U.S. PRESIDENT'S MALARIA INITIATIVE

PMI | Africa IRS (AIRS) Project

Indoor Residual Spraying (IRS 2) Task Order Six

AIRS ETHIOPIA ENTOMOLOGICAL MONITORING FINAL REPORT

JUNE 2016 –DEC 2016

RE-SUBMITTED & APPROVED: FEBRUARY 16, 2017

CONTENT

LIST OF FIGURES

LIST OF TABLES

ACRONYMS

AIRS	Africa Indoor Residual Spraying
CB	Community-based
CDC	Centers for Disease Control and Prevention
DB	District-based
DDT	Dichlorodiphenyltrichloroethane
FMOH	Federal Ministry of Health
HLC	Human Landing Catch
Kdr	Knockdown Resistance
IR	Insecticide Resistance
IRS	Indoor Residual Spray
PSC	Pyrethrum Spray Catch
PMI	President Malaria Initiative
SNNPR	Southern Nations Nationalities and People Region
SOP	Spray Operator
WHO	World Health Organization

1. EXECUTIVE SUMMARY

1.1 BACKGROUND

Entomological monitoring was conducted from June – December 2016 and included monthly collection of data on vector density and species composition to help understand the abundance, seasonal patterns, biting behavior, and parity of Anopheles mosquitoes, and to assess the impact of indoor residual spraying (IRS) on entomological indicators. Pyrethrum spray catches (PSC), human landing catches (HLC) and CDC light traps were carried out in two intervention (sprayed) sites and one control (not sprayed) site. The intervention sites were in Gobu Sayo and Seka Chekorsa districts. One site from Ilugelan District, Ejaji, served as an unsprayed control site. HLC was used in two households in each sentinel site for two nights per month. PSC was used to sample indoor resting mosquitoes in 20 houses in each of the study sites every month. CDC light traps were installed in two houses adjacent to houses selected for HLC in each of the three sentinel sites. In 2016, insecticide susceptibility using WHO tube tests and CDC bottle bioassays were conducted in 11 sentinel sites and three malarious districts, respectively, to determine the response of the main malaria vector to different insecticides used for IRS. Furthermore, wall bioassays were conducted to monitor the decay rate of pirimiphos-methyl (Actellic 300 CS) in four selected districts. The wall bioassay tests were conducted in 12 houses per site with a total of 48 houses sampled.

1.2 RESULTS

Vector density: 8,501 female Anopheles mosquitoes comprising six species were collected. The most abundant species were *An. gambiae* s.l. (26.8%), *An. coustani* (51.7%) and *An. pharoensis* (21.5%). Overall, the main vector of malaria in this study, *An. gambiae* s.l., reached its peak at variable times between June and September, with densities dropping from October onwards. In the control site, peak density was achieved in August. *An. gambiae* s.l. was most abundant during the peak rainy period (June – August) in all sites. *An. coustani* was the dominant species collected from August onwards. Indoor resting density and human biting rates as measured by PSCs and HLCs, respectively, dropped after IRS in both intervention sites but increased and peaked in August in the control site.

Resting habit: The resting habits of *An. gambiae* s.l. were variable by site. *An. gambiae* s.l. tended to exhibit endophilic tendencies in Seka Chekorsa intervention site while it was more exophilic in Gobu Seyo and Ejaji sites when we compared fed versus half gravid and gravid mosquitoes in PSC collections before spraying. The density of *An. gambiae* s.l. resting indoors reduced from 5.95 pre-spray to 0.64 mosquitos per house per day after IRS in the intervention sites. There was a slight increase in *An. gambiae* s.l. density in the control site (3.1 pre-spray vs. 4.11 post spray).

Feeding time and location: *An. gambiae* s.l. tended to feed more outdoors than indoors showing exophagic tendency in the Seka Chekorsa intervention site (75.5%) but less so in Gobu Sayo (51.0%). In the control site in Ejaji the vector tended to show slight endophagic tendencies (52.6%). *An. gambiae* s.l. engaged in biting throughout the night, but peak biting was variable between sites, with Gobu Sayo and Seka Chekorsa recording pre-midnight biting activity (19.00 – 23.00 hours). In Ejaji control site a higher proportion of host-seeking *An. gambiae* s.l. was collected from 23.00 – 4.00 hours.

Parity rate: Monthly parous rates for *An. gambiae* s.l. were variable between sites throughout the period of study with generally high rates recorded before IRS in Gobu Sayo intervention sites (80.4%). Parous rates were reduced for the first two months after IRS in the intervention sites (56.4 and 64.5%) but increased to 78.7% and 81.8% in September and November, respectively. In the Ejaji control site

parous rates remained high during the whole period of study (90.4 – 96.6%). Data from Seka Chekorsa intervention site did not show a clear pattern likely due to low numbers of Anopheles dissected.

Susceptibility test: The susceptibility of *An. gambiae* s.l. to seven insecticides recommended for malaria vector control was tested using the WHO tube test in eleven sites. The results showed that the vector was fully susceptible to propoxur and pirimiphos-methyl in eight and nine sites, respectively. Resistance and possible resistance to pirimiphos-methyl were recorded in one site each. Possible resistance to propoxur was reported from three sites. Suspected resistance and resistance to bendiocarb were observed in one site each. An. gambiae s.l. was resistant to dichlorodiphenyltrichloroethane (DDT) and permethrin in all sites tested. Resistance to deltamethrin was reported in all sites except one site where the vector was susceptible. The vector was shown to be fully susceptible to malathion in three sites; possibly resistant in four sites and resistant in another four sites. Susceptibility tests using CDC bottle bioassays were conducted in three sites with two different insecticides; namely, deltamethrin, and permethrin. In the three sites a synergist (PBO) was used. An. gambiae s.l. was resistant to permethrin and deltamethrin in the three sites. However, pre-treatment with PBO fully restored susceptibility at the diagnostic dosage to deltamethrin in all three sites indicating that an oxidase mechanism of resistance is probably involved. In Amibara site, pre-test PBO exposure restored susceptibility to deltamethrin with 100% knock down but not for permethrin. It is highly likely that permethrin resistance may be mediated by other mechanisms in addition to oxidases.

Wall bioassay test: The overall mean mortality of *An. arabiensis* for time zero was 99.7% one to two days after spraying with pirimiphos-methyl. There was no significant difference in mortality of mosquitoes between community-based (CB) IRS and district-based (DB) IRS model sites on all wall surfaces sprayed. The results over the five months were not consistent. In Tiro Afeta, pirimiphos-methyl performed generally well on all wall surfaces with an average mortality rate of 84.3% after five months. However, in Chewaka, mortality of susceptible mosquitoes was 77.4% and 78.3% on mud and dung wall surfaces, respectively, after five months. A mean mortality of 73.3% was recorded five months after IRS for the studied areas. Overall, pirimiphos-methyl tended to perform better on painted wall surfaces and less so on mud and dung.

Sporozoite Elisa: Sporozoite rates of 0.3% and 1.2%, respectively, were recorded for *P. vivax* and *P. falciparum* circumsporozoite protein (CSP) using the ELISA test for 334 *An. gambiae* s.l. samples tested from the three sites during the pre-spray period. The sporozoite rates during the post-spray period were as follows: 0. 11% and 0.64% for *P. vivax* and *P. falciparum* from 926 tested *An. gambiae* s.l.

Molecular identification of *An. gambiae* s.l. and determination of kdr allelic frequency: A total of 662 An. gambiae s.l. selected randomly from specimens collected from 10 study sites (from six regions) were identified to species using species-specific PCR. The results of the molecular analysis showed that An. arabiensis was the only sibling species of the gambiae complex in all the study sites. The result of the analysis showed that only the West African kdr allele (L1014F) was represented in populations of An. gambiae s.l. tested from the 10 study sites in six regions of the country. The kdr east allele L1014S was not present in the samples tested.

Conclusions: The vector monitoring studies conducted indicated that the main malaria vector An. gambiae s.l. reached peak density in August in the control site. Based on these results, conducting IRS in early June with long-lasting insecticides would most probably provide sufficient protection. Indoor resting densities as well as human biting rates declined after IRS in both intervention sites from July onwards compared to the control site, most likely due to the effect of insecticide sprayed. Though the main vector was found to be fully susceptible to pirimiphos-methyl and propoxur in most of the test sites, the emergence of resistance in a few sites requires further confirmation and close monitoring so appropriate measures are taken to limit its spread.

2. INTRODUCTION

Entomological activities are essential for proper targeting and planning of IRS. They often include monitoring of IRS impact on vector density, behavior, and composition; evaluating the susceptibility level of the local vectors to different insecticides; and understanding the potential mechanisms of resistance. Entomological activities also are vital in determining the residual life of different insecticides on different types of wall surfaces under various environmental conditions. Entomological study results from susceptibility tests provide empirical evidence that inform selection of insecticides for IRS in addition to other operational criteria.

In 2016, AIRS Ethiopia continued routine entomological monitoring studies and insecticide resistance (IR) testing in order to monitor the efficacy of IRS on malaria transmission in the project areas. Specific objectives of the 2016 entomological work were to:

- Determine Anopheles species composition;
- Monitor vector density and behavior before and after spray operations;
- Assess susceptibility of the main malaria vector to different insecticides;
- Assess quality of spray operations and decay rate of insecticides;
- Train university staff on basic malaria entomology as a capacity building effort.

In addition to vector density and behavior studies in three sentinel sites, this report incorporates other entomological monitoring activities performed by the PMI AIRS Project in 2016 including:

- Vector density and behavior studies by collaborating universities (Annex B);
- Insecticide susceptibility tests using the WHO tube test & CDC bottle bioassay (Annex C);
- Wall bioassays for IRS quality check (Annex D);
- Training of staff from collaborating universities on basic malaria entomology (Annex E);
- Determination of insecticide decay rate in selected sites in Ethiopia (Annex F).

3. Monitoring Vector Behavior and Density

3.1 Introduction

In 2016, AIRS Ethiopia selected three sentinel sites to undertake a number of entomological studies, including vector population dynamics and behavior. The project selected two intervention (sprayed) sites and one control (not sprayed) site to collect data on comprehensive entomological indicators that included vector behavior and density. The intervention sites were in Gobu Sayo District and Seka Chekorsa District. One site from Ilugelan District, Ejaji, was selected as an unsprayed control site. Gobu Sayo and Ejaji are located in Western Oromia 50 kilometers (km) from each other. Seka Chekorsa is in Southwest Oromia about 300 km from the two sites. The intervention sites were sprayed with Actellic 300 CS in June 2016.

The AIRS entomology team conducted data collection in Gobu Sayo and Ejaji, and the project contracted Jimma University to work in Seka Chekorsa sentinel site. Data collection started in June 2016 and continued until December 2016. IRS was conducted in Gobu Sayo and Seka Chekorsa sites from June 28-30, 2016. This report covers the work that was performed from June 2016 to Dec 2016.

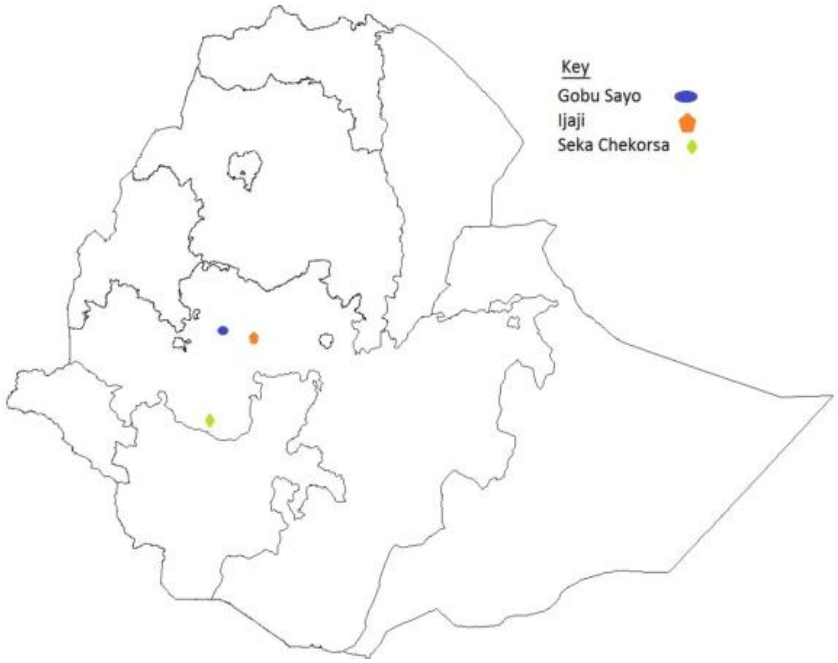
3.2 Objectives of AIRS Ethiopia Entomological Monitoring

- Identify the *Anopheles* mosquitoes present in the two intervention areas and one control area, indoor resting density, man biting rate(s), and biting cycles;
- Determine vector density, distribution, and seasonality in the intervention and control areas;
- Provide quality assurance of the IRS program through the World Health Organization (WHO) cone/wall bioassay;
- Determine the extent of indoor feeding (endophagy) and indoor resting (endophily) tendencies;
- Determine parity as an entomological indicator to ascertain if the age composition of the mosquito population was affected as an indication whether or not the IRS intervention affects vector dynamics and their ability to transmit malaria in the intervention areas.

4. METHODS

Six rounds of entomological data collection were conducted in the three sentinel sites shown in Figure 1. The first collection was performed in June 2016 and continued at one-month intervals up to December 2016. However, in Oct 2016 due to security concerns entomological activities were not conducted. Excel software was used to produce summary tables and graphics. The methods and procedures used to collect the entomological data from the three sentinel sites are described below.

FIGURE 1: 2016 SENTINEL SITES FOR MONITORING MOSQUITO DENSITY AND BEHAVIOR



Figure

4.1 HUMAN LANDING CATCHES

Human landing catches (HLC) were conducted in two houses in each sentinel site for two nights per month; thus data being collected for four trap nights per site per month. One mosquito collector was seated indoors and another seated outdoors from 6 p.m. to 6 a.m. to collect blood-seeking mosquitoes. Outdoor mosquito collection was carried out about eight meters from each of the two sampled houses. A team of two collectors was assigned a six-hour shift. A total of four collectors per house per night covered 12 hours of collections from 6 p.m. to 6 a.m. (6 p.m. - midnight and midnight - 6 a.m). Outdoor and indoor collectors switched sites every hour. Collectors adjusted their clothing so that the legs were exposed up to the knees. When a mosquito was felt, collectors quickly turned on the torch, collected the mosquito with the sucking tube and transferred it to a paper cup. One cup was used for each hour of collection. Hourly temperature and humidity were recorded. At the end of the collection, mosquitoes were transported to the field lab and identified using taxonomic keys (Gilles and Coetzee, 1987).

4.2 Pyrethrum Spray Catch

Pyrethrum Spray Catch (PSC) was used to sample indoor resting mosquitoes in 20 houses in each of the study sites every month. Collections were carried out in the morning between 6:00 a.m. and 7:30 a.m. Before the PSC was performed, all occupants were cordially asked to move out of the house. The team recorded information from the head of household or an adult member about the number of people who slept in the house the previous night and the number of treated nets used. The floor was then covered with white sheets and the eaves, windows, and other mosquito escape routes around the house were sprayed as were the walls and roof space inside the house with Baygon (knockdown spray). Ten minutes after spraying, collectors gathered all the mosquitoes that were knocked down from the sheets and sorted them by species. The abdominal status of all female *Anopheles* mosquitoes was determined, and individual specimens were recorded as unfed, blood-fed, half-gravid, and gravid females.

4.3 CDC Light Traps

Centers for Disease Control and Prevention (CDC) light traps were installed in two houses adjacent to the houses selected for HLC in each of the three sentinel sites, and collection was done for two nights every month. The CDC light-traps were suspended in a bedroom 1.5 meters high from the floor and about 50 centimeters from a human sleeping under a bed net. The light traps were fitted with an incandescent bulb. The traps were set from 6 p.m. to 6 a.m. Mosquitoes were collected from the traps the next morning and sorted at the field lab.

4.4 Identification of Malaria Vectors

Anopheles mosquitoes collected through HLC, PSC, and CDC light traps were preliminarily identified to the species level morphologically. All *Anopheles* specimens that were not dissected were labeled and stored individually in Eppendorf tubes on silica gel for further processing by Jimma University including, sporozoite ELISA and molecular identification of the *An. gambiae* complex and kdr allele assays.

4.4.1 Sporozoite ELISA

Dried head and thorax of the preserved Anopheles were carefully separated from the abdomen and tested for *P. falciparum* and *P.vivax* circumsporozoite proteins (CSPs) simultaneously following (Wirtz et al 1992). Each 96-well microtiter plates were coated separately with 50 µl of *P. falciparum, P. vivax*-210 and *P. vivax*-247 monoclonal antibodies (MAB), respectively. It was incubated at room temperature for at least 30 minute. Contents of plates were drained, washed three times with PBS-Tween 20, filled with 200 µl blocking buffer (BB) and incubated for 1hr at room temperature. During the incubation period, mosquitoes were ground individually in 50 µl boiled casein containing Igepal CA-630 and the final volume brought to 250 µl with BB. BB was removed from plates and 50 µl of each mosquito triturate was added to each of the three test wells. CSP positive samples and laboratory reared *An. arabiensis* were used as positive and negative controls, respectively. Plates were incubated for two hours and washed with PBS-Tween 20 twice. 50 µl aliquots of homologous peroxidase-conjugated MAB (0.05 µg/50 µl BB) were added to each triplicate well in the plates and incubated for one hour. Plates were washed three times with PBS-Tween 20, 100 µl ABTS peroxidase substrate added per well and incubated for 30 and or 60 min. Plates were read both visually and using an absorbance reader.

4.4.2 Molecular Identification of Anopheles gambiae Complex and *kdr* Allele Detection Using Polymerase Chain Reaction

Sub-samples of both survived and dead mosquitoes following bioassays were randomly selected by region, site and insecticide tested for molecular identification of the gambiae complex and kdr allele detection. Genomic DNA from the sub-samples of both survived and dead mosquitoes following WHO bioassay tests conducted in eight sites was extracted following the procedure described by Collins et al., (1987). DNA was re-suspended in 25 ml sterile TE-buffer (10 mM Tris–HCl pH 8, 1 mM EDTA). Molecular identification of *An. gambiae* s.l was carried out using species-specific polymerase chain reaction (PCR) techniques including the primers for *An. gambiae* s.s., *An. arabiensis* and *An. quadriannulatus* by adapting the method used by Scott et al., (1993). Genomic DNA was mixed with the primers AR (specific for *An. arabiensis*), AG (specific for *An. gambiae* s.s.), QD (specific for *An. quadriannulatus*) and UN (common for both species) in a 25 µl reaction. Amplification reactions contained 1 µl of DNA, 1.5 mM $MgCl_2$, 10 mM Tris-HCl (pH 8.4), 50 mM KCl, 0.1% Triton X-100, 200 µM of dNTP's (Amersham, Buckinghamshire, United Kingdom), 80 nM of primers UN and AR, 40 nM of primer GA and 0.25 U of Silverstar DNA polymerase (Eurogentec, Seraing, Belgium). The PCR was carried out as described in Scott et al., (1993). The amplified products were checked on a 2% agarose gel, stained with ethidium bromide, and visualized on the Image Master VDS (Amersham Pharmacia, Uppsala, Sweden. Sekoru colony of *Anopheles arabiensis* strain from the Vector Biology and Control Research Unit, Tropical and Infectious Diseases Research Center, Jimma University was used as a control.

The detection of L1014S or L1014F kdr alleles was based on protocols developed by Martinez-Torres et al., (1998) and Ranson et al., (2000). The following primers were used to detect the L1014F allele (AS-PCR Agd3) - Agd1 (5'-atagattccccgaccatg-3'), Agd2 (5'-agacaaggatgatgaacc- 3'), Agd3 (5'-aatttgcattacttacgaca-3') and Agd4 (5'-ctgtagtgataggaaattta-3'), whereas primers Agd1, Agd2, Agd4 and Agd5 (5'-tttgcattacttacgactg-3') were used to detect the presence of L1014S allele (AS-PCR Agd5). Amplification was performed in a 50 µl reaction containing 2 µl of template DNA, 1 × Qiagen PCR buffer, 0.5 mM MgCl2, 100 nM of each primer, 200 µM of dNTP's, and 1U of Taq DNA polymerase (Taq PCR core kit, Qiagen, Hilden, Germany). The thermo-cycling conditions were: initial 94°C denaturation for five minutes, 10 cycles of one minute denaturation at 94°C, 30 seconds annealing at 54°C and 30 seconds extension at 72°C, followed by 30 cycles of one minute denaturation at 94°C, 30 seconds annealing at 47°C and 30 seconds extension at 72°C, and a final extension at 72°C for 10 minutes. Amplification products were checked on a 2% agarose gel and visualized after ethidium bromide staining.

4.5 Determination of Parity

Unfed females belonging to *An. gambaie* s.l., presumably *An. arabiensis,* from HLC were dissected for ovary parity under a dissecting microscope to determine parity rate based on coiling of ovarian tracheoles (Detinova 1962). Mosquitoes were kept in wet petri dishes and dissected within 12 hours after capture.

5. RESULTS

5.1 ANOPHELES SPECIES DIVERSITY AND ABUNDANCE

During the six months of the study, a total of 8,501 adult female *Anopheles* mosquitoes were collected using PSC, HLC and CDC light traps. A summary of the species collected is shown in Table1. Detailed data are included in Annex A.

An. gambiae s.l., *An. coustani* and *An. pharoensis* were common in all three sites. *An. squamosus* was collected from Gobu Seyo and Seka Chokorsa sites. *An. demelloni* and *An. natalensis* were collected from Gobu Sayo site only. In addition to the *Anopheles*, 62,460 *Culex* mosquitoes were collected through the different collection techniques.

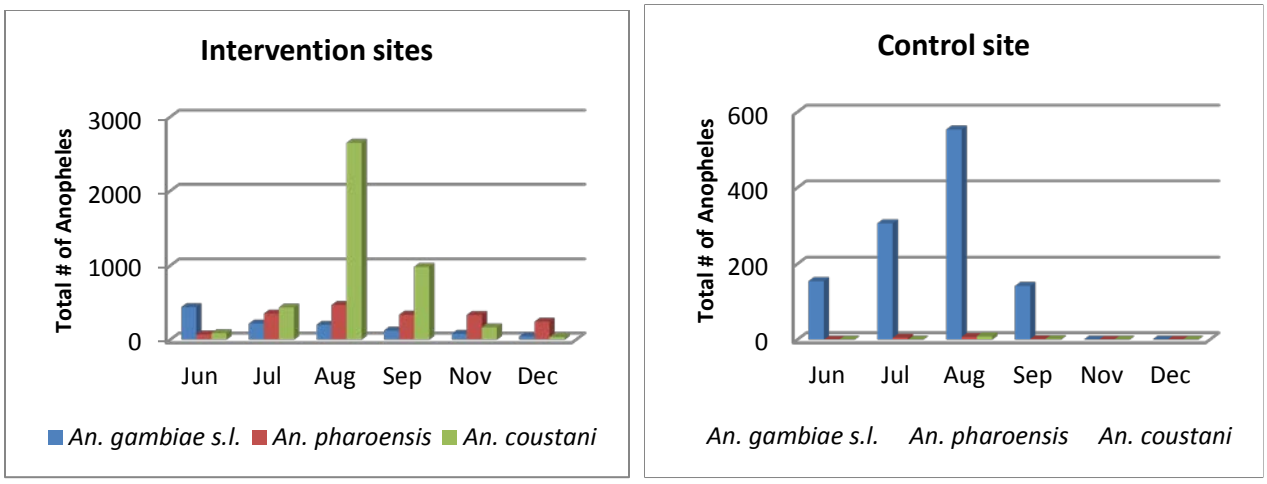
TABLE 1: ANOPHELES SPECIES COLLECTED IN THREE SENTINEL SITES, JUNE-DEC 2016

Site	*An. gambiae* s.l.	*An. pharoensis*	*An. coustani*	*An. demeilloni*	*An. squamosus*	*An. natalensis*
Gobu Seyo	857	1622	4027	13	9	4
Seka Chekorsa	254	185	332	0	13	0
Ejaji	1158	14	13	0	0	0
Total	2269	1821	4372	13	22	4

5.2 COMPARISON OF *ANOPHELES* DENSITY BETWEEN INTERVENTION AND CONTROL SITES

As indicated in Figure 2 the results of the six-month entomological study of *An. gambiae* s.l. showed that the density decreased after spraying in the intervention sites but increased in the control site. However, other *Anopheles* species (*An. pharoensis* & *An. coustani*) showed marked increase in density after spraying from July - September.

FIGURE 2: *ANOPHELES* COLLECTIONS IN INTERVENTION AND CONTROL SITES



NB: June: Pre-spray; July to Dec: Post IRS

5.3 PYRETHRUM SPRAY CATCH

Tables 2, 3 and 4 show the PSC results, which indicate that vector density was high pre-spray (June) compared to subsequent months after spraying (July – December) in the intervention sites. In Gobu Sayo intervention site, mean indoor resting density of female *An. gambiae* s.l. pre-spray was 5.95 mosquitoes per house per day. To assess the impact IRS had on vector density, mean *An. gambiae* s.l. before IRS was compared with six months data post IRS, assuming that IRS would be effective for at least five months based on residual life data in Ethiopia. Vector density declined from 5.95 mosquitos per house per day during pre-spray to 0.64 mosquitos per house per day post spray. The mean density dropped in July, one month after spraying, and remained low throughout the subsequent five months. In the other intervention site, Seka Chekorsa, the mean indoor resting density of female *An. gambiae* s.l. per house per day was 0.85 during the pre-spray period. Following spraying, a drop in the mean vector indoor resting density was observed with 0.2 female *An. gambiae* s.l. per house per day recorded after spray. In the control site of Ejaji, the mean indoor resting density per house per day increased during the same period from 3.1 pre-spray to 4.11 after spray.

In indoor resting collections using PSC, the proportion of half-gravid and gravid mosquitoes is expected to be higher than fed mosquitoes if the vector's resting habit is endophilic. In Gobu Sayo, very few gravid mosquitoes were found two months after spray (Figure 3). In Seka Chekorsa, the proportion of gravid mosquitoes was high pre-spray and reduced to zero after spraying (Figure 4). In the control site, the proportion of gravid mosquitoes remained high throughout the study period (Figure 5).

TABLE 2: PSC COLLECTIONS, GOBU SAYO INTERVENTION SITE, JUNE-DEC 2016

Time	# of houses	# of Occupants	*# of LLINs	*An. gambiae* s.l. Collected	Abdominal/Blood Digestion stages				Total (HG+G)	Proportion of gravid (HG+G/ HG+G+F)	Female per house	# Fed per human host
		Human			UF^	F^	HG^	G^				
Jun	20	70	8	119	18	60	21	20	41	0.41	5.95	0.86
Jul	20	81	16	18	5	13	0	0	0	0	0.9	0.16
Aug	20	78	20	20	3	10	7	0	7	0.41	1	0.13
Sep	20	77	20	7	2	2	2	1	3	0.6	0.35	0.03
Nov	20	87	18	10	0	5	2	3	5	0.5	0.5	0.06
Dec	20	79	10	9	1	5	1	2	3	0.4	0.45	0.06

NB: ^ UF – un-fed, F-fed, HG-half-gravid, G – gravid

TABLE 3: PSC COLLECTIONS, SEKA CHEKORSA INTERVENTION SITE, JUNE-DEC 2016

Time	# of houses	# of Occupants	*# of LLINs	*An. gambiae* s.l. Collected	Abdominal/Blood Digestion stages				Total (HG+G)	Proportion of gravid (HG+G/ HG+G+F)	Female per house	# Fed per human host
		Human			UF^	F^	HG^	G^				
June	20	82	42	17	0	2	5	10	15	0.9	0.85	0.024
July	20	85	42	2	0	2	0	0	0	0	0.1	0.024
Aug	20	86	42	0	0	0	0	0	0	0	0	0
Sept	20	83	42	11	1	7	3	0	3	0.3	0.55	0.084
Nov	20	69	42	3	0	0	2	1	3	1	0.15	0
Dec	20	69	42	0	0	0	0	0	0	0	0	0

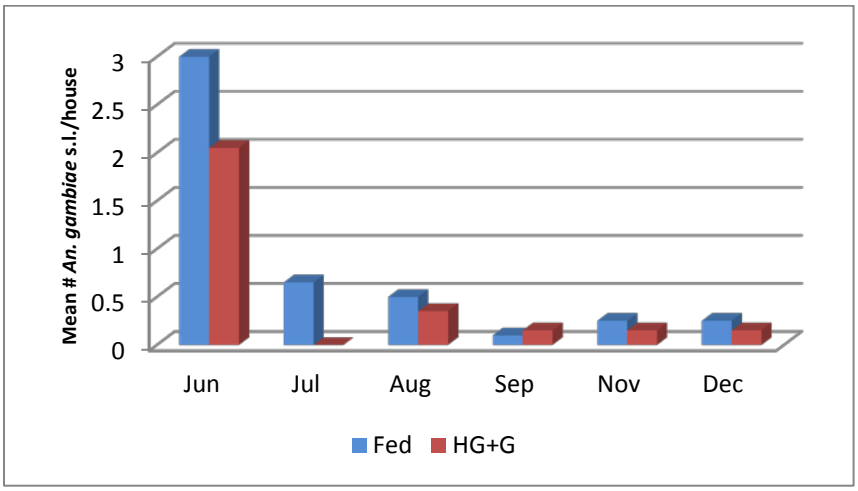
NB: ^ UF – un-fed, F-fed, HG-half-gravid, G - gravid

TABLE 4: PSC COLLECTIONS, EJAJI CONTROL SITE, JUNE-DEC 2016

Time	# of houses	# of Occupants	*# of LLINs	*An. gambiae* s.l. Collected	Abdominal/Blood Digestion stages				Total (HG+G)	Proportion of gravid (HG+G/ HG+G+F)	Female per house	# Fed per human host
		Human			UF^	F^	HG^	G^				
June	20	78	7	91	1	45	31	4	35	0.44	3.9	0.58
July	20	75	8	151	5	48	49	49	98	0.67	7.55	0.64
Aug	20	89	13	213	10	113	52	38	90	0.44	10.65	1.27
Sept	20	75	18	47	1	32	11	3	14	0.30	2.35	0.43
Nov	20	75	18	0	0	0	0	0	0	0	0	0
Dec	20	89	9	1	0	1	0	0	0	0	0	0

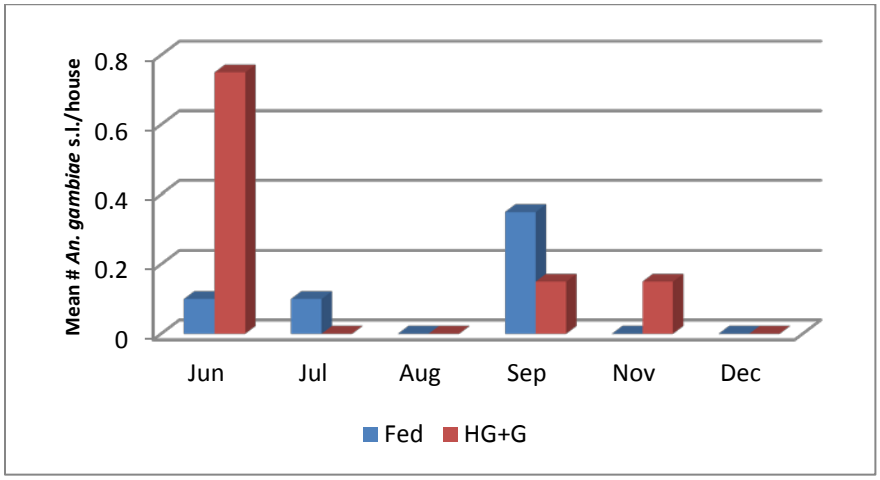
NB: ^ UF – unfed, F-fed, HG-half-gravid, G - gravid

FIGURE 3: PSC COLLECTIONS GOBU SAYO INTERVENTION SITE, JUNE-DEC 2016



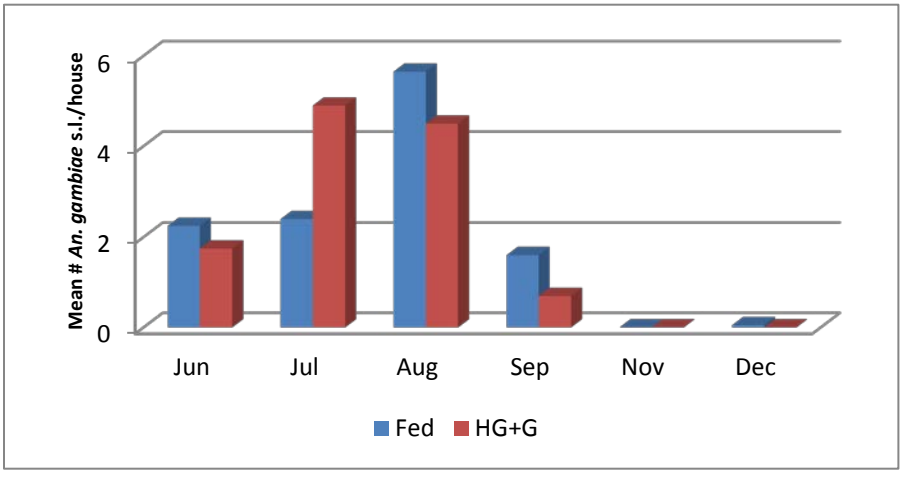
NB: June: Pre-spray; July to Dec: Post IRS

FIGURE 4: PSC COLLECTIONS SEKA CHEKORSA INTERVENTION SITE, JUNE-DEC 2016



NB: June: Pre-spray; July to Dec: Post IRS

FIGURE 5: PSC COLLECTIONS, EJAJI CONTROL SITE, JUNE-DEC 2016



5.4 HUMAN LANDING CATCH

HLC collection was done once a month in each site in two houses for two consecutive nights for a total of four nights each month. During the study period, a total of 4,605 *Anopheles* mosquitoes were collected while attempting to feed on human baits. Of these, 1,354 were *An. gambiae* s.l., 1,027 *An. pharoensis,* 2,203 *An. coustani,* 13 *An. squamosus,* six *An. demeilloni* and two *An. natalensis*. The proportion of indoor to outdoor collection for the main vector, *An. gambiae* s.l., in the intervention area was 347 (44.3%) vs. 437 (55.7%), respectively, indicating a slight tendency towards outdoor feeding (exophagy). The difference in biting tendencies was not statistically significant (p=0.53). In the control site, attempts to bite indoors was higher than outdoors for *An. gambiae* s.l. though it was not statistically significant (p=0.87). *An. pharoensis, An. coustani, An. squamosus*, *An. demeilloni* and *An. natalensis* also preferred to bite outdoors at higher rates than *An. gambiae* s.l. Details on HLC collections by sentinel site are provided in Tables 5, 6, and 7 and Figures 6, 7 and 8.

TABLE 5: HLC IN GOBU SAYO, INTERVENTION SITE, JUNE-DEC 2016

Time	*An. gambiae* s.l.					*An. pharoensis*			*An. coustani*			*An. demeilloni*			*An. squamosus*			*An. natalensis*			Total *Anopheles* collected		
	In	MBR Indoor	Out	MBR Outdoor	Tot.	In	Out	Tot.	In	Out	Tot.	In	Out	Tot.	In	Out	Tot.	In	Out	Tot.	In	Out	Grand total
June	122	30.5	128	32	250	9	20	29	1	42	43	0	2	2	0	0	0	0	0	0	132	192	324
July	64	16	69	17.25	133	40	147	187	20	226	246	0	0	0	0	1	1	0	0	0	124	443	567
Aug	61	15.25	33	8.25	94	53	139	192	79	1064	1143	0	3	3	0	0	0	0	2	2	193	1241	1434
Sept	28	7	35	8.75	63	40	122	162	33	383	416	0	0	0	0	0	0	0	0	0	101	540	641
Nov	10	2.5	34	8.5	44	102	187	289	9	82	91	0	1	1	0	0	0	0	0	0	121	304	425
Dec	22	5.5	15	3.75	37	74	147	221	2	19	21	0	0	0	0	0	0	0	0	0	98	181	279

TABLE 6: HLC IN SEKA CHEKORSA, INTERVENTION SITE, JUNE-DEC 2016

Time	*An. gambiae* s.l.					*An. pharoensis*			*An. coustani*			*An. demeilloni*			*An. squamosus*			Total *Anopheles* collected		
	In	MBR Indoor	Out	MBR Outdoor	Total	In	Out	Total	In	Out	Total	In	Out	Total	In	Out	Total	In	Out	Grand total
June	9	2.25	33	8.25	42	5	20	25	2	14	16	0	0	0	0	0	0	16	67	83
July	9	2.25	30	7.5	39	13	52	65	9	31	40	0	0	0	1	1	2	32	114	150
Aug	8	2	12	3	20	5	12	17	20	75	95	0	0	0	1	2	3	34	101	233
Sept	9	2.25	31	7.75	40	6	28	34	19	58	77	0	0	0	1	6	7	35	123	158
Nov	5	1.25	15	3.75	20	5	12	17	5	20	25	0	0	0	0	0	0	15	47	62
Dec	0	0	2	0.5	2	1	3	4	2	8	10	0	0	0	0	0	0	3	13	16

TABLE 7: HLC IN EJAJI, CONTROL SITE, JUNE-DEC 2016

Time	*An. gambiae* s.l.					*An. pharoensis*			*An. coustani*			*An. demeilloni*			*An. squamosus*			Total *Anopheles* collected		
	In	MBR Indoor	Out	MBR Outdoor	Total	In	Out	Total	In	Out	Total	In	Out	Total	In	Out	Total	In	Out	Grand total
June	29	7.25	20	5	49	0	0	0	0	0	0	0	0	0	0	0	0	29	20	49
July	99	24.75	49	12.25	148	2	2	4	0	0	0	0	0	0	0	0	0	101	51	152
Aug	125	31.25	157	39.25	282	3	2	5	0	7	7	0	0	0	0	0	0	128	166	294
Sept	47	11.75	44	11	91	1	0	1	0	2	2	0	0	0	0	0	0	48	46	94
Nov	0	0	0	0	0	0	0	0	0	2	2	0	0	0	0	0	0	0	2	2
Dec	1	0.25	1	0.25	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

FIGURE 6: HLC IN GOBU SAYO, INTERVENTION SITE, JUNE-DEC 2016

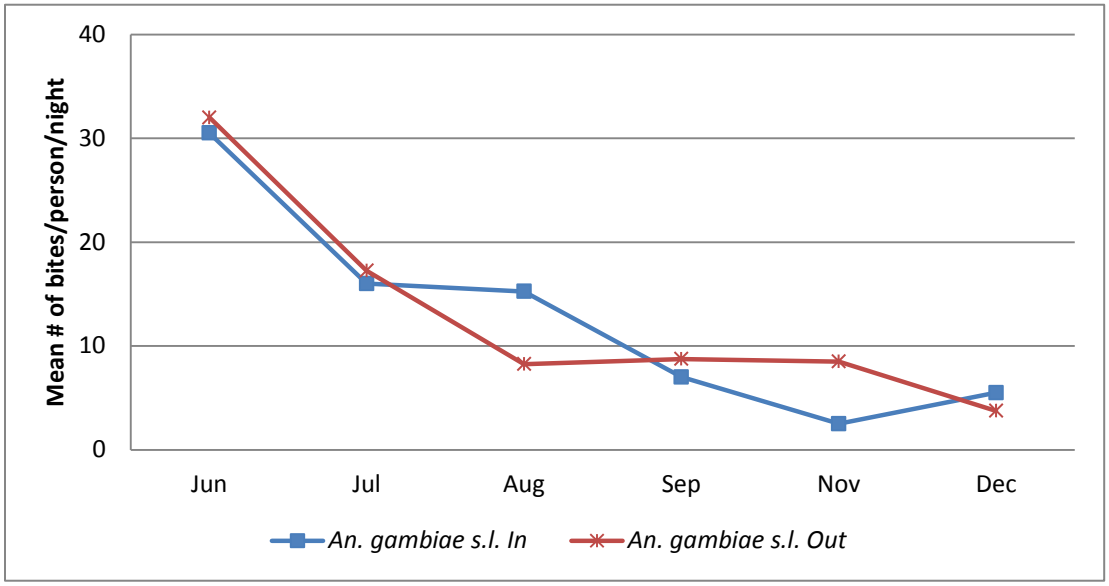


FIGURE 7: HLC IN SEKA CHEKORSA, INTERVENTION SITE, JUNE-DEC 2016

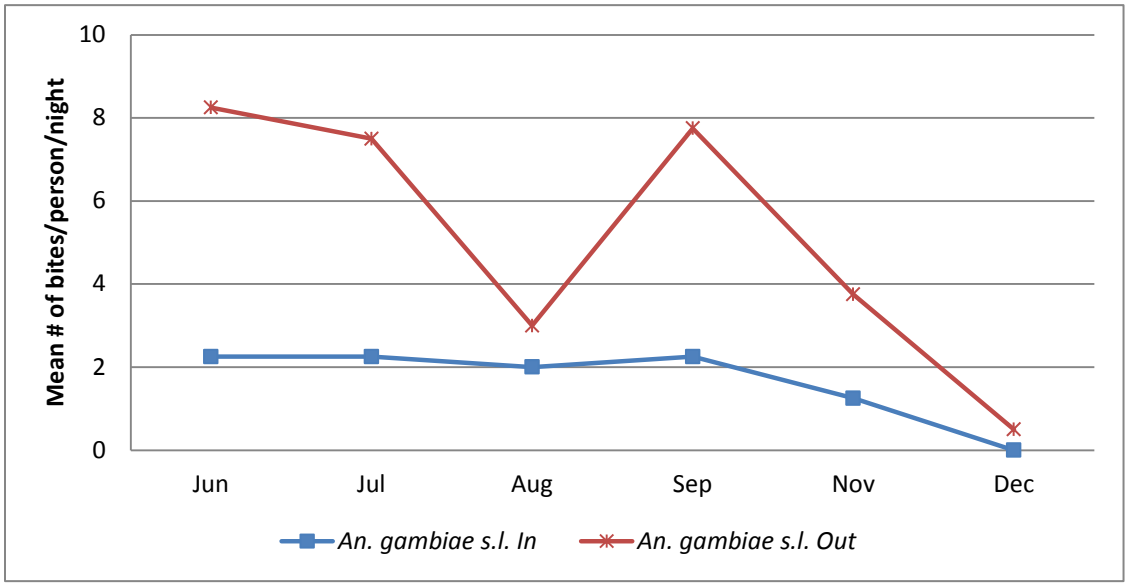
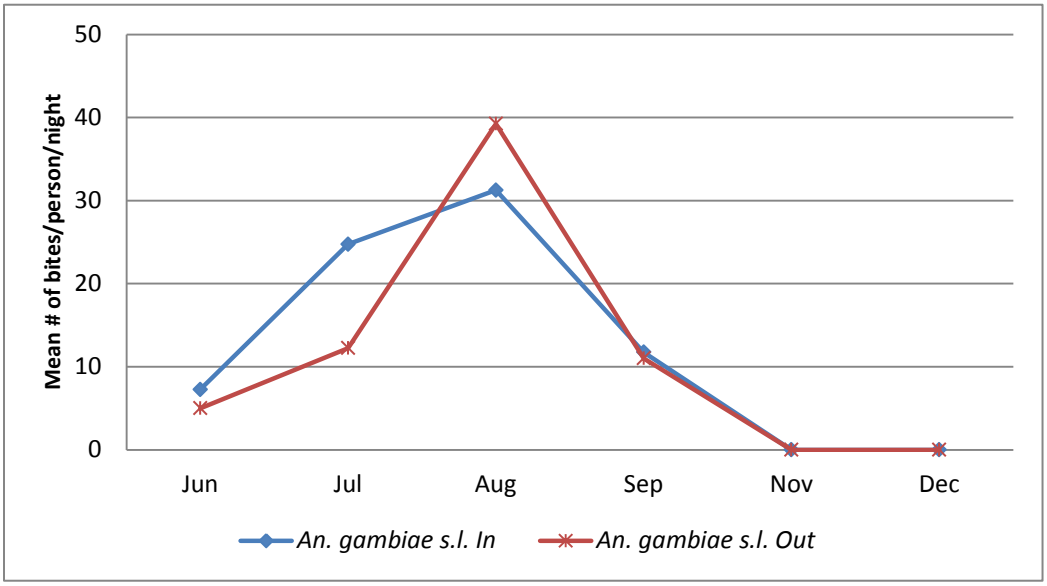


FIGURE 8: HLC IN EJAJI, CONTROL SITE, JUNE-DEC 2016



In Gobu Sayo, the indoor mean biting rate decreased from 30.5 bites per person per night before IRS (June) to 11.56 bites per person per night after spraying (p=0.009) from July- December. In the same site, the mean outdoor biting rate decreased from 32 bites per person per night before IRS to 11.6 bites per person per night post IRS (p=0.005). However, in the control site, Ejaji, during the same time period the mean indoor biting rates increased from 7.3 to 17 bites per person per night during the period that coincided with IRS in the intervention sites and the mean outdoor biting increased from 5 before IRS to 15.7 bites per person per night; the difference in increase over time was not statistically significant (P>0.05). The difference in the biting rate observed between the control and intervention sites (i.e. increase in the control and decrease in the intervention site post IRS) might be explained by the impact of IRS.

In Seka Chekorsa sentinel site, the mean indoor biting rate decreased from 2.3 pre-spray (June) to 1.9 bites per person per night post-spray (July – December). Similarly, the mean outdoor biting rates also decreased from 8.3 to 5.6 bites per person per night over the same period. The biting rates before IRS were not statistically different from post IRS (p>0.05) both indoors and outdoors. Biting tendency of *An. gambiae* s.l. in the intervention sites, Gobu Seyo and Seka Chekorsa, on average was variable. In Gobu Sayo District, on average, *An. gambiae* s.l. was found to bite indoors and outdoors in almost equally proportions. In Gobu Sayo district the mean outdoor biting rate over the survey period was 19.6 bites per person per night outdoor and 19.2 indoor (P>0.05). In Seka Chekorsa the mean outdoor biting rate was 7.7 and the indoor biting rate was 2.5 (P<0.05). However, in the control site, on average, the biting rate of *An. gambiae* s.l. was higher indoors as compared to outdoors. The mean biting rates were 16.9 and 18.9 bites per person per night outdoors and indoors, respectively. The difference in the outdoor and indoor biting rates was not statistically significant in the control site (P>0.05)

The main malaria vector, *An. gambiae* s.l. engaged in biting throughout the night, but peak biting tended to vary between sites. In Gobu Sayo and Seka Chekorsa pre-midnight biting activity was recorded, with peak activity occurring from 20.00 - 21.00 hours (Figure 9 and 10). In Ejaji, control site, a high

proportion of host-seeking *An. gambiae* s.l. tended to bite from 23.00 – 4.00 hours and decreased progressively towards dawn (Figure 11).

FIGURE 9: BITING TREND OF *AN. GAMBIAE* S.L. GOBU SAYO, INTERVENTION SITE

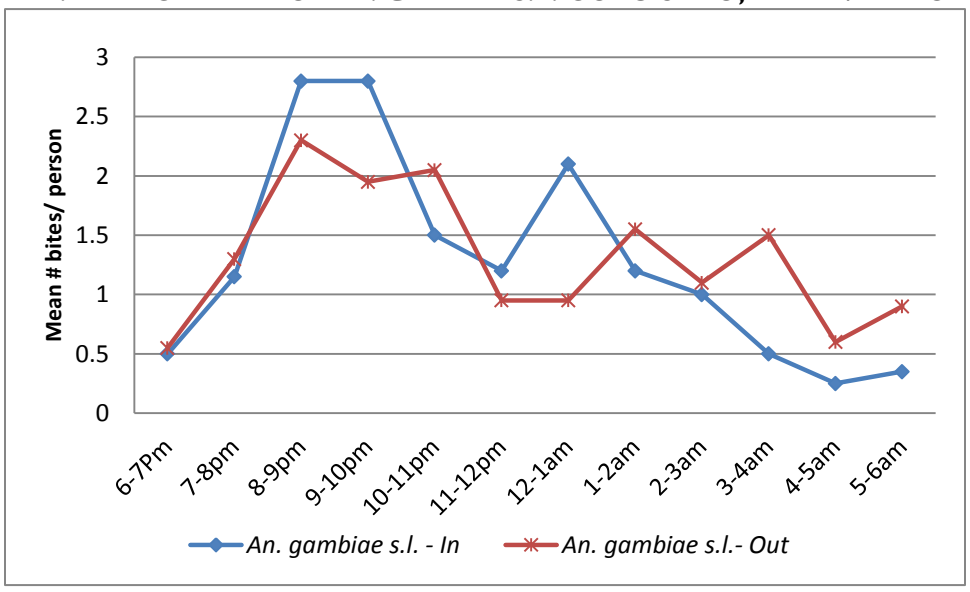


FIGURE 10: BITING TREND IN *AN. GAMBIAE* S.L. SEKA CHEKORSA, INTERVENTION SITE

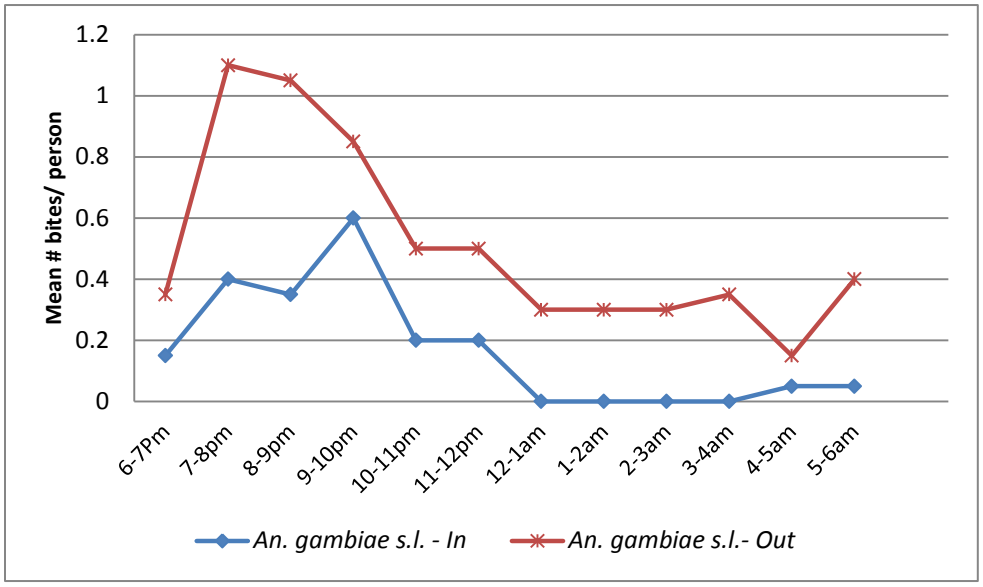
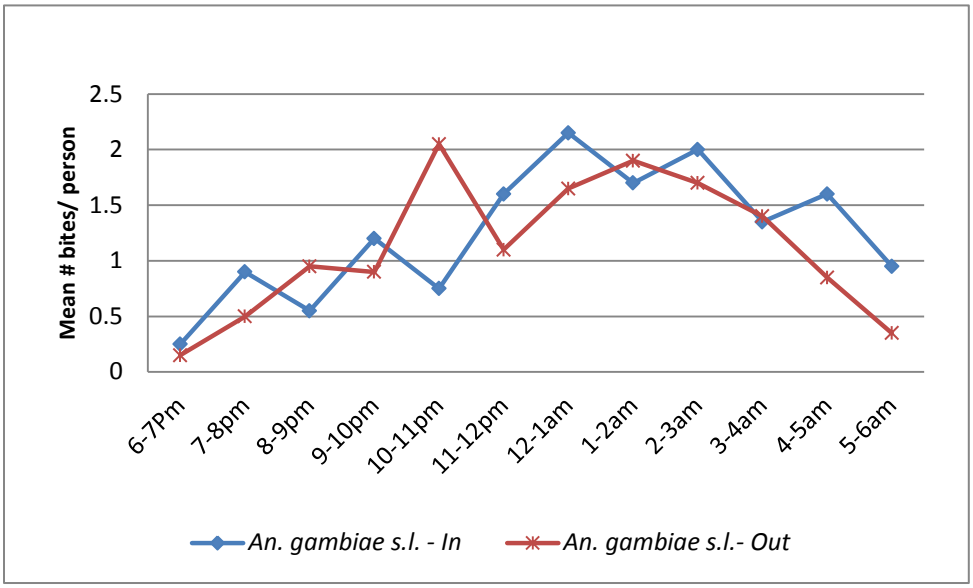


FIGURE 11: BITING TREND OF *AN. GAMBIAE* S.L. EJAJI, CONTROL SITE



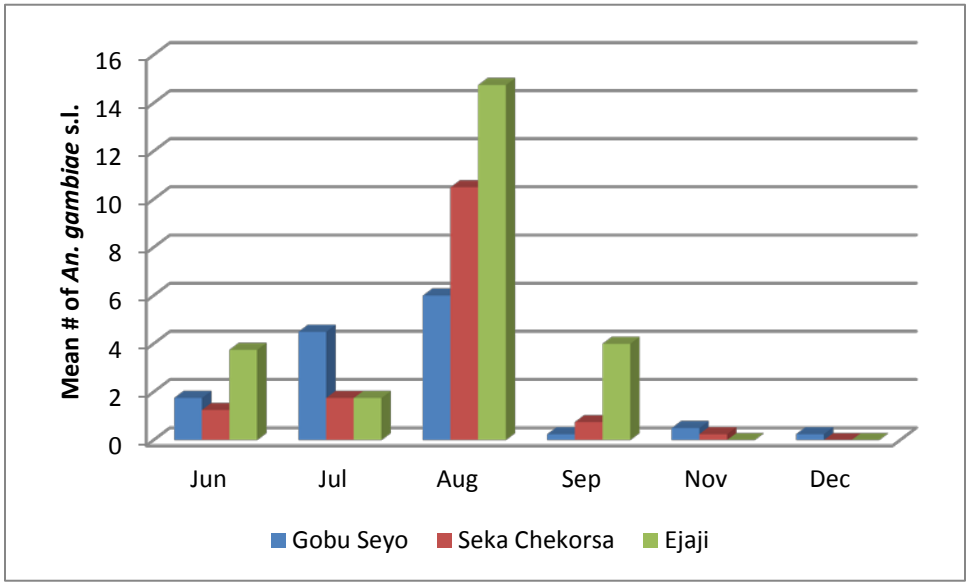
5.5 CDC LIGHT TRAPS

An. gambiae s.l. comprised 6.8% (n= 208) of the total female Anopheless collected from Gobu Sayo, Seka Chekorsa (intervention) and Ejaji (control) sites using CDC light traps indoors (Table 8). There was a clear pattern in monthly CDC light trap collections of *An. gambiae* s.l. from all three sites after spraying, which differs with PSC and HLC collections that showed a reduction after spray. There is no clear explanation as to why such variations were observed based on sampling technique.

TABLE 8: INDOOR CDC LIGHT TRAP COLLECTIONS, INTERVENTION AND CONTROL SITES

Time	Gobu Sayo (Intervention)						Seka Chekorsa (Intervention)				Ejaji (Control site)	
	An. gambiae s.l.	*An. pharoensis*	*An. coustani*	*An demeilloni*	*An. natalensis*	Total	*An. gambiae* s.l.	*An. pharoensis*	*An. coustani*	Total	*An. gambiae* s.l.	Total
Pre-spray	7	4	28	1	0	40	5	0	0	5	15	15
July	18	98	144	3	2	265	7	2	6	15	7	7
Aug	24	254	1356	2	2	1638	42	1	41	84	59	59
Sept	1	135	477	1	1	615	3	0	12	15	16	16
Nov	2	25	44	0	0	71	1	0	4	5	0	0
Dec	1	14	2	0	0	17	0	1	0	1	0	0

FIGURE 12: *AN. GAMBIAE* S.L. INDOOR CDC LIGHT TRAP COLLECTIONS IN THREE SITES

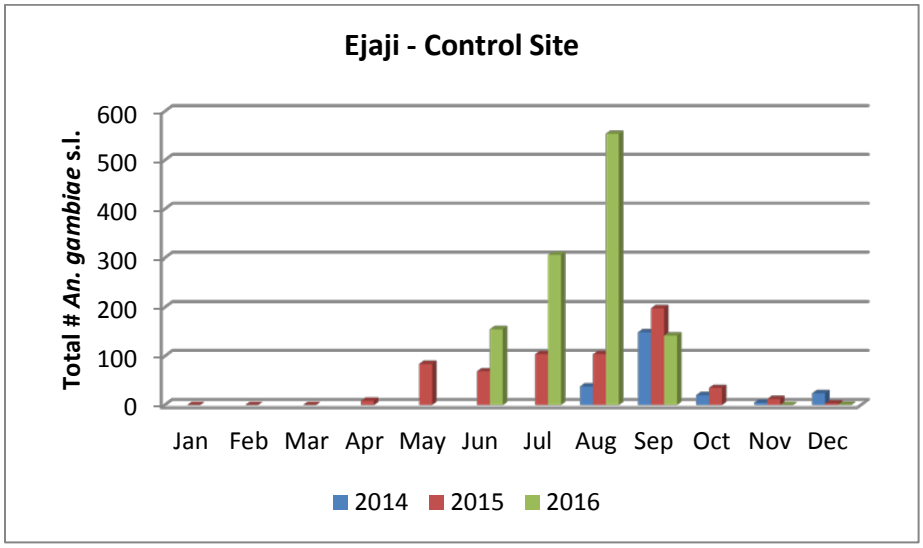


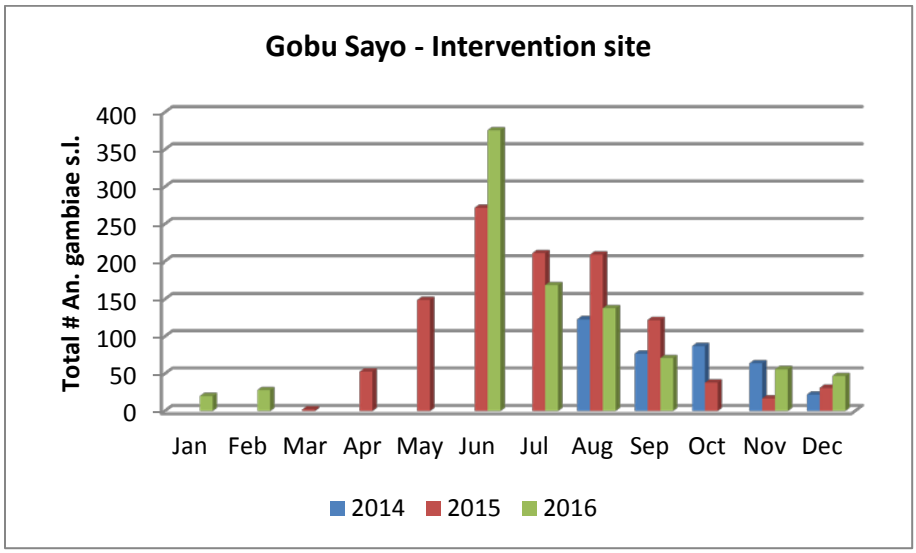
NB: Gobu Sayo and Seka Chekorsa: Intervention sites; Ejaji: Control site

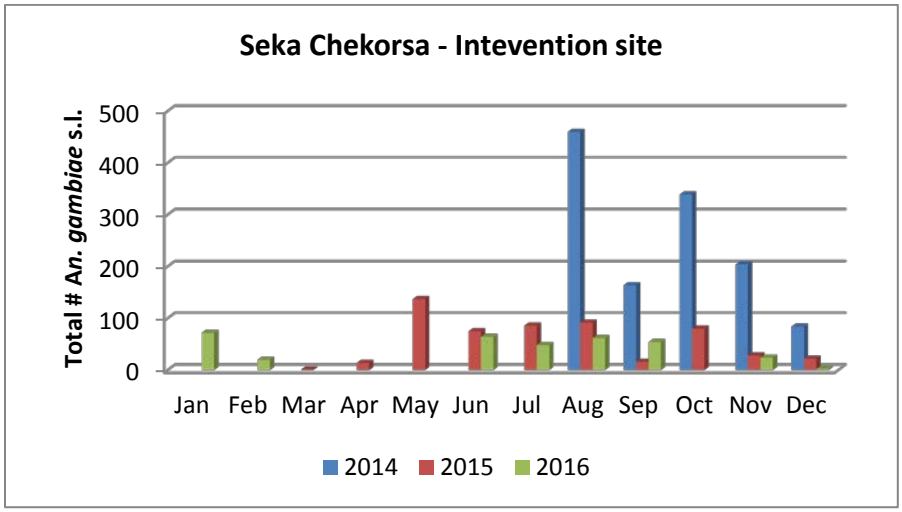
5.6 TREND OF *ANOPHELES GAMBIAE* S.L. DENSITY FOR THREE YEARS (2014 – 2016)

Year round data has been collected so far for one year (March 2015 – Feb 2016) and is continuing for a second year (June 2016 – May 2017). In 2014, vector density data was collected for five months (August - December 2014). Even though the data is not complete for each month and year, it is evident based on vector density data so far collected that *An. gambiae* s.l. starts proliferation in April and reaches a peak between June and August, with the peak density varying between sites. In Ejaji control site and Seka Chekorsa peak density was recorded in August and in Gobu Sayo it was recorded in June (Figure 13). Overall, active breeding of malaria vectors occurs in June and July, and the start of the IRS campaign should be targeted before the peak breeding activity in late May or early June. The use of an insecticide with long lasting action is therefore critical.

FIGURE 13: TREND OF *ANOPHELES GAMBIAE* S.L. IN THREE SENTINEL SITES 2014 – 2016


Ejaji - Control Site
Total # An. gambiae s.l.
600
500
400
300
200
100
0
Jan Feb Mar Apr May Jun Jul Aug Sep Oct Nov Dec
2014 2015 2016


Gobu Sayo - Intervention site
Total # An. gambiae s.l.
400
350
300
250
200
150
100
50
0
Jan Feb Mar Apr May Jun Jul Aug Sep Oct Nov Dec
2014 2015 2016


Seka Chekorsa - Intevention site
Total # An. gambiae s.l.
500
400
300
200
100
0
Jan Feb Mar Apr May Jun Jul Aug Sep Oct Nov Dec
2014 2015 2016

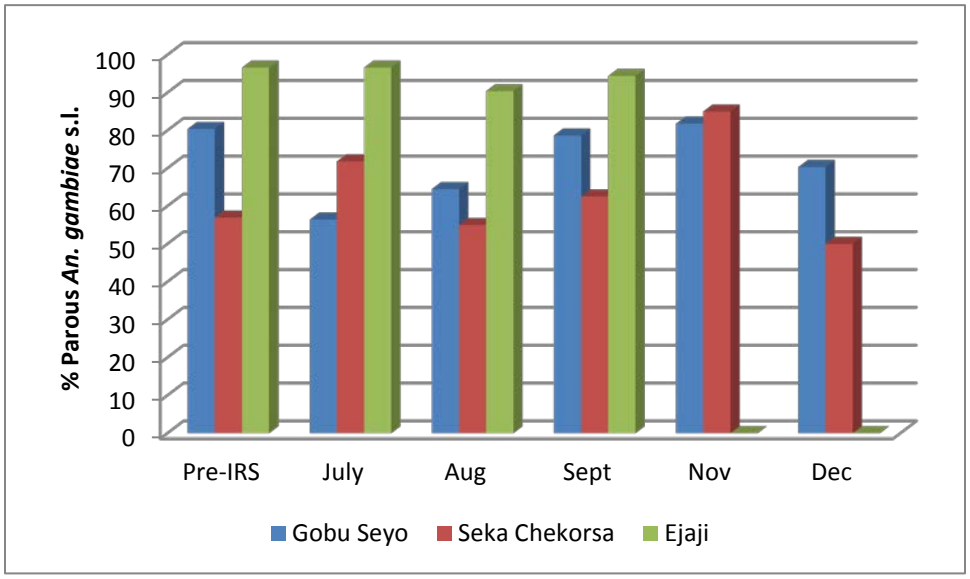
5.7 DETERMINATION OF PARITY

Ovary dissection was performed on all unfed female mosquitoes captured during the pre- and post-spray from HLC collections to determine parity rates. In Gobu Sayo and Seka Chekorsa intervention sites, the parity rate ranged from 55.0 – 85.0% after spray (Table 9). In Ejaji control site, the parous rate was 96.6% pre-spray and remained high above 90% post spray (Figure 14).

TABLE 9: PARITY RATES OF *ANOPHELES GAMBIAE* S.L. IN THREE STUDY SITES

Time	Gobu Sayo				Seka Chekorsa				Ejaji			
	An. gambiae s.l.	# Dissected	Parous	% Parous	*An. gambiae* s.l.	# Dissected	Parous	% Parous	*An. gambiae* s.l.	# Dissected	Parous	% parous
Pre-spray	250	250	201	80.4	42	42	24	57	59	58	56	96.6
July	133	133	75	56.4	39	39	28	71.8	148	147	142	96.6
Aug	94	93	60	64.5	20	20	11	55	282	281	254	90.4
Sept	63	61	48	78.7	40	40	25	62.5	91	90	85	94.4
Nov	44	44	36	81.8	20	20	17	85	0	0	0	0
Dec	37	37	26	70.3	2	2	1	50	0	0	0	0

FIGURE 14: PARITY RATES OF *AN. GAMBIAE* S.L. IN THREE STUDY SITES



NB: Gobu Sayo and Seka Chekorsa: Intervention sites; Ejaji: Control site

5.8 MOLECULAR AND ELISA TESTS

5.8.1 PRE-SPRAY SPOROZOITE ELISA TEST

A total of 334 *An. gambiae* s.l. were tested for *Plasmodium* circumsporozoite protein before spray operations. One specimen was positive for *P. vivax* and four specimens for *P. falciparum* circumsporozoite protein (Table 10).

TABLE 10: SPOROZOITE RATES OF *AN. GAMBIAE* S.L. COLLECTED FROM THREE SITES BEFORE SPRAY OPERATION

Site	Number tested	Pv-210	PV-247	Pf
		positive	**positive**	**Positive**
Seka Chekorsa	43	0	0	0
Gobu Seyo	247	0	1	4
Ejaji	44	0	0	0
Total	334	0	1	4

5.8.2 POST-SPRAY SPOROZOITE ELISA

A total of 926 *An. gambiae* s.l. were tested for *Plasmodium* circumsporozoite proteins. Out of the analyzed specimens six were positive for *P. falciparum* and one for *P. vivax* circumsporozoite proteins (Table 11). The entomological inoculation rate (infective bites per person per night) for *An. gambiae* s.l. derived based on specimens that tested positive for *Plasmodium* circumsporozoite proteins (*P. falciparum* and *P. vivax*) is shown in Table 12.

Of the 660 *An. coustani* and 1,006 *An. pharoensis* tested none was positive for *P. falciparum* or *P. vivax* circumsporozoite proteins.

TABLE 11: SPOROZOITE RATES OF *ANOPHELES GAMBIAE* S.L. COLLECTED FROM THREE SITES AFTER SPRAY OPERATION

Site	Number tested	Pv-210	PV-247	Pf
		positive	**positive**	**positive**
Seka Chekorsa	80	0	0	0
Gobu Seyo	290	0	0	4
Ejaji	556	0	1	2
Total	926	0	1	6

TABLE 12: ENTOMOLOGICAL INOCULATION RATE FOR *AN. GAMBIAE* S.L.

Month	Gobu Seyo			Seka Chekorsa			Ejaji		
	MBR	SR	EIR+(per person per	MBR	SR	EIR(per person	MBR	SR	EIR (per person per

			night)			per night)			night)
June	62.5	1.5	0.93	11	0	0	12.25	0	0
July	33.25	0	0	9.8	0	0	37	0	0
Aug	23.5	1.9	0.45	5	0	0	70.5	0.4	0.28
Sept	15.75	ND	ND	10	ND	ND	22.75	1.6	0.36
Nov	11	4.8	0.53	5	ND	ND	2	ND	ND
Dec	9.25	ND	ND	0.5	ND	ND	0	ND	ND

+EIR: Infective bites per person per night

5.8.3 MOLECULAR IDENTIFICATION OF AN. GAMBIAE S.L.

A total of 662 *An. gambiae* s.l. selected randomly from specimens collected from 10 study sites in six regions of the country were identified to species using species-specific PCR. The results of the molecular analysis showed that *An. arabiensis* was the only species of the complex represented (Table 13).

TABLE 13: PCR IDENTIFICATION OF *AN. GAMBIAE* S.L. SAMPLES FOLLOWING BIOASSAY TESTS IN 10 SITES

Region	Site	# *An. gambiae* s.l. assayed	# *An. gambiae* s.l. specimen not amplified	# *An. arabiensis*
Afar	Amibara	79	6	73
Amhara	Metema	34	6	28
Gambella	Abobo	80	14	66
Oromyia	Asendabo	68	8	60
	Babile	51	5	46
	Nono	50	15	35
	Ziway	60	3	57
SNNPR	Arbaminch	80	14	66
Tigray	Alamata	80	7	73
	Humara	80	10	70
	Total	**662**	**88**	**574**

5.8.4 STATUS OF KDR RESISTANCE FOR DELTAMETHRIN AND DDT

AIRS Ethiopia provided support in supplies and logistics to Jimma University to conduct molecular analysis to determine the mechanism of resistance in *An. gambiae* s.l. In 2016, some 562 female Anopheles mosquitoes collected from 10 sites were analyzed for *kdr* mutations using the polymerase

chain reaction (PCR). The *kdr* West and East mutations were assessed in surviving and dead *An. gambiae* s.l. randomly selected following bioassay tests. Of the specimens analyzed, only 444 (79%) specimens were amplified. The result of the analysis showed that only the West African *kdr* allele (L1014F) was common in populations of *An. gambiae* s.l. tested from the 10 study sites in six regions of the country (Table 14). The *kdr* allele L1014S (kdr east) was absent in the samples tested.

TABLE 14: RESULTS OF PCR ANALYSIS FOR *KDR* RESISTANCE MECHANISM

Site	Insecticide	Bioassay phenotype	# mosquito assayed	Homozygous resistant (RR)	Heterozygous (RS)	Homozygous	*kdr* allele frequency	
						Wild type (SS)	**R**	**S**
Amibara	DDT	Alive	30	6	6	13	0.36	0.64
		Dead	10	0	1	4	0.1	0.9
	Deltamethrin	Alive	24	3	3	8	0.33	0.68
		Dead	9	0	0	5	0	1
Metema	DDT	Alive	11	1	3	4	0.31	0.69
		Dead	8	0	1	4	0.2	0.8
	Deltamethrin	Alive	1	1	0	0	1	0
		Dead	0	0	0	0	0	0
Gambella	DDT	Alive	19	0	2	1	0.33	0.67
		Dead	9	1	0	2	0.33	0.67
	Deltamethrin	Alive	30	11	7	3	0.73	0.27
		Dead	8	0	1	5	0.08	0.92
Asendabo	DDT	Alive	28	9	11	6	0.56	0.44
		Dead	0	0	0	0	0	0
	Deltamethrin	Alive	25	3	12	6	0.43	0.57
		Dead	7	1	1	3	0.3	0.7
Babile	DDT	Alive	13	7	2	0	0.89	0.11
		Dead	9	9	0	0	1	0
	Deltamethrin	Alive	15	14	0	0	1	0
		Dead	9	8	1	0	0.94	0.167
Nono	DDT	Alive	12	2	3	3	0.44	0.56
		Dead	9	0	3	3	0.25	0.75
	Deltamethrin	Alive	8	7	0	0	1	0
		Dead	6	2	2	2	0.5	0.5
Ziway	DDT	Alive	30	9	10	11	0.47	0.53
		Dead	10	0	2	8	0.1	0.9
	Deltamethrin	Alive	9	2	4	3	0.44	0.56
		Dead	8	0	1	7	0.06	0.94
Arbaminch	DDT	Alive	28	12	7	7	0.6	0.4

		Dead	10	1	3	4	0.31	0.69
	Deltamethrin	Alive	22	12	8	2	0.73	0.27
		Dead	6	2	1	3	0.41	0.59
Alamata	DDT	Alive	30	10	12	4	0.62	0.38
		Dead	9	0	4	3	0.29	0.71
	Deltamethrin	Alive	20	6	5	4	0.57	0.43
		Dead	10	0	1	3	0.13	0.87
Humera	DDT	Alive	27	14	6	4	0.71	0.29
		Dead	74	0	2	5	0.14	0.86
	Deltamethrin	Alive	24	7	6	5	0.56	0.44
		Dead	9	2	2	4	0.38	0.62
	DDT	Alive	**228**	**70**	**62**	**53**	**0.51**	**0.49**
		Dead	**148**	**11**	**16**	**33**	**0.32**	**0.68**
	Total		**376**	**81**	**78**	**86**	**0.46**	**0.54**
	Deltamethrin	Alive	**178**	**66**	**45**	**31**	**0.62**	**0.38**
		Dead	**72**	**15**	**10**	**32**	**0.35**	**0.65**
	Total		**250**	**81**	**55**	**63**	**0.55**	**0.45**

6. VECTOR RESTING DENSITY IN VERANDAHS

Mosquito sampling was conducted to establish whether or not vector biting and possible transmission of malaria take place in verandahs. Early morning hand collection of mosquitoes was conducted in 12 selected houses with verandahs in Ejaji and Gobu Sayo (Gambela Tare kebele) for two months (July and August 2016). The results showed that only one *Anopheles gambiae* s.l. was collected resting in verandahs (Table 15).

TABLE 15: RESULTS OF MOSQUITO SAMPLING IN VERANDAHS

Time	Site	# Houses			# Collected	
		Sampled Houses	**Positive for Anopheles**	**Positive for culicines**	***An. gambiae* s.l.**	**Culicines**
July 2016	Ejaji	12	0	6	0	16
	Gobu Sayo	12	0	2	0	3
	Total	24	0	8	0	19
Aug 2016	Ejaji	12	1	6	1	21
	Gobu Sayo	12	0	0	0	0
	Total	24	1	6	1	21

7. Discussion and Lessons Learned

I. The results of the AIRS entomological study suggest that the mosquito population is already on the rise by the time spraying starts at the end of June and that implementation of IRS in late May just at the beginning of the rains should be considered. Unfortunately, due to the timing of insecticide delivery, the campaign is scheduled to begin in late June in 2017.

II. The reduction in indoor resting density, overall human landing catches and parity rates post spray in the intervention site as compared to the control site showed that IRS tended to achieve desired impact in controlling malaria vectors.

III. The early evening biting (6:00 p.m. – 10:00 p.m.) of the malaria vectors in the three study sites is an indication that transmission may occur before people go to bed hence compromising the protection afforded by LLINs.

8. RECOMMENDATIONS

- Based on the temporal distribution of vectors it is recommended to implement IRS late in the month of May with insecticides with long residual efficacy.

- Since the density of *An. pharoensis* and *An. coustani* were found to increase after spraying and assessment of their role in malaria transmission is necessary, there also is a need to monitor the susceptibility status of these secondary vectors to currently-used insecticide for IRS.

- The participation of the NMCP in entomological monitoring activities should be strengthened to ensure ownership and quality through supervision.

- Training of staff and facilitation of 25 sentinel sites selected by the FMOH/ NMCP for monitoring IR should be prioritized to provide a countrywide picture of vector susceptibility in response to the insecticide resistance monitoring and management strategy (IRMMS).

REFERENCES

Martinez-Torres D , Chandre F , Williamson MS , Darriet F , Berge JB , Devonshire AL , Guillet P , Pasteur N , Pauron D , 1998 . Molecular characterization of pyrethroid knockdown resistance (kdr) in the major malaria vector *Anopheles gambiae* s.s. Insect Mol Biol 7: 179 – 184.

Ranson H , Jenson B , Vulule JM , Wang X , Hemingway J , Collins FH , 2000 . Identification of a point mutation in the voltage gated sodium channel gene of Kenyan *Anopheles gambiae* associated with resistance to DDT and pyrethroids . Insect Mol Biol 9: 491 – 497.

Scott et al, 1993. Identification of singe specimens of the *Anopheles gambiae* complex by the polymerase chain reaction. Am J Trop Med Hyg 49(4); 520-529.

Wirtz RA, Sattabongkot J, Hall T, Burkot TR, Rosenberg R, 1992 Development and Evaluation of an Enzyme-Linked Immunosorbent Assay for Plasmodium vivax-VK247 Sporozoites. J Med Entomol 29: 854–7.

ANNEX A: SUMMARY RESULTS OF MOSQUITO COLLECTIONS

TABLE A 1: *ANOPHELES* SPECIES IN GOBU SAYO (INTERVENTION SITE)

Time	*An. gambiae* s.l.				*An. pharoensis*				*An. coustani*				*An. demeilloni*				*An. squamosus*			*An. natalensis*			Culicine			
	PSC	CDC	HLC	Total	PSC	CDC	HLC	Total	PSC	CDC	HLC	Total	PSC	CDC	HLC	Total	CDC	HLC	Total	CDC	HLC	Total	PSC	CDC	HLC	Total
June	119	7	250	376	0	4	29	33	0	28	43	71	0	0	2	2	1	0	1	0	0	0	36	1452	367	1855
July	18	18	133	169	0	98	187	285	0	144	246	390	1	3	0	4	3	1	4	1	0	1	18	11507	466	11991
Aug	20	24	94	138	4	254	192	450	12	1356	1143	2511	0	2	3	5	2	0	2	1	2	2	70	22020	1069	23159
Sept	7	1	63	71	6	135	162	303	3	477	416	896	0	1	0	1	1	1	2	1	0	1	81	9386	895	10362
Nov	10	2	44	56	1	25	289	315	0	44	91	135	0	0	1	1	0	0	0	0	0	0	7	751	354	1112
Dec	9	1	37	47	1	14	221	236	1	2	21	24	0	0	0	0	0	0	0	0	0	0	6	45	101	152

TABLE A 2: *ANOPHELES* SPECIES IN SEKA CHEKORSA (INTERVENTION SITE)

Time	*An. gambiae* s.l.				*An. pharoensis*				*An. coustani*				*An. squamosus*				Culicine			
	PSC	CDC	HLC	Total	PSC	CDC	HLC	Total	PSC	CDC	HLC	Total	PSC	CDC	HLC	Total	PSC	CDC	HLC	Total
June	17	5	42	64	8	5	25	38	0	0	16	16	0	0	0	0	23	42	168	233
July	2	7	39	48	1	2	65	68	0	6	40	46	0	0	2	2	101	67	173	341
Aug	0	42	20	62	0	1	17	18	3	41	95	139	0	0	3	3	15	145	123	283
Sept	11	3	40	54	0	0	34	34	0	12	77	89	0	0	7	7	17	29	182	228
Nov	3	1	20	24	3	0	17	20	2	4	25	31	0	0	1	1	12	18	60	90
Dec	0	0	2	2	2	1	4	7	1	0	10	11	0	0	0	0	13	15	13	41

TABLE A 3: *ANOPHELES* SPECIES IN EJAJI (CONTROL SITE)

Time	*An. gambiae* s.l.				*An. pharoensis*				*An. coustani*				Culicine			
	PSC	CDC	HLC	Total	PSC	CDC	HLC	Total	PSC	CDC	HLC	Total	PSC	CDC	HLC	Total
June	91	15	49	155	0	0	0	0	0	0	0	0	158	35	130	633
July	151	7	148	306	1	0	4	5	0	0	0	0	773	117	906	1796
Aug	213	59	282	554	2	0	5	7	2	0	7	9	459	257	1274	2429
Sept	47	4	91	142	1	0	1	2	0	0	2	2	1370	329	1374	3073
Nov	0	0	**0**	0	0	0	0	0	0	0	2	2	836	522	889	2247
Dec	1	0	**0**	1	0	0	0	0	0	0	0	0	228	150	436	5320

ANNEX B: VECTOR DENSITY AND BEHAVIORAL STUDIES CONDUCTED BY UNIVERSITIES

As per the approved work plan of 2016, further entomological monitoring was conducted in collaboration with Gondar, Jimma, Jigjiga, Arbaminch, Addis Ababa, and Mekelle universities in sites selected based on the FMOH's insecticide resistance monitoring and management plan. Key entomological indicators, including vector distribution and seasonality; vector feeding time and location; and vector resting behavior were monitored. Vector density, distribution, and seasonality were monitored for five months from July until Nov 2016. The collection methods were similar to the three PMI-supported sentinel sites including PSC, HLC and CDC light traps. The study sites included: Goro (Oromia) for Addis Ababa, Sile (SNNPR) for Arbaminch, Alamata (Tigray) for Mekelle, Metema (Amhara) for Gondar, and Babile (Oromia) for Jigjiga Universities.

1. ANOPHELES SPECIES DIVERSITY AND ABUNDANCE

During the five months of the study, a total of 1,663 adult female *Anopheles* mosquitoes were collected using PSC, HLC and CDC light traps. Detailed data are included in Tables B1, B2, B3, B4 and B5. The species composition of collected mosquitoes follows:

- 1,456 *An. gambiae* s.l.
- 107 *An. coustani/ziemanni*
- 42 *An. pharoensis*
- *19 An. natalensis*
- 15 *An. pretoriensis*
- 10 *An. demeilloni*
- 8 *An. tenebrosus*
- 2 *An. christyi*
- 2 *An. implexus*
- 1 *An. funestus*
- 1 *An. flavicosta*.

In addition, 2,660 *Culex* mosquitoes were captured through the different collection techniques. *An. gambiae* s.l. was the most prevalent species in all five sites. *An. coustani* and *An. pharoensis* were collected from Goro and Alamata sites. *An. christyi* was collected from Goro, *An. demeilloni*

was collected from Alamata, *An. tenebrous*, *An. funestus* and *An. flavicosta* were collected at Sile site only. *An. pretoriensis* and *An. implexus* were collected from Babile site. The results of the study show the presence of a rich diversity of *Anopheles* in the country.

Figure B1. Distribution of the major *Anopheles* species in five sites sampled

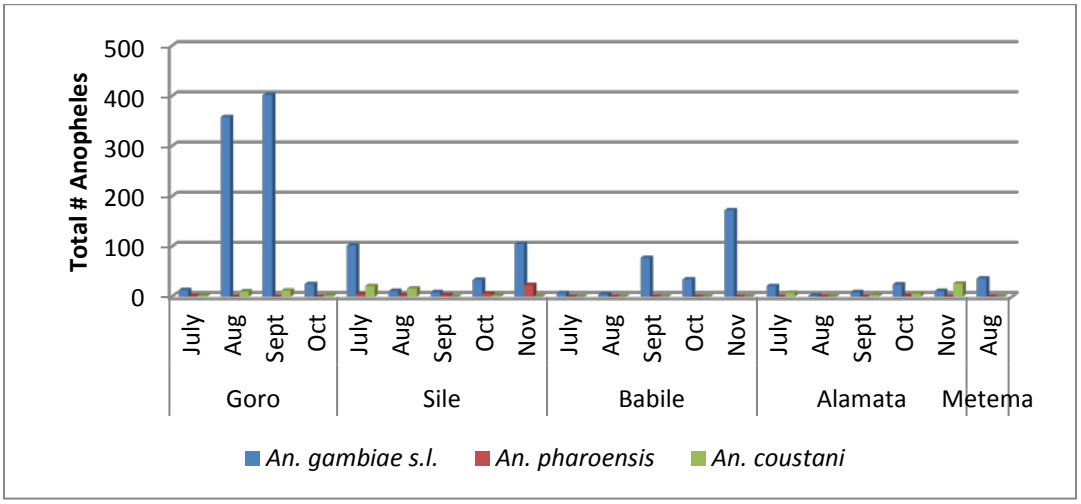


Table B1: *Anopheles* Species collected in Goro site (Oromia region)

Time	*An. gambiae* s.l.				*An. pharoensis*				*An. coustani*						Culicine			
	PSC	CDC	HLC	Total	PSC	CDC	HLC	Total	PSC	CDC	HLC	Total	HLC	Total	PSC	CDC	HLC	Total
July	1	2	10	13	0	0	1	1	1	0	1	2	0	0	0	2	33	35
Aug	4	31	323	358	0	0	0	0	0	3	7	10	1	1	0	52	111	163
Sept	31	33	333	402	0	0	0	0	0	3	8	12	0	1	0	21	62	83
Oct	0	5	20	25	0	0	0	0	0	0	2	2	0	0	3	5	10	18
Total	36	71	686	798	0	0	1	1	1	6	18	26	1	2	3	80	216	299

Table B2: *Anopheles* Species collected in Alamata site (Tigray region)

Time	***An. gambiae* s.l.**				***An. pharoensis***		***An. coustani***				***An. demeilloni***				**Culicine**			
	PSC	CDC	HLC	Total	HLC	Total	PSC	CDC	HLC	Total	PSC	CDC	HLC	Total	PSC	CDC	HLC	Total
July	13	0	8	21	0	0	0	1	6	7	3	3	0	6	3	8	27	38
Aug	0	2	0	2	0	0	0	0	0	0	0	0	0	0	8	4	20	32
Sept	0	2	7	9	0	0	0	1	2	3	1	1	1	3	13	2	1	16
Oct	0	2	22	24	1	1	0	0	6	6	0	0	0	0	11	3	12	26
Nov	2	0	9	11	0	0	0	0	26	26	1	0	0	1	8	3	7	18
Tot	15	6	46	67	1	1	0	2	40	42	5	4	1	10	43	20	67	130

Table B3: *Anopheles* Species collected in Metema site (Amhara region)

Time	*An. gambiae* s.l.				Culicine			
	PSC	CDC	HLC	Total	PSC	CDC	HLC	Total
Aug	17	15	4	36	95	51	26	172
Total	17	15	4	36	95	51	26	172

Table B4: *Anopheles* Species collected in Babile site (Oromia region)

Time	*An. gambiae* **s.l.**				*An. pretoriensis*			*An. natalensis*				*An. Implexus*		**Culicine**			
	PSC	CDC	HLC	Total	CDC	HLC	Total	PSC	CDC	HLC	Total	HLC	Total	PSC	CDC	HLC	Total
June	0	1	6	7	0	0	0	0	0	0	0	0	0	0	0	0	0
July	1	4	0	5	0	0	0	0	0	0	0	0	0	101	4	0	105
Sept	34	8	35	77	2	2	4	0	0	0	0	2	2	4	0	12	16
Oct	6	2	26	34	1	1	2	0	0	1	1	0	0	17	11	33	71
Nov	52	39	81	172	7	2	9	0	6	12	18	0	0	2	0	1	2
Total	93	54	148	295	10	5	15	0	6	13	19	2	2	124	15	46	194

Table B5: *Anopheles* Species collected in Sile site (SNNPR)

Time	*An. gambiae* s.l.				*An. pharoensis*			*An. coustani*				*An. tenebrosus*			*An. funestus*		*An. flavicosta*		Culicine			
	PSC	CDC	HLC	Total	PSC	HLC	Total	PSC	CDC	HLC	Total	CDC	HLC	Total	PSC	Total	PSC	Total	PSC	CDC	HLC	Total
July	66	11	25	102	1	4	5	0	5	16	21	1	3	4	1	1	1	1	664	41	161	930
Aug	0	2	9	11	0	3	3	0	3	13	16	0	0	0	0	0	0	0	299	2	96	435
Sept	0	4	5	9	0	3	3	0	0	0	0	0	1	1	0	0	0	0	118	19	30	1365
Oct	3	1	29	33	0	6	6	0	0	2	2	0	0	0	0	0	0	0	46	3	73	138
Nov	24	10	71	105	0	6	6	0	0	0	0	0	3	3	0	0	0	0	85	4	80	169
Total	93	28	139	260	1	22	23	0	8	31	39	1	7	8	1	1	1	1	1212	69	440	1865

ANNEX C: 2016 VECTOR SUSCEPTIBILITY TESTING

INTRODUCTION

Entomological monitoring is one of the key activities that PMI is supporting nationally in Ethiopia. When the PMI-funded IRS project, led by Research Triangle Institute, began its entomological monitoring activities in 2008, it detected a high level of vector resistance to DDT. The insecticide had been used for IRS for five decades. As a result, in 2009, Ethiopia's government-funded national IRS program and the PMI IRS Project switched to the use of a pyrethroid class of insecticides. However, 2010–2011 studies on IR by PMI/ Research Triangle Institute, the WHO, the FMOH, and Jimma, Mekelle, Dilla, and Addis Ababa universities showed that resistance to deltamethrin and other pyrethroids had spread to many parts of the country. This finding called on the national IRS program to reconsider the use of pyrethroids. In 2013 the national IRS program sprayed bendiocarb and propoxur, and the PMI-funded IRS project used bendiocarb in its target districts. In 2014, PMI AIRS again sprayed bendiocarb. In 2015, PMI AIRS in collaboration with FMOH, piloted Actellic 300 CS in eight project districts and the use of this insecticide was expanded to 36 project districts in 2016.

Nationwide entomological monitoring needs to be done to obtain a complete picture of IR in the country. PMI is supporting expanded entomological work to generate data on key entomological variables, in particular, the vectors' resistance to different insecticides. These data will guide and help to refine vector control activities of the PMI AIRS Project in Ethiopia and the national IRS program. Ethiopia developed and launched the national insecticide resistance monitoring and management strategy (IRMMS) in 2016, which will guide the rational use of insecticide-based malaria control interventions, including IRS, with a view of minimizing insecticide selection pressure.

In 2012 and 2013, AIRS Ethiopia had five sites for longitudinal monitoring of IR. This number increased to eight fixed sentinel sites in 2014 and 2015. In 2016 the number further increased to 11 sites, including eight previous fixed sites with Jimma, Addis Ababa, Mekelle and Gondar universities conducting studies at two sites each, and Arbaminch and Jigjiga universities in one study site each. One site was done by the PM AIRS Project team.

METHODOLOGY

MOSQUITO COLLECTION AND REARING

The entomology team used mosquitoes reared from field-collected larvae or pupae. All efforts were made to collect larvae and pupae from various breeding sites so that the mosquitoes tested were fully representative of the vector population in the area. Mosquitoes were morphologically identified at an adult stage and only mosquitoes that appeared to be the main vector, *An. gambiae* s.l., were selected for

the resistance test. Identification was double checked with dead mosquitoes after the test. Non *An. gambiae* were excluded from the count.

WHO TUBE TEST FOR SUSCEPTIBILITY

The teams used standard WHO tube test methodology to test susceptibility of the main vector, *An. gambiae* s.l., for an array of insecticides (WHO, 2013). Three to four replicates of 25 non-blood-fed, two- to three-day-old mosquitoes were exposed to insecticide-impregnated papers for one hour. Similarly, control mosquitoes were exposed to oil-impregnated papers. The number of knocked down mosquitoes were recorded during the exposure time at intervals of 15, 30, 45, and 60 minutes and for another hour after mosquitoes were transferred to holding tubes. A mosquito is considered knocked down if it lay on its side on the floor of the exposure tube and was consequently unable to fly (WHO, 2013). Mortality counts were taken after 24 hours of the holding period. Cotton wool soaked in 10% sugar solution was placed on top of the holding tube and optimum temperature and relative humidity was kept from a damp towel placed on top of holding boxes where tubes were kept.

CDC BOTTLE ASSAY TESTS

The CDC bottle assay (Brogdon et al. 2010) was also used during the peak mosquito population season to test the susceptibility of the main vector to different insecticides recommended for use in vector control. Mosquitoes reared from field-collected larvae or pupae were used for the tests. Efforts were made to collect larvae and pupae from various breeding sites so that the mosquitoes tested were fully representative of the vector population in the area.

Stock solution was prepared for each insecticide tested by diluting technical grade insecticide in 50 ml of acetone. Each bottle was internally coated with one ml of stock solution. The control bottle was coated with one ml of acetone. The bottles were covered with mats and kept overnight in a dark place to dry. The assay was run by introducing 15-25 mosquitoes to each bottle using an aspirator. As soon as the mosquitoes were transferred to bottles, a timer was set and knockdown recorded at 30 minutes diagnostic time. Mosquitoes that survived the diagnostic dosage and time were assumed to be resistant to the insecticide tested. The following insecticides were tested using CDC bottle bioassays: 21ug/bottle for permethrin and 12.5ug/bottle for deltamethrin.

DATA ANALYSIS

Interpretation of the status of susceptibility or resistance was based on the WHO 2013 classification criteria. If the 24-hour mortality rate was higher than 98%, the vector was considered fully susceptible to the insecticide; between 80-98%, the vector was classified as suspected resistant; and if mortality was below 80%, the vector was classified as resistant. When the control mortality was between 5-20%, the average observed mortality was corrected using Abbott's formula (Abbott, 1925). When the control mortality was above 20% the test result was discarded and the test was repeated.

RESULTS AND DISCUSSION

The main malaria vector, *An. gambiae* s.l., was tested for susceptibility to seven insecticides recommended for malaria vector control using the WHO tube test in 11 sites. The results showed that the vector was fully susceptible to pirimiphos-methyl and propoxur in nine and eight sites, respectively.

Resistance and possible resistance to pirimiphos-methyl were recorded in one site each. Possible resistance to propoxur was reported from three sites. Suspected resistance and resistance to bendiocarb were recorded in one site each. *An. gambiae* s.l. was resistant to DDT and permethrin in all sites tested. Susceptibility to deltamethrin was reported in only one of the 11 sites tested. The vector was shown to be fully susceptible to malathion in three sites; possibly resistant in four sites and resistant in another four sites.

Susceptibility tests using CDC bottle bioassays were conducted in three sites (Amibara, Ziway Dugda and Nono) with two different insecticides; namely, deltamethrin and permethrin, by the AIRS team. In all three sites the vector was resistant to permethrin at 1x and 2x (17- 83%); possibly resistant at 5x and 10x in Amibara and Nono sites but susceptible at 5x and 10x in Ziway Dugda site. The vector was also found to be resistant to deltamethrin at 1x in Nono site and possibly resistant at 2x in Ziway Dugda and Nono sites and susceptible at 5x and 10x in all sites. In all sites mortality was 100% at 1x for deltamethrin after the vector was treated with PBO, indicating that an oxidase mechanism of resistance is involved. For the vector exposed to permethrin after PBO treatment, mortality ranged from 86.8-100% at 1x (Table C3).

Tables C1 and C2 show resistance results to the insecticides tested in 2016 and in previous years (2012 – 2015), respectively. The malaria vector remains highly resistant to DDT and the pyrethroids.

TABLE C1. INSECTICIDE RESISTANCE RESULTS FROM ELEVEN SITES IN 2016

Insecticide	% Mortality (dead/exposed)										
	Omonada (Oromia)	**Nono (Oromia)**	**Ziway (Oromia)**	**Babile(Oromia)**	**Bahirdar(Amhara)**	**Metema (Amhara)**	**Alamata (Tigray)**	**Humera (Amhara)**	**Arbaminch (SNNPR)**	**Abobo (Gambella)**	**Amibara (Afar)**
DDT	10 (10/100) (R)	29(29/100) (R)	27(27/100) (R)	11(11/100) (R)	ND	73.5(75/102) (R)	25(34/100) (R)	33(33/100) (R)	51(51/100) (R)	37 (37/100) (R)	67(67/100) (R)
Deltamethrin	8 (8/100) (R)	51.5(50/97) (R)	76(76/100) (R)	22(22/100) (R)	80.6(79/98) (R)	99.1(105/106) (S)	50(50/100) (R)	33.7(39/100) (R)	41(41/100) (R)	33 (33/100) (R))	53(53/100) (R)
Malathion	83 (83/100) (R)	100(100/100) (S)	98(98/100) (S)	96(96/100)(POR)	72.8(75/103) (R)	81.9(50/61) (R)	96(72/75) (POR)	25(25/100) (R)	100(100/100) (S)	95 (95/100) (POR)	95.7(96/100) (POR)
Pirimiphos-methyl	98 (98/100) (S)	100(100/100) (S)	99(99/100) (S)	85(85/100) (R)	100(25/25) (S)	100(103103) (S)	92(69/75) (POR)	100(100/100) (S)	100(100/100) (S)	100 (100/100) (S)	100(100/100) (S)
Bendiocarb	80 (80/100) (R)	98(98/100) (S)	100(100/100) (S)	100(100/100) (S)	100(75/75) (S)	100(104/104) (S)	97.3(73/75) (POR)	100(100/100) (S)	100(100/100) (S)	98 (98/100) (S)	99(99/100) (S)
Propoxur	100 (100/100) (S)	95(85/100) (POR)	94(94/100) (POR)	100(100/100) (S)	97(97/100) (POR)	100(103/103) ((S)	100(100/100) (S)	100(100/100) (S)	100(100/100) (S)	100 (100/100) (S)	100(100/100) (S)
Permethrin	5 (5/100) (R)	52.7(55/101) (R)	57(57/100) (R)	16(16/100) (R)	79.4(81/102) (R)	89.9(98/109) (R)	26.7(20/75) (R)	69(69/100) (R)	84(84/100) (R)	20 (20/100) (R)	68.4(71/100) (R)

Note: - ND-Not done due to scarcity of mosquitoes.

TABLE C2: COMPARISON OF IR STATUS OF AN. GAMBIAE S.L. IN 2012, 2013, 2014, 2015 AND 2016 IN 8 FIXED SAMPLING SITES

Insecticides	IR MONITORING SITES and % test mortality rates																													
	Omonada					Zwai					Chewaka				Bahrdar					Halaba		Alamata			Gambela			Amibara		
	2012	2013	2014	2015	2016	2012	2013	2014	2015	2016	2012	2013	2014	2015	2012	2013	2014	2015	2016	2012	2015	2014	2015	2016	2014	2015	2016	2014	2015	2016
DDT	3.8	9	6.8	4	10	13	26	6.2	ND	27	3	22	6	14.4	6	16	9.3	10.8	ND	0	25	25	40	25	12.5	24	37	18.8	48	67
Lambda cyhalothrin	25.7	15	39	9	ND	ND	ND	4.3	ND	ND	ND	44	11	53.7	ND	ND	24	10.7	ND	ND	21	58	35	ND	14.7	24	ND	46.2	34.9	ND
Deltamethrin	12.8	26	42	32	8	27	36	10.7	32	76	12	51	46	48.5	44	20	25	25.3	80.6	1	43	44	45	50	18.1	11	33	45.4	49.2	53
Fenitrothion	99.1	97	100	100	ND	99	-	100	100	ND	100	100	100	100	100	100	100	100	ND	100	100	100	100	ND	100	100	ND	100	100	95.7
Malathion	66.1	81	73	83	83	90	90	92.9	ND	98	58	71	94	95.7	26	33	89	43	72.8	48	96	89	100	96	95.5	88	95	100	100	100
Pirimiphos-methyl	100	100	100	98	98	ND	100	100	100	99	ND	100	100	100	100	100	100	100	100	ND -	100	100	100	92	100	100	100	100	100	100
Propoxur	98	98	100	100	100	100	100	100	100	94	96	100	100	100	100	96	99	99	97	99	100	98	100	100	100	100	100	100	100	100
Bendiocarb	93	92	86.4	95	80	100	100	100	100	100	90	100	100	100	87	75	87	87	100	98	100	96	100	97.3	92	100	98	100	100	99
Permethrin	10.9	22	16	22	5	ND	-	2.9	13	57	ND	ND	31	20	-	-	66	9	79.4		25	10	89	26.7	28.4	28	20	19.1	60.9	68.4
Alpha cypermethrin	24.8	-	35	50	ND	ND	32	5	ND	ND	ND	ND	ND	17.4	50	43	61	9.3	ND	ND	16	19	53	ND	11.4	86	ND	86.6(	70.3	ND
Etofenprox	8.7	-	55	4	ND	ND	20	28.7	ND	ND	ND	ND	24	9.9	23	55	23	17.3	ND	ND	43	77	80	ND	14.7	ND	ND	72.5	78.9	ND

Note: - ND-Not done due to scarcity of mosquitoes.

TABLE C3: SUMMARY OF CDC BOTTLE ASSAY RESULTS CONDUCTED IN 2016

Region	District	Type of insecticide	% Mortality after 30 minutes (Dead/Exposed)			
Oromia			1X	2X	5X	10X
	Ziway Dugda	Permethrin (Intensity)	17 (16/94)	68.4 (65/95)	100 (94/94)	100 (81/81)
		Deltamethrin (Intensity)	91.4 (85/93)	96.7 (89/92)	100 (97/97)	100 (99/99)
		Deltamethrin + PBO	100 (99/99)			
		Permethrin + PBO	94.6 (87/92)			
	Nono	Permethrin (Intensity	43.2 (41/95)	78.6 (66/84)	95.2 (80/84)	95.7 (88/92)
		Deltamethrin (Intensity)	87.8 (86/98)	89 (73/82)	97.5 (78/80)	100 (98/98)
		Deltamethrin + PBO	100 (80/80)			
		Permethrin + PBO	100 (90/90)			
Afar	Amibara	Permethrin (Intensity)	64 (48/75)	83.3 (50/60)	96.7 (58/60)	
		Deltamethrin (Intensity)	94.9 (75/79)	100 (59/59))	100 (60/60)	
		Deltamethrin + PBO	100 (60/60			
		Permethrin + PBO	86.8 (66/76)			

REFERENCES

Abbott W.S.1925. A method of computing the effectiveness of insecticide. *J. Econ. Ent.* 18(2), pp. 265-267.

Brogdon WG, McAllister JC: Simplification of mosquito bioassays through use of time-mortality determinations in glass bottles. J Am Mosq Control Assoc 1998, 14(2):159-164

World Health Organization. 1981. Instructions for determining the susceptibility or resistance of adult mosquitoes to organochlorine, organophosphate and carbamate insecticides. Diagnostic test. Document WHO/VBC/81.806. WHO, Geneva.

World Health Organization 1998. Test procedures for insecticide resistance monitoring in malaria vectors, bio-efficacy and persistence of insecticides on treated surfaces. *(WHO/CDS/CPC/MAL/98.12)*, WHO, Geneva.

ANNEX D: WALL BIOASSAY FOR SPRAY QUALITY ASSURANCE AND MONITORING DECAY RATE

1. WALL BIOASSAY FOR QUALITY CHECK

PMI AIRS Ethiopia conducts routine entomological monitoring in selected sites to provide data for decision making. Data generated is used to justify decisions such as the type of insecticide and selection of target areas. It also helps to assess the quality of the vector control intervention as well as its efficacy. The project implemented the following entomology activities in collaboration with local universities:

- Vector density and species composition in intervention areas
- Vector behavior
- Wall bioassay to assess quality of insecticide application and insecticide decay rates
- Insecticide resistance monitoring.

The project supports the Oromia Regional Health Bureau's insectary in Adama through procurement of supplies and care of lab animals for mosquito feeding, maintenance of insectary equipment, and payment of temporary staff. Further support is provided to insectaries at Jimma University and Addis Ababa University. The insectaries serve as a source of susceptible *An. arabiensis* mosquitoes for entomological monitoring activities undertaken by the AIRS team.

2. DETERMINATION OF QUALITY OF SPRAYING AND DECAY RATE

The PMI AIRS Ethiopia team conducted cone bioassay tests for quality check and decay rate in four sites; two each in DB IRS and CB IRS districts. Spraying in all project districts was done using Actellic 300 CS.

The tests were performed in 12 houses per site purposefully selected to represent different wall types and structures sprayed by different SOPs. A total of 48 houses were sampled in the four The tests were carried out using known susceptible *An. arabiansis* colonies reared in Addis Ababa and Jimma University insectaries. Larvae were reared to adults; two- to three-day-old, sugar-fed adults were exposed to the sprayed walls in the selected houses.

Results of the wall bioassay tests conducted one to two days after spraying with pirimiphos-methyl are shown in Table D1. Mortality of susceptible *An. arabiensis* was 100% for all wall surfaces tested in Shebe

Sombo District and all painted wall surfaces in all four villages. In Chewaka and Bako Tibe districts, mortality of the susceptible mosquitoes was 98.3% and 98.9% for mud wall surfaces, respectively. The mortality of susceptible mosquitoes on dung wall surfaces was 98.7% in Tiro Afeta District but 100% in Bako Tibe, Chewaka and Shebe Sombo. There was no difference in mortality of mosquitoes between CB IRS and DB IRS model sites on all wall surfaces sprayed. As indicated in Table E1, the overall mean mortality of *An. arabiensis* for time zero was 99.7% (one to two days after IRS).

In Chewaka, pirimiphos-methyl mortality rate was 77.4% and 78.3% after five months with susceptible mosquitoes on mud and dung wall surfaces, respectively. The results over the months were not consistent. In Tiro Afeta, pirimiphos-methyl performed well on all wall surfaces with average mortality rate of 84.3% after five months. A mean mortality of 73.3% was recorded five months after IRS. The overall test results are shown in Table D1.

TABLE D1. DECAY RATE OF PIRIMIPHOS-METHYL SPRAYED IN FOUR INTERVENTION SITES IN 2016

Time	% Mortality (dead/exposed) of *An. arabiensis*																Overall mean
	Chewaka				Tiro-Afeta				Bako-Tibe				Shebe-Sombo				
	Dung	Mud	Painted	Mean	Dung	Mud	Painted	Mean	Dung	Mud	Painted	Mean	Dung	Mud	Painted	Mean	
T0 (June 2016)	100(60/60)	98.3(173/176)	100(118/118)	99.6	98.7(148/150)	100(150/150)	100(60/60)	99.6	100(60/60)	98.9(177/179)	100(117/117)	99.6	100(30/30)	100(180/180)	100(150/150)	100	99.7
T1 (July 2016)	98.4(60/61)	93.4(169/181)	100(121/121)	97.3	100(121/121)	100(180/180)	100(60/60)	100	94.9(56/59)	96.7(176/182)	100(120/120)	97	100(31/31)	100(185/185)	100(152/152)	100	98.6
T2 (Aug 2016)	80.6(54/67)	79.9(159/199)	99.2(131/132	86.6	90.5(105/116)	100(165/165)	100(58/58)	96.8	63.3(42/66)	83.5(162/194)	97.8(131/134)	82	93.5(29/31)	91.3(178/195)	99.4(167/168)	95	95.7
T3(Sept 2016)	81.7(49/60)	73.8(135/183)	96.8(12/125)	84.1	64.7(75/116)	74.3(133/179)	100(58/58)	79.7	62.3(38/61)	78.3(144/184)	96(12/126)	79	100(29/29)	75.9(136/179)	84.7(127/150)	87	82.4
T5(Nov) 2016)	78.3(47/60)	77.4(144/186)	99.1(121/122)	84.9	85.1(97/114)	85.4(146/171)	82.5(52/63)	84.3	61(37/61)	74.8 (151/2012)	97(129/133)	77.6	ND	68.3(127/186)	63.1(94/149)	65.7	78.1

REFERENCES

Abbott W.S.1925. A method of computing the effectiveness of insecticide. *J. Econ. Ent.* 18(2), pp. 265-267.

ANNEX E: TRAINING ON BASIC MALARIA ENTOMOLOGY FOR SIX UNIVERSITIES

The PMI AIRS Project in collaboration with the FMOH conducted entomology training at the Malaria Control Training Center, Adama Town, from June 7-14, 2016, with participants drawn from six universities. The PMI AIRS Project provides support to six universities (Arbaminch, Mekele, Gonder, Addis Ababa, Jigjiga and Jimma) in the country to conduct vector density and behavior studies and insecticide susceptibility monitoring at selected sentinel sites with the overall aim of building local capacity. A total of 26 participants, including four coordinators and 22 entomology assistants, attended the training which covered the following topics:

- Malaria entomology and malaria transmission
- Malaria vector sampling methods
- Identification of malaria vectors
- Vector incrimination
- WHO tube bioassay
- Cone bioassay.

The project provided support to one staff from ORHB to attend the environmental compliance training organized by the PMI AIRS Project in Accra, Ghana, April 1 – 4, 2016. The project further supported the participation of one staff from the FMOH in the AIRS organized regional entomology held in Harare, Zimbabwe, from June 27 – July 4, 2016.

ANNEX F: DETERMINATION OF INSECTICIDE DECAY RATE IN ETHIOPIA

PMI AIRS Ethiopia in collaboration with Jimma and Addis Ababa universities conducted studies to determine the decay rate of different insecticides in experimental huts in two sites (Ziway and Sekoru) from November 2015 – June 2016. The objectives of the study were to:

- Determine the decay rates of bendiocarb, propoxur, and pirimiphos-methyl;
- Determine the effect of different wall surfaces on the persistence of insecticides;
- Determine whether or not insecticide decay rates vary by site.

Ten experimental huts were constructed at each of the study sites with wall surfaces representative of the situation within the communities: painted, smooth mud, rough mud and dung surfaces. Two huts served as controls (sprayed with water) while the remaining eight were treated with bendiocarb (400mg/m^2), propoxur (1000mg/m^2), propoxur (2000mg/m^2) and pirimiphos-methyl (1000mg/m^2) in replicates. Four filter papers (Whatman # 1) were placed on each of the wall surfaces and the concentration of the insecticide was tested by High Performance Liquid Chromatography (HPLC) at Jimma University. To determine the decay rate, two- to five-day-old susceptible *An. arabiensis* were exposed to the different wall surfaces in intervals of one month from November 2015 to June 2016.

The results of this study showed that the mean mortality of *An. arabiensis* exposed to bendiocarb sprayed surfaces was less than 80% one month post IRS on all wall surfaces except for painted wall surfaces where it remained active for four months with mortality >97% recorded in both sites (Table F1). The mean mortality of *An. arabiensis* to propoxur 1000mg/m^2 was found to be 80% after two months, except on dung and painted wall surfaces where 85% mortality was reported at four months in both sites.

Overall mean mortality on surfaces sprayed with propoxur 2000gm/m^2 and pirimiphos-methyl was greater than 80% five months after spraying in both sites. Mortality *of An. arabiensis* exposed to dung and painted wall surfaces spayed with propoxur 2000 mg/m^2 was 83% at Sekoru site seven months post spray (Figure F1). Similarly, Actellic 300 CS was still active on smooth mud wall surfaces at Ziway (80%) and Sekoru (83.1%) sites seven months after spraying (Table F2).

TABLE F1. MEAN PERCENT MORTALITY OF *AN. ARABIENSIS*

Time	Mean % mortality of *An. arabiensis*											
	Bendiocarb WP (400mg/m²)			Propoxur WP (1000mg/m²)			Propoxur WP (2000mg/m²)			Actellic 300 CS (1000mg/m²)		
	Sokoru	Ziway	Mean	Sokoru	Ziway	Mean	Sokoru	Ziway	Mean	Sokoru	Ziway	Mean
T0 (Nov 2015)	95.9	83.8	89.9	100	94.6	97.3	100	100	100	100	99.6	99.8
T1 (Dec 2015)	74.2	64.2	69.2	96.3	82.5	89.4	100	100	100	97.6	99.2	98.4
T2 (Jan 2016)	38.5	47.2	42.9	81.2	79.2	80.2	100	98.7	99.35	93.3	95.4	94.4
T3 (Feb 2016)	37.8	48.8	43.3	39.6	48.8	44.2	90.1	85.8	87.95	89.6	79.8	84.7
T4 (Mar 2016)	62.2	35.8	49.0	65.8	69.2	67.5	87.6	85.0	86.3	92.0	97.5	94.8
T5 (April 2016)	24.9	35	30.0	42.3	42.3	42.3	91	88.0	89.5	89.2	87.7	88.5
T6 (May 2016)	32.5	17.5	25.05	14.7	21.7	18.2	73.7	63.8	69	68.5	32.2	50.2
T7 (June 2016)	ND	ND	ND	ND	ND	ND	83.1	65.8	74.5	63.0	83.3	73.2

TABLE F2. PERCENT MORTALITY OF *AN. ARABIENSIS* ON DIFFERENT WALL SURFACES AT 6 AND 7 MONTHS POST SPRAY

Time	Insecticide	Mean % mortality of *An. arabiensis*									
		Sokoru					Ziway				
		Rough	Smooth	Dung	Painted	Mean	Rough	Smooth	Dung	Painted	Mean
T6 (May 2016)	Propoxur WP (1000mg/m²)	18.3	27.0	8.5	4.9	14.7	5.0	3.3	53.3	28.3	22.5
	Propoxur WP (2000mg/m²)	55.7	45.9	93.2	100	73.1	23.3	36.7	95	100	63.8
	Actellic 300 CS (1000mg/m²)	67.8	83.1	54.8	68.3	68.5	73.3	80	6.7	10.0	42.5
	Bendiocarb WP (400mg/m²)	18.3	30.0	19.7	62.1	32.5	8.3	16.7	5.0	40.0	17.5
T7 (June 2016)	Propoxur 2000mg/m²	ND	ND	82.8	83.3	83.1	ND	ND	76.3	53.3	65.8
	Actellic 300 CS 1000mg/m²	ND	63.0	ND	ND	63	ND	83.3	ND	ND	73.2

FIGURE F1. RESULTS OF DECAY RATE MONITORING IN 2 SITES IN ETHIOPIA