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

MAY 2017 – APRIL 2018

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ACRONYMS

AIRS	Africa Indoor Residual Spraying
BBI	Bovine Blood Meal Index
CDC	Centers for Disease Control and Prevention
DDT	Dichlorodiphenyltrichloroethane
ELISA	Enzyme-linked Immunosorbent Assay
EPHI	Ethiopia Public Health Institute
FMOH	Federal Ministry of Health
HBI	Human Blood Meal Index
HLC	Human Landing Catch
IRMMS	Insecticide Resistance Monitoring and Management Strategy
IRS	Indoor Residual Spray
LLIN	Long-lasting Insecticidal Net
ND	No Data
PBO	Piperonyl Butoxide
PCR	Polymerase Chain Reaction
PMI	President's Malaria Initiative
POR	Possibility of Resistance
PSC	Pyrethrum Spray Catch
R	Resistance
RR	Homozygous Resistant
RS	Heterozygous Resistant
S	Susceptible
SNNPR	Southern Nations Nationalities and People Region
SS	Wild Type Susceptible
WHO	World Health Organization

EXECUTIVE SUMMARY

BACKGROUND

The President's Malaria Initiative (PMI) Africa Indoor Residual Spraying (AIRS) project conducted entomological monitoring from May 2017 through April 2018. The activity included monthly collection of data on vector density and species composition to help understand the abundance, seasonal patterns, biting behavior, sporozoite infection, blood meal sources, 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 Centers for Disease Control and Prevention (CDC) light traps were carried out in three PMI AIRS project sites (Abaya and Nono districts in Oromia region, and Bambasi in Benishangul-Gumuz region) and four non-PMI/AIRS project sites. All three PMI AIRS monitoring districts were sprayed with pirimiphos-methyl (Actellic 300 CS. Monitoring was also conducted in Dangur-Benshangul, an agricultural development district, by Addis Ababa University. Insecticide susceptibility using World Health Organization (WHO) tube tests was conducted in 12 sentinel sites to determine the response of the main malaria vector to different insecticides used for IRS. Susceptibility testing was also conducted on the new-generation insecticides clothianidin (2 districts) and chlorfenapyr (1 district), using WHO tube test and CDC bottle bioassay methods, respectively. The project also conducted wall bioassays to evaluate the quality of spray and monitor the decay rate of pirimiphos-methyl IRS in four selected districts; a total of 48 houses, 12 houses per sentinel site, were monitored for residual life. Molecular species identification, detection of alleles of knockdown resistance (*kdr*), and detection of blood meal sources and sporozoite infections were conducted by Jimma University.

RESULTS

Vector density and species composition: During the 12 rounds (months) of the study, a total of 1,838 adult female *Anopheles* mosquitoes were collected using PSC, HLC, and CDC light traps. *An. gambiae* s.l. accounted for 42.9 %(n=789) of all *Anopheles* collected and it was the most prevalent species in all the three sites. On average, vector density was low when baseline data were collected in the month of May; it increased in the month of June immediately after the IRS operation and then decreased for three subsequent months post IRS (July, August, and September) most likely due to the impact of the IRS. May is usually a dry season and the low vector density noted at baseline could be attributed to the absence of breeding sites. June is when the rainy season starts in most places in Ethiopia. However, the more likely reason for the increase in vector density in June was that spraying started in that month and entomological surveillance data were collected before the IRS activity had an impact on the overall vector population.

Feeding time and location: Based on the data collected from May 2017 through April 2018, the main malaria vector, *An. gambiae* s.l. (presumably *An. arabiensis*), tended to bite throughout the night with peak biting times varying by site. In Abaya, biting activity was generally low and did not vary markedly from 7:00 pm to 2:00 am. In Bambasi, peak biting activity was recorded after midnight, whereas in Nono, early biting activity was witnessed, with peak biting activity occurring between 8:00 pm and 9:00 pm. The proportion of indoor to outdoor collection for the main vector, *An. gambiae* s.l., in the three areas was 146 (27.28) and 389 (72.71%), respectively, indicating a tendency toward outdoor feeding (exophagy).

Parity rate: Due to the varying number of mosquitoes obtained in different months and sites, as well as small sample sizes, no clear trend was observed in the parity rate.

Susceptibility test: *An. arabiensis* populations from all 12 test sites were fully susceptible to pirimiphos-methyl. They were also susceptible to bendiocarb and to propoxur in 11 of 12 sites. In Omonada, suspected resistance to bendiocarb and resistance to propoxur was noted with a test mortality of 91% and 86%, respectively. The population of *An. arabiensis* from all study sites was resistant to permethrin with a percent mortality of 10%–89%. It also was resistant to deltamethrin in 11 sites; the exception was the BahirDar site, where suspected resistance to deltamethrin was recorded with a test mortality rate of 93%. Low resistance intensity to deltamethrin was recorded in Babile, moderate resistance intensity in Abobo, Halaba, and Selekleka, and high resistance intensity in Abaya, Omonada, Zeway, Amibara, and Humera. Low resistance intensity to permethrin was recorded in four sites (Omonada, Babile, Jinka, and Amibara), and moderate resistance intensity to permethrin was recorded in six sites (Abaya, Zeway, Abobo, Selekleka, Humera, and Halaba). No high intensity resistance to permethrin was noted. Pre-exposure to piperonyl butoxide (PBO) restored susceptibility to deltamethrin in seven of nine sites (98.7%–100% mortality) and permethrin in six of nine (98%–100% mortality) sites. Pre-exposure to PBO improved test mortality rates compared with no pre-exposure at the remaining tests sites but didn't restore susceptibly to deltamethrin in two of the nine sites (59.2%–96% mortality) or to permethrin in three of the nine sites (82.9%–93.3% mortality). Molecular tests conducted on surviving and dead *An. arabiensis* from susceptibility tests of DDT and deltamethrin revealed higher frequency of resistant *kdr* gene in surviving mosquitoes. The number of mosquitoes with homozygous resistant *kdr* gene was more than the heterozygous resistant gene.

Mosquito net cone bioassay: All (100%) laboratory-reared *An. arabiensis* were killed when exposed to new long-lasting insecticidal nets (LLINs): deltamethrin-treated PermaNet® 2 and alphacypermethrin-treated MAGNet™ nets. In contrast, average mortality of wild *An. gambiae* s.l. exposed to these two LLINs was low, 78.8% for PermaNet® 2.0 nets and 81.5% for MAGNet™ nets.

Wall bioassay test: High *An. arabiensis* mortalities were observed at time zero and one month after spray,. The mean mortality of mosquitoes in Bako was below 80% on mud surfaces two months after spray, and four months on both dung and painted surfaces. The residual life was four and six months for mud and painted surfaces, respectively, in Chewaka. But the residual life remained high in Goro and Nono, where mean mortality after four months was 97.8% and 99.5%, respectively. Cone bioassay test mortality rates dropped below 80% six months after spraying in Goro and were right on 80% in Nono for mud surfaces at six months. Mortality on mud wall surfaces was generally lower than on dung and painted surfaces.

Sporozoite ELISA: A total of 5,423 *An. gambiae* s.l. from 13 sites were ELISA tested for circumsporozite proteins. *Plasmodium vivax* infections were detected in one out of 342 specimens (0.3%) from Arorsha, Alamata and two out of 805 specimens (0.25%) from Kurkura, Alamata. A single specimen out of 992 from Shelle in Arbaminch was positive for *P. falciparum* infection giving the area a 0.1% infection rate. The overall infection rate of *P. vivax* for the 13 sites was 0.06% (3/5423) while that of *P. falciparum* was 0.02%.

Blood meal ELISA: The number of *Anopheles gambiae* s.l. that took blood meals from bovine hosts were higher than the number feeding off humans suggesting the preference of this species to feed more on cattle. The human blood index ranged from the low of 9.8% in Shelle-ArbaMinch to the highest 55.6% in Abaya. The lowest bovine blood index (34.2%) was from Babile while the highest (68.3%) was from Arorsha. Mixed blood meals were common in all sites.

Molecular identification of *An. gambiae* s.l.: Out of 887 *An. gambiae* s.l. mosquitoes collected from 11 study sites and characterized using polymerase chain reaction, 835 (94.1%) were successfully identified as *An. arabiensis*. The remaining mosquitoes failed to amplify for this species and *An. amharicus* probably because of deterioration and low quality of DNA or mis-identification of mosquitoes in the field.

CONCLUSIONS

Based on the rainy season and vector density, early June appears to be the right time to conduct IRS. Insecticide resistance data collected in 2017 indicated that the primary vector, *An. arabiensis*, is fully susceptible to pirimiphos-methyl. The vector is also susceptible to carbamates in 10 of the 11 sites tested. These two classes of insecticides can potentially be used for IRS in Ethiopia. Resistance to pyrethroids is widespread and the strength of resistance is increasing; the extent of this resistance on the efficacy of interventions, especially on LLINs, needs to be assessed.

1. INTRODUCTION

In Ethiopia, the President's Malaria Initiative (PMI) Africa Indoor Residual Spraying (AIRS) project conducts a number of entomological activities to provide data for vector control programming. Its activities include monitoring the temporal and spatial variation in vector density and behavior, vector composition, susceptibility to different insecticides; synergist assays to assess the involvement of metabolic resistance mechanisms; intensity assays to assess the strength of resistance; and cone bioassays to assess the quality of spray operations. The results from susceptibility tests provide information on the levels of resistance of local vectors to approved insecticides for indoor residual spraying (IRS) countrywide in line with the Insecticide Resistance Monitoring and Management Strategy (IRMMS), and they form the basis for selection of insecticides for IRS. The PMI AIRS project is further engaged in capacity building to enhance the knowledge and skills of entomologists/technicians at different levels, enabling them to carry out entomological monitoring activities in selected sentinel sites across the country. Currently, the project works closely with six national universities, the Federal Ministry of Health (FMOH), and the Ethiopia Public Health Institute (EPHI).

This entomological final report summarizes activities conducted by AIRS Ethiopia from May 2017 to April 2018 including:

- Pre/post IRS vector monitoring in three new sentinel sites (Abaya and Nono in Oromia region, and Bambasi in Benishangul-Gumuz region) and vector behavioral studies in five sites by collaborating universities;
- Vector behavior studies in Dangur (Benishangul-Gumuz region), an agricultural development site;
- Insecticide susceptibility tests using the World Health Organization (WHO) tube test;
- Net-bioassay using the WHO cone test;
- Assessment of quality of spray operations and decay rate of pirimiphos-methyl (Actellic 300 CS); and;
- Training of staff from six universities, the FMOH, and the EPHI on WHO tube test guidelines as part of the project's capacity-building effort.

From May 2017 through April 2018, AIRS Ethiopia carried out entomological monitoring studies, insecticide resistance testing, and cone bioassays (wall and mosquito net assays) in order to monitor the efficacy of vector control on malaria transmission in the PMI/AIRS project and non-project areas. The objectives of the entomological studies were to:

- Determine *Anopheles* species composition;
- Monitor vector density and behavior;
- Assess susceptibility of the main malaria vector to different insecticides;
- Assess quality of spray operations and decay rate of insecticides;
- Assess the insecticidal activity of the mosquito nets through cone assays; and
- Train university staff on basic malaria entomology as part of project capacity building.

2. METHODOLOGY

2.1 STUDY SITES

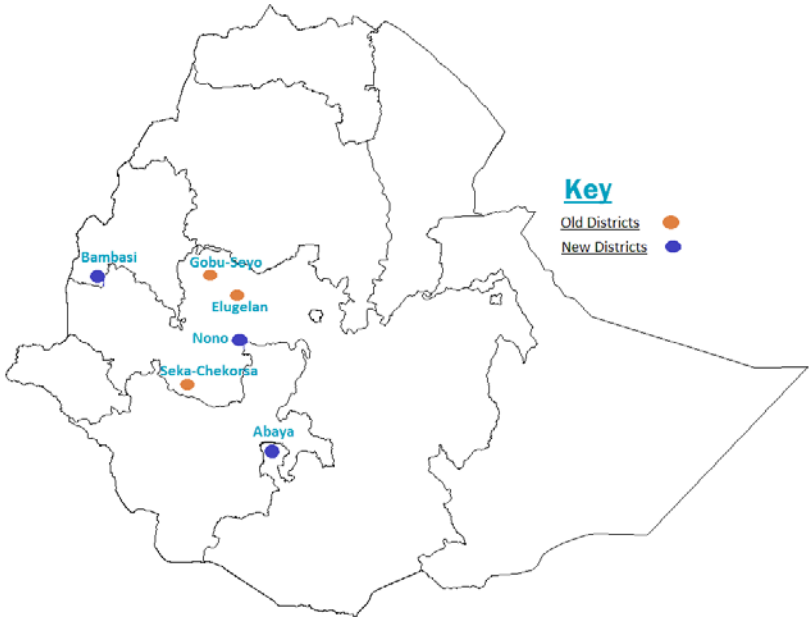
2.1.1 VECTOR DENSITY AND BEHAVIORAL STUDY (JUNE 2016–MAY 2017)

Entomological data were collected from Elu-gelan, Gobu Sayo, and Seka Chekorsa districts as part of the 12-month longitudinal study that started in June 2016. The data collected from January through May 2017 were incorporated into the PMI AIRS annual entomology report for 2016 and are not part of this report.

2.1.2 VECTOR DENSITY AND BEHAVIORAL STUDIES IN AIRS PROJECT DISTRICTS

Following the completion of the longitudinal study described above in Section 2.1.1, entomological monitoring activities began in May 2017 in three new intervention districts, Abaya and Nono in Oromia region and Bambasi in Benishangul-Gumuz region (Figure 1). Twelve rounds of entomological data collection were conducted on a monthly basis in the three sentinel sites from May 2017 to April 2018.

Figure 1: Old and New Sentinel Sites for Monitoring Vector Density and Behavior



2.2 Collection Methods

Three mosquito collection methods were implemented during the study period.

2.2.1 Human Landing Catches

Human landing catches (HLC) were conducted in the same two houses in each sentinel site for two nights per month. The two nights were different for the two houses; thus, data were collected on four nights per site per month. One mosquito collector was seated indoors and another seated outdoors from 6:00 pm to 6:00 am to collect blood-seeking mosquitoes. Outdoor mosquito collection was carried out about eight meters from the sampled houses. A team of two collectors was assigned to six-hour shifts, meaning a total of four collectors per house per night covered the 12 hours of collections from 6:00 pm to 6:00 am. Outdoor and indoor collectors switched houses every hour. Collectors adjusted their clothing so that their legs were exposed up to the knees. When they felt a mosquito, they quickly turned on the torch, collected the mosquito with the sucking tube, and transferred the mosquito 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 (Verrone 1962; Gilles and Coetzee 1987).

2.2.2 Pyrethrum Spray Catch

Pyrethrum spray catch (PSC) was used to sample indoor resting mosquitoes in the same 20 houses in each of the study sites every month. Collections were carried out in the morning between 6:00 am and 7:30 am. 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 had slept in the house the previous night and the number of treated nets present. 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 an aerosol (Baygon) knockdown spray. Ten minutes after spraying, collectors gathered from the sheets all the mosquitoes that had been knocked down and sorted them by species. The abdominal status of all female anophelines was determined, and individual specimens were recorded as unfed, blood-fed, half-gravid, or gravid females.

2.2.3 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 above the floor and about 50 centimeters away from a human sleeping under a bed net. The light traps were fitted with an incandescent bulb. The traps were set from 6:00 pm to 6:00 am. Mosquitoes were collected from the traps the next morning and sorted at the field lab.

2.3 Identification of Malaria Vectors, Infections, and Blood Meal Sources

Anopheles mosquitoes collected through HLC, PSC, and CDC light traps were preliminarily identified to the species level morphologically. All *Anopheles* specimens were labeled and stored individually in Eppendorf tubes on silica gel for further processing to detect sporozoite infection, identify the source of blood meals and species identification through polymerase chain reaction by Jimma University.

2.3.1 Sporozoite ELISA

Sporozoite ELISA technique was used to detect circumsporozoite proteins in *Anopheles* mosquitoes known or suspected to be vectors of malaria in Ethiopia as described by Wirtz *et al.* (1987).

2.3.2 BLOOD MEAL ELISA

Sources of blood meals of *An. gambiae* s.l. were assessed using blood meal ELISA following the method developed by Beier *et al.* (1988) and classified as human, bovine or mixed (human and bovine). Based on the results, the human blood index (HBI) and the bovine blood index (BBI) were determined. When mixed blood meals were detected, it was added to both human and bovine data.

2.3.3 MOLECULAR IDENTIFICATION OF ANOPHELES GAMBIAE S.L.

Sub samples of *Anopheles gambiae* s.l. from entomological monitoring was investigated using polymerase Chain reaction method as described by Scott et al. (1993).

2.4 DETERMINATION OF PARITY

Ovaries of unfed *An. arabiensis* females from HLC were dissected under a dissecting microscope to determine parity rate based on coiling of ovarian tracheoles (Detinova 1962). Ovaries on a slide with a drop of water were removed from the abdomen using dissecting needles.

2.5 SUSCEPTIBILITY TEST WITH DISCRIMINATING CONCENTRATIONS

The standard WHO tube test was used to test susceptibility of *An. arabiensis* to seven insecticides recommended for malaria vector control (deltamethrin, permethrin, pirimiphos-methyl, bendiocarb, propoxur, malathion, and DDT) (WHO 2016). The tests took place in 12 sites (Abaya, Abobo, Amibara, Babile, Bahirdar, Halaba, Humera, Metema, Omonada, Selekleka, Jinka and Ziway Dugda). Four replicates of about 25 non-blood-fed, 2–3-day-old female mosquitoes were exposed to insecticide-impregnated papers for one hour. Control mosquitoes were exposed to oil-impregnated papers. The number of knocked-down mosquitoes were recorded after 60 minutes of exposure. Mortality counts were taken after 24 hours of 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.

2.6 INTENSITY ASSAYS

An. arabiensis from all 12 sentinel sites that were tested against diagnostic concentrations of deltamethrin and permethrin were resistant or had suspected resistance to the two insecticides. The same vector populations were tested against high concentrations of deltamethrin and permethrin, 5x and 10x, using the WHO tube test in a stepwise manner to assess the strength or intensity of resistance. Mosquitoes reared from field-collected larvae or pupae were used for the tests. Four replicates of about 25 non-blood-fed, 2–3-day-old mosquitoes were exposed to 5x or 10x insecticide-impregnated papers for one hour. Simultaneously, control mosquitoes were exposed to oil-impregnated papers. Mortality counts were taken after 24 hours of the holding period. The test was conducted according to the WHO test procedure for intensity assays and the result was interpreted as low, moderate, or high resistance (WHO 2016).

2.7 SYNERGIST ASSAYS

When pyrethroid resistance has been detected in *An. arabiensis*, a synergist assay is used to assess the involvement of metabolic resistance mechanisms in the expression of resistance phenotypes. The assay measures the effect of pre-exposure to a synergist on the expression of insecticide resistance. Synergists are available for certain metabolic detoxification enzyme groups including esterases, oxidases, and glutathione *S*-transferases. In all areas, we used the synergist piperonyl butoxide (PBO), which can synergize the effects of pyrethroid insecticides by reducing or nullifying the detoxifying capabilities of

enzymes, primarily monooxygenases for pyrethroid insecticides. Partial or complete mitigation of the expression of a resistant phenotype implies that a monooxygenase-based detoxification system is primarily responsible for the resistance in the absence of PBO. The test was conducted according to the WHO test procedure for synergist assays (WHO 2016).

2.8 MOLECULAR DETECTION OF KDR ALLELES

Surviving and dead *An. arabiensis* from DDT and deltamethrin susceptibility tests were examined by PCR to detect *kdr* alleles. The primers used were Agd1, Agd2, Agd3, Agd4 and Agd5 to detect the west and east African point mutations (Martinez-Torres, 1998; Ranson et al., 2000). The proportion of mosquitoes with homozygous resistant (RR), heterozygous resistant (RS) and wild type susceptible (SS) alleles was determined.

2.9 NET ASSAY

Bioassays were performed to measure the response of *An. gambiae* s.l. to pyrethroid-treated impregnated nets. Five 30cm x 30cm pieces of net were cut from five sides of new long-lasting insecticidal nets (LLINs) (PermaNet® 2.0, impregnated with deltamethrin (55mg/m^2) and MAGNet™, impregnated with alphacypermethrin (5.8g/kg±15%)) and used in the test.

Five wild, non-blood-fed, 2–5-day-old female *An. gambiae s.l.* mosquitoes were placed in a standard WHO cone and exposed to a piece of net for three minutes. Up to four cones at a time were attached to a piece of net. Knock-down was recorded 60 minutes after exposure and mortality after a 24-hour holding period. Cotton wool soaked in 10% sugar solution was provided during the holding period. The procedure was repeated until a total of 50 mosquitoes were exposed to each piece of net. Mosquitoes exposed to untreated nets were used as controls. Susceptible *An. arabiensis* mosquitoes from Adama Insectary were used as reference.

2.10 QUALITY ASSURANCE AND DECAY RATE

Cone bioassay tests were conducted to determine the quality of IRS and rate of decay of insecticide on the sprayed walls in four sites, two each from district-based and community-based IRS districts within two weeks of the start of IRS and continued monthly. All project districts were sprayed with pirimiphos-methyl (Actellic 300 CS) from June 12, 2017, for 31 days.

The tests were performed in 12 houses per site, which were purposely selected to represent different wall types and structures sprayed by different spray operators. A total of 48 houses were sampled in the four sites. The tests were carried out using known susceptible *An. arabiensis* colonies reared at the Adama Malaria Control Laboratory and Jimma University insectaries; 2–3-day-old sugar-fed adults were exposed to the sprayed walls in the selected houses. Three cones were fixed per house at the height of 0.5 m, 1.0 m, and 1.5 m and 10 mosquitoes were introduced to each cone , giving a total of 30 test mosquitoes per house. Mosquitoes were then exposed for 30 minutes and the number of knocked down mosquitoes were counted immediately after transferring to paper cups. Another count was also taken after 30 minutes. Temperature and relative humidity were recorded at every house where mosquitoes were exposed.

3. RESULTS

3.1 ANOPHELES SPECIES DIVERSITY, BEHAVIOR, AND ABUNDANCE

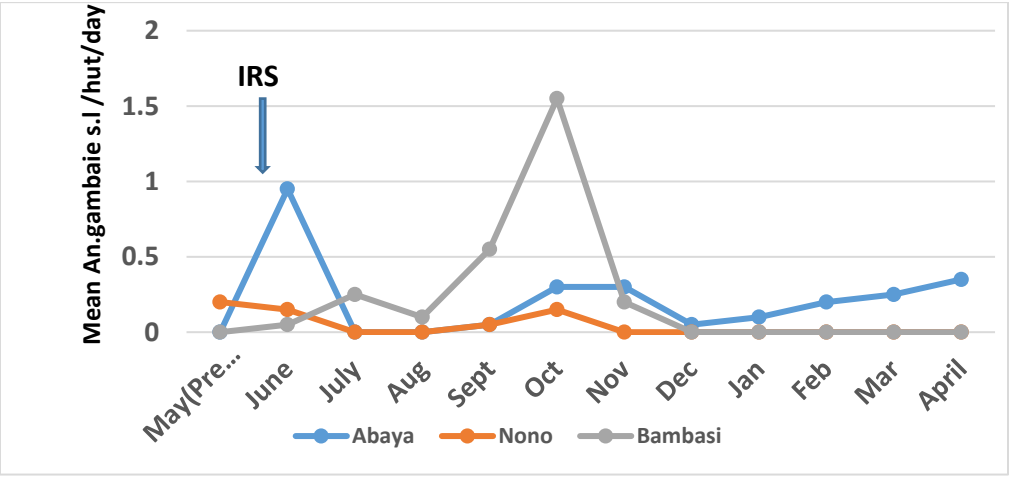
3.1.1. SPECIES COMPOSITION AND SEASONALITY

During the 12 rounds of the vector surveillance from May 2017 to December2018, a total of 1,838 adult female *Anopheles* mosquitoes were collected using PSC, HLC, and CDC light traps. *An. arabiensis* (n=789) was the most prevalent species in all the three sites (see Annex A). In addition, 5,572 *Culex* mosquitoes were captured using the three different collection techniques.

3.1.1.1 PYRETHRUM SPRAY CATCH

Pyrethrum spray catch (PSC) results (Annex A and Figure 2) indicate that mean indoor resting vector density was generally low in the new surveillance sites: fewer than two mosquitoes per house per day were recorded during the surveillance period. Pre-spray mean indoor resting density of female *An. gambiae* s.l. ranged from 0 per house per day in Abaya and Bambasi to 0.2 female *An. gambiae* s.l. per house per day in Nono. The overall mean indoor resting densities at 12 months post spray were 0.23, 0.03, and 0.25 female *An. gambiae* s.l. per house per day in Abaya, Nono, and Bambasi sentinel sites, respectively. Vector density appeared to have declined immediately after spraying, remained low for three months (July–September) and increased in October and November). Overall lower vector density was recorded in May (pre-spray) than in post spray months. This is most likely explained by absence of breeding sites in May (dry season) compared to the subsequent months of June to October (wet season). This is most likely explained by absence of breeding sites in May (dry season) compared to the subsequent months of June to October (wet season). An increasing trend in indoor density was observed in Abaya from January through April 2018, which could be attributed to the presence of rain in April in the area unlike other sites.

Figure 2: PSC Collections in Abaya, Nono, and Bambasi Intervention Sites (May 2017 – April 2018)[1]

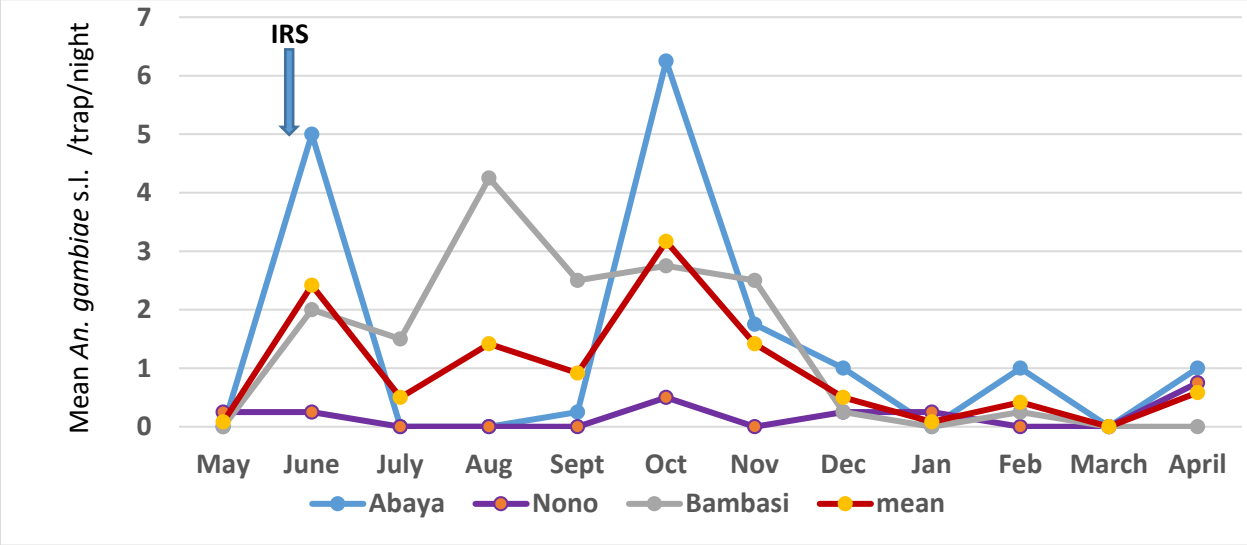


[1] Data from Nono is not apparent on the graph past December because the results are identical to Bambasi (no mosquitoes recorded) and the trend lines are overlapping.

3.1.1.2 CDC LIGHT TRAPS

Figure 3 summarizes CDC light trap results. The mean density of female *An. gambiae* s.l. mosquitoes collected in the month of May (pre-spray) was 0.25 mosquito per trap nights in Nono. No vector mosquito was collected from either Abaya or Bambasi sentinel sites in May. The vector density remained low without much fluctuation in Nono throughout the monitoring period. In Bambasi, *An. gambiae* s.l. density increased until it peaked in August, after which it declined possibly because the rainy season had ended. In Abaya, vector density sharply increased in June when IRS was in progress, then declined and remained low for three months after spraying possibly due to the impact of IRS. It suddenly increased in October possibly due to the decay rate and loss of efficacy of the sprayed insecticide. The decline in density in the months of November and December and low vector density observed through April could be attributed to the end of the wet season and absence of breeding sites.

Figure 3: CDC Light Trap Collections in Abaya, Nono, and Bambasi Intervention Sites (May 2017 – April 2018)



3.1.1.3 HUMAN LANDING CATCH

During the study period, a total of 937 *Anopheles* mosquitoes were collected as they attempted to feed on human bait located indoors and outdoors. Of these, 535 were *An. gambiae* s.l., 535 *An. coustani,* 104 *An. pharoensis,* and 13 *An. demeilloni.* Figures 4–6 break down the indoor to outdoor collections for the main vector, *An. gambiae* s.l., in the three sentinel sites. Indoor and outdoor totals were 146 (27.3%) and 389 (72.7%), respectively, indicating a tendency for outdoor areas feeding (exophagy).

In the Abaya sentinel site (Figure 4), the indoor mean biting rate increased from 1.5 bites per person per night pre IRS (May) to 4.25 bites per person per night one week post spraying, before IRS could have an impact on the vector population. The indoor mean biting rates dropped for three subsequent months post IRS (July–September) with a range of 0–0.25 bites per person per night. The indoor biting rate increased again in the month of October, four months after spraying, most likely due to decay and reduced efficacy of IRS. A similar trend was observed in the mean outdoor biting rates with a higher frequency of biting than indoors, except in July and August. The biting rates were low during the dry season from January to March; however, an increase in April was observed due to the short rain season that created additional breeding sites.

In the Nono sentinel site (Figure 5), the indoor biting rates decreased for two consecutive months post IRS (June and July) probably due to the impact of IRS; the rates then increased from August through September. There was a gradual but continuous decrease in vector density from October to December, which stayed low until March. There was an immediate upsurge in vector density in April because of the rain in that month. The mean outdoor biting rate followed a similar pattern to the indoor rate, except that its peak was one month later, in September instead of August. The mean outdoor biting rates dropped starting in October and remained low until March, which was likely attributable to the ending of rainy season in November and decreased number of breeding habitats. However, in April the rate was slightly increased at the start of the small rainy season in April.

In the Bambasi sentinel site (Figure 6), the mean indoor and outdoor biting rates increased in June, with the beginning of the rainy season and before the impact of IRS was observed. Biting rates slightly decreased in the months post IRS (July and August). An increase in the mean biting rates was then observed in September and October, three and four months after spraying. We did not find a plausible explanation as to why the effect of IRS on the mean biting rates in Bambasi and Nono was lower and shorter lived than in Abaya. During the dry season from November to April the biting rates were low.

Figure 7 shows the number of *An. gambiae* s.l. collected monthly at the three sites using the three sampling methods.

Figure 4: HLC Collections in Abaya Intervention Site (May 2017 –April 201)

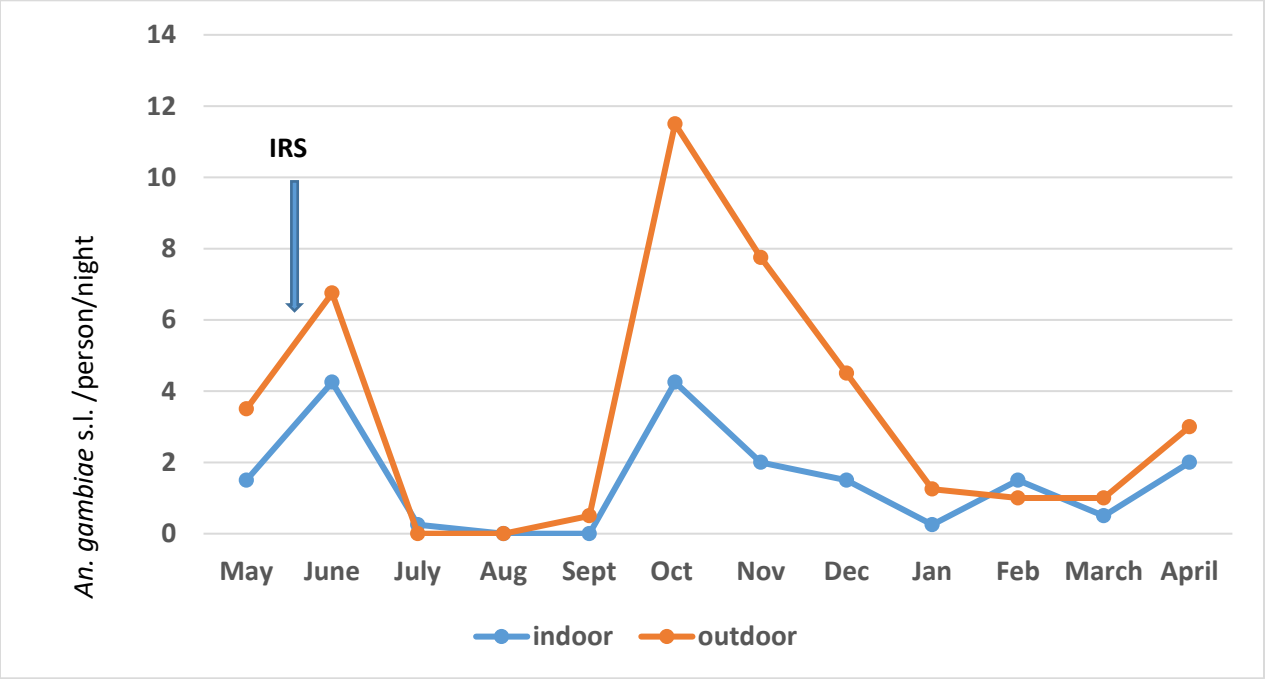


Figure 5: HLC Collections in Nono Intervention Site (May 2017 – April 2018)

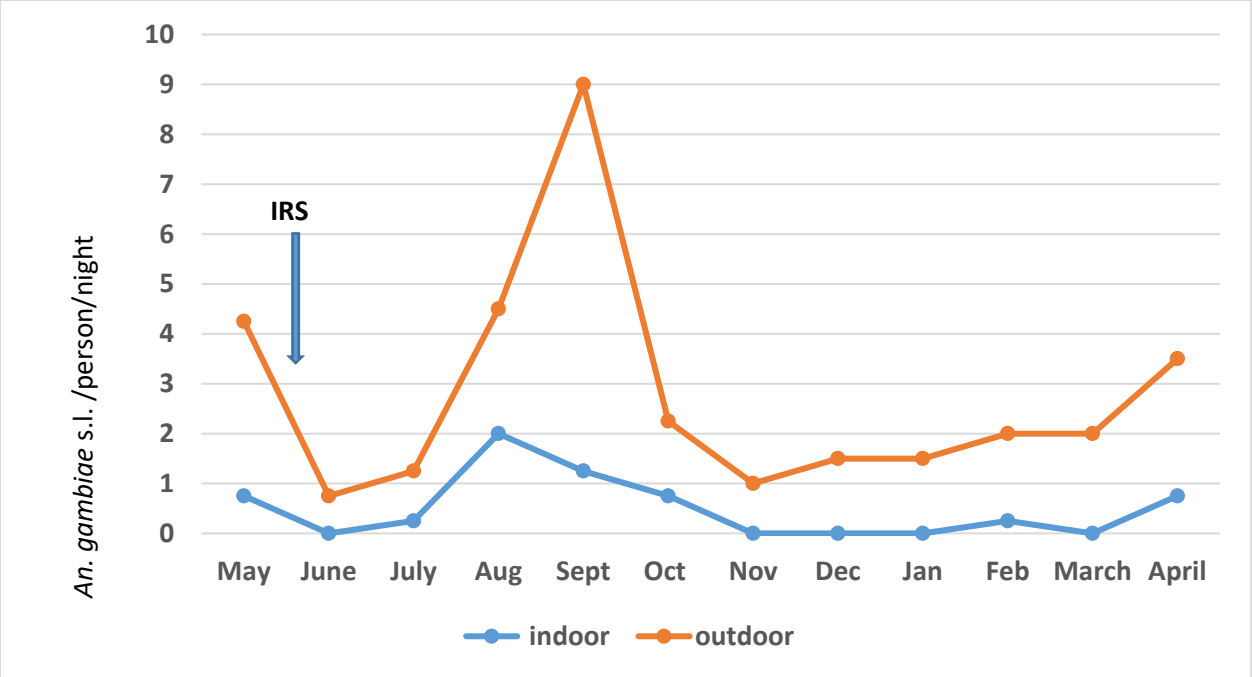
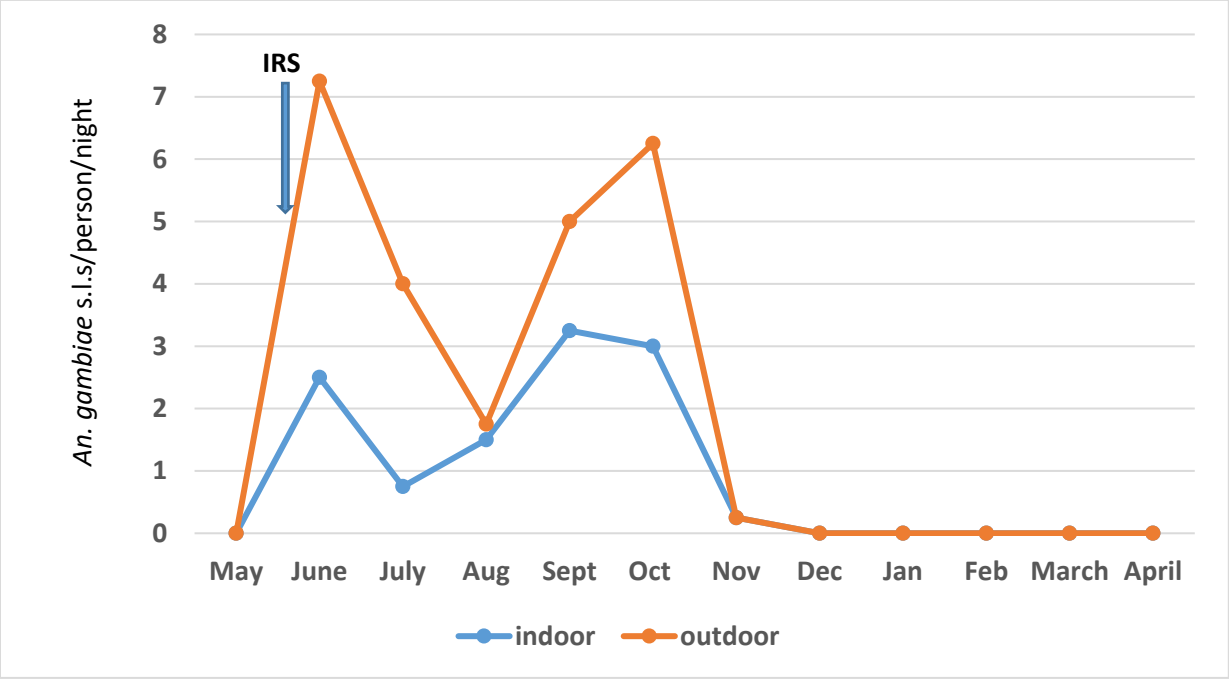
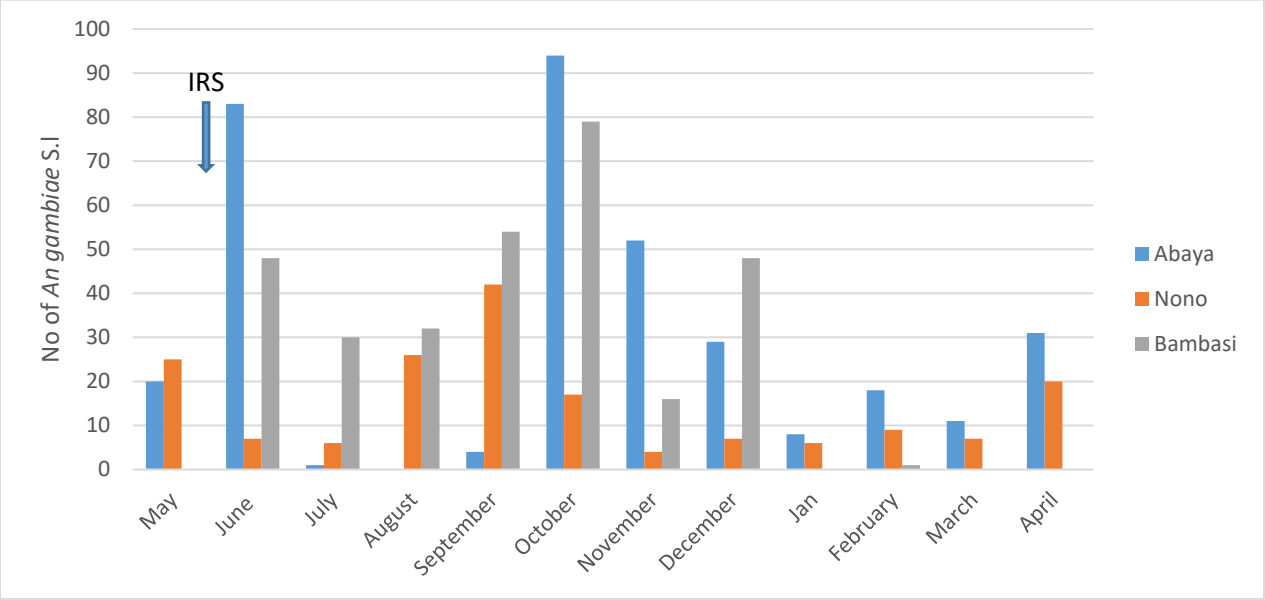


Figure 6: HLC Collections in Bambasi Intervention Site (May 2017 –April 2018)2



[2] Data from indoor collections is not apparent on the graph past December because the results are identical to outdoor collections (no mosquitoes recorded) and the trend lines are overlapping.

Figure 7: An. gambiae s.l. Collections in Abaya, Nono, and Bambasi Intervention Sites (May 2017 – April 2018)



Note: The three sites were sprayed using Actellic 300 CS.

3.2 Feeding Time and Location

Based on data collected from May 2017 to April 2018, the main malaria vector, *An. gambiae* s.l., tended to bite throughout the night with peak biting times varying between sites (Figures 8–10). In Abaya, biting activity was generally low and did not vary markedly from 7:00 pm to 2:00 am. The frequency of biting declined consistently after 2:00 am indoors; outdoors, it fluctuated somewhat. In Nono, early biting activity peaked between 8:00 and 9:00 pm, whereas in Bambasi, peak biting activity was recorded after midnight. Biting continued in all the three sites at low rates until 6:00 am, when collections stopped.

Figure 8: Biting Trends of An. gambiae s.l. in Abaya (May 2017 – April 2018)

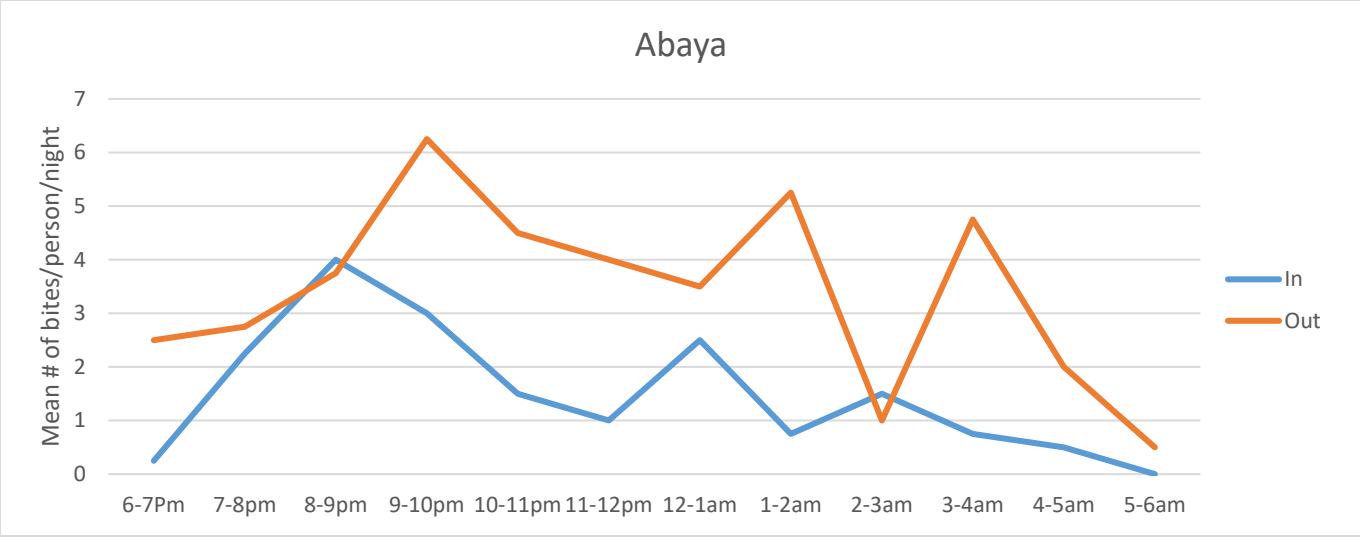


Figure 9: Biting Trends of An. gambiae s.l. in Bambasi (May 2017 – April 2018)

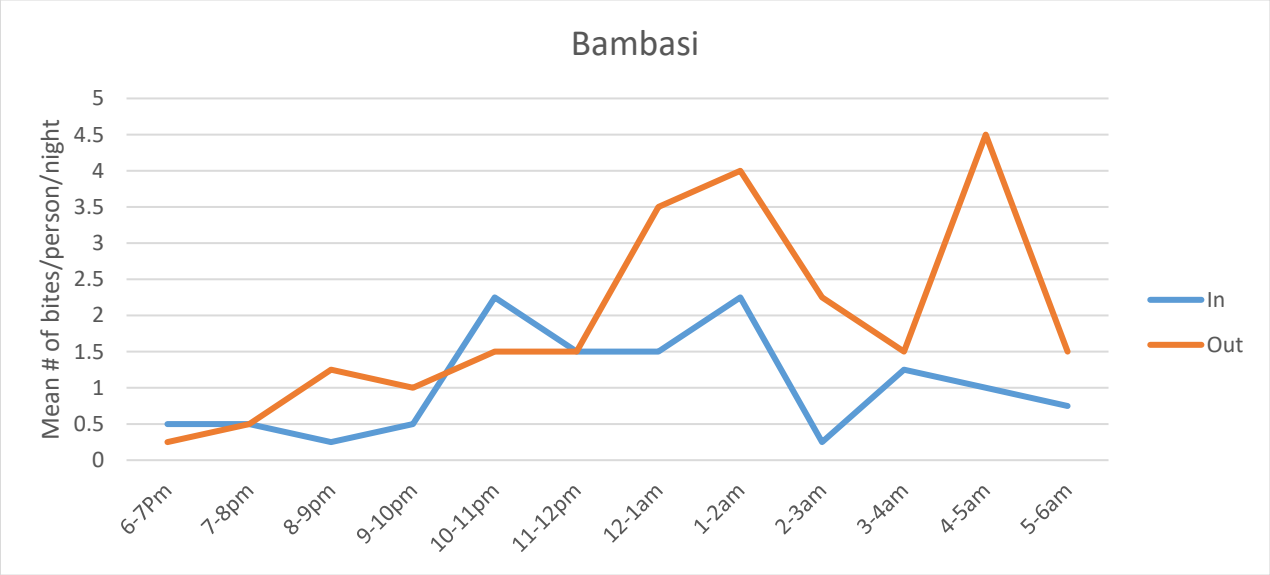
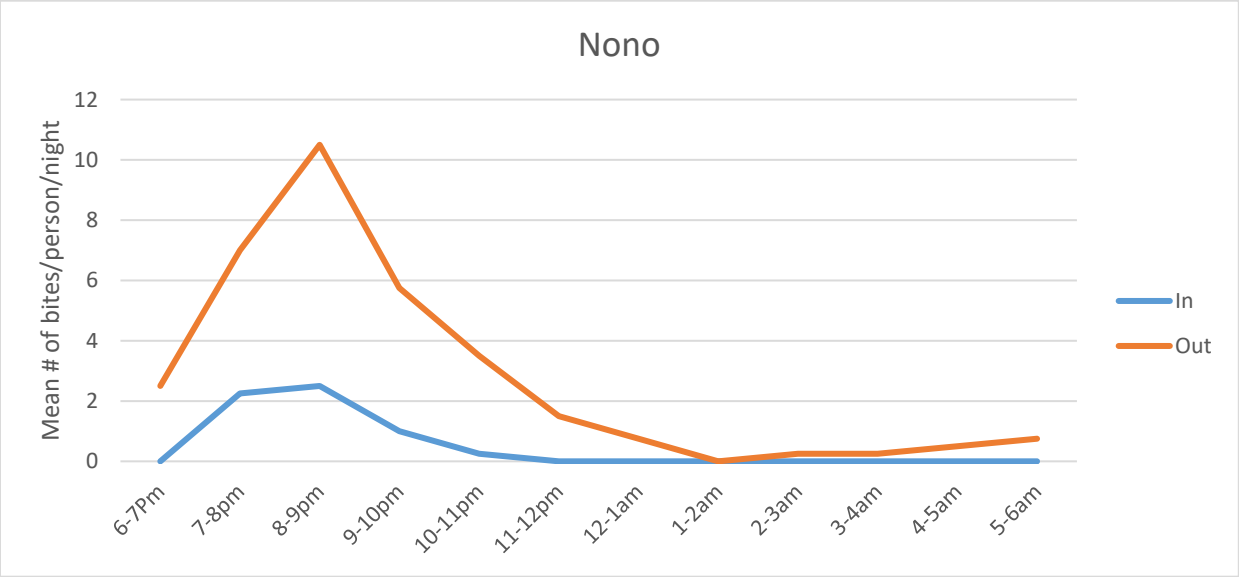


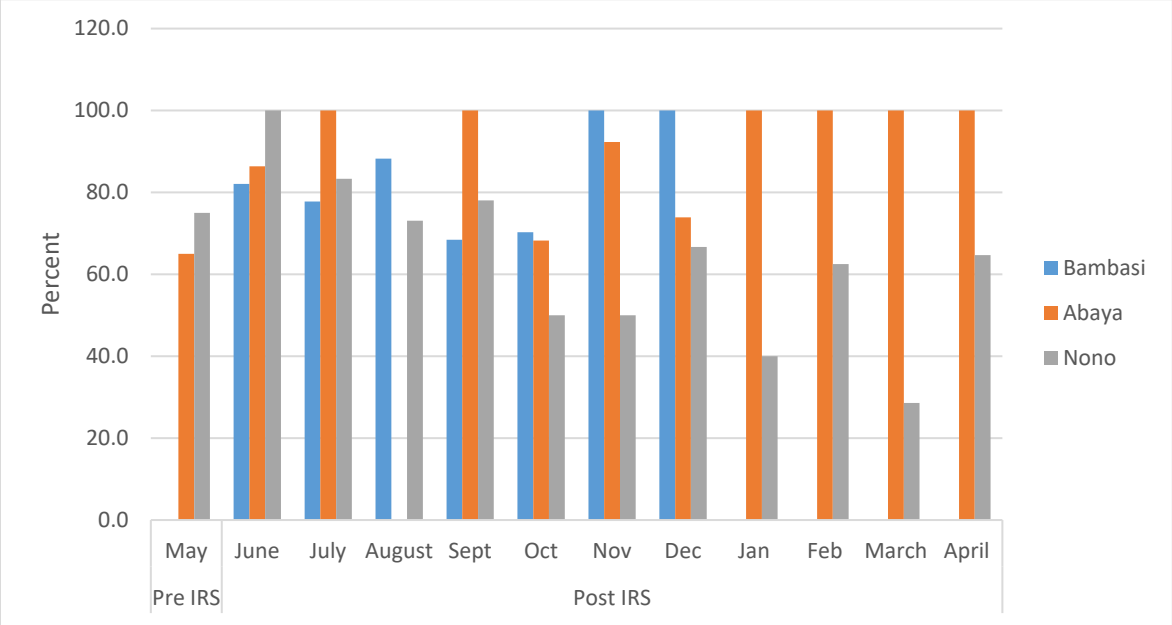
Figure 10: Biting Trends of An. gambiae s.l. in Nono (May 2017 – April 2018)



3.3 PARITY RATES

Ovary dissection was performed on all unfed female mosquitoes captured from HLC collections to determine parity rates (Annex B). The parity rate did not reflect any change between pre and post IRS. The number of mosquitoes collected was too low (ranging from 0 to 63 in all the sites and in different months) to conclude with confidence as to why the change was not significant (Figure 11).

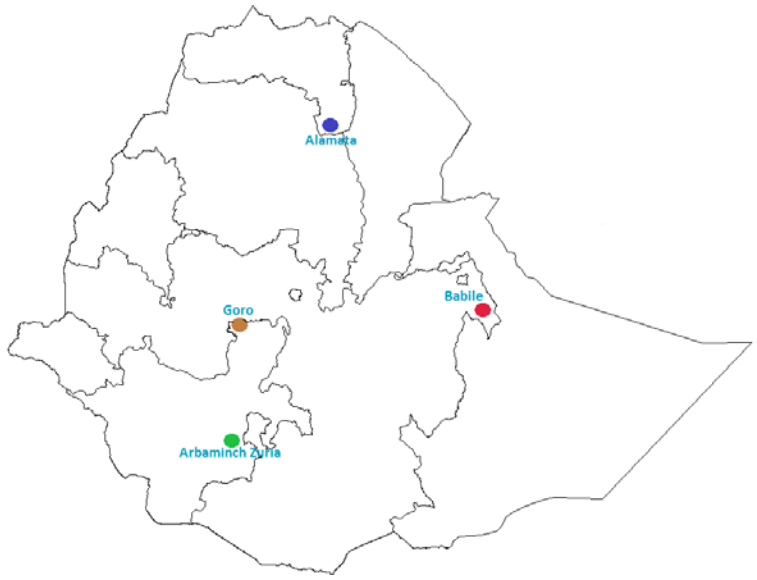
Figure 11: Parity Rates of Anopheles gambiae s.l. in Entomology Study Sites (May 2017 – April 2018)



3.4 Vector Density and Behavioral Studies Conducted in Non-AIRS Project Districts

As per the approved work plan of 2017, AIRS Ethiopia conducted entomological monitoring in collaboration with Jimma, Jigjiga, Arbaminch, Addis Ababa, and Mekelle universities at sites selected based on the FMOH's IRMMS. The study sites were the government IRS sites of Alamata (Tigray), Arbaminch (SNNPR), Babile (Oromia), and Goro (Oromia) as shown in Figure 12. IRS was carried out in Goro in June 2017 and in the other three sites in August 2017. Key entomological indicators, including vector density, distribution, and seasonality; vector feeding time and location; and vector resting behavior were assessed for six months (July–Dec 2017) using PSC, CDC light trap, and HLC collection methods..

Figure 12: Sentinel Sites for Vector Density Monitoring in Collaboration with Universities (July–Dec 2017)



A total of 4,660 adult female *Anopheles* mosquitoes were collected using the three collection methods (Table 1). *An. gambiae* s.l. was the most prevalent species in the four sites. *An. coustani* was collected from both Goro and Alamata, while *An. pharoensis*, *An. demeilloni, An. pretoriensis,* and *An. maculipalpis* were collected from Alamata only. Table 1 indicates the number and types of mosquito species collected in the non-AIRS project districts.

Table 1: Total Number of Mosquitoes Collected in Four Sentinel Sites of Universities (July–Dec 2017)

Species	Number collected
An. gambiae s.l.	4,660
An. pharoensis	90
An. demeilloni	41
An. coustani/ ziemanni	109
An. pretoriensis	1
An. maculipalpis	1
Culex spp	4,064

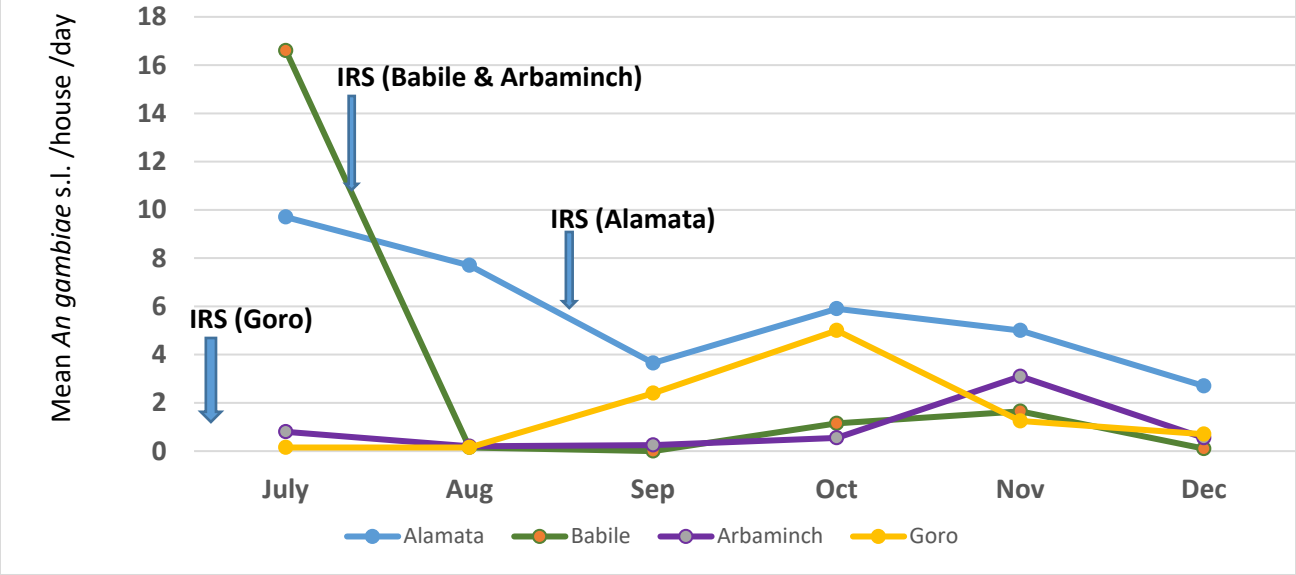
3.4.1 Pyrethrum Spray Collections

As shown in Figure 13, PSC results indicate that vector density was higher in the intervention sites of Babile and Arbaminch pre-spray (July) than in subsequent months (August–December). To assess the impact of IRS on vector density, mean *An. gambiae* s.l. pre spray was compared with five months of data post spray. For more detailed data, see Annex C.

In Babile, mean indoor resting density of female *An. gambiae* s.l. declined from 16.6 mosquitoes per house per day during the pre-spray period to 1.02 mosquitoes per house per day post spray. The mean density dropped dramatically in August, one month after spraying, and remained low throughout the subsequent five months. In Arbaminch, the mean indoor resting density of female *An. gambiae* s.l. per house per day was 0.8 during the pre-spray period. Following spraying, the mean vector indoor resting density dropped quickly with only 0.2 female *An. gambiae* s.l. per house per day recorded one month after spray. However, in November, four months after spray, female *An. gambiae* s.l. per house per day in Arbaminch increased to 3.1 – residual life seems to have lasted only three months. This might be attributed either to spray quality (under-dosing) or to factors related to wall type walls or other environmental factor/s that might degrade the sprayed insecticide quicker than expected.

In Alamata, where IRS took place in August, the mean indoor resting density per house per day during pre-spray was 8.7; this declined to 4.3 post spraying. In areas where baseline data collected in July after mosquito density had increased, a drop in mosquito density was observed immediately after spraying, most likely due to that spraying. In the Goro sentinel site, data collection started in July post spray. Goro was sprayed in June. There, vector density was low in July and August,increased in September, and peaked in October.

Figure 13. PSC Collections Arbaminch, Babile, Goro, and Alamata Intervention Sites (July–Dec 2017)

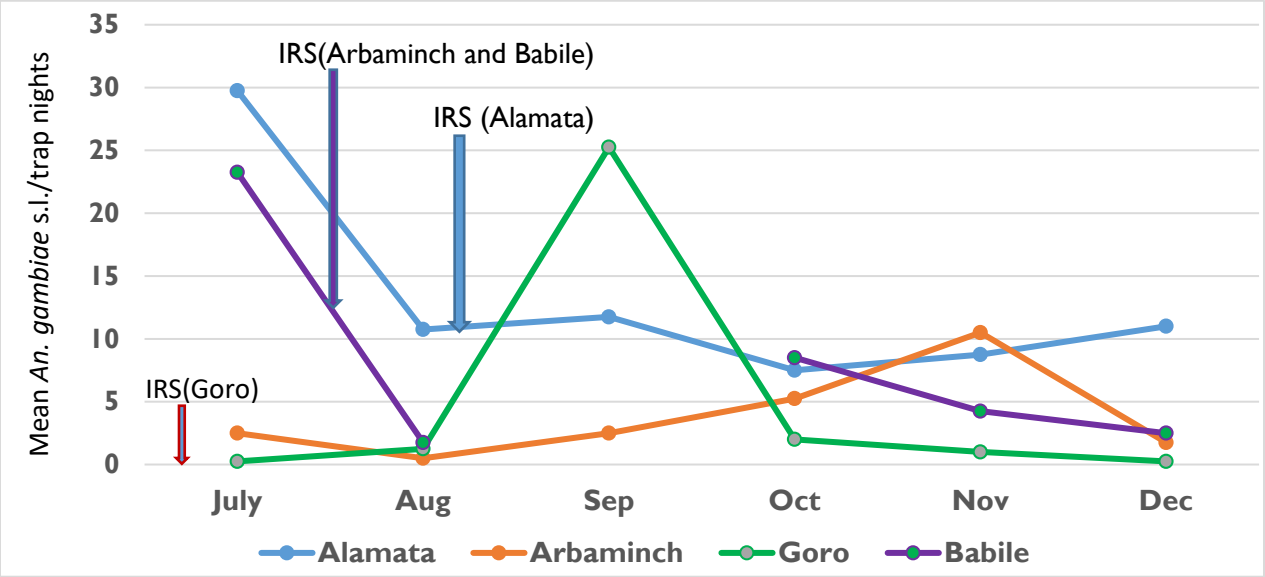


3.4.2 CDC Light Trap Collection

An. gambiae s.l. comprised 85.5% (n=691) of the total female *Anopheles* mosquitoes collected using CDC light traps in the non-PMI AIRS IRS sentinel sites. Vector density declined in August irrespective of the spray status (Figures 14). The density either gradually decreased or stayed around the same in Alamata and Babile. The situation was different in Arbaminch, where the vector density slightly decreased one month after spraying and showed an increasing trend for three subsequent months. There is no clear

explanation as to why such variation was observed. For more detailed data, see Annex C. In Goro, vector density was low for two consecutive months post spray, July and August, peaking in September.

Figure 14. CDC Light Trap Collections in Arbaminch, Babile, Goro, and Alamata Intervention Sites (July–Dec 2017)



3.4.3 Human Landing Catch

A total of 2,663 *Anopheles* mosquitoes were collected while attempting to feed on human bait. Of these, 2,585 were *An. gambiae* s.l., 28 An. *pharoensis, and* 50 *An. coustani.* The proportion of indoor to outdoor collection for the main vector, *An. gambiae* s.l., in the four areas were 706 (27.3%) and 1,879 (72.7%), respectively, indicating a high tendency toward outdoor feeding (exophagy). The difference in biting tendencies was statistically significant ($p<0.0001$).

In Arbaminch, the outdoor biting activity declined in August, one month after IRS and July baseline, but it gradually increased in the susequent months and peaked in November (Figure 15). The indoor biting rates were consistently low with minimum fluctuation in this site . In Goro (where, again, IRS took place in June), the biting activity was low for two consecutive months after IRS in July and August (Figure 16). It increased in September, then dropped considerably in October and stayed low until December. A similar bitting pattern was observed both indoors and outdoors in Goro, though the frequncy of biting was slighly higher outdoors. In Babile, the biting activity of *An gambiae s.*l. declined both indoors and outdoors in August then increased for two consecutive months in September and October outdoors and only for one month indoors, after which it declined (Figure 17). In Alamata, where IRS took place in August, the outdoor biting acivity was high in August and September, then dropped in October and remined low until December (Figure 18).

Figure 15. Biting Trends of *An. gambiae* s.l. in Arbaminch (July–Dec 2017)

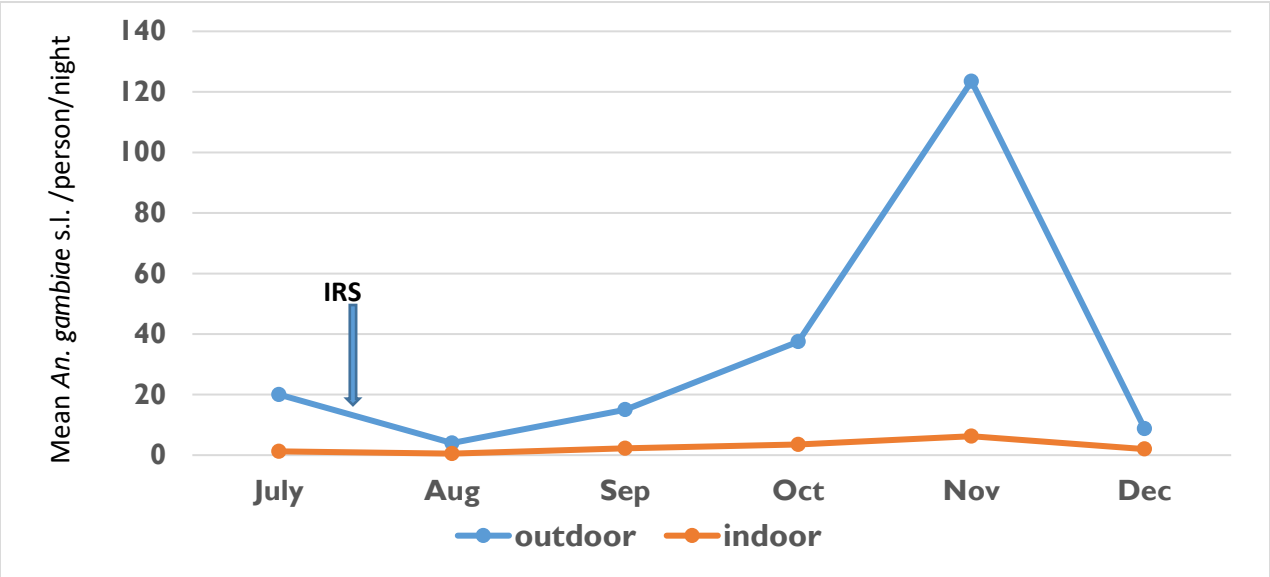


Figure 16. Biting Trends of *An. gambiae* s.l. in Goro (July–Dec 2017)

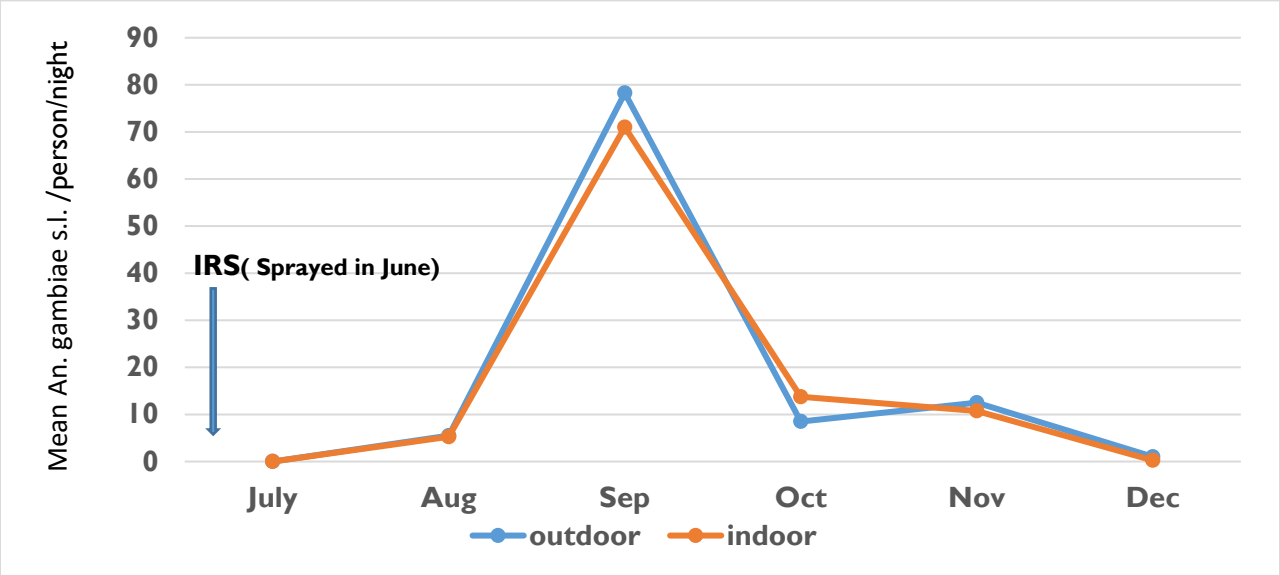


Figure 17. Biting Trends of *An. gambiae* s.l. in Babile (July–Dec 2017)

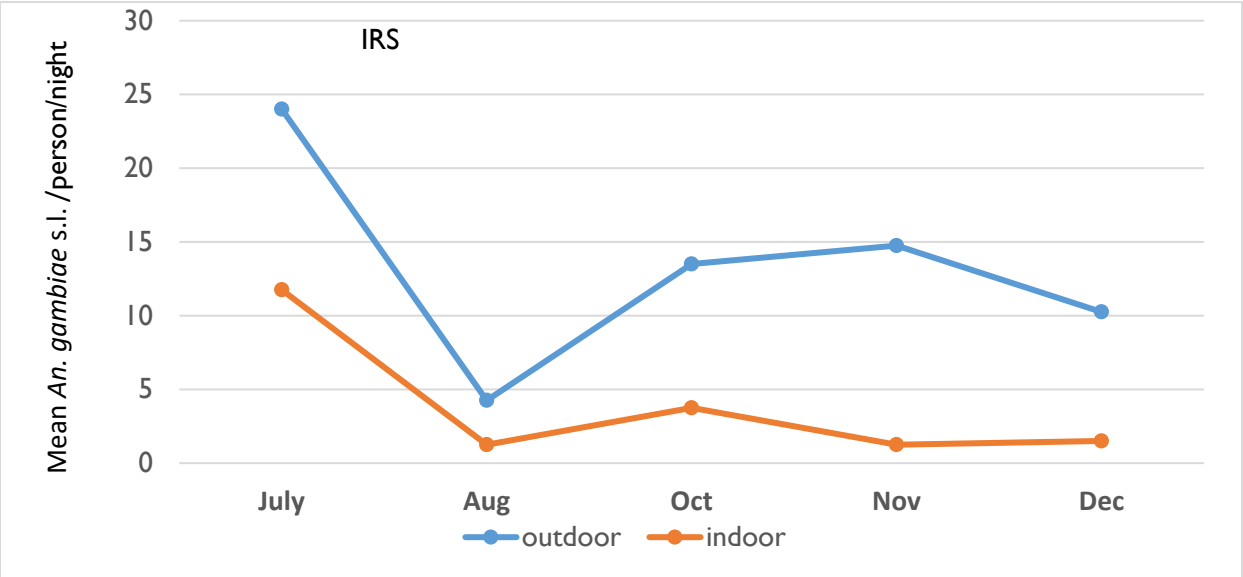
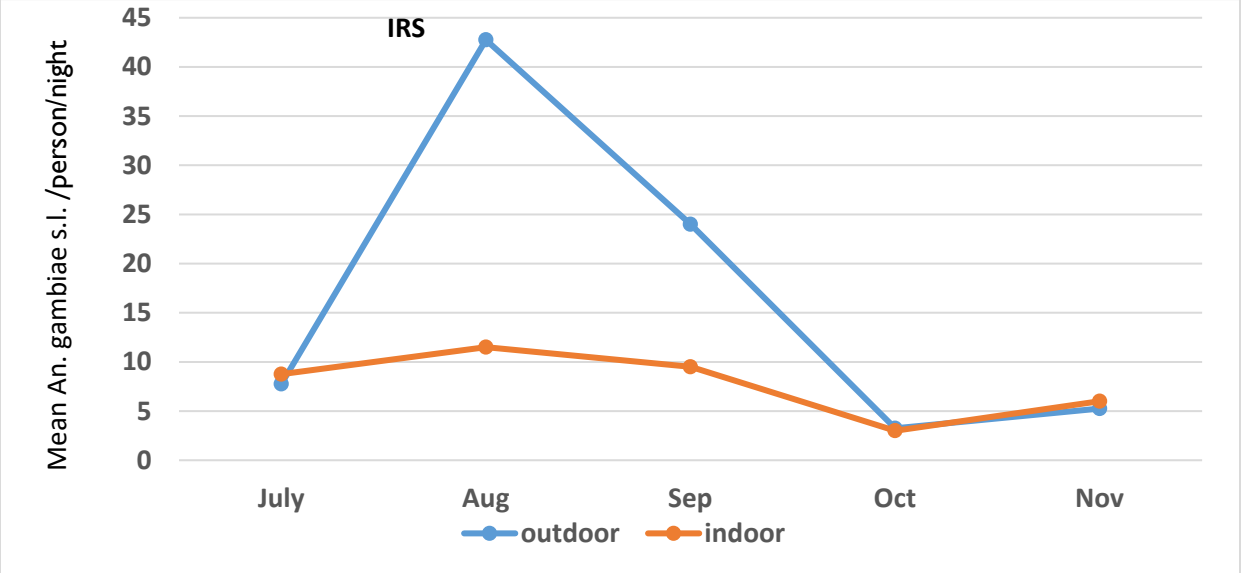


Figure 18. Biting Trends of *An. gambiae* s.l. in Alamata (July–Dec 2017)



3.5 Monitoring Malaria Vector Behavior in Agricultural Development Areas

Entomological studies were conducted in the large-scale Dangur agricultural development area in Benishangul-Gumuz region. The agricultural site experiences a substantial population influx during peak activity season and the study aimed to understand the potential risk of malaria transmission to this migrant population. Vector biting behavior and species diversity were determined over the period of the study (July–December 2017). IRS was conducted in the site with FMOH support in July 2017.

Collection used the HLC method, carried out in both dwellings and in the field. The collection from houses was conducted simultaneously both indoors and outdoors throughout the night (6:00 pm–6:00 am). Four individuals were involved per house per night; two of them collected from dusk to midnight and the other two from the midnight to dawn. Additional (outdoor) collection was done in the field, where there are no houses, to know the biting activities of mosquitoes in such settings. Collection was followed by morphological identification of the mosquitoes to the species level using dissecting microscope and identification keys (Verrone 1962).

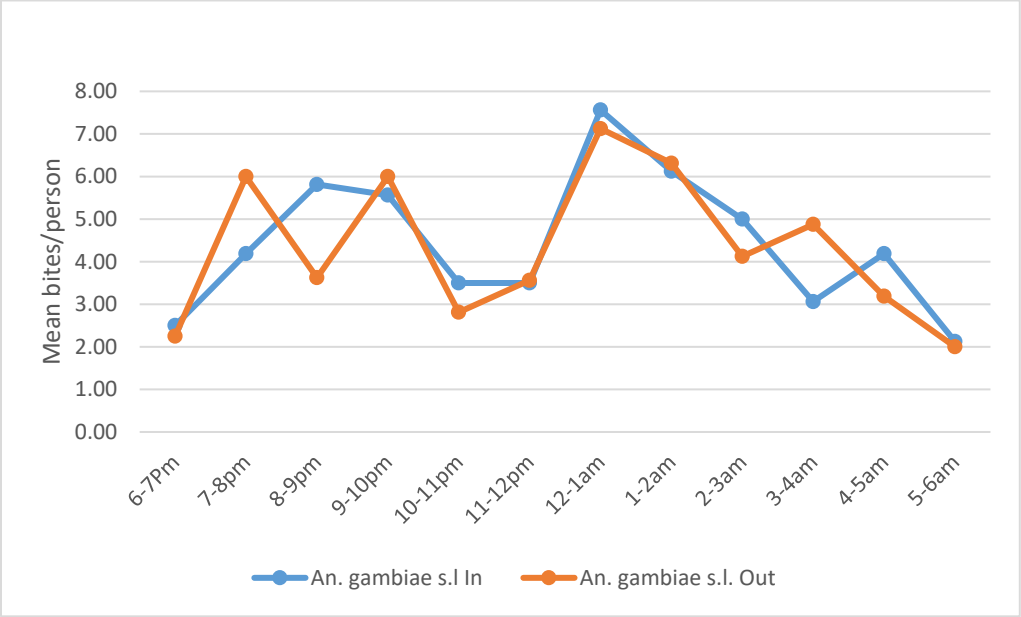
A total of 1,887 adult female *Anopheles* mosquitoes belonging to six different species were collected from eight farm areas. As Table 2 shows, *An. gambiae* s.l. was the predominant species at 88.3% (n=1,667). Approximately the same number of mosquitoes were collected both indoors 50.2% (n=837) and outdoors 49.8% (n=830); that is, there was equivalent exhophagic and endophagic behavior with only a slight endophagic tendency. A total of 861 *Culex* mosquitoes were also collected. Annexes D and E give more detailed data about the house and field collections.

Table 2: Total Anopheles Mosquitoes Collected in the Dangur Agricultural Development Area

Species	**Number of Mosquitoes Collected**
An. gambiae **s.l.**	1,667
An. pharoensis	37
An. demeilloni	89
An. coustani/ ziemanni	80
An. pretoriensis	7
An. natalensis	7

In general the peak biting rates were observed in September at collection sites of Dangur district. The collections also showed a high tendency of *An. gambiae* s.l. to bite throughout the night, with peak activity at 9.00–10.00 pm and 12:00 am–1:00 am (Figure 19).

Figure 19. Biting Trends of An. gambiae s.l. in Dangur Agricultural Development Site (July–Dec 2017)



3.6 ELISA AND MOLECULAR TEST RESULTS

3.6.1 SPOROZOITE ELISA

A total of 5,423 *An. gambiae* s.l. from 13 entomological monitoring sites were examined for sporozoite infections. Of these, one specimen was positive for *Plasmodium vivax* (Pv-210**),** two for PV-247 and another one for *P. falciparum* circumsporozoite proteins giving an overall infection rate of 0.02%, 0.04%, and 0.02%, respectively (Table 3). The *P. vivax* positive *An. gambiae* s.l. originated from Arorsha and Kurkura in Alamata and the positive *P. falciparum* from Shelle in Arbaminch.

Table 3. Sporozoite Infection Rates of An. gambiae s.l.

Site, District	Number Tested	PV210 (%+ve)	PV-247 (%+ve)	Pf (%+ve)
Abaya, West Guji	279	0	0	0
Arorsha, Alamata	342	0	1 (0.3)	0
Kurkura, Alamata	805	1 (0.12)	1 (0.12)	0
Babile, East Hararghe	876	0	0	0
Bambasi	257	0	0	0
Biftu Jalala, Nono	178	0	0	0
Bore Tika, Seka	22	0	0	0
Chano Mille, Arbaminch	28	0	0	0
Shelle, Arbaminch	992	0	0	1 (0.1)
Arbaminch Zuria	23	0	0	0
Ejaji, Ilugelan	56	0	0	0
Gobu Sayo, East Wollega	442	0	0	0
Goro, Southwest Shoa	1123	0	0	0
Total	5423	1 (0.02)*	2 (0.04)*	1 (0.02)

*Overall infection rate was 0.06%.

3.6.2 BLOOD MEAL SOURCES OF AN. GAMBIAE S.L.

The origin of blood meals of *An. gambiae* s.l. populations was investigated from 10 sites using bovine and human antigens. The majority of mosquitoes in all the sites were fed blood from cattle, and hence, the bovine blood index was higher than that of the human blood index (Table 4). The result is similar to published materails that have presented evidence of the preference of this species for bovine blood meals in Ethiopia. In some parts of the country cattle are kept in human dwellings and mostly in compouds very close to houses allowing mosquitoes to feed on both hosts. Mixed blood meals were found in all tested populations of mosquitoes, but most of the mixed meals were from Kurkura and Babile. The number of unidentified blood meals from these two sites was large, suggesting the presence of other sources of blood meals such as camels. Sites with less than 20% of mosquitoes with human blood were Shelle, Goro, and Bambasi.

Table 4. Blood Meal Sources of An. gambiae s.l. and Indices

Site	Origin of Blood Meals					HBI (%)	BBI (%)
	Number tested	Human only	Bovine only	Mixed	Unidentified		
Abaya, West Guji	9	1	1	4	3	55.6	55.6
Arorsha, Alamata	63	4	20	23	16	42.9	68.3
Ejaji, Ilugelan	12	0	3	4	5	33.3	58.3
Gobo Sayo, E/Wollega	85	4	23	15	43	22.4	44.7
Goro, SW Shoa	120	3	33	16	68	15.8	40.8

Site	Origin of Blood Meals					HBI (%)	BBI (%)
	Number tested	Human only	Bovine only	Mixed	Unidentified		
Kurkura, Alamata	389	8	117	111	153	30.6	58.6
Babile, East Hararge	275	33	50	44	148	28.0	34.2
Biftu Jalala, Nono	9	0	3	2	4	22.2	55.6
Shelle, Arbaminch	61	1	36	5	19	9.8	67.2
Bambasi	44	0	22	8	14	18.2	68.2

3.6.3 Molecular Species Identification

Overall 887 *An. gambiae* s.l. from 11 sites in six regions were PCR tested for species identification. Positive results were obtained for 835 (94.1%) specimens, all of them *An. arabiensis* (Table 5).

Table 5. PCR Identified An. gambiae s.l.

Region	Site	Number of *An. gambiae* s.l. assayed	Number of *An. arabiensis* (%)
Tigray	Selekleka	100	89 (89)
	Alamata	49	46 (93.9)
Amhara	Humera	100	94 (94)
Oromia	Asendabo	100	94 (94)
	Ziway	79	75 (94.9)
	Abaya	80	72 (90)
	Babile	100	95 (95)
SNNPR	Arbaminch	37	37 (100)
	Halaba	75	71(94.7)
Afar	Amibara	79	79 (100)
Gambella	Abobo	88	83 (94.3)
	Total	**887**	**835 (94.1)**

4. INSECTICIDE RESISTANCE STUDIES

4.1. SUSCEPTIBILITY TESTS WITH DISCRIMINATING CONCENTRATION

The percent mortality of mosquitoes exposed to different insecticides varied from site to site. All (100%) mosquitoes exposed to pirimiphos-methyl in all the study sites were killed except in Selekleka, which was 98% (Table 6). The mortality of mosquitoes exposed to bendiocarb and propoxur was 98–100% in all sites except Omonada, where the mortality was 91% (probable resistance) for bendiocarb and 86% (resistance) for propoxur. For DDT, the percent mortality of mosquitoes in all the sites was less than 90% (range: 17% (Babile) to 89% (Jinka)), which showed no DDT resistance reversal although the insecticide had not been used since 2010. The test result for malathion showed susceptibility in Babile (100%); suggested resistance (93–97%) in Abaya, Omonada, Jinka, and Abobo; and resistance (65–85%) in Bahirdar, Metema, and Selekleka. For deltamethrin, there was resistance in all sites except in Bahirdar and Jinka, where mortality was 93% (suggestive of resistance). Permethrin resistance was recorded in all the 12 test sites. Results were interpreted according to the WHO test procedure manual published in 2016.

Table 6. Results of Insecticide Susceptibility Using WHO Tube Test for Discrimination Concentration in 2017

Type of Assay	Insecticide	Percent Mortality of Wild *An. gambiae* s.l.											
		Oromia		Gambella		Afar		Amhara		Tigray		SNNPR	
		Abaya	Omonada	Ziway	Babile	Abobo	Amibara	Metema	Bahirdar	Selekleka	Humera	Halaba	Jinka
Discriminating dose	DDT	59.2(60/ 101) (R)	25 (25/100) (R)	22 (22/ 100) (R)	17 (17/100) (R)	50 (50/ 100) (R)	40 (40/ 100) (R)	ND	67(67/ 100) (R)	65(65/ 100) (R)	30(30/ 100) (R)	17.8(18/ 101) (R)	89(89/100) (R)
	Malathion	97 (97/ 100) (POR)	93 (93/ 100) (POR)	ND	100 (100/100)(S)	93 (93/ 100) (POR)	ND	85 (85/100) (R)	78(78/100) (R)	65(65/100) (R)	91(91/100) (POR)	ND	96(96/100) (POR)
	Pirimiphos-methyl	100 (100/ 100) (S)	100 (101/ 101) (S)	100 (100/100) (S)	100 (100/100)(S)	100 (100/ 100 (S)	100 (100/ 100) (S)	100 (120/120) (S)	100(115/115) (S)	98(98/100) (S)	100 (100/100) (S)	100 (100/100) (S)	100 (100/100) (S)
	Bendiocarb	100 (100/100) (S)	91 (91/100) (POR)	100 (100/100) (S)	99 (99/100) (S)	100 (100/100) (S)	100 (100/100) (S)	100 (110/110) (S)	100(101/101) (S)	99(99/100) (S)	100 (100/100) (S)	100 (100/100) (S)	100 (100/100) (S)
	Propoxur	100 (100/100) (S)	86 (86/100) (R)	100 (100/100) (S)	98 (98/100) (S)	100 (100/100) (S)	100 (100/100) (S)	100 (113/113) (S)	100(102/102) (S)	99(99/100) (S)	100 (100/100) (S)	100 (100/100) (S)	100 (100/100) (S)
	Deltamethrin	32 (32/100) (R)	20.4 (21/103) (R)	10 (10/100) (R)	49 (49/100) (R)	29.7 (30/101) (R)	13 (13/100) (R)	88.7(94/10) (R)	93(93/100) (POR)	30(30/100) (R)	52(52/100) (R)	22(22/100) (R)	98(98/100) (POR)
	Permethrin	46 (46/100) (R)	33.7 (34/101) (R)	36 (36/100) (R)	50 (58/116) (R)	30 (30/100) (R)	61 (61/100) (R)	89(89/100) (R)	85(85/100) (R)	76(76/100) (R)	70(70/100) (R)	48(48/100) (R)	88(88/100) (R)

Note: R=resistance, ND=no data, POR=possible resistance, S=susceptible

4.2 Susceptibility Tests for Determining Intensity of Resistance

Intensity assays of deltamethrin and permethrin at 1X, 5X, and 10X concentrations were carried out in the 12 sites. For deltamethrin at 5X, the percent mortality of *An. gambiae* s.l. was less than 90% in all sites except Babile and Selekleka, where mortality was 99% (S) and 95% (POR), respectively. At 10X concentration, the mortality of the population from Abobo, Selekleka, and Halaba sites was 99–100%, which the WHO criteria place in the moderate intensity of resistance classification. Results from the remaining five sites (Abaya, Omonada, Zeway, Amibara, and Humera) fell into the high resistance intensity category for deltamethrin (Table 7).

For permethrin, *An. gambiae* s.l. from Omonada, Babile, Jinka, and Amibara showed susceptibility at 5X, which is classified as low intensity resistance. Moderate intensity resistance to permethrin was observed in Abaya, Zeway, Abobo, Selekleka, Humera, and Halaba.

Annex F shows the results of intensity assays of deltamethrin and permethrin at 1X, 5X, and 10X concentrations in the 12 sites.

Table 7. Result of Resistance Intensity for Deltamethrin and Permethrin in Different Districts

District	Deltamethrin			Permethrin		
	Low resistance	**Moderate resistance**	**High resistance**	**Low resistance**	**Moderate resistance**	**High resistance**
Abaya			X		X	
Omonada			X	X		
Zeway			X		X	
Babile	X			X		
Abobo		X			X	
Amibara			X	X		
Metema	Not done					
Bahirdar						
Selekleka		X			X	
Humera			X		X	
Halaba		X			X	
Jinka	Vector was susceptible			X		

4.3 SYNERGIST ASSAY

Table 8 shows the results of synergist assays for deltamethrin and permethrin done in nine of the 11 sites. Pre-exposure to PBO fully restored susceptibility to deltamethrin in seven of the nine sites (98.7–100% mortality) and permethrin in six of the nine (98–100% mortality). In general, pre-exposure to PBO improved test mortality rates compared with no pre-exposure. High intensity pyrethroid resistance was demonstrated through the intensity assay; this resistance appears to be mediated mainly by metabolic oxidase enzymes in most sites.. PBO pre-exposure restored susceptibility to deltamethrin in all districts except Omonada (59.2% mortality) and Selekleka (96% mortality). Similarly, it restored susceptibility to permethrin in most sites, with the exception of Abaya, Abobo, and Omonada, where the percent mortality of mosquitoes after pre-exposure was 93.3%, 86.7%, and 82.9%, respectively.

Table 8. Results of Insecticide Susceptibility Using WHO Tube Test for Synergist Assay

Type of Assay	Insecticide	Percent Mortality of Wild *An. gambiae* s.l.										
		Oromia				Gambela	Afar	Amhara		Tigray		SNNPR
		Abaya	Omonada	Ziway	Babile	Abobo	Amibara	Metema	Bahirdar	Selekleka	Humera	Halaba
Synergist Assay	Deltamethrin + PBO	100 (75/75)	59.2 (45/76)	98.7 (74/75)	100 (75/75)	100 (75/75)	98.7 (74/75)			96(72/75)	98.7(74/75)	99(74/75)
	Deltamethrin alone	49.3 (37/75)	19.7 (15/76)	24 (18/75)	73.3(55/75)	34.7 (26/75	44 (33/75)			60(45/75)	32(24/75)	19(14/75)
	PBO alone	0 (0/75)	0 (0/75)	2 (2/75)	2.7(73/75)	0 (0/75)	6.7 (5/75)			3(2/75)	4(3/75)	0(0/75)
	Control	0 (0/75)	0 (0/75)	2.7 (0/75)	0(0/75)	0 (0/75)	1.3 (1/75)			0(0/75)	1.3(1/75	0(0/75)
	Permethrin + PBO	93.3 (70/75)	82.9 (63/76)	98.7 (74/75)	100(75/75)	86.7 (65/75)	98.7 (74/75)			100(75/75)	100(75/75)	100(75/75)
	Permethrin alone	72 (54/75)	20.8 (16/77)	26.7 (20/75)	40(30/75)	37.3 (28/75)	46.7 (35/75)			76(57/75)	70.7(53/75)	53(40/75)
	PBO alone	0 (0/75)	0 (0/75)	0 (0/75)	0(0/75)	0 (0/75)	4 (3/75)			4(3/75)	2.7(2/75)	0(0/75)
	Control	0 (0/75)	0 (0/75)	0 (0/75)	0(0/75)	0 (0/75)	0 (0/75)			0(0/75)	4(3/75)	0(0/75)

4.4 SUSCEPTIBILITY TESTS FOR CLOTHIANIDIN

Clothianidin was tested to determine the baseline susceptibility status of wild *An. gam*biae s.l. from Abaya district and susceptible *An. arabiensis* from the Adama insectary. The tests were conducted using an interim diagnostic dosage provided by Sumitomo Chemicals. Clothianidin (SumiShield 50 WG) was treated at 13.2 mg a.i per filter paper and test mortality recorded every day up to a seven-day holding period (Table 9).

Table 9. Results of Insecticide Susceptibility for Clothianidin Insecticide

Site	Holding period	Wild *An. gambiae* s.l.			Control		Susceptible *An. arabiensis*			Control	
		# Exposed	% Mortality (observed)	% Mortality (corrected)	# Exposed	% Mortality	# Exposed	% Mortality	% Mortality (corrected)	# Exposed	% Mortality
Adama	Day 1	100	**57**	57	49	0	100	**48**	45.8	49	4(2/49)
	Day 2	100	**75**	75	49	0	100	**82**	81.3	49	4(2/49)
	Day 3	100	**80**	80	49	0	100	**92**	91.5	49	6.1(3/49)
	Day 4	100	**93**	49	49	2(1/49)	100	**98**	97.8	49	10.2(5/49)
	Day 5	100	**97**	97.5	49	18.4(9/49)	100	**98**	98.8	49	10.2(5/49)
	Day 6	100	**97**	97.5	49	24.5(12/49)	100	**100**	100	49	12.2(6/49)
	Day 7	100	98	96.8	49	36.7 (18/49)	100	100	100	49	16.3 (8/49)
Abaya	Day 1	100	**42**	42	50	0	100	**58**	58	50	0
	Day 2	100	**66**	66	50	0	100	**81**	81	50	0
	Day 3	100	**86**	86	50	0	100	**95**	95	50	2(1/50)
	Day 4	100	**89**	89.4	50	6(3/50)	100	**100**	100	50	14(7/50)
	Day 5	100	**91**	90.2	50	8(4/50)	100	**100**	100	50	14(7/50)
	Day 6	100	**99**	98.9	50	12(6/50)	100	**100**	100	50	14(7/50)
	Day 7	100	99	98.8	50	18 (9/50)	100	100	100	50	14 (7/50)

4.5 SUSCEPTIBILITY TEST FOR CHLORFENAPYR

Chlorfenapyr was tested to determine its diagnostic concentration against susceptible *An. arabiensis* from the Adama insectary and wild *An. gambiae* in Abaya district for 12.5, 25, 50, 100, 200µg, negative control, positive control at the same time using CDC bottle bioassays. Unfortunately, the dose supplied by CDC was miscalculated and only a tenth of the dose was used, resulting in dose of 1.25, 2.5, 5, 10, and 20 µg per bottle. The test results were read every 24 hours of the holding period (held at 21–28˚C and 54–66% RH) for three days (Table 10). There was significant variation in percent mortality between the laboratory-reared *An. arabiensis* and the wild *An. gambiae* s.l. The underdosing is the most plausible reason why the wild mosquitoes survived even the highest concentration of insecticide, 20µg. We plan to repeat the test with the correct dose to see if this result is reproducible.

Table 10. Results of Insecticide Susceptibility for Chlorfenapyr Insecticide

Holding Period	Total exposed	Laboratory-reared *An. arabiensis*						Wild *An. gambiae* s.l.					
		Chlorfenapyr concentration/ bottle						Chlorfenapyr concentration/ bottle					
		1.25µg	2.5µg	5.0µg	10.0µg	20.0µg	Control	1.25µg	2.5µg	5.0µg	10.0µg	20.0µg	Control
		100	100	100	100	100	100	100	100	100	100	100	100
Day 1	# Dead after 1 days	45	74	91	94	98	9	1	30	64	73	87	0
	% Observed mortality	45	74	91	94	98	9	1	30	64	73	87	0
	% Corrected mortality	36.6	71.4	90.1	93.4	97.8	-	-	-	-	-	-	-
Day 2	# Dead after 1 days	65	87	99	100	100	14	1	36	78	85	93	0
	% Observed mortality	65	87	99	100	100	14	1	36	78	85	93	0
	% Corrected mortality	75.6	84.9	98.8	100	100	-	-	-	-	-	-	-
Day 3	# Dead after 3 days	79	91	99	100	100	14	3	40	86	89	96	0
	% Observed mortality	79	91	99	100	100	14	3	40	86	89	96	0
	% Corrected mortality	75.6	89.5	98.9	100	100	-	-	-	-	-	-	-

4.6. ALLELIC FREQUENCY OF KNOCKDOWN RESISTANCE

DNA isolates of 887 *An. arabiensis* from insecticide susceptible tests of DDT and deltamethrin and preserved as dead and survivors from 11 sites in five regions were PCR assayed to check for resistant (R)/susceptible (S) alleles of the *kdr* gene (Table 11). Of these, 704 samples were successfully amplified for the West African *kdr* mutation; the rest failed. The genes were classified as RR, RS, and SS. Close to 63% (440/704) of the vector population carried the SS gene, 35% carried RR, and the remaining 2% carried RS. Similar to past published and unpublished reports, both alleles are found in dead and surviving mosquitoes (Table 11). In addition to *kdr*, there are likely other resistance mechanisms at play.

Table 11. *1014*F *kdr* Allele Frequencies in Field Populations of *An. arabiensis* from Eleven Sites (five regions) in Ethiopia (2017)

Region	Site	Bioassay Phenotype	# Mosquitoes Assayed	RR	RS	SS	*kdr* Allele Frequency	
							R	S
Tigray	Alamata (mosquito samples from 2016 test)	DDT survivors	15	2	0	11	0.15	0.85
		DDT dead	10	2	0	5	0.29	0.71
		Delta survivors	14	4	0	9	0.31	0.69
		Delta dead	10	0	0	6	0.00	1.00
	Humera	DDT survivors	40	17	0	4	0.81	0.19
		DDT dead	10	5	0	2	0.71	0.29
		Delta survivors	40	6	0	29	0.17	0.83
		Delta dead	10	1	0	9	0.10	0.90
	Selekleka	DDT survivors	40	33	0	3	0.92	0.08
		DDT dead	10	0	0	8	0.00	1.00
		Delta survivors	40	14	0	11	0.56	0.44
		Delta dead	10	3	4	0	0.71	0.29
Afar	Amibara	DDT survivors	29	0	0	29	0.00	1.00
		DDT dead	10	0	0	8	0.0	1.00
		Delta survivors	30	0	0	27	0.00	1.00
		Delta dead	10	0	0	9	0.00	1.00
Oromia	Omonada	DDT survivors	40	27	1	4	0.86	0.14
		DDT dead	10	2	0	0	1.00	0.00
		Delta survivors	40	4	0	32	0.11	0.89
		Delta dead	10	0	0	8	0.00	1.00
	Babile	DDT survivors	40	25	1	11	0.69	0.31
		DDT dead	10	1	1	6	0.19	0.81
		Delta survivors	40	26	0	6	0.81	0.19
		Delta dead	10	5	0	4	0.56	0.44
	Zeway	DDT survivors	30	0	0	27	0.00	1.00
		DDT dead	10	0	0	9	0.00	1.00
		Delta survivors	30	1	0	25	0.04	0.96
		Delta dead	9	0	0	7	0.00	1.00
	Abaya	DDT survivors	20	9	0	7	0.56	0.44

Region	Site	Bioassay Phenotype	# Mosquitoes Assayed	RR	RS	SS	*kdr* Allele Frequency	
							R	S
		DDT dead	10	0	0	6	0.00	1.00
		Delta survivors	40	7	2	17	0.31	0.69
		Delta dead	10	1	0	8	0.11	0.89
	Jinka	DDT survivors	15	0	0	12	0.00	1.00
		DDT dead	10	0	0	10	0.00	1.00
		Delta survivors	2	0	0	1	0.00	1.00
		Delta dead	10	0	0	10	0.00	1.00
	Halaba	DDT survivors	25	3	3	16	0.20	0.80
		DDT dead	10	1	0	9	0.10	0.90
		Delta survivors	30	5	0	21	0.19	0.81
		Delta dead	10	1	0	9	0.10	0.90
Gambella	**Abobo**	DDT survivor	28	15	1	5	0.74	0.26
		DDT dead	10	0	0	0	ND	ND
		Delta survivors	40	23	0	0	1.00	0.00
		Delta dead	10	8	0	0	1.00	0.00
		Total	**887**	**251**	**13**	**440**		
Proportion from 704 successfully analyzed				**0.35**	**0.02**	**0.63**		
Summary				**RR**	**RS**	**SS**		
DDT survivors			**322**	**131 (0.49)**	**6 (0.03)**	**129 (0.48)**		
DDT Dead			**110**	**11 (0.15)**	**1 (0.01)**	**63 (0.84)**		
Delta survivors			**346**	**90 (0.33)**	**2 (0.01)**	**178 (0.66)**		
Delta Dead			**109**	**19 (0.15)**	**4 (0.04)**	**70 (0.75)**		

The frequency of the homozygous kdr gene in DDT survivors was 49 % (n=266) much higher than in the dead mosquitoes that was only 15% (n= 84). The frequency of heterozygous kdr was 3% and 1% in DDT survivors and dead mosquitoes, respectively. Higher frequency of wild susceptible gene was found in mosquitoes dead after exposure to DDT than survivors 84% and 48%, respectively. A similar trend was observed in mosquitoes that were dead and survived after exposure to deltamethrin.

5. NET BIOASSAYS

The study showed 100% mortality in laboratory-reared *An. arabiensis* exposed to both PermaNet® 2.0 and MAGNet™ nets. Average mortality of wild *An. gambiae* s.l. exposed to PermaNet® 2.0 and MAGNet™ LLINs in the four sites (Adama, Ziway, Amibara, and Halaba) was, however, low and ranged from 69% to 88% and 73% to 96% for the respective net brands (Table 12). This variation in mortality between insectary-reared and wild-collected mosquitoes could be attributable to a difference in the susceptibility status of the mosquitoes. All tested nets were new and obtained from the respective district stores.

Table 12. Bioassay Results on PermaNet and MAGnet for Adama, Ziway, Amibara, and Halaba Districts

District	Net type	# Mosquitoes Tested	# Knocked Down after 60 Minutes	# Dead after 24 Hours	% Mortality	Control (untreated net)		
						# Exposed	# Dead	% Mortality
			Susceptible *An. arabiensis*					
Adama	PermaNet® 2	100	95	100	100	20	0	0
	MAGNet™	100	84	100	100	20	0	0
			Wild *An. gambiae* s.l.					
Adama	PermaNet® 2	100	70	81	81	20	0	0
	MAGNet™	100	60	74	74	20	0	0
Ziway	PermaNet® 2	100	69	88	88	20	0	0
	MAGNet™	100	55	96	96	20	0	0
Amibara	PermaNet® 2	100	59	69	69	20	0	0
	MAGNet™	100	52	73	73	20	0	0
Halaba	PermaNet® 2	1001	35	77	77	20	0	0
	MAGNet™	100	42	83	83	20	0	0

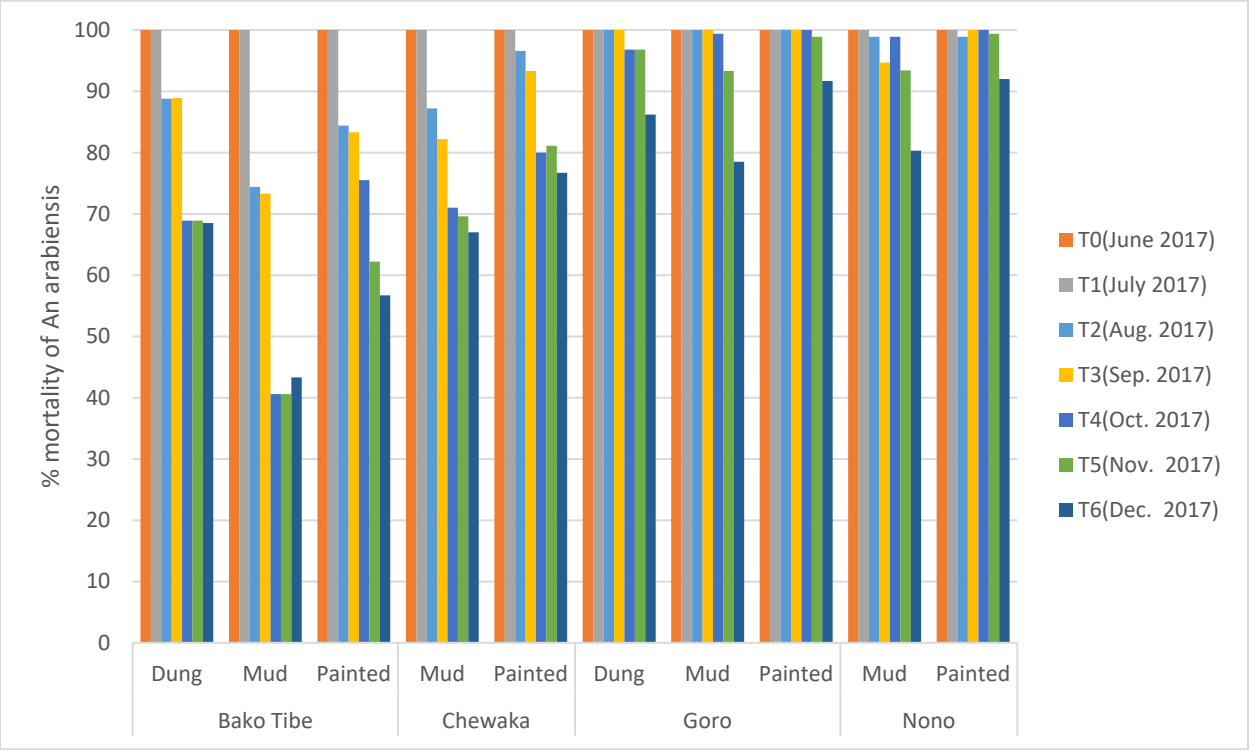
6. Quality of Spraying and Insecticide Decay Rate

Results of the cone wall bioassay tests conducted at different times, starting from zero, after spraying with pirimiphos-methyl are shown in Table 13 and Figure 20. The high *An. arabiensis* mortalities recorded at time zero and one month might be an indication that the application of the insecticide on wall surfaces was satisfactory. The average mortality of mosquitoes in Bako Tibe and Chewaka was below 80% at two and four months after spray, respectively, but remained high in Goro (98.7%) and Nono (99.5%) even after four months. On mud surfaces, pirimiphos-methyl (Actellic 300 CS) lasted five months in Goro and at least six months in Nono. Mortality on mud wall surfaces was generally lower than on dung and painted surfaces; this difference was statistically significant in Bako Tibe and Chewaka ($P<0.005$, data not shown). The variation in mortality might be due to the variation in wall surfaces or spray quality, as well as other environmental factors. For more detail, see Annex G.

Table 13. Results of Quality Assurance of 2017 IRS and Actellic Decay Rate Monitoring

Time	% Mortality of *An. arabiensis*														
	Bako Tibe				Chewaka			Goro				Nono			Overall mean
	Dung	Mud	Painted	Mean	Mud	Painted	Mean	Dung	Mud	Painted	Mean	Mud	Painted	Mean	
T0 (June 2017)	100 (90/90)	100 (180/180)	100 (90/90)	100	100 (270/270)	100 (90/90)	100	100 (120/120)	100 (150/150)	100 (90/ 90)	100	100 (180/180)	100 (180/180)	100	100
T1 (July 2017)	100 (90/90)	100 (180/180)	100 (90/90)	100	100 (270/270)	100 (90/90)	100	100 (132/132)	100 (157/157)	100 (101/101)	100	100 (193/193)	100 (186/186)	100	100
T2 (Aug 2017)	88.8 (79/89)	74.4 (131/176)	84.4 (76/90)	83	87.2 (231/265)	96.6 (84/87)	90	100 (134/134)	100 (168/168)	100 (104/104)	100	98.9 (197/199)	98.9 (197/199)	99	94
T3 (Sep 2017)	88.9 (80/90)	73.3 (132/180)	83.3 (75/90)	82	82.59 (223/270)	93.3 (84/90)	88	100 (133/133)	100 (162/162)	100 (100/100)	100	94.7 (178/188)	100 (191/191)	97	93
T4 (Oct 2017)	68.9 (62/90)	40.6 (73/180)	75.5 (68/90)	62	71 (193/270)	80(72/90)	76	96.8 (123/127)	99.4 (156/157)	100(93/93)	98.7	98.9 (185/187)	100 (183/183)	99.5	84
T5 (Nov 2017)	68.9 (62/90)	40.6 (73/180)	62.2 (56/90)	57	69.6 (188/270)	81.1 (73/90)	75	96.8 (121/125)	93.3 (147/158)	98.9 (94/95)	96.3	93.4 (170/182)	99.4 (182/183)	96	81
T6 (Dec 2017)	68.5 (61/89)	43.3(78/180)	56.7 (51/90)	56.2	67 (180/270)	76.7 (69/90)	71.8	86.2 (107/124)	78.5 (124/158)	91.7 (89/97)	85.5	80.3 (151/188)	92 (173/188)	86.2	75

Figure 20. Results of Pirimiphos-Methyl Decay Rate Monitoring



7. SUMMARY

- Entomological monitoring data collected in Abaya, Nono, and Bambasi showed that IRS had an impact on vector density.
- Bovine blood was found to be the predominant source of blood meal for *An. gambiae* s.l., presumably *An. arabiensis* in Ethiopia.
- *P. falciparum* and *P. vivax* infected *An. gambiae* s.l. were found in Shelle and Alamata, respectively
- *An. arabiensis* was the only species of *An. gambiae* s.l. identified in molecular tests.
- Pyrethroid resistance appears to be mediated by metabolic oxidase enzymes in most sites, except Omonada.
- The data generated showed that the local vector was susceptible to clothianidin at the Abaya site and probably resistant at the Adama site. The local vector also did not die when exposed to doses of up to 20µg of chlorfenapyr per bottle. This calls for further testing using the correct doses of chlorfenapyr to evaluate the susceptibility of wild vectors.
- High intensity pyrethroid resistance was demonstrated through intensity assays and net bioassays.
- The results from the susceptibility studies showed that the vector is susceptible to pirimiphos-methyl, but there is an indication of the emergence of resistance to malathion, propoxur, and bendiocarb; hence, further monitoring remains important. Both susceptible and resistant alleles exist in the DDT and deltamethrin survived and dead *An. arabiensis.* However, the wild type susceptible gene was more predominant in dead mosquitoes than th survivors.
- Quality of spraying has been demonstrated with high mortalities of the vector on sprayed surfaces with overall mortality of 93% recorded three months post spraying, except for one site (Bako Tibe) where the residual life of the pirimiphos-methyl was observed to be less than 80% at two months post-spraying. We suspect this is due to higher porosity of mud surfaces in this site, to be confirmed with additional assays.

8. Training on Updated WHO Tube Test Guidelines

AIRS Ethiopia, in collaboration with the FMOH, conducted a five-day training on updated WHO tube test guidelines. Fifteen experts attended the training, which was held on June 5–9, 2017, at Adama Malaria Control Training Center (Table 14). The training covered the following topics:

- Quality check of impregnated papers
- WHO tube test with discriminating doses
- WHO tube test with intensity and synergist assay
- Test procedure for clothianidin insecticide

Table 14. Participants Trained on WHO Tube Test Guidelines

Participant Affiliation	# Trainees Total		
	Male	Female	Total
Jimma University	1	0	1
Arbaminch University	1	0	1
Gondar University	1	0	1
Jigjiga University	1	0	1
Mekele University	1	0	1
Addis Ababa University	2	0	2
FMOH	3	1	4
EPHI	3	1	4
Total	13	2	15

The project further provided support to two staff from two universities (Jimma and Arbaminch) to attend a regional laboratory training course organized by Notre Dame University in partnership with the Centre Recherche Entomologique de Cotonou, and hosted by the National Institute for Medical Research, Mwanza, in Tanzania on September 11– 22, 2017.

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ANNEX A: ANOPHELES SPECIES COLLECTED IN ABAYA AND NONO (OROMIA REGION), AND BAMBASI (BENISHANGUL-GUMUZ REGION)

Site	Time	*An. gambiae* s.l.				*An. pharoensis*				*An. coustani*				*An. demeilloni*				Culicine			
		PSC	CDC	HLC	Total	PSC	CDC	HLC	Total	PSC	CDC	HLC	Total	PSC	CDC	HLC	Total	PSC	CDC	HLC	Total
Abaya	May (Pre IRS)	0	0	20	20	0	0	2	2	0	0	1	1	0	0	0	0	60	6	268	334
	June (Post IRS)	19	20	44	83	0	0	7	7	0	0	0	0	0	0	0	0	88	49	244	381
	July (Post IRS)	0	0	1	1	0	1	16	17	1	0	1	2	0	0	0	0	54	93	293	440
	Aug (Post IRS)	0	0	0	0	0	1	2	3	0	0	0	0	0	0	0	0	59	65	232	356
	Sept (Post IRS)	1	1	2	4	0	0	2	2	0	0	0	0	0	0	0	0	9	10	44	63
	Oct (Post IRS)	6	25	63	94	0	0	3	3	0	0	0	0	0	0	0	0	58	19	301	378
	Nov (Post IRS)	6	7	39	52	0	0	10	10	0	0	3	3	0	0	0	0	29	10	237	276
	Dec (Post IRS)	1	4	24	29	0	0	7	7	0	0	3	3	0	0	0	0	10	11	38	59
	Jan (Post IRS)	2	0	6	8	0	1	36	37	0	0	0	0	0	0	0	0	25	10	26	61
	Feb (Post IRS)	4	4	10	18	0	0	4	4	0	0	0	0	0	0	0	0	63	3	11	77
	March (Post IRS)	5	0	6	11	0	0	1	1	0	0	0	0	0	0	0	0	82	19	154	255
	April (Post IRS)	7	4	20	31	0	0	2	2	0	0	0	0	0	0	0	0	110	12	361	483

Site	Time	*An. gambiae* s.l.				*An. pharoensis*				*An. coustani*				*An. demeilloni*				Culicine			
		PSC	CDC	HLC	Total	PSC	CDC	HLC	Total	PSC	CDC	HLC	Total	PSC	CDC	HLC	Total	PSC	CDC	HLC	Total
Nono	May (Pre IRS)	4	1	20	25	0	0	0	0	0	0	2	2	0	0	0	0	99	3	45	147
	June (Post IRS)	3	1	3	7	0	0	0	0	0	0	0	0	0	0	0	0	83	18	16	117
	July (Post IRS)	0	0	6	6	0	0	0	0	0	0	1	1	0	0	0	0	19	29	54	102
	Aug (Post IRS)	0	0	26	26	0	0	1	1	0	0	2	2	0	0	0	0	55	44	249	348
	Sept (Post IRS)	1	0	41	42	0	0	0	0	0	2	10	12	0	0	0	0	123	10	213	346
	Oct (Post IRS)	3	2	12	17	0	0	0	0	0	0	15	15	0	0	0	0	82	12	103	197
	Nov (Post IRS)	0	0	4	4	0	0	0	0	0	0	2	2	0	0	0	0	17	4	41	62
	Dec (Post IRS)	0	1	6	7	0	0	0	0	0	0	8	8	0	0	0	0	17	7	59	83
	Jan (Post IRS)	0	1	5	6	0	0	0	0	0	0	3	3	0	0	0	0	7	17	32	56
	Feb (Post IRS)	0	0	9	9	0	0	0	0	0	0	0	0	0	0	0	0	9	9	9	27
	March (Post IRS)	0	0	8	8	0	0	0	0	0	0	0	0	0	0	0	0	10	3	12	25
	April (Post IRS)	0	3	17	20	0	0	0	0	0	0	0	0	0	0	0	0	14	5	27	46
Bambasi	May (Pre IRS)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	28	0	0	28
	June (Post IRS)	1	8	39	48	0	0	1	1	0	4	6	10	0	0	0	0	16	11	8	35
	July (Post IRS)	5	6	19	30	0	30	1	31	0	33	9	42	0	0	0	0	9	27	8	44
	Aug (Post IRS)	2	17	13	32	0	28	5	33	2	23	62	87	1	0	0	1	11	46	20	77
	Sept (Post IRS)	11	10	33	54	0	12	2	14	3	21	39	63	0	7	2	9	13	41	21	75
	Oct (Post IRS)	31	11	37	79	1	70	0	71	8	220	106	334	0	13	3	16	70	255	100	425

Site	Time	*An. gambiae* s.l.				*An. pharoensis*				*An. coustani*				*An. demeilloni*				Culicine			
		PSC	**CDC**	**HLC**	**Total**	**PSC**	**CDC**	**HLC**	**Total**	**PSC**	**CDC**	**HLC**	**Total**	**PSC**	**CDC**	**HLC**	**Total**	**PSC**	**CDC**	**HLC**	**Total**
	Nov (Post IRS)	4	10	2	16	1	2	2	5	12	37	9	58	11	26	2	39	24	36	31	91
	Dec (Post IRS)	0	1	0	1	0	3	0	3	1	19	0	20	11	4	0	15	12	10	6	28
	Jan (Post IRS)	0	0	0	0	0	0	0	0	0	9	1	10	11	6	4	21	7	3	2	12
	Feb (Post IRS)	0	1	0	1	0	1	0	1	0	5	2	7	3	1	2	6	9	16	1	26
	March (Post IRS)	0	0	0	0	0	0	0	0	0	2	0	2	0	0	0	0	5	3	1	9
	April (Post IRS)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	3
Total		**116**	**138**	**535**	**789**	**2**	**149**	**104**	**255**	**27**	**375**	**285**	**687**	**37**	**57**	**13**	**107**	**1386**	**919**	**3267**	**5572**

ANNEX B: GAMBIAE S.L. IN THREE STUDY SITES

Time	Abaya				Nono				Bambasi			
	An. gambiae s.l.	# Dissected	Parous	% Parous	*An. gambiae* s.l.	# Dissected	Parous	% Parous	*An. gambiae* s.l.	# Dissected	Parous	% parous
May (Pre IRS)	20	20	13	65	20	20	15	75	0	0	0	0
Jun (Post IRS)	44	44	38	86.4	3	3	3	100	39	39	32	82.1
Jul (Post IRS)	1	1	1	100.0	6	6	5	83.3	19	19	16	84.2
Aug (Post IRS)	0	0	0	0.0	26	26	19	73	13	13	12	92.3
Sep (Post IRS)	2	2	2	100.0	41	41	32	78.05	33	33	21	63.6
Oct (Post IRS)	63	63	43	68.3	12	12	6	50	37	37	26	70.3
Nov(Post IRS)	39	39	36	92.3	4	4	2	50	2	2	2	100
Dec (Post IRS)	24	23	17	74	6	6	4	67	2	2	2	100
Jan(Post IRS)	6	6	6	100	5	5	2	40	0	0	0	0.0
Feb(Post IRS)	10	10	10	100	9	8	5	62.5	0	0	0	0.0
March(Post IRS)	6	6	6	100	8	7	2	29	0	0	0	0.0
April(Post IRS)	20	20	19	95	17	17	11	64.7	0	0	0	0.0

ANNEX C: ANOPHELES SPECIES COLLECTED IN GORO, BABILE, ARBAMINCH, AND ALAMATA SITES (JULY–DEC 2017)

Site	Time	*An. gambiae* s.l.				*An. Pharoensis*				*An. Coustani*				Culex			
		PSC	CDC	HLC	Total	PSC	CDC	HLC	Total	PSC	CDC	HLC	Total	PSC	CDC	HLC	Total
Goro (Oromia)IRS conducted in June	July	3	1	0	4	0	0	0	0	0	0	1	1	0	9	12	21
	Aug	3	5	43	51	0	0	0	0	0	0	5	5	2	47	236	285
	Sep	48	101	597	746	0	0	0	0	0	1	17	18	4	52	113	169
	Oct	100	8	89	197	0	0	0	0	0	0	3	3	2	36	92	130
	Nov	21	4	93	118	0	0	0	0	0	0	1	1	0	3	0	3
	Dec	14	1	5	20	0	0	0	0	0	0	1	1	1	0	0	1
S/Total		**189**	**120**	**827**	**1136**	**0**	**0**	**0**	**0**	**0**	**1**	**28**	**29**	**9**	**147**	**453**	**609**
Babile (Oromia) IRS conducted before Aug collections	July	332	93	145	570	0	0	0	0	0	0	0	0	5	4	0	9
	Aug	3	7	22	32	0	0	0	0	0	0	0	0	12	5	11	28
	Sep	Not done															
	Oct	23	34	69	126	0	0	0	0	0	0	0	0	0	0	13	13
	Nov	33	17	64	114	0	0	0	0	0	0	0	0	0	0	0	0
	Dec	2	10	47	59	0	0	0	0	0	0	0	0	0	0	0	0
S/Total		**393**	**161**	**347**	**901**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**17**	**9**	**24**	**50**

Site	Time	*An. gambiae* s.l.				*An. Pharoensis*				*An. Coustani*				Culex			
		PSC	CDC	HLC	Total	PSC	CDC	HLC	Total	PSC	CDC	HLC	Total	PSC	CDC	HLC	Total
Arbaminch (SNNPR)IRS conducted before Aug collections	July	16	10	85	111	0	0	0	0	0	0	0	0	119	14	146	279
	Aug	4	2	18	24	0	0	0	0	0	0	0	0	257	3	87	347
	Sep	5	10	69	84	0	0	0	0	0	0	0	0	298	45	298	641
	Oct	11	21	164	196	0	0	7	7	0	0	0	0	238	20	190	448
	Nov	62	42	519	623	0	2	11	13	0	0	1	1	455	70	313	838
	Dec	11	7	43	61	0	0	9	9	0	0	0	0	200	29	206	435
S/Total		**109**	**92**	**898**	**1099**	**0**	**2**	**27**	**29**	**0**	**0**	**1**	**1**	**1567**	**181**	**1240**	**2988**
Alamata (Tigray) IRS conducted after Aug collections	July	194	119	66	379	0	42	0	42	0	13	3	16	64	70	28	162
	Aug	154	43	217	414	1	0	0	1	0	0	5	5	7	36	9	52
	Sep	73	47	134	254	2	7	0	9	0	2	4	6	13	13	4	30
	Oct	118	30	25	173	0	3	1	4	0	12	5	17	0	22	22	44
	Nov	100	35	24	159	0	1	0	1	0	17	2	19	4	11	12	27
	Dec	54	44	47	145	1	3	0	4	0	14	2	16	3	48	51	102
S/Total		**693**	**318**	**513**	**1524**	**4**	**56**	**1**	**61**	**0**	**58**	**21**	**79**	**91**	**200**	**126**	**417**
Total		**1384**	**691**	**2585**	**4660**	**4**	**58**	**28**	**90**	**0**	**59**	**50**	**109**	**1684**	**537**	**1843**	**4064**

ANNEX D: MOSQUITOES COLLECTED IN DANGUR DISTRICT FROM DWELLINGS BY HLC (JULY–DEC 2017)

Farm		Time	*An. gambiae* s.l.		*An. pharoensis*		*An. coustani*		*An. demeilloni*		*An. pretoriensis*		*An. natalensis*		Culex spp.	
Type	# Farms		In	Out	In	Out	In	Out	In	Out	In	Out	In	Out	In	Out
Large scale		July	62	29	0	0	0	0	0	1	1	5	0	2	10	19
		Aug	191	128	2	5	1	1	0	2	0	0	0	0	31	22
		Sep	409	449	2	2	5	4	0	1	0	0	0	0	7	5
		Oct	70	101	5	4	1	7	7	4	0	0	0	0	21	31
		Nov	19	31	5	1	0	1	12	13	0	0	0	0	8	12
		Dec	10	10	2	4	1	2	4	10	0	0	0	0	7	8
Small Scale		July	7	8	0	0	0	0	0	0	0	0	0	0	179	295
		Aug	12	19	0	1	0	0	0	0	0	1	0	0	6	4
		Sep	40	37	0	0	0	13	0	0	0	0	0	0	6	5
		Oct	16	14	0	0	1	7	1	9	0	0	0	0	25	30
		Nov	1	2	0	0	0	1	1	2	0	0	0	0	1	1
		Dec	0	2	0	0	0	0	5	4	0	0	0	0	0	2
Total			**837**	**830**	**16**	**17**	**9**	**36**	**30**	**46**	**1**	**6**	**0**	**2**	**301**	**434**

ANNEX E: MOSQUITOES COLLECTED FROM FIELD OUTDOORS USING HLC (JULY–DEC 2017)

Farm		Time	Outdoor HLC					
Type	# Farms		*An. gambiae* s.l.	*An. pharoensis*	*An. coustani*	*An. demeilloni*	*An. natalensis*	Culex spp
Large Scale	4	Sep	13	0	3	0	0	16
		Oct	15	0	2	5	1	45
		Nov	10	1	8	0	0	14
		Dec	4	3	3	0	0	0
Small Scale	4	Sep	30	0	5	0	0	25
		Oct	2	0	9	5	4	19
		Nov	4	0	5	3	0	7
		Dec	0	0	0	0	0	0
Total			78	4	35	13	5	126

ANNEX F: RESULTS OF INSECTICIDE SUSCEPTIBILITY USING WHO TUBE TEST FOR INTENSITY ASSAY IN 2017

Intensity Assay	Insecticide	Percent mortality of Wild *An. gambiae* s.l.											
		Oromia				Gambella	Afar	Amhara		Tigray		SNNPR	
		Abaya	Omonada	Ziway	Babile	Abobo	Amibara	Metema	Bahirdar	Selekleka	Humera	Halaba	Jinka
Intensity assay	Deltamethrin 1X	32 (32/100) (R)	20.4 (21/103) (R)	10 (10/100) (R)	49 (49/100) (R)	29.7 (30/101) (R)	13 (13/100) (R)	88.7 (94/106)(R)	93 (93/100) (POR)	30(30/100) (R)	52 (52/100) (R)	22 (22/100) (R)	98 (98/100) (S)
	Deltamethrin 5X	63 (63/100)	27(27/100)	81 (81/100)	99 (99/100)	76 (76/100)	88 (88/100)	ND		95(95/100) (POR)	82(82/100) (R)	89 (89/100) (R)	
	Deltamethrin 10X	85 (85/100)	67(67/100)	97 (97/100)	NA	100 (100/100)	96 (96/100)			100 (100/100)	84(84/100)	99 (99/100)	
	Permethrin 1X	46 (46/100) (R)	33.7 (34/101) (R)	36 (36/100) (R)	50 (58/116) (R)	30 (30/100) (R)	61 (61/100) (R)	89 (89/100)(R)	85 (85/100)(R)	76(76/100) (R)	70(70/100) (R)	48 (48/100) (R)	88 (88/100) (R)
	Permethrin 5X	62 (60/97)	100(100/100)	90 (90/100)	99 (99/100)	85 (85/100)	99 (99/100)	ND		97(97/100)	75(75/100)	96.3 (93/100)	100 (100/100)
	Permethrin 10X	100 (100/100)	NA	99 (99/100)	NA	100 (100/100)	NA			99(99/100)	99(99/100)	98 (/98/100)	

ANNEX G: RESULTS OF CONE WALL BIOASSAY CONDUCTED AT FOUR SITES ON SPRAYED WALLS IN 2017

Time	Exposure height from the floor	% Mortality (dead/exposed) of *An. arabiensis*									
		Bako Tibe			Chewaka		Nono		Goro		
		Dung	Mud	Painted	Mud	Painted	Mud	Painted	Dung	Mud	Painted
T0 (June 2017)	High(1.5m)	100 (30/30)	100 (60/60)	100 (30/30)	100(90/90)	100(30/30)	100(60/60)	100(60/60)	100(40/40)	100(50/50)	100(30/30)
	Middle(1m)	100 (30/30)	100 (60/60)	100 (30/30)	100(90/90)	100(30/30)	100(60/60)	100(60/60)	100(40/40)	100(50/50)	100(30/30)
	Low(0.5m)	100 (30/30)	100 (60/60)	100 (30/30)	100(90/90)	100(30/30)	100(60/60)	100(60/60)	100(40/40)	100(50/50)	100(30/30)
	Mean	100 (90/90)	100 (180/180)	100 (90/90)	100(270/270)	100(90/90)	100(180/180)	100(180/180)	100(120/120)	100(150/150)	100(90/90)
T1 (July 2018)	High(1.5m)	100 (30/30)	100 (60/60)	100 (30/30)	100(90/90)	100(30/30)	100(63/63)	100(61/61)	100(42/42)	100(55/55)	100(36/36)
	Middle(1m)	100 (30/30)	100 (60/60)	100 (30/30)	100(90/90)	100(30/30)	100(64/64)	100(64/64)	100(45/45)	100(52/52)	100(32/32)
	Low(0.5m)	100 (30/30)	100 (60/60)	100 (30/30)	100(90/90)	100(30/30)	100(66/66)	100(61/61)	100(45/45)	100(50/50)	100(33/33)
	Mean	100 (90/90)	100(180/180)	100 (90/90)	100(270/270)	100(90/90)	100(193/193)	100(186/186)	100(132/132)	100(157/157)	100(101/101)
T2 (Aug 2017)	High(1.5m)	86.67(26/29)	71.18(42/59)	86.67(26/30)	92.13(82/89)	100(30/30)	100(63/63)	98.55(68/69)	100(42/42)	100(57/57)	100(37/37)
	Middle(1m)	93.33(28/30)	81.25(48/59)	76.67(23/30)	91.01(81/89)	92.59(25/27)	97.1(67/69)	100(65/65)	100(44/44)	100(55/55)	100(32/32)
	Low(0.5m)	83.3(25/30)	70.68(41/58)	90(27/30)	78.16(68/87)	96.67(29/30)	100(67/67)	98.46(64/65)	100(48/48)	100(56/56)	100(35/35)
	Mean	88.76 (79/89)	74.43(131/176)	84.44(76/90)	87.16(231/265)	96.55(84/87)	100(197/199)	98.9(197/199)	100(134/134)	100(168/168)	100(104/104)
T3 (Sept 2017)	High(1.5m)	90(27/30)	83.33(50/60)	90(27/30)	87.78(79/90)	93.33(28/30)	94.92(56/59)	100(66/66)	100(49/49)	100(55/55)	100(33/33)
	Middle(1m)	90 (27/30)	70(42/60)	90(27/30)	86.67(78/90)	96.67(29/30)	93.65(59/63)	100(62/62)	100(42/42)	100(50/50)	100(33/33)
	Low(0.5m)	86.67 (26/30)	66.67(40/60)	70(21/30)	73.33(66/90)	90(27/30)	90(63/66)	100(63/63)	100(42/42)	100(57/57)	100(34/34)
	Mean	88.9(80/90)	73.33(132/180)	83.33(75/90)	82.59(223/270)	93.33(84/90)	94.68(178/188)	100(191/191)	100(133/133)	100(162/162))	100(100/100)
T4 (Oct 2017)	High(1.5m)	90.9(27/30)	41.67(25/60)	80(24/30)	70(63/90)	93.33(28/30)	100(65/65)	100(64/64)	97.6(41/42)	100(52/52)	100(32/32)
	Middle(1m)	63.33(19/30)	46.67(28/60)	80(24/30)	80(72/90)	80(24/30)	98.38(61/62)	100(60/60)	100(42/42)	100(54/54)	100(31/31)
	Low(0.5m)	53.33(16/30)	33.33(20/60)	66.67(20/30)	64.44(58/90)	66.67(20/30)	98.33(59/60)	100(59/59)	96.85(40/43)	98(50/51)	100(30/30)
	Mean	68.89(62/90)	40.56(73/180)	75.56(68/90)	71.48(193/270)	80(72/90)	98.9(185/187)	100(183/183)	96.85(123/127)	99.36(156/157)	100(93/93)
T5 (Nov. 2017)	High(1.5m)	100 (30/30)	40(24/60)	60 (18/30)	75.56(68/90)	80(24/30)	100(63/63)	100(61/61)	100(43/43)	92.59(50/54)	100(31/31)
	Middle(1m)	60 (18/30)	48.33(29/60)	83.33(25/30)	74.44(67/90)	90(27/30)	90.5(57/63)	100(63/63)	97.5(39/40)	94.54(52/55)	100(33/33)
	Low(0.5m)	46.67(14/30)	33.33(20/60)	43.33(13/30)	58.89(53/90)	73.33(22/30)	89.3(50/56)	98.3(58/59)	92.85(39/42)	91.83(45/49)	96.77(30/31)
	Mean	68.89(62/90)	40.56(73/180)	62.22(56/90)	69.63(188/270)	81.11(73/90)	93.44(170/182)	99.45(182/183)	96.8(121/125)	93.03(147/158)	98.94(94/95)

Time	Exposure height from the floor	% Mortality (dead/exposed) of *An. arabiensis*									
		Bako Tibe			Chewaka		Nono		Goro		
		Dung	Mud	Painted	Mud	Painted	Mud	Painted	Dung	Mud	Painted
T6 (Dec. 2017)	High(1.5m)	76.67(23/30)	43.33(26/60)	63.33(19/30)	73.33(66/90)	66.67(20/30)	88.63(54/61)	96.67(58/60)	97.56(40/41)	90.38(47/52)	96.87(31/32)
	Middle(1m)	65.5(19/29)	48.33(29/60)	60(18/30)	75.56(68/90)	86.67(26/30)	78.05(50/64)	93.75(60/64)	83.3(35/42)	77.78(42/54)	90.9(30/33)
	Low(0.5m)	63.33(19/30)	38.33(23/60)	46.67(14/30)	51.11(46/90)	76.67(23/30)	74.6(47/63)	85.93(55/64)	78.04(32/41)	67.3(35/52)	87.5(28/32)
	Mean	68.5(61/89)	43.33(78/180)	56.67(51/90)	66.67(180/270)	76.67(69/90)	80.3(151/188)	92.02(173/188)	86.29(107/124)	78.48(124/158)	91.75(89/97)
Total	High(1.5m)	92.34(193/209)	68.49(287/419)	82.85(174/210)	85.53(538/629)	90.48(190/210)	97.69(424/434)	99.32(438/441)	99.33(297/299)	97.6(366/375)	99.56(230/231)
	Middle(1m)	81.81(171/210)	70.64(296/419)	84.29(177/210)	86.80(546/629)	92.27(191/207)	93.93(418/445)	99.08(434/438)	97.29(287/295)	95.94(355/370)	98.66(221/224)
	Low(0.5m)	76.19(160/210)	63.16(264/418)	73.80(155/210)	71.12(471/627)	86.19(181/210)	94.06(412/438)	97.45(420/431)	95.02(286/301)	93.97(343/365)	97.78(220/225)
	Mean	83.3(524/629)	67.43(847/1256)	80.32(506/630)	84.49(1555/1885)	89.63(562/627)	95.22(1254/1317)	98.6(1292/1310)	97.2(870/895)	95.9(1064/1110)	98.67(671/680)