015**



**FIGURE 16. AN. GAMBIAE S.L., HOURLY BITING RATE (HLC), SEGOU, JUNE–DECEMBER 2015**



**FIGURE 17. AN. GAMBIAE S.L., HOURLY BITING RATE (HLC), BLA, JUNE–DECEMBER 2015**



MBR/ person/ night indoors and outdoors from both IRS and control sites are provided in Table 4. Before the IRS, the indoor and outdoor biting rates of *An. gambiae* s.l. were similar. After spraying, the indoor and outdoor MBR was approximately the same in Koulikoro (IRS site 1) and Kati (control site 1), and in Baroueli (IRS site 2) and Segou (control site 2). The MBR/ person/ night increased in all sites after IRS compared to before IRS, most probably associated with the increase of vector breeding sites and density with rainfall (Annex A Table A-3).

**TABLE 4. AN. GAMBIAE S.L. MEAN MBR INDOOR AND OUTDOOR, HLC, JUNE–DECEMBER 2015**

	Indoor	Outdoor	In:Out Ratio
<b>Pre IRS</b>			
Koulikoro IRS site 1	14.50	15.50	0.48 : 0.51
Baroueli IRS site 2	0.50	0.25	0.66 : 0.33
Kati control site 1	9.50	13.50	0.44 : 0.55
Segou control site 2	0.25	0.25	0.50 : 0.50
Bla control site 3	0	0	
<b>Post IRS</b>			
Koulikoro IRS site 1	29.95	29.60	0.50 : 0.48
Baroueli IRS site 2	0.7	0.35	0.66 : 0.33
Kati control site 1	45.3	43.15	0.51 : 0.48
Segou control site 2	1.95 <sup>a</sup>	1 <sup>b</sup>	0.66 : 0.33
Bla control site 3	4.05 <sup>a</sup>	6.25 <sup>b</sup>	0.39 : 0.60

Note: MBR indoor and outdoor with different superscript are statistically significant from each other.

HLC and CDC light traps provide comparable data in Table 5 and 6. However, the number of mosquitoes/trap/night collected in IRS and control sites by HLC was significantly higher than the number collected by CDC light traps.

**TABLE 5. NUMBER OF MOSQUITOES COLLECTED (HLC), ALL SITES, JULY–DECEMBER 2015**

Species	<i>An gambiae s.l.</i>	<i>An funestus s.l.</i>	<i>An pharoensis</i>	<i>An rufipes</i>	<i>An zemani</i>	Total
<b>Pre IRS</b>						
IRS site 1	120	0	0	3	0	123
IRS site 2	3	0	0	0	0	3
Control site 1	92	0	0	0	0	92
Control site 2	2	0	0	0	0	2
Control site 3	0	0	0	0	0	0
<b>Post IRS</b>						
IRS site 1	1191	2	14	0	1	1208
IRS site 2	21	0	0	0	0	21
Control site 1	1769	0	60	0	0	1829
Control site 2	59	0	0	0	0	59
Control site 3	206	0	4	0	0	210

**TABLE 6. NUMBER OF MOSQUITOES COLLECTED (CDC LIGHT TRAPS), ALL SITES, JULY–DECEMBER 2015**

Species	<i>An. gambiae s.l.</i>	<i>An. funestus</i>	<i>An. pharoensis</i>	<i>An. rufipes</i>	<i>An. zemani</i>	Total
<b>Pre IRS</b>						
IRS site 1	63	0	0	0	0	63
IRS site 2	1	0	0	0	0	1
Control site 1	8	0	0	0	0	8
Control site 2	2	0	0	0	0	2
Control site 3	0	0	0	0	0	0
<b>Post IRS</b>						
IRS site 1	535	0	8	1	2	546
IRS site 2	6	0	0	0	0	6
Control site 1	906	0	18	0	0	924
Control site 2	10	0	0	0	5	15
Control site 3	55	1	8	0	0	64

## 3.2 VECTOR INSECTICIDE RESISTANCE

### 3.2.1 VECTOR SUSCEPTIBILITY TEST

Vector susceptibility tests were conducted using non-blood-fed adult female *An. gambiae s.l.* reared from larvae and pupae collected in Koulikoro (IRS site 1), Baroueli (IRS site 2), and Bla (control site 3). These tests were to determine the susceptibility level of the vector population collected from targeted districts. High levels of resistance were found to permethrin, deltamethrin, and DDT. *An. gambiae s.l.*

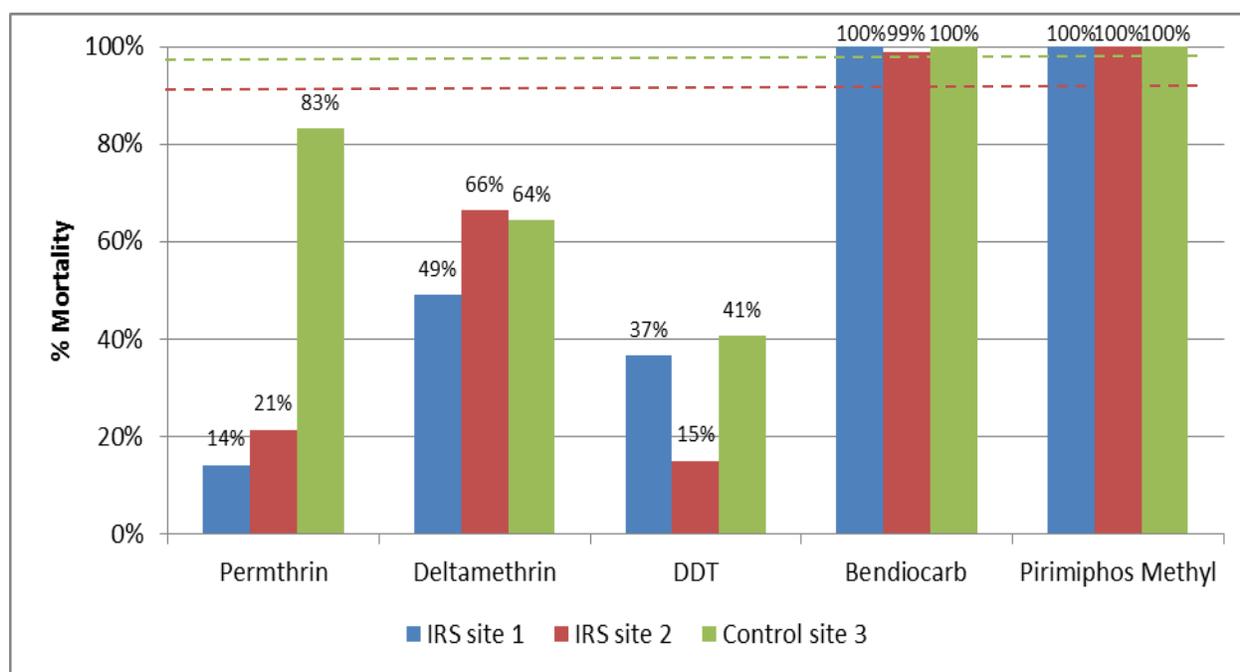
were fully susceptible to bendiocarb and pirimiphos-methyl in all sites (Table 7, Figure 18, Annex C Table C-1).

**TABLE 7. WHO SUSCEPTIBILITY ASSAYS WITH AN. GAMBIAE S.L., 2015**

District	Site	Permethrin		Deltamethrin		DDT		Bendiocarb		Pirimiphos-methyl	
		%	#	%	#	%	#	%	#	%	#
Koulikoro	IRS site 1	14% R	100	49% R	100	37%	104	100% S	100	100% S	100
Baroueli	IRS site 2	21% R	103	66% R	104	15%	101	99% S	100	100% S	101
Bla	Control site 3	83% R	101	64% R	101	41%	101	100% S	104	100% S	101

Note: %: Mortality percentage; #: number tested; R: resistance; S: susceptible

**FIGURE 18. 2015 WHO SUSCEPTIBILITY ASSAYS WITH AN. GAMBIAE S.L., ALL SITES**



### 3.2.2 RESISTANCE INTENSITY ASSAY

*An. gambiae* s.l. from Koulikoro (IRS site 1), Baroueli (IRS site 2), and Bla (control site 3) were tested with CDC bottle assay to determine the intensity of IR against permethrin and deltamethrin at 1X, 2 X, 5X, and 10X concentrations. As expected, test mortality increased as the insecticide concentration increased for both permethrin and deltamethrin. In all sites, the mortality was <70% when *An. gambiae* s.l. was exposed to permethrin at 10 times the diagnostic dosages. When *An. gambiae* s.l. was exposed to deltamethrin at 10 times the diagnostic dosages in IRS sites 1 and 2 and control site 3, the mortality was respectively 94%, 91%, and 84% (Table 8). The results show the presence of high-intensity resistance to pyrethroids. The intensity of resistance was higher to permethrin than to deltamethrin.

**TABLE 8. PYRETHROID RESISTANCE INTENSITY, KOULIKORO, BAROUELI, AND BLA**

Dose	Permethrin						Deltamethrin					
	IRS site 1		IRS site 2		Control site 3		IR site 1		IRS site 2		Control site 3	
	%	#	%	#	%	#	%	#	%	#	%	#
1X	19% R	100	9% R	104	4% R	101	70% R	101	78% R	103	34% R	102
2X	9% R	100	20% R	103	20% R	101	51% R	101	80% R	102	73% R	102
5X	14% R	100	38% R	103	38% R	102	80% R	103	75% R	102	71% R	101
10X	66% R	100	60% R	103	45% R	102	94% PR	102	91% PR	103	84% R	101

#: Mortality percentage; #: number tested; R: resistance; PR: Possible resistance

### 3.2.3 MIXED FUNCTION OXIDASES RESISTANCE MECHANISM

*An. gambiae* s.l. from Koulikoro (IRS site 1), Baroueli (IRS site 2), and Bla (control site 3) were tested with CDC bottle assays to determine the role of MFOs in pyrethroid resistance. The data in Table 9 indicate that MFOs play a significant role in phenotypic resistance to permethrin in two sites, and deltamethrin in two sites ( $P < 0.05$ ). The use of PBO exposure before permethrin significantly increased mortality in IRS site 2 and control site 3 but did not restore full susceptibility. Likewise, use of PBO before deltamethrin exposure increased significantly mortality in IRS site 1 and control site 3 but did not fully restore susceptibility (Table 9).

**TABLE 9. 2015 MFO RESISTANCE MECHANISM, KOULIKORO, BAROUELI, AND BLA**

District	Site	Permethrin				Deltamethrin			
		Insecticide only		+ PBO		Insecticide only		+ PBO	
		%	#	%	#	%	#	%	#
Koulikoro	IRS site 1	19% R	100	27% R	93	70% R	101	93%* PR	102
Baroueli	IRS site 2	9% R	104	61%* R	102	78% R	103	71% R	80
Bla	Control site 3	4% R	101	54%* R	101	34% R	102	78%* R	100

Note: #: Mortality percentage; #: number tested; R: resistance; PS: Possible resistance; \*:  $p < 0.05$  (Z-test for difference in proportions). The control mortality for each test was  $< 5\%$ .

### 3.3 QUALITY OF SPRAYING AND INSECTICIDE DECAY RATE

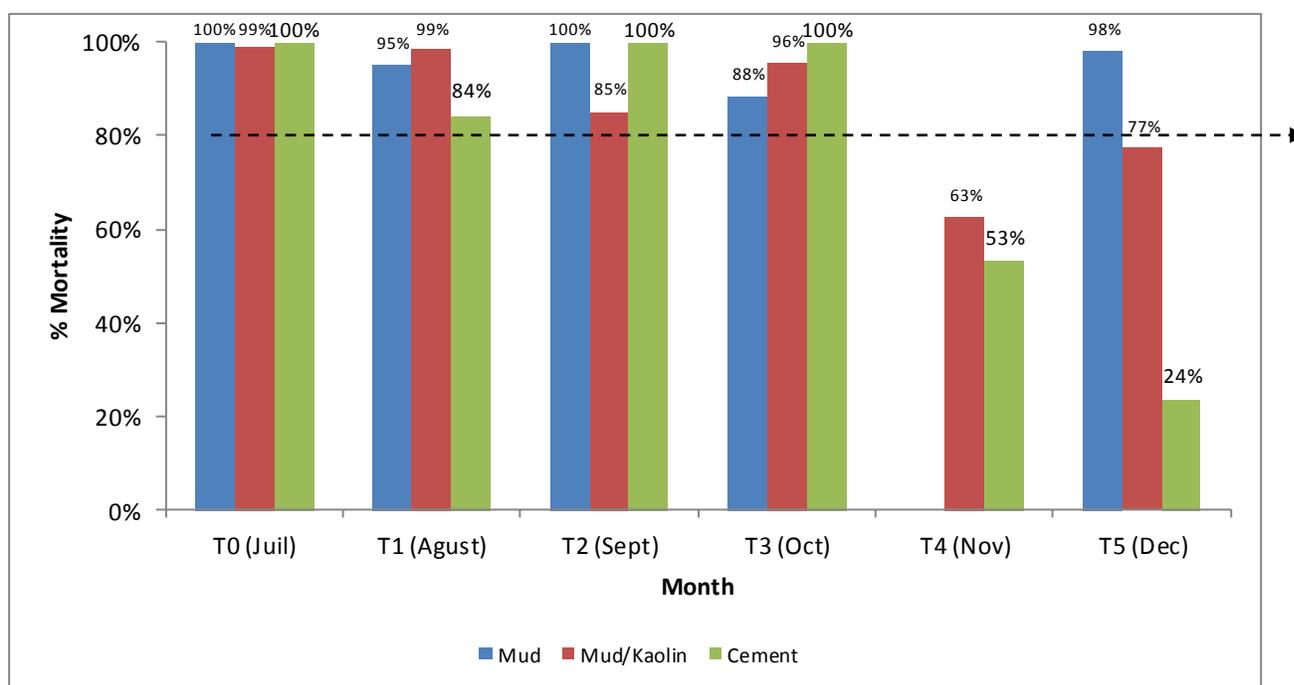
Pirimiphos-methyl 300CS (organophosphate) was sprayed in Koulikoro (IRS site 1) and Baroueli (IRS site 2) districts. At the beginning of the IRS campaign, cone bioassays were done to assess the quality of spraying at four sentinel sites (Tienfala and N'Dentila in Koulikoro district, Konobougou and Tigui in Baroueli district). The assessment helped check the efficacy and homogeneity of insecticide treatment. Mosquitoes of the *An. gambiae* KISUMU strain, which is susceptible to pirimiphos-methyl, were reared at the AIRS Mali insectary and were used to assess the quality of spraying. Bioassays were performed 24 hours after IRS, following WHO procedures. Cone bioassays were conducted in 20 sprayed structures (rooms) in the two districts within 24 hours of spraying to assess the quality of spraying and then the structures were monitored on a monthly basis to determine the insecticide decay rate. In each district, 10 structures were sampled and used for the tests.

The cone bioassay tests conducted in the two IRS targeted districts showed that the quality of spraying was acceptable and that 24 hours (time 0=T0) after spraying, the test mortality rates of susceptible mosquitoes on mud, kaolin mud, and cement surfaces ranged from 99% to 100% (Figure 19).

There were no differences in test mortality rates of mosquitoes exposed to the sprayed walls at three different heights at baseline, which was 99%-100% (Tables 10 and 11). This indicates that the spraying was relatively homogeneous.

Three months after spraying (time 3=T3), the test mortality rates were higher than 80% for all substrates (88% on mud, 96% on kaolin mud, 100% on cement). Four months (time 4=T4) after spraying, the test mortality rates were less than the 80% WHO threshold on kaolin mud at 63% and cement at 53%. Mud was not tested in month four. Five months after spraying (time 5=T5) the test mortality were still higher than 80% on mud surface at 98%. Mortality for cement decreased further to 24%, while for kaolin mud was 77% (only slightly below WHO cut-off). According to WHO, cut-offs of 80% indicate a residual lifespan of months on cement and kaolin mud and >5 months on mud. The residual life of only three months recorded on these substrates is shorter than observed in other AIRS countries, where up to 10 months was observed (Ghana). This might have been due to chemical breakdown of the insecticide or physical characteristics resulting in the insecticide not being bioavailable on the surface and warrants further investigation. The houses in Koulikoro and Baroueli typically have mud walls with some covered on the surface with kaolin.

**FIGURE 19. WHO CONE TEST RESULTS, AN. GAMBIAE KISUMU MORTALITY AFTER 30 MINUTES EXPOSURE TO PIRIMIPHOS-METHYL, KOULIKORO AND BAROUELI**



**TABLE 10. SUMMARY OF QUALITY ASSURANCE TESTS, WHO CONE BIOASSAY, KOULIKORO AND BAROUELI DISTRICTS**

Test Date	District	Test Site	# Houses Tested	# Mosquitoes Tested	Knockdown n 30 Min	% Knockdown 30 Min	# Dead after 24 Hrs.	% Test Mortality
07-03-2015	Koulikoro	Tienfala	5	158	52	33	158	100
07-04-2015	Koulikoro	Kolebougou	5	168	75	45	168	100

07-4-2015	Baroueli	Konobougou	5	163	18	11	159	<b>98</b>
07-5-2015	Baroueli	Tigui	5	155	56	36	155	<b>100</b>
Total			20	644	203	32	640	<b>99</b>

**TABLE II. CONE BIOASSAY TEST SUMMARY RESULTS**

Cone Position	# Houses	# Tested	# Knocked down 30 Min	% Knockdown 30 Min	# Dead after 24 Hrs.	% Test Mortality
Top	18	215	65	30%	214	<b>100</b>
Middle	18	215	55	26%	214	<b>100</b>
Bottom	18	214	83	39%	212	<b>99</b>
Total test	NA	644	203	32%	640	<b>99</b>

### 3.4 VECTOR INSECTICIDE RESISTANCE MOLECULAR CHARACTERIZATION

A total of 50 mosquitoes for each site (Koulikoro, Baroueli, and Bla) were used for molecular characterization of IR. For each site, 25 live and 25 dead mosquitoes following exposure to permethrin and deltamethrin in WHO susceptibility assays were chosen; for all other insecticides 12–13 live and 12–13 dead mosquitoes were chosen.

#### 3.4.1 RESISTANCE RELATED TO WEST AFRICA *KDR* LI014F MUTATION

The West Africa *kdr* LI014F mutation was found to be at a high frequency in all three sites and ranged from 90% to 96% in *An. coluzzii*, 95% to 96% in hybrid *An. coluzzii*, and *An. gambiae* s.s., and 72% to 92% in *An. gambiae* s.s., and 83% to 100% in *An. arabiensis*. High allelic frequencies were found in both live and dead mosquitoes (Tables 12 and 13).

**TABLE 12. LI014F RESISTANCE GENOTYPES AND FREQUENCY, KOULIKORO AND BAROUELI**

Koulikoro/ Baroueli	<i>An. coluzzii</i>			<i>f(kdr)</i>	Hybrid <i>An. coluzzii</i> and <i>An. gambiae</i> s.s.			<i>f(kdr)</i>	<i>An. gambiae</i> s.s.			<i>f(kdr)</i>	<i>An. arabiensis</i>			
	LI014F genotypes				LI014F genotypes				LI014F genotypes				LI014F genotypes			
	RR	RS	SS		RR	RS	SS		RR	RS	SS		RR	RS	SS	
Permethrin Alive	1	0	0	<b>1</b>	7	0	0	<b>1</b>	16	2	0	<b>0.84</b>	0	0	0	n/a
Permethrin Dead	5	0	0	<b>1</b>	8	0	0	<b>1</b>	8	1	0	<b>0.94</b>	0	0	0	n/a
Deltamethrin Alive	4	1	1	<b>0.75</b>	5	2	0	<b>0.85</b>	10	1	2	<b>0.8</b>	0	0	0	n/a
Deltamethrin Dead	3	0	0	<b>1</b>	10	0	0	<b>1</b>	10	0	0	<b>1</b>	2	1	0	<b>0.83</b>
<b>Total</b>	13	1	1	<b>0.9</b>	30	2	0	<b>0.96</b>	44	4	2	<b>0.92</b>	2	1	0	<b>0.83</b>

**TABLE 13. LI014F RESISTANCE GENOTYPES AND FREQUENCY, BLA**

Bla	<i>An. coluzzii</i>			<i>f(kdr)</i>	Hybrid <i>An. coluzzii</i> and <i>An. gambiae</i> s.s.			<i>f(kdr)</i>	<i>An. gambiae</i> s.s.			<i>f(kdr)</i>	<i>An. arabiensis</i>			<i>f(kdr)</i>
	LI014F genotypes				LI014F genotypes				LI014F genotypes				LI014F genotypes			
	RR	RS	SS		RR	RS	SS		RR	RS	SS		RR	RS	SS	
Permethrin Alive	4	1	0	<b>0.9</b>	4	0	0	<b>1.0</b>	2	0	0	<b>1.0</b>	0	0	0	n/a
Permethrin Dead	5	0	0	<b>1.0</b>	6	0	0	<b>1.0</b>	1	0	0	<b>1.0</b>	1	0	0	<b>1.0</b>
Deltamethrin Alive	1	0	0	<b>1.0</b>	4	1	0	<b>0.9</b>	3	2	2	<b>0.57</b>	0	0	0	n/a
Deltamethrin Dead	3	0	0	<b>1.0</b>	4	1	0	<b>0.9</b>	1	0	0	<b>1.0</b>	4	0	0	<b>1.0</b>
<b>Total</b>	13	1	0	<b>0.96</b>	18	2	0	<b>0.95</b>	7	2	2	<b>0.72</b>	5	0	0	<b>1.0</b>

### 3.4.2 EAST AFRICA KNOCK-DOWN RESISTANCE LI014S MUTATION

The East Africa *kdr* LI014S resistance mutation was found at low frequency only in *An. gambiae* s.s. and *An. coluzzii* from the three sites. The allelic frequency was <10% in *An. gambiae* s.s. (7% in IRS site 1 and 2 and 4% in control site 3) and <15% in *An. coluzzii* (13% in IRS site 1 and 2). This mutation was not found in *An. arabiensis* or hybrid *An. coluzzii* and *An. gambiae* s.s. This mutation was found only in live mosquitoes from IRS site 1 and control site 3 but found in live and dead mosquitoes in IRS site 2 (Tables 15–17).

**TABLE 14. LI014S RESISTANCE GENOTYPES AND FREQUENCY, KOULIKORO AND BAROUELI**

Koulikoro/ Baroueli	<i>An. coluzzii</i>			<i>f(kdr)</i>	Hybrid <i>An. coluzzii</i> and <i>An. gambiae</i> s.s.			<i>f(kdr)</i>	<i>An. gambiae</i> s.s.			<i>f(kdr)</i>	<i>An. arabiensis</i>			<i>f(kdr)</i>
	LI014S genotypes				LI014S genotypes				LI014S genotypes				LI014S genotypes			
	RR	RS	SS		RR	RS	SS		RR	RS	SS		RR	RS	SS	
Permethrin Alive	0	0	1	<b>0</b>	0	0	7	<b>0</b>	1	1	16	<b>0.08</b>	0	0	0	n/a
Permethrin Dead	1	0	4	<b>0.2</b>	0	0	8	<b>0</b>	0	0	9	<b>0</b>	0	0	0	n/a
Deltamethrin Alive	0	2	4	<b>0.16</b>	0	0	7	<b>0</b>	0	1	12	<b>0.03</b>	0	0	0	n/a
Deltamethrin Dead	0	0	3	<b>0</b>	0	0	10	<b>0</b>	1	1	8	<b>0.15</b>	0	0	3	<b>0</b>
<b>Total</b>	1	2	12	<b>0.13</b>	0	0	32	<b>0</b>	2	3	45	<b>0.07</b>	0	0	3	<b>0</b>

**TABLE 15. LI014S RESISTANCE GENOTYPES AND FREQUENCY, BLA, 2015**

Bla	<i>An. coluzzii</i>				Hybrid <i>An. coluzzii</i> and <i>An. gambiae</i> s.s.				<i>An. gambiae</i> s.s.				<i>An. arabiensis</i>			
	LI014S genotypes			f(kdr)	LI014S genotypes			f(kdr)	LI014S genotypes			f(kdr)	LI014S genotypes			f(kdr)
	RR	RS	SS		RR	RS	SS		RR	RS	SS		RR	RS	SS	
Permethrin Alive	0	0	5	0	0	0	4	0	0	1	1	0.25	0	0	0	0
Permethrin Dead	0	0	5	0	0	0	6	0	0	0	1	0.0	0	0	1	0
Deltamethrin Alive	0	0	1	0	0	0	5	0	0	0	7	0.0	0	0	0	0
Deltamethrin Dead	0	0	3	0	0	0	5	0	0	0	1	0.0	0	0	4	0
<b>Total</b>	<b>0</b>	<b>0</b>	<b>14</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>20</b>	<b>0</b>	<b>0</b>	<b>1</b>	<b>10</b>	<b>0.04</b>	<b>0</b>	<b>0</b>	<b>5</b>	<b>0</b>

The higher intensity of resistance to pyrethroids detected in the IRS sites 1 and 2 and control site 3 may partially be due to the presence of LI014F and LI014S mutations and MFOs.

### 3.4.3 ACE-IR MUTATION

The *ace IR* mutation responsible for carbamates and organophosphates resistance was not detected in the three tested sites. This data confirms the result of WHO susceptibility tests, which show full susceptibility of these vectors populations to carbamates and organophosphates.

## 3.5 SPOROZOITE INFECTION AND PARITY RATE AND BLOOD MEAL ORIGIN

Mosquitoes collected monthly were processed by ELISA to determine the *Plasmodium falciparum* sporozoite infection rate and blood meal origin.

### 3.5.1 SPOROZOITE INFECTION RATE

*An. gambiae* s.l. monthly *P. falciparum* sporozoite infection rate per site is indicated in Tables 16 and 17. Pre-IRS infection rates in June were similar ( $p=0.51$ ) in Koulikoro (IRS site 1) and Kati (control site 1), and in Baroueli (IRS site 2) and Segou (control site 2) ( $p=0.54$ ) (Table 16). The highest infection rates were observed in September (two months post-IRS) for both IRS and control sites. During this period, the infection rate in IRS site 1 (8.1%; 95% CI 1.3-14.8) was significantly lower ( $P=0.02$ ) than in control site 1 (23.0%; 95% CI 12.4-33.5). However, IRS site 2 (6.7%; 95% CI 0.0-19.3) was not significantly different to control site 2 (3.9%; 95% CI 0.0-9.2) ( $p=0.65$ ), although low numbers of *An. gambiae* s.l. were processed at this time.

The Bla infection rate in September 2015 (4.9%; 95% CI 0.0-10.3) was approximately the same ( $p=0.70$ ) as compared to 2014 (6.9%; 95% CI 0.0-16.1). This could be due to low numbers of *An. gambiae* s.l. being processed, 61 mosquitoes in 2015 and 29 mosquitoes in 2014 (Table 16 and Annex E Table E-1). Between September and December the IRS sites combined had a sporozoite rate that was lower than the unsprayed sites ( $P=0.02$ ).

The lower infection rate during the high transmission period in IRS site 1 compared to control site 2 could be due to the impact of IRS.

**TABLE 16. AN. GAMBIAE S.L. PLASMODIUM. FALCIPARUM (SPOROZOITE) INFECTION RATES, ALL SITES, HLC, JUNE–SEPTEMBER**

Site	June			August			September		
	# tested	# SPZ (+)	SPZ rate	# tested	# SPZ (+)	SPZ rate	# tested	# SPZ (+)	SPZ rate
IRS site 1	171	8	0.05	100	2	0.02	62	5	0.08
IRS site 2	4	1	0.25	0	0	0	15	1	0.07
Control site 1	91	6	0.07	99	8	0.08	61	14	0.23
Control site 2	2	1	0.50	10	0	0.00	51	2	0.04
control site 3	0	0	0	22	1	0.05	61	3	0.05

# tested: Number tested; # SPZ (+): Number of positive sporozoite; SPZ rate: sporozoite rate

Jun: Pre IRS, Aug: one month post IRS; Sept: two months post IRS, Oct: three months post IRS, Nov: four months post IRS, and Dec: five months post IRS.

**TABLE 17. AN. GAMBIAE S.L. PLASMODIUM. FALCIPARUM (SPOROZOITE) INFECTION RATES, ALL SITES, HLC, OCTOBER–DECEMBER**

Site	October			November			December		
	# tested	# SPZ (+)	SPZ rate	# tested	# SPZ (+)	SPZ rate	# tested	# SPZ (+)	SPZ rate
IRS site 1	200	2	0.01	34	2	0.06	0	0	0
IRS site 2	6	0	0	0	0	0	0	0	0
Control site 1	204	6	0.03	38	3	0.08	2	0	0.00
Control site 2	3	0	0.00	0	0	0	0	0	0
control site 3	11	0	0.00	0	0	0	2	0	0.00

# tested: Number tested; # SPZ (+): Number of positive sporozoite; SPZ rate: sporozoite rate

Jun: Pre IRS, Aug: one month post IRS; Sept: two months post IRS, Oct: three months post IRS, Nov: four months post IRS, and Dec: five months post IRS.

*An. gambiae* s.l. Entomological Inoculation Rate (EIR) nightly and monthly per site is indicated in Tables 18–22. Eight collectors gathered mosquitoes for two nights per month at each site. The highest EIRs were observed in September 2015 (two months post IRS) for both IRS and control sites (Tables 18 and 20). During this period, the EIR appears to be two times lower in Koulikoro (IRS site 1) than in Kati (control site 1), while the numbers collected in Baroueli (IRS site 2) and Segou (control site 2) were too low for comparison (Tables 19 and 21).

During the high malaria transmission period in September 2015 (two months post IRS), the monthly EIR in Bla was 30.8 compared with 7.5 in September 2014, almost four times higher (although n=29 in 2014). This could be due to the withdrawal of IRS in 2015, but the sample size is insufficient for firm conclusions to be drawn (Table 22 and Annex Table E-3).

The lower EIR during the high transmission period in IRS sites 1 and 2 compared to control sites 1 and 2 could be due to the impact of IRS.

**TABLE 18. EIR, MONTHLY COLLECTIONS, KOULIKORO**

<b>Month</b>	<b>Total <i>An. gambiae</i> s.l. Collected</b>	<b>Biting Rate</b>	<b>SPZ Rate</b>	<b>Nightly EIR</b>	<b>Monthly EIR</b>
Jun	120	15	0.05	<b>0.70</b>	<b>21.05</b>
Aug	165	20.625	0.02	<b>0.41</b>	<b>12.38</b>
Sep	577	72.125	0.08	<b>5.82</b>	<b>174.50</b>
Oct	414	51.75	0.01	<b>0.52</b>	<b>15.53</b>
Nov	34	4.25	0.06	<b>0.25</b>	<b>7.50</b>
Dec	1	0.125	0.00	<b>0.00</b>	<b>0.00</b>

**TABLE 19. EIR, MONTHLY COLLECTIONS, BAROUELI**

<b>Month</b>	<b>Total <i>An. gambiae</i> s.l. Collected</b>	<b>Biting Rate</b>	<b>SPZ Rate</b>	<b>Nightly EIR</b>	<b>Monthly EIR</b>
Jun	3	0.38	0.25	<b>0.09</b>	<b>2.81</b>
Aug	0	0.00	0.00	<b>0.00</b>	<b>0.00</b>
Sep	14	1.75	0.07	<b>0.12</b>	<b>3.50</b>
Oct	6	0.75	0.00	<b>0.00</b>	<b>0.00</b>
Nov	1	0.13	0.00	<b>0.00</b>	<b>0.00</b>
Dec	0	0.00	0.00	<b>0.00</b>	<b>0.00</b>

**TABLE 20. EIR, MONTHLY COLLECTIONS, KATI**

<b>Month</b>	<b>Total <i>An. gambiae</i> s.l. Collected</b>	<b>Biting Rate</b>	<b>SPZ Rate</b>	<b>Nightly EIR</b>	<b>Monthly EIR</b>
Jun	97	12.13	0.07	<b>0.80</b>	<b>23.98</b>
Aug	501	62.63	0.08	<b>5.06</b>	<b>151.82</b>
Sep	438	54.75	0.23	<b>12.57</b>	<b>376.97</b>
Oct	788	98.50	0.03	<b>2.90</b>	<b>86.91</b>
Nov	37	4.63	0.08	<b>0.37</b>	<b>10.95</b>
Dec	5	0.63	0.00	<b>0.00</b>	<b>0.00</b>

**TABLE 21. EIR, MONTHLY COLLECTIONS, SEGOU**

<b>Month</b>	<b>Total <i>An. gambiae</i> s.l. Collected</b>	<b>Biting Rate</b>	<b>SPZ Rate</b>	<b>Nightly EIR</b>	<b>Monthly EIR</b>
Jun	2	0.25	0.50	<b>0.13</b>	<b>3.75</b>
Aug	8	1.00	0.00	<b>0.00</b>	<b>0.00</b>
Sep	46	5.75	0.04	<b>0.23</b>	<b>6.76</b>
Oct	6	0.75	0.00	<b>0.00</b>	<b>0.00</b>

Nov	2	0.25	0.00	<b>0.00</b>	<b>0.00</b>
Dec	0	0.00	0.00	<b>0.00</b>	<b>0.00</b>

**TABLE 22. EIR, MONTHLY COLLECTIONS, BLA**

Month	Total <i>An. gambiae</i> s.l. Collected	Biting Rate	SPZ Rate	Nightly EIR	Monthly EIR
Jun	0	0.00	0.00	<b>0.00</b>	<b>0.00</b>
Aug	22	2.75	0.05	<b>0.13</b>	<b>3.75</b>
Sep	167	20.88	0.05	<b>1.03</b>	<b>30.80</b>
Oct	11	1.38	0.00	<b>0.00</b>	<b>0.00</b>
Nov	4	0.50	0.00	<b>0.00</b>	<b>0.00</b>
Dec	2	0.25	0.00	<b>0.00</b>	<b>0.00</b>

### 3.5.2 PARITY RATE

*An. gambiae* s.l. collected by HLC parity rate before and after IRS are shown in Table 23. Before IRS, the parity was approximatively the same ( $p=0.29$ ) in Koulikoro (IRS site 1) as compared to Kati (control site 1). After IRS, the parity was  $>60\%$  and approximatively the same ( $p=0.41$ ) in IRS site 1 and control site 1, but it was significantly lower ( $p=0.03$ ) in Baroueli (IRS site 2) than in Segou (control site 2).

The Bla post-IRS parity rate (46.15) in 2015 was significantly lower ( $p=0.001$ ) than the 2014 parity rate (78.79%) (Table 23 and Annex D Table D-1). This is in contrast to the hypothesis that the mean age and parity rate would increase following IRS withdrawal.

**TABLE 23. AN. GAMBIAE S.L. PARITY RATE (HLC) PRE- AND POST-IRS, ALL SITES, JUNE-DECEMBER**

	# Dissected	# Parous	% Parous	Confidence Interval 95%
<b>Pre IRS</b>				
IRS site 1	38	27	<b>71.05%</b>	51.9 : 85.5
IRS site 2	2	2	<b>100.00%</b>	100 : 100
Control site 1	28	23	<b>82.14%</b>	68.0 : 96.3
Control site 2	2	0	<b>0</b>	0
Control site 3	0	0	<b>0</b>	0
<b>Post IRS</b>				
IRS site 1	366	260	<b>71.04%</b>	68.1 : 77.2
IRS site 2	6	4	<b>66.67%</b>	28.9 : 104.4
Control site 1	443	303	<b>68.40%</b>	64.1 : 72.7
Control site 2	34	32	<b>94.12%</b>	86.2 : 102.0
Control site 3	104	48	<b>46.15%</b>	36.6 : 55.7

### 3.5.3 BLOOD MEAL ORIGIN

The monthly Human and Bovine Blood Meal Index per site are indicated in Tables 24–28. Pre- and post-IRS, *An. gambiae* s.l. fed on humans and bovines in similar proportions in both IRS and control sites. The proportion that fed on humans (including mixed human/bovine) was surprisingly low at 42% (123/295) in Koulikoro, with 38% having fed on an unidentified host (non-human or bovine). It would be of interest to determine whether there are differences in host preference and indoor/outdoor biting between *An. gambiae* s.s., *An. arabiensis*, *An. coluzzii*, and hybrids.

**TABLE 24. HUMAN AND BOVINE BLOOD MEAL INDEX, KOULIKORO, MONTHLY COLLECTIONS**

Month	# Tested	Human (+)		Bovine (+)		Mixed Human/ Bovine(+)		Others	
		#	%	#	%	#	%	#	%
Jun	47	2	4.3%	34	72.3%	10	21%	1	2.1%
Aug	32	1	3.1%	18	56.3%	13	40.6%	0	0.0%
Sep	40	5	12.5%	5	12.5%	6	15.0%	24	60.0%
Oct	193	56	29.0%	38	19.7%	19	9.8%	80	41.5%
Nov	27	13	48.1%	1	3.7%	7	25.9%	7	25.9%
Dec	3	1	33.3%	0	0.0%	2	66.7%	0	0.0%
Total Aug-Dec	295	76	25.8%	62	21.0%	47	15.9%	111	37.6%

**TABLE 25. HUMAN AND BOVINE BLOOD MEAL INDEX, BAROUELI, MONTHLY COLLECTIONS**

Month	# Tested	Human (+)		Bovine (+)		Mixed Human/ Bovine(+)		Others	
		#	%	#	%	#	%	#	%
Jun	19	0	0.0%	11	57.9%	8	42%	0	0.0%
Aug	11	4	36.4%	1	9.1%	2	18.2%	4	36.4%
Sep	45	10	22.2%	14	31.1%	10	22.2%	11	24.4%
Oct	17	9	52.9%	4	23.5%	3	17.6%	1	5.9%
Nov	16	1	6.3%	6	37.5%	3	18.8%	6	37.5%
Dec	3	0	0.0%	2	66.7%	2	66.7%	0	0.0%
Total Aug-Dec	92	24	26.1%	27	29.3%	20	21.7%	22	23.9%

**TABLE 26. HUMAN AND BOVINE BLOOD MEAL INDEX, KATI, MONTHLY COLLECTIONS**

Month	# Tested	Human (+)		Bovine (+)		Mixed Human/ Bovine(+)		Others	
		#	%	#	%	#	%	#	%
Jun	79	2	2.5%	28	35.4%	43	54%	6	7.6%
Aug	81	29	35.8%	15	18.5%	15	18.5%	22	27.2%
Sep	36	4	11.1%	0	0.0%	2	5.6%	30	83.3%
Oct	200	59	29.5%	39	19.5%	39	19.5%	63	31.5%
Nov	33	8	24.2%	1	3.0%	16	48.5%	8	24.2%
Dec	5	2	40.0%	1	20.0%	2	40.0%	5	100.0%
Total Aug-Dec	355	102	28.7%	56	15.8%	74	20.8%	128	36.1%

**TABLE 27. HUMAN AND BOVINE BLOOD MEAL INDEX, SEGOU, MONTHLY COLLECTIONS**

Month	# Tested	Human (+)		Bovine (+)		Mixed Human/ Bovine(+)		Others	
		#	%	#	%	#	%	#	%
Jun	14	0	0.0%	4	28.6%	11	79%	0	0.0%
Aug	34	0	0.0%	24	70.6%	8	23.5%	2	5.9%
Sep	36	2	5.6%	4	11.1%	4	11.1%	26	72.2%
Oct	72	23	31.9%	9	12.5%	3	4.2%	37	51.4%
Nov	6	2	33.3%	1	16.7%	1	16.7%	2	33.3%
Dec	2	0	0.0%	1	50.0%	0	0.0%	1	50.0%
Total Aug-Dec	150	27	18.0%	39	26.0%	16	10.7%	68	45.3%

**TABLE 28. HUMAN AND BOVINE BLOOD MEAL INDEX, BLA, MONTHLY COLLECTIONS**

Month	# Tested	Human (+)		Bovine (+)		Mixed Human/ Bovine(+)		Others	
		#	%	#	%	#	%	#	%
Jun	32	2	6.3%	17	53.1%	9	28%	4	12.5%
Aug	92	8	8.7%	25	27.2%	2	2.2%	57	62.0%
Sep	92	41	44.6%	12	13.0%	27	29.3%	12	13.0%
Oct	233	35	15.0%	74	31.8%	14	6.0%	110	47.2%
Nov	1	0	0.0%	0	0.0%	1	100.0%	0	0.0%
Dec	4	0	0.0%	2	50.0%	1	25.0%	4	100.0%
Total Aug-Dec	422	84	19.9%	113	26.8%	45	10.7%	183	43.4%

## 4. CONCLUSIONS

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*An. gambiae* s.l. was the primary malaria vector species group and *An. funestus* s.l. the secondary group present. There was a predominance of *An. gambiae* s.s. in Baroueli district and the hybrid *An. coluzzii* and *An. gambiae* s.s. in Koulikoro and Bla districts. The high frequency of hybrids was unexpected and suggests that there is not a barrier to mating between *An. gambiae* s.s. and *An. coluzzi* in this area. However, we need to demonstrate that this result is quality assured, and that the high hybridization rate (never seen anywhere else at this rate) is valid. We have since arranged to collaborate with Dr. Gregory Lanzano and Dr. Yoosook Lee of UC-Davis to conduct quality assurance of the samples from 2015. The collaborators opined that the Scott and Favia assays used in Mali can give confusing results, especially at sites that include hybrid individuals. A more reliable method is the Divergence Island SNP (DIS) assay that was developed by the UC-Davis team. Whereas the Scott and Favia assays are based on only a single genetic marker on the X chromosome the DIS assay includes 17 markers that are located over each of the 3 chromosomes. It is much more accurate and it has the advantage of distinguishing first generation (F1) hybrids from backcross hybrids, many of which would be undetectable using the Favia assay. A sub-sample of extracted DNA from 2015 of 30-50 samples at each of Koulikoro, Kati, Bla, Niono, Kita, Selingue, Bougoni, Djenne, and Bandiagara are being sent for quality assurance of species identification using the DIS technique. This work is being done as part of ongoing UC-Davis research into hybridization and will not require additional funding from PMI. These results will provide a true picture of the hybridization events occurring in Mali and will help to inform decision making for entomological monitoring in 2016.

Resistance tests showed:

- Widespread resistance of *An. gambiae* s.l. to DDT, permethrin, and deltamethrin in the two targeted IRS districts: Koulikoro, Baroueli, and control site Bla.
- Full susceptibility of *An. gambiae* s.l. to bendiocarb (carbamate) and pirimiphos-methyl in Koulikoro, Baroueli, and Bla.

There is presence of high intensity resistance to pyrethroids with \*10 times diagnostic concentration killing <100% at all sites. The intensity of resistance was higher to permethrin than to deltamethrin. Resistance to pyrethroids in Koulikoro, Baroueli, and Bla was partially due to a high frequency of L1014F *kdr* mutation and elevated MFOs. Presence of L1014F *kdr* was not associated with increased survival in susceptibility tests.

Quality assurance cone bioassay tests conducted in two IRS targeted districts showed that the quality of spraying was acceptable with 100% mortality shortly after spraying. The residual efficacy of pirimiphos-methyl CS was less than five months on mud, and three months on cement and kaolin/mud according to the WHO cut-off of 80% mortality.

Peak vector density and biting rates were observed two to three months after IRS and the biting rate appeared to be lower in IRS sites as compared to control sites. The major vectors in both IRS and control sites were biting at similar rates indoors and outdoors, with the peak in biting late at night between 10 p.m. and 4:00 a.m. The importance of outdoor biting will depend on local cultural night time practices.

The comparison of HLC and CDC light traps produced similar data in terms of biting times, but the number of mosquitoes collected was greater for HLC than for CDC light traps. Also, due to high cost and the difficulty in having dry ice for outdoor trapping, HLC is a cost-effective method in Mali.

Blood-meal analysis showed that the proportion of *An. gambiae* s.l. that fed on humans was low at <50%, with many having fed on cattle or unidentified animal hosts.

Both IRS sites had low densities of *An. gambiae* s.l. resting indoors by morning. The low density during the peak in IRS sites as compared to control sites could be due to the impact of IRS.

Post IRS, human biting rates peaked during September and October and declined in November and December. During the peak, the IRS sites had a human biting rate of 67–78 bites per night in Koulikoro (IRS site 1) and 1–2 in Baroueli (IRS site 2). The biting rates in the unsprayed villages were 96–101 in Kati (control site 1), 4–7 in Segou (control site 2), and 16–26 in Bla (control site 3). During this period, the EIR appeared to be two times lower in Koulikoro (IRS site 1) than in Kati (control site 1). In Baroueli, where IRS was conducted, the EIR was <4 infective bites per month even during the peak transmission season. However, in Koulikoro (IRS site 1), there was a high EIR of 174 infective bites per month during September, only two months after spraying. Nevertheless, the EIR at IRS sites appeared to be lower than in the respective unsprayed sites.

In Bla district, where IRS was withdrawn and SMC plus LLIN universal coverage introduced in 2015, there were early signs of a possible increase in transmission, with a fourfold EIR increase compared to 2014; however, the sample size was insufficient for direct comparison.

# ANNEX A: DENSITY AND RAINFALL

**TABLE A-I. DENSITY OF ANOPHELINE (*AN. GAMBIAE* S.L.) AND CULICINE (*CULEX* AND *AEDES*) AND PARITY RATE OF *AN. GAMBIAE* S.L. CAPTURED BY PSC, KOULIKORO AND KATI**

District	Koulikoro IRS site I		Kati Non IRS site I	
Spray status	Spray		Non-spray	
Mosquito	Anopheline	Culicine	Anopheline	Culicine
<b>Baseline: Before IRS (July)</b>				
Number of female capture	56	24	121	350
Frequency of female capture	17%	7%	26%	74%
Number of room	20	20	20	20
<b>Density/House/Night</b>	<b>2.80</b>	<b>1.20</b>	<b>6.05</b>	<b>17.50</b>
<b>One month after IRS (August)</b>				
Number of female capture	58	21	275	259
Frequency of female capture	73%	27%	51%	49%
Number of room	20	20	20	20
<b>Density/House/Night</b>	<b>2.90</b>	<b>1.05</b>	<b>13.75</b>	<b>12.95</b>
<b>Two months after IRS (September)</b>				
Number of female capture	56	9	603	117
Frequency of female capture	86%	14%	84%	16%
Number of room	20	20	20	20
<b>Density/House/Night</b>	<b>2.80</b>	<b>0.45</b>	<b>30.15</b>	<b>5.85</b>
<b>Three months after IRS (October)</b>				
Number of female capture	144	9	446	53
Frequency of female capture	94%	6%	89%	11%
Number of room	20	20	20	20
<b>Density/House/Night</b>	<b>7.20</b>	<b>0.45</b>	<b>22.30</b>	<b>2.65</b>
<b>Four months after IRS (November)</b>				
Number of female capture	18	31	22	63
Frequency of female capture	37%	63%	26%	74%
Number of room	20	20	20	20
<b>Density/House/Night</b>	<b>0.90</b>	<b>1.55</b>	<b>1.10</b>	<b>3.15</b>

<b>Five months after IRS (December)</b>				
Number of female capture	0	3	0	69
Frequency of female capture	0%	100%	0%	100%
Number of room	20	20	20	20
<b>Density/House/Night</b>	<b>0.00</b>	<b>0.15</b>	<b>0.00</b>	<b>3.45</b>

**TABLE A-2. DENSITY OF ANOPHELINE (AN. GAMBIAE S.L.), AND CULICINE (CULEX AND AEDES) AND PARITY RATE OF AN. GAMBIAE S.L. CAPTURED BY PSC, BAROUELI, SEGOU, AND BLA**

District	Baroueli IRS site 1		Segou Non-IRS site 2		Bla Non-IRS site 3	
Spray status	Spray		Non-spray		Non-spray	
Mosquito	Anopheline	Culicine	Anopheline	Culicine	Anopheline	Culicine
<b>Baseline: Before IRS (July)</b>						
Number of female capture	47	35	26	16	35	92
Frequency of female capture	57%	43%	62%	38%	28%	72%
Number of room	20	20	20	20	20	20
<b>Density/House/Night</b>	<b>2.35</b>	<b>1.75</b>	<b>1.30</b>	<b>0.80</b>	<b>1.75</b>	<b>4.60</b>
<b>One month after IRS (August)</b>						
Number of female capture	15	4	36	34	240	579
Frequency of female capture	79%	21%	51%	49%	29%	71%
Number of room	20	20	20	20	20	20
<b>Density/House/Night</b>	<b>0.75</b>	<b>0.20</b>	<b>1.80</b>	<b>1.70</b>	<b>12.00</b>	<b>28.95</b>
<b>Two months after IRS (September)</b>						
Number of female capture	48	0	234	20	703	331
Frequency of female capture	100%	0%	92%	8%	68%	32%
Number of room	20	20	20	20	20	20
<b>Density/House/Night</b>	<b>2.40</b>	<b>0.00</b>	<b>11.70</b>	<b>1.00</b>	<b>35.15</b>	<b>16.55</b>
<b>Three months after IRS (October)</b>						
Number of female capture	19	4	73	12	235	65
Frequency of female capture	83%	17%	86%	14%	78%	22%
Number of room	20	20	20	20	20	20
<b>Density/House/Night</b>	<b>0.95</b>	<b>0.20</b>	<b>3.65</b>	<b>0.60</b>	<b>11.75</b>	<b>3.25</b>
<b>Four months after IRS (November)</b>						
Number of female capture	7	9	9	22	55	28
Frequency of female capture	44%	56%	29%	71%	66%	34%

Number of room	20	20	20	20	20	20
<b>Density/House/Night</b>	<b>0.35</b>	<b>0.45</b>	<b>0.45</b>	<b>1.10</b>	<b>2.75</b>	<b>1.40</b>
<b>Five months after IRS (December)</b>						
Number of female capture	4	1	2	43	4	10
Frequency of female capture	80%	20%	4%	96%	29%	71%
Number of room	20	20	20	20	20	20
<b>Density/House/Night</b>	<b>0.20</b>	<b>0.05</b>	<b>0.10</b>	<b>2.15</b>	<b>0.20</b>	<b>0.50</b>

**TABLE A-3. RAINFALL IN KOULIKORO AND BAROUELI, APRIL–DECEMBER 2015**

<b>Month</b>	<b>IRS site 1</b>	<b>IRS site 2</b>
April	10.2	0
May	9.5	11
June	140.3	32
July	265.6	157
August	208.4	201
September	170.6	192
October	40.5	20
November	16	0
December	0	0
Total	861.1	613
Mean rainfall	95.7	68.1

# ANNEX B: VECTOR SEASONALITY

**TABLE B-1. AN. GAMBIAE S.L. SEASONALITY (HLC), KOULIKORO AND KATI, JULY-DECEMBER**

	Koulikoro		Kati	
	IRS site 1 Indoor	IRS site 1 Outdoor	Control site 1 Indoor	Control site 1 Outdoor
June	14.50	15.50	9.50	13.50
August	27.25	14.00	66.50	58.75
September	67.50	76.75	56.25	53.25
October	50.75	52.75	100.75	96.25
November	4.00	4.50	2.75	6.50
December	0.25	0	0.25	1

**TABLE B-2. AN. GAMBIAE S.L. SEASONALITY (HLC), BAROUELI, SEGOU, AND BLA, JULY-DECEMBER**

Biting	Baroueli		Segou		Bla	
	IRS site 2 Indoor	IRS site 2 Outdoor	Control site 2 Indoor	Control site 2 Outdoor	Control site 3 Indoor	Control site 3 Outdoor
June	0.50	0.25	0.25	0.25	0.00	0.00
August	0.00	0.00	1.75	0.25	2.50	3.00
September	2.00	1.50	7.00	4.50	16.00	25.75
October	1.25	0.25	0.50	0.25	1.25	1.50
November	0.25	0	0.5	0	0	1
December	0	0	0	0	0.5	0

# ANNEX C: SUSCEPTIBILITY TEST RESULTS

**TABLE C-1. SUSCEPTIBILITY TEST RESULTS AFTER 2015 SPRAYING**

District	Site	Insecticide Tested	No of Mosquitoes Exposed	Not Dead	% Test Mortality	95% Confidence Interval
Koulikoro	Koulikoro	Permethrin	100	14	14	7.87 : 22.37
		Deltamethrin	100	49	49	38.86 : 59.19
Baroueli	Baroueli	Permethrin	103	22	21	13.89 : 30.53
		Deltamethrin	104	69	66	56.41 : 75.31
Bla	Bla	Permethrin	101	84	83	74.42 : 89.87
		Deltamethrin	101	65	64	54.20 : 73.63
Koulikoro	Koulikoro	DDT	104	38	37	27.31 : 46.55
Baroueli	Baroueli	DDT	101	15	15	8.55 : 23.31
Bla	Bla	DDT	101	41	41	30.93 : 50.82
Koulikoro	Koulikoro	Bendiocarb	100	100	100	96.37 : 100.00
Baroueli	Baroueli	Bendiocarb	100	99	99	94.55 : 99.97
Bla	Bla	Bendiocarb	104	104	100	96.51 : 100.00
Koulikoro	Koulikoro	Pirimiphos-methyl	100	100	100	96.37 : 100.00
Baroueli	Baroueli	Pirimiphos-methyl	101	101	100	96.41 : 100.00
Bla	Bla	Pirimiphos-methyl	101	101	100	96.41 : 100.00

# ANNEX D. CONE TEST RESULTS

**TABLE D-1. TYPE OF SURFACES TESTED WITH WHO CONE TEST, KOULIKORO AND BAROUELI, JULY-DECEMBER**

		# Mud	# Mud/Kaolin	# Cement
T0	IRS Site 1	1	8	1
	IRS Site 2	1	9	0
T1	IRS Site 1	1	3	1
	IRS Site 2	1	9	0
T2	IRS Site 1	1	7	1
	IRS Site 2	1	6	0
T3	IRS Site 1	1	6	1
	IRS Site 2	1	2	0
T4	IRS Site 1	0	5	1
	IRS Site 2	0	2	0
T5	IRS Site 1	1	6	1
	IRS Site 2	1	7	0

**TABLE D-2. WHO CONE TEST RESULTS, AN. GAMBIAE KISUMU STRAIN MORTALITY AFTER 30 MINUTES EXPOSURE TO PIRIMIPHOS-METHYL, KOULIKORO AND BAROUELI, JULY-DECEMBER**

		T0 (Jul)	T1 (Aug)	T2 (Sept)	T3 (Oct)	T4 (Nov)	T5 (Dec)
<b>Mud</b>	<b># Tested</b>	201	62	120	165		202
	<b># Dead</b>	201	59	120	146		198
	<b>% Mortality</b>	<b>100%</b>	<b>95%</b>	<b>100%</b>	<b>88%</b>		<b>98%</b>
<b>Mud+kaolin</b>	<b># Tested</b>	378	337	237	136	126	299
	<b># Dead</b>	374	332	202	130	79	231
	<b>% Mortality</b>	<b>99%</b>	<b>99%</b>	<b>85%</b>	<b>96%</b>	<b>62.70%</b>	<b>77%</b>
<b>Cement</b>	<b># Tested</b>	32	32	30	31	32	34
	<b># Dead</b>	32	27	30	31	17	8
	<b>% Mortality</b>	<b>100%</b>	<b>84%</b>	<b>100%</b>	<b>100%</b>	<b>53.13%</b>	<b>24%</b>

# ANNEX E. VECTOR INFECTION RATE

**TABLE E-1. AN. GAMBIAE S.L. PLASMODIUM. FALCIPARUM (SPOROZOITE) INFECTION RATES, ALL SITES, JULY–SEPTEMBER 2014**

HLC	July			August			September		
	District	# tested	# SPZ (+)	SPZ rate	# tested	# SPZ (+)	SPZ rate	# tested	# SPZ (+)
IRS site 1	19	1	0.052	27	0	0	29	2	0.07

# tested: Number tested; # SPZ (+): Number of positive sporozoite; SPZ rate: sporozoite rate; Jul: Pre IRS, Aug: one month Post IRS; Sept: two month Post IRS.

**TABLE E-2. AN. GAMBIAE S.L. PLASMODIUM. FALCIPARUM (SPOROZOITE) INFECTION RATES, ALL SITES, OCTOBER–NOVEMBER 2014**

HLC	October			November		
	District	# tested	# SPZ (+)	SPZ rate	# tested	# SPZ (+)
IRS site 1	12	1	0.08	30	0	0

# tested: Number tested; # SPZ (+): Number of positive sporozoite; SPZ rate: sporozoite rate; Oct: three month Post IRS, Nov: four month Post IR.

**TABLE E-3. EIR, MONTHLY COLLECTIONS, BLA, 2014**

Month	Total <i>An. gambiae</i> s.l. collected	biting rate	SPZ rate	nightly EIR	monthly EIR
July	20	2.50	0.05	0.12	3.60
Aug	21	2.62	0	0	0
Sep	29	3.62	0.07	0.25	7.50
Oct	12	1.50	0.08	0.12	
Nov	5	0.62	0	0	0

**TABLE E-4. AN. GAMBIAE S.L. PLASMODIUM. FALCIPARUM (SPOROZOITE) INFECTION RATES, ALL SITES, HLC, CDC LIGHT TRAPS AND PSC, JUNE–SEPTEMBER 2015**

HLC	June			August			September		
	District	# tested	# SPZ (+)	SPZ rate	# tested	# SPZ (+)	SPZ rate	# tested	# SPZ (+)
IRS site 1	196	10	0.05	156	4	0.03	162	11	0.07
IRS site 2	46	1	0.02	10	0	0.00	21	1	0.05
Control site 1	192	6	0.03	244	15	0.06	184	24	0.13
Control site 2	27	1	0.04	10	0	0.00	53	2	0.04
control site 3	38	1	0.03	80	5	0.06	80	5	0.06

# tested: Number tested; # SPZ (+): Number of positive sporozoite; SPZ rate: sporozoite rate. Jun: Pre IRS, Aug: one month Post IRS; Sept: two month Post IRS, Oct: three month Post IRS, Nov: four month Post IRS and Dec: five month Post IRS.

**TABLE E-5. AN. GAMBIAE S.L. PLASMODIUM. FALCIPARUM (SPOROZOITE) INFECTION RATES, ALL SITES, HLC, CDC LIGHT TRAPS AND PSC, OCTOBER–DECEMBER 2015**

District	October			November			December		
	# tested	# SPZ (+)	SPZ rate	# tested	# SPZ (+)	SPZ rate	# tested	# SPZ (+)	SPZ rate
IRS site 1	468	23	<b>0.05</b>	52	3	<b>0.06</b>	3	0	<b>0.00</b>
IRS site 2	23	2	<b>0.09</b>	0	0	<b>0.00</b>	4	0	<b>0.00</b>
Control site 1	508	22	<b>0.04</b>	65	6	<b>0.09</b>	7	1	<b>0.14</b>
Control site 2	76	0	<b>0.00</b>	15	0	<b>0.00</b>	2	0	<b>0.00</b>
control site 3	262	15	<b>0.06</b>	55	3	<b>0.05</b>	6	1	<b>0.17</b>

# tested: Number tested; # SPZ (+): Number of positive sporozoite; SPZ rate: sporozoite rate

Jun: Pre IRS, Aug: one month Post IRS; Sept: two month Post IRS, Oct: three month Post IRS, Nov: four month Post IRS and Dec: five month Post IRS.

**TABLE E-6. AN. GAMBIAE S.L. PLASMODIUM. FALCIPARUM (SPOROZOITE) INFECTION RATES, ALL SITES, HLC, SEPTEMBER–DECEMBER 2015**

HLC	September-December		
District	# tested	# SPZ (+)	SPZ rate
IRS site 1 and 2	317	10	<b>0.03</b>
Control site 1 and 2	359	25	<b>0.07</b>

IRS sites vs Control sites: p=0.02

# tested: Number tested; # SPZ (+): Number of positive sporozoite; SPZ rate: sporozoite rate

# ANNEX F. VECTOR PARITY RATE

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**TABLE F-1. AN. GAMBIAE S.L. PARITY RATE (HLC) PRE- AND POST-IRS, BLA JULY-DECEMBER, 2014**

	<b># Dissected</b>	<b># Parous</b>	<b>% Parous</b>	<b>Confidence Interval 95%</b>
Pre IRS				
Bla	13	11	<b>84.62%</b>	65.0: 104.2
Post IRS				
Bla	33	26	<b>78.79%</b>	64.8 : 92.7

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