THE PMI AFRICA IRS (AIRS) PROJECT

INDOOR RESIDUAL SPRAYING (IRS 2) TASK ORDER SIX

PMI AIRS LIBERIA

ENTOMOLOGICAL MONITORING

FINAL REPORT

DECEMBER 1, 2016 – NOVEMBER 30, 2017

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ACRONYMS

AIRS	Africa Indoor Residual Spraying
CDC	Centers for Disease Control and Prevention
ELISA	Enzyme-Linked Immunosorbent Assay
gCHV	general Community Health Volunteers
HBR	Human Biting Rate
HLC	Human Landing Catch
IRS	Indoor Residual Spraying
Kdr	Knock-Down Resistance
LLIN	Long-Lasting Insecticide-Treated Nets
MIS	Malaria Indicator Survey
NMCP	National Malaria Control Program
PBO	Piperonyl Butoxide
PCR	Polymerase Chain Reaction
PMI	President's Malaria Initiative
PSC	Pyrethrum Spray Collection
RR	Mutant Homozygote
RS	Mutant Heterozygote
SS	Susceptible Homozygote
WHO	World Health Organization

EXECUTIVE SUMMARY

In 2017, entomological monitoring in Liberia was conducted in four sentinel sites in Bong, Grand Bassa, Margibi, and Montserrado counties. Multiple collection methods, including human landing catches (HLCs), pyrethrum spray catches (PSCs), and Centers for Disease Control and Prevention (CDC) light traps, were used to capture information on the spatial and temporal composition and distribution of malaria vector mosquito species. Entomological monitoring was also conducted at 10 sites along a transect from Yekepa in Nimba County to Kollieman Town in Montserrado County as part of a two-year study to potentially identify more suitable, alternative sentinel sites. Insecticide susceptibility tests and insecticide resistance assays were conducted at an additional five sentinel sites in five counties to assess vector susceptibility to pyrethroid insecticides.

Across the four sentinel sites, *Anopheles gambiae* s.l. was the most dominant malaria vector found at all sites, with peak densities occurring in May or June and ranging from 10 – 27 mosquitoes per room per day as determined by PSCs. Numbers of *An. gambiae* s.l. mosquitoes collected using HLCs method were low across sites, which made comparisons of indoor versus outdoor biting activity challenging. However, it is noteworthy that a greater number of *An. gambiae* s.l. were collected outdoors than indoors in Frank Town, where the human biting Rrate (HBR) was highest among the sites (1.68 bites per person per night outdoors, 0.98 bites per person per night indoors). Molecular identification of a subsample of mosquitoes collected is planned for 2018. However, recently completed analyses of mosquitoes collected in 2015 – 2016 revealed that the majority are *An. coluzzii* (79%), 21% *An. gambiae*.

The transect study produced similar results, with *An. gambiae* s.l. comprising the majority of vector mosquitoes collected with peak densities observed in May. However, *Anopheles funestus* s.l. was found at most of the sites, with the highest numbers found at Jenepleta, Koyah and Zeanzue. Samples from these sites will be processed through ELISA to determine the sporozoite rate. The transect study will continue into 2018 with additional collections in May and October 2018. Following laboratory analysis, results will be evaluated to decide whether any of the sites should be added as sentinel sites for monitoring and resistance testing.

Insecticide susceptibility tests revealed that *An. gambiae* s.l. is resistant to deltamethrin at all sentinel sites and to alphacypermethrin at all sites except Sergent Kolly. Deltamethrin resistance intensity was found to be 10 times the diagnostic dosage at Saint John, Tubmanburg and Cari sites. Synergist assays showed that exposure to piperonyl butoxide (PBO) restored *An. gambiae* s.l. susceptibility to alphacypermethrin but not deltamethrin in Bong County, and failed to restore deltamethrin susceptibility in mosquitos tested at other sites. These data suggest multiple mechanisms of pyrethroid resistance.

Entomological monitoring activities continue to provide important information on malaria vector species composition and seasonality in Liberia. High pyrethroid resistance intensity and the likelihood of multiple resistance mechanisms suggest the need for an expanded geographical range for insecticide resistance monitoring activities to inform the decision-making process for prioritizing areas and planning for deployment of malaria vector control interventions in Liberia.

1. INTRODUCTION

Malaria is endemic in Liberia, with nationwide prevalence remaining unchanged at 45% among children aged 6-59 months, from 2011 to 2016 (2011 MIS, 2016 MIS). According to the 2016 MIS, malaria prevalence is more than two-fold greater in rural areas (62%) than in urban areas (30%). While indoor residual spraying (IRS) was implemented from 2009 through 2013, long-lasting insecticide treated nets (LLINs) are currently the only vector control strategy in Liberia.

Entomological monitoring and IRS were conducted in Bong County from 2009 through 2013, with entomological monitoring continuing through 2017. Under the President's Malaria Initiative (PMI) Africa Indoor Residual Spraying (AIRS) project, vector monitoring was conducted in two main sentinel sites starting in 2012: Tomato Camp in Bong County and Frank Town in Montserrado County, which were last sprayed in 2013 and 2012, respectively. In 2016, VectorLink added two sentinel sites, following the recommendation of the National Malaria Control Program (NMCP): Jeneta in Margibi County and Bokay Town in Grand Bassa County. Bokay Tow was used as sentinel site in 2012 and was sprayed previously while Jeneta has never been sprayed or served as a monitoring site. In 2017, monthly entomological surveillance was conducted in all four sites.

Entomological monitoring was conducted to determine the malaria vector species composition, density, seasonality, behavior, and susceptibility to pyrethroid insecticides. *Anopheles* mosquitoes were sampled using HLCs, PSCs and CDC light trap collections. Insecticide susceptibility tests were conducted from December 2016 to November 2017 at five sites in five counties (Nimba, Bong, Bomi, Grand Bassa, and Montserrado) as indicated in the map (Figure 1). Testing in Margibi was planned for 2017, but will not be conducted until 2018.

FIGURE 1: AIRS LIBERIA ENTOMOLOGICAL MONITORING SITES AND INSECTICIDE SUSCEPTIBILITY SITES, DEC 2016 TO NOV 2017

In addition to the routine activities at established sentinel sites, two periodic collections were conducted as part of a transect study in 10 selected villages in four counties (Nimba, Bong, Margibi and Montserrado). These collections provided additional information on the species composition and abundance of malaria vectors in Liberia, and produced preliminary data that could guide further study site selection. The AIRS in-country entomologist (also referred to as the technical manager) and NMCP vector control unit staff completed the collections, with support from general community health volunteers (gCHVs) hired in the study sites.

2. OBJECTIVES

- Determine insecticide susceptibility of *An. gambiae* s.l., the primary local malaria vector
- Determine the spatial and temporal composition and distribution of anopheline vector species
- Maintain and support a functional insectary
- Build local capacity in entomological surveillance methods and techniques

3. MATERIALS AND METHODS

3.1 ENTOMOLOGICAL MONITORING

AIRS supported entomological monitoring activities at four sites in 2017. Tomato Camp (Kpaai District) sits at a higher altitude than Jeneta (Five District), Frank Town (Careysburg District), and Bokay Town (District One). However, all four sites are hot and humid rural areas. The vegetation is a scanty canopy, with extensive clearing of the forests. House walls are made of mud, and roofs are corrugated iron sheets or grass, and farming is the residents' main activity. Potential mosquito breeding sites are present in and around villages, mainly during the rainy season (May to October). Human-made mosquito breeding sites such as borrow pits and brick pits are common in this area. The breeding sites are rain-dependent, shallow, transient water pools that disappear in the dry season (December to March).

3.1.1 ADULT MOSQUITO COLLECTIONS

Three mosquito collection methods were used to collect adult mosquitoes in the four sentinel sites: PSC, CDC light traps and HLC. PSC collections occurred monthly from December 2016 to November 2017 in 20 houses per study site during two consecutive days. Because of low mosquito densities in Bokay Town, PSC collections were suspended in July 2017. The PSC technique was used to collect indoor resting mosquitoes between 6:30 AM and 8:00 AM at each study site using a commercial pyrethroid insecticide spray called Hewelon[1]. In the houses selected for spraying, white cloth/sheets were placed on the floor from wall to wall. The teams sprayed the aerosol insecticide in the house after closing windows and doors. A 10-minute knock-down period was allowed and the sheets were collected. The teams then identified and recorded mosquitoes from each house.

CDC light traps were used to collect mosquitoes on two consecutive nights per month using eight traps (four inside and another four outside) from 6:00 PM to 6:00 AM in each study village. The light traps were set up in selected houses where people slept under a mosquito net. The consent of the head of each household was acquired beforehand. The traps were placed toward the sleepers' legs and hung them approximately 150 cm above the ground, depending on whether the person slept on the bed or the floor. The outdoor CDC light traps were set up near the houses with the indoor traps away from the main door. The outdoor traps were not baited

HLCs, performed indoors and outdoors, were conducted in two locations per village, on one night, every two months. HLCs were used to collect mosquitoes landing on human baits between 6:00 PM and 6:00 AM. With legs exposed to attract host-seeking mosquitoes, two human baits sat indoors and another pair sat outdoors. The pairs switched between outdoors and indoors on an hourly basis. The collectors used flashlights and a tubing aspirator to collect mosquitoes after they had landed on the human baits' legs but before the mosquitoes could bite. The teams transferred the mosquitoes to paper cups topped with mosquito net.

The HBR was calculated by dividing the number of mosquitoes collected per site by the number of collection nights times the number of collectors. The endophagic rate is the proportion of mosquitos that feeds indoors, and the exophagic rate is the proportion that feeds outdoors.

[1] Hewlon ingredients: Rich-D-Transallethrin 0.1%, Tetramethrin 0.2%, Bata-Cypermethrin 0.3%,), manufactured by Guangdong QUNME Industrial Daily Chemicals Co., Ltd. China,

All mosquitoes were identified morphologically. The *Anopheles* species identified (Gillies and Coetzee 1987) were preserved on silica gel for laboratory processing to identify their infection status by the Enzyme-Linked Immunosorbent Assay (ELISA). Following analysis with ELISA, a subsample of mosquitoes were selected for testing by polymerase chain reaction (PCR) to determine the vector population's composition. The outcomes of this study will provide more information on vector composition, distribution and infection rates.

The AIRS team used chi-square tests to determine the differences between two selected variables such as number of mosquitoes collected indoors and outdoors per site and percentage mortality obtained with and without PBO for synergist assays.

3.2 Transect Study Conducted in 10 Sites

The team conducted a transect study in Liberia to provide additional insight on vector abundance, composition, behavior and sporozoite rates outside the routine monitoring sites. The goal was to gather information for decision-making in the selection of new sites for routine vector monitoring and insecticide resistance surveillance in the country. A team comprising three NMCP staff and the Abt entomologist conducted entomological monitoring activities in 10 villages along a transect from Yekepa in Nimba County to Kollieman Town in Montserrado County (Figure 2) at the beginning of the rainy season in May 2017. We performed a second round of vector surveillance at the same sites at the end of the rainy season in October 2017.

FIGURE 2: LOCATION OF PMI AIRS LIBERIA TRANSECT STUDY SITES PROSPECTED IN MAY AND OCTOBER 2017

The team collected adult mosquitoes in 20 houses per village, using the PSC method, from 06:00 AM to 08:00 AM. Collections using CDC light traps were also completed in four houses, both indoors and outdoors (eight traps per site), from 06:00 PM to 06:00 AM. All mosquitoes were identified morphologically. The *Anopheles* species identified (Gillies and Coetzee 1987) were preserved on silica gel for laboratory processing to identify their infection status by the Enzyme-Linked Immunosorbent Assay (ELISA). Following analysis with ELISA, a subsample of mosquitoes will be analyzed by polymerase chain reaction (PCR) to determine the vector population's composition. The outcomes of this study will provide more information on vector composition, distribution and infection rates.

3.3 INSECTICIDE SUSCEPTIBILITY TESTS

From December 2016 to November 2017, NMCP staff and the AIRS entomologist conducted insecticide resistance intensity assays at five sites (see Annex 1). They used CDC bottle tests and World Health Organization (WHO) tube tests for susceptibility tests using the diagnostic and intensity assay concentrations. The main goal of the tests was to monitor the intensity of resistance of *An. gambiae* s.l. mosquitoes to pyrethroids (deltamethrin and alpha-cypermethrin). The results will help to plan for malaria vector control and to map insecticide resistance status across the country.

In the counties visited, the AIRS team and gCHVs collected *An. gambiae* s.l. larvae mainly in rice fields, brick pits, and water pools from 10:00 AM to 02:00 PM. The team reared *Anopheles* larvae in a field insectary, where they monitor relative humidity and temperature to keep mosquitoes in suitable conditions for the tests.

3.3.1 SUSCEPTIBILITY TESTS USING WHO METHOD

AIRS purchased filter papers impregnated with different pyrethroid (deltamethrin, alphacypermethrin) from Malaysia and used them to assess the susceptibility status of *An. gambiae* s.l. For each insecticide to be tested, insecticide impregnated papers were inserted in four exposure tubes. The exposure tube for the control contained a paper impregnated with oil only. Blank filter papers were introduced in four replicate holding tubes and one control tube. Two- to five-day old female *An. gambiae* s.l. mosquitoes were transferred in the holding tubes for one hour before transferring them to the exposure tubes. The knockdown was recorded per time interval for one hour. After one hour of exposure in exposure tubes, mosquitoes were transferred back in the holding tubes and fed them sugar solution. The mortality was recorded 24 hours after the test. The team preserved a subsample of dead mosquitoes and all the survivors in labeled Eppendorf tubes.

3.3.2 CDC BOTTLE ASSAY AT DIAGNOSTIC DOSE AND INTENSITY ASSAY

The susceptibility of *An. gambiae* s.l. mosquitoes to the pyrethroid insecticides deltamethrin 1X and alpha-cypermethrin 1X was determined using the CDC bottle-test technique. Mosquito mortality was recorded at 15-minute intervals up to 120 minutes (two hours). Four replicates and one control with 25 mosquitos each for each insecticide used for the tests. In total, 250 wild females *An. gambiae* s.l. were tested for two hours of exposure to deltamethrin and to alpha-cypermethrin. Mosquitoes that die at the diagnostic time (30 minutes) were considered to be susceptible. Mosquitoes that survived beyond that time represented the proportion of resistant individuals in the population of *An. gambiae* s.l. tested. In Bentor, Montserrado County, two tests (Delta1, Delta2) were conducted using deltamethrin and two others using alpha-cypermethrin (Alpha1, Alpha2).

For the resistance intensity assays, CDC Atlanta provided PMI AIRS the vials containing the technical grade insecticides concentrations (1X, 2X, 5X and 10X). The dilution was done with 50ml acetone per tube. For the intensity assays, the team coated four test bottles with 1ml of different concentrations of the prepared insecticide solutions (deltamethrin and alpha-cypermethrin). The team coated the control bottles with 1 ml acetone only. The team kept them overnight to dry before use. About 25 females *An. gambiae* s.l. two- to five-days old were transferred into each of the tubes. The mortality was recorded per time interval up to two hours.

3.3.3 SYNERGIST ASSAY USING CDC AND WHO TUBE TEST METHOD

The data collected from 2014 up to 2017 have shown that in Liberia, *An. gambiae* s.l. populations were resistant to deltamethrin and alphacypermethrin. Based on that, AIRS used both the WHO and CDC methods to conduct synergist assays with PBO on the two insecticides using two- to five-day-old adult *An. gambiae* s.l. female mosquitos which emerged from pupae.

For the CDC method, PBO vial tube contents were diluted with 50ml acetone solution. One bottle was coated with 1ml of PBO solution for use as the synergist-exposure bottle. A second bottle was coated with 1 ml of acetone to serve as a synergist-control bottle. These were left to dry overnight. A subsample of 125 females *An. gambiae* s.l. were introduced into the synergist-exposure bottle for one hour. Another 125 females *An. gambiae* s.l. transferred for one hour into the synergist-control bottle coated with acetone only. We transferred the mosquitoes in two different cages labeled "PBO" and none "PBO." After one hour, we transferred the mosquitoes to two holding cages: one for the synergist control mosquitoes and another for the synergist-exposure mosquitoes. Four replicate tests were done for PBO and non-PBO, based on the CDC bioassays method, resulting in eight bottles - four bottles for PBO and four bottles for non-PBO. In each insecticide coated and control bottle, we introduced 25 females using a mouth aspirator and recorded the mortality every 15-min, up to two hours.

For the WHO method, we used WHO filter papers impregnated with PBO to conduct synergist tests. After the one-hour exposure period to PBO (no PBO for insecticide alone tests), we transferred mosquitoes to exposure tubes and completed the WHO tube tests as per the standard procedure.

For both CDC and WHO methods, when control mortality was higher than 5%, mortality data were corrected with Abbott's formula. With more than 20% mortality among control mosquitoes, the tests were discarded.

3.3.4 LABORATORY ANALYSES

Samples collected through entomological activities from the three permanent sentinel sites and through the transect study were analyzed using ELISA to determine sporozoite infection rates and by PCR for molecular species identification. Of the approximately 1,000 *An. gambiae* s.l. mosquito samples preserved after the 2015-2016 susceptibility tests, VectorLink sent 200 surviving and dead mosquitoes to the University of Witwatersrand, South Africa, for molecular identification to the species level using primers for *An. gambiae*, *An. arabiensis*, *An. merus,* and *An. quadriannulatus.* In addition, the University conducted molecular diagnosis of the insecticide resistance method, using hydrolysis probes for knock-down resistance (*kdr)*-mutation to analyze a subsample of *An. gambiae* s.l. that was exposed to pyrethroids and analyzed another subsample exposed to organophosphate or carbamate insecticides for Ace-1^R mutation.

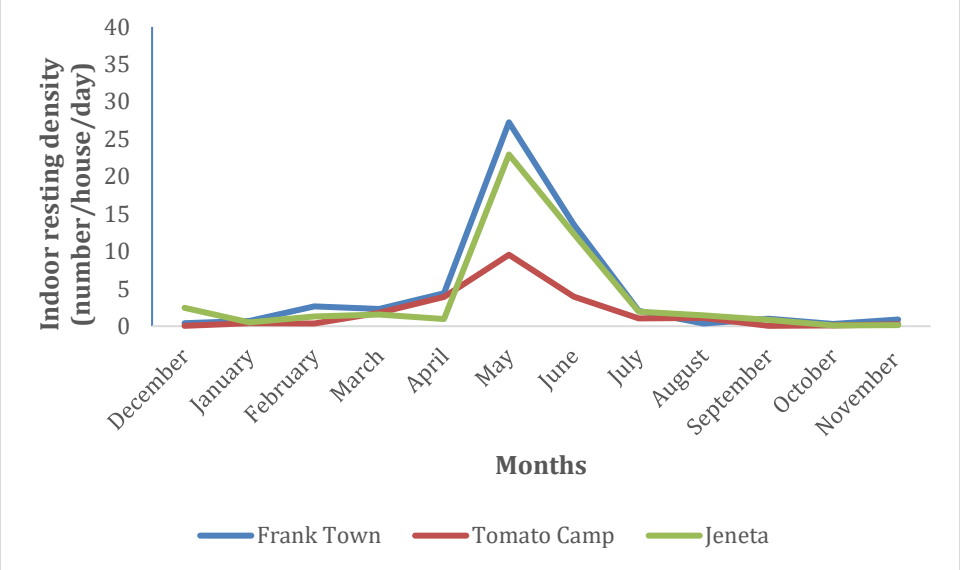
In 2018, VectorLink plans to develop local capacity in order to process the remaining of the samples.

4. RESULTS AND DISCUSSION

4.1 INDOOR RESTING DENSITY

From December 2016 to November 2017, PSCs collected a total of ## female *An. gambiae* s.l. (including 1,118 in Frank Town, 451 in Tomato Camp, and 931 in Jeneta). An additional ### other Anopheles collected are summarized in Annex 2. The annual peak vector indoor resting densities were observed in May-June in all three sentinel sites (Figure 3), with 27, 23, and 10 *An. gambiae* s.l. collected per room per day in Frank Town, Jeneta, and Tomato Camp, respectively, and the density was generally lower from July through April in all sentinel sites, when compared to the rest of the study period. A sharp decline in vector abundance observed from July to September, was attributable to frequent and abundant rainfall. Such rainfall can wash out *An. gambiae* s.l. breeding sites

FIGURE 3. INDOOR RESTING DENSITIES OF *AN. GAMBIAE* S.L. AS COLLECTED BY PSCS, IN FRANK TOWN, TOMATO CAMP AND JENETA SENTINEL SITES (DECEMBER 2016 – NOVEMBER, 2017)



The majority of *An. gambiae* s.l. collected by PSC were blood-fed, comprising ≥80% of mosquitoes collected in all three sentinel sites (Figures 4 – 7). Few other mosquito species (including *An. funestus)* were collected, in Frank Town, Tomato Camp, and Jeneta.

FIGURE 4: ANNUAL DISTRIBUTION OF *AN. GAMBIAE* S.L. MOSQUITOES BY ABDOMINAL STAGE, COLLECTED BY PSCS IN FRANK TOWN, DEC 2016-NOV 2017

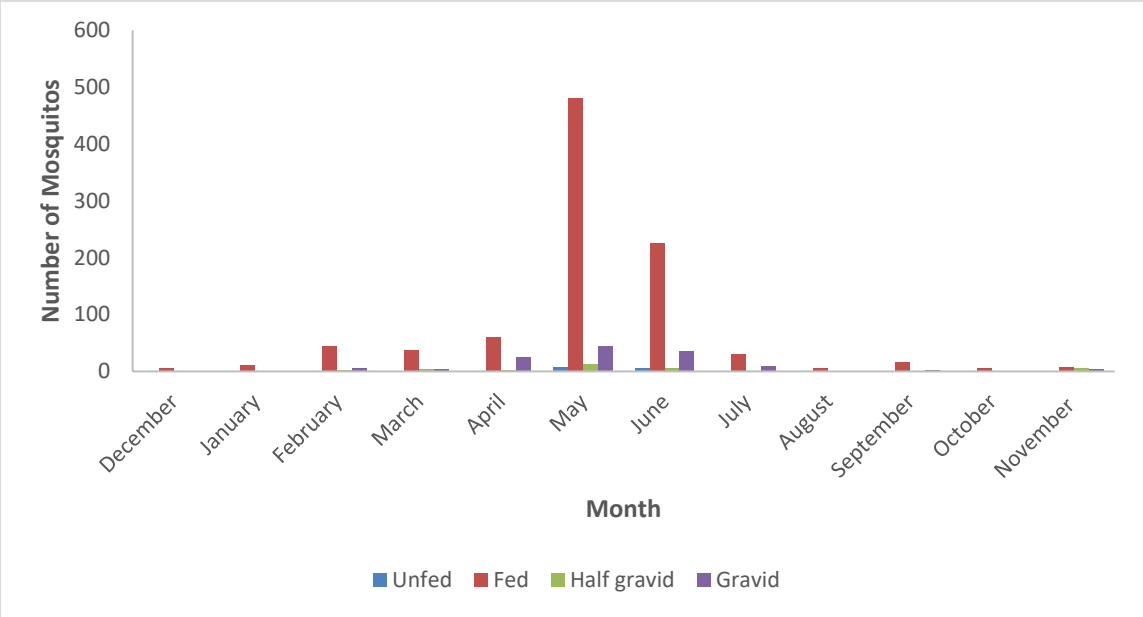


FIGURE 5: ANNUAL DISTRIBUTION OF *AN. GAMBIAE* S.L. MOSQUITOES BY ABDOMINAL STAGE, COLLECTED BY PSCS IN TOMATO CAMP, DEC 2016-NOV 2017

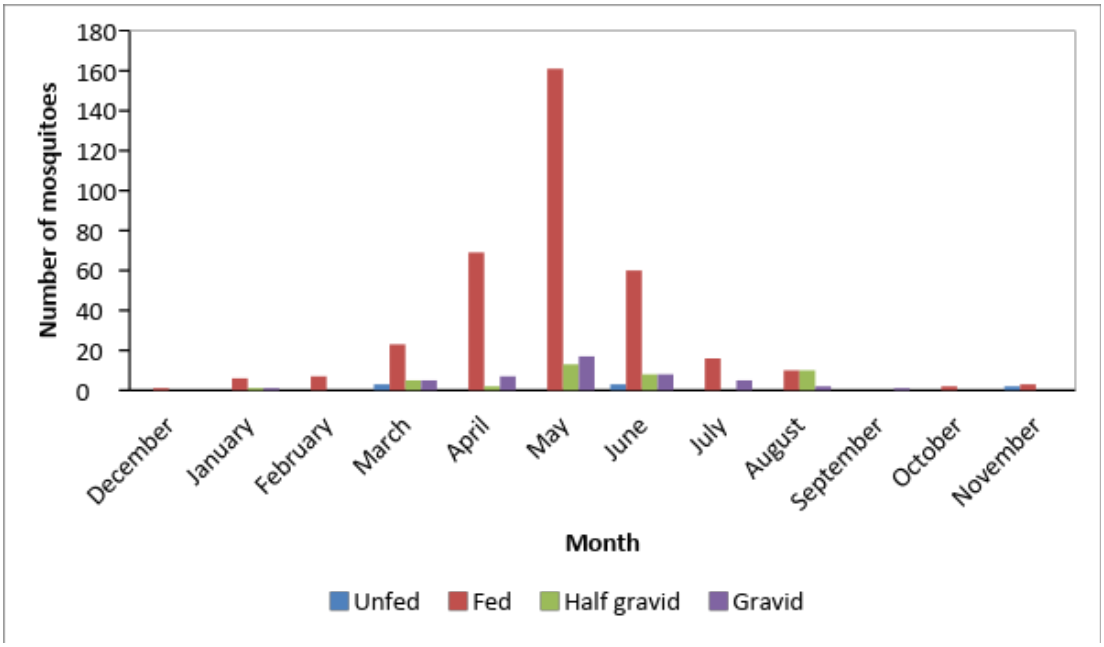


FIGURE 6: ANNUAL DISTRIBUTION OF *AN. GAMBIAE* S.L. MOSQUITOES BY ABDOMINAL STAGES COLLECTED BY PSC IN JENETA, DEC 2016 TO NOV 2017

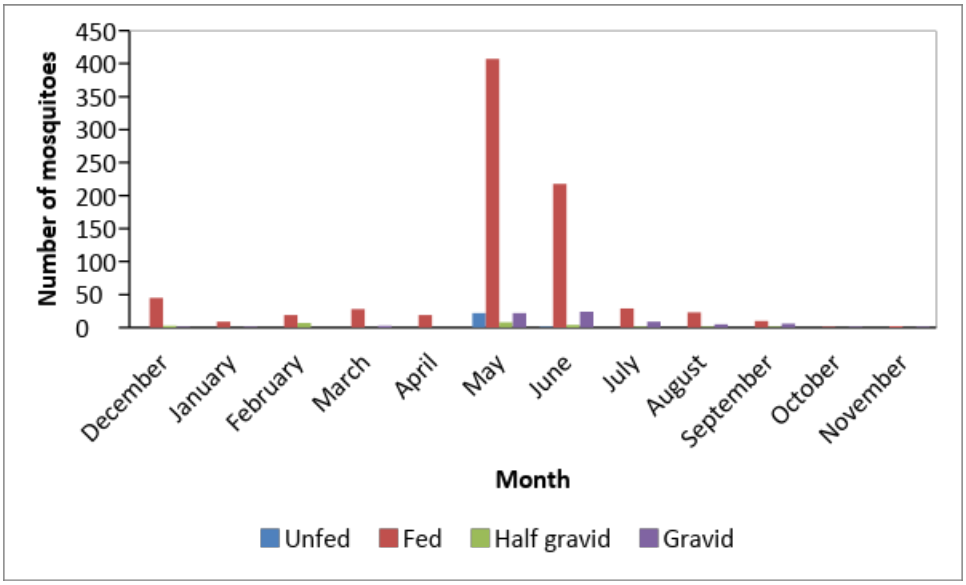
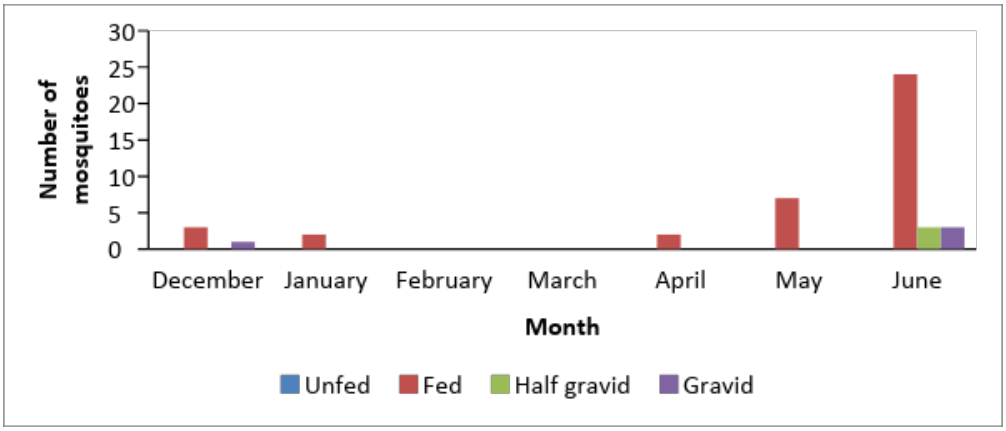


FIGURE 7: ANNUAL DISTRIBUTION OF *AN. GAMBIAE* S.L. MOSQUITOES BY ABDMINAL STAGES COLLECTED BY PSC METHOD IN BOKAY TOWN, DEC 2016 TO JUN 2017



4.2 HUMAN LANDING COLLECTIONS

In total, the team collected 193 *An. gambiae* s.l. in the four sentinel sites using HLC (Table 1). In Frank Town, where we collected more than 60% of *An. gambiae* s.l. by HLC, there was a higher tendency toward outdoor biting. However, the biting rates were too low in all other sites to establish any pattern on biting behavior of the vector.

TABLE 1: NUMBER OF *AN. GAMBIAE* S.L. COLLECTED INDOORS AND OUTDOORS BY HLC IN TOMATO CAMP, FRANK TOWN, AND JENETA, JAN-NOV 2017

Site	Indoor N (*)	Outdoor N (*)	Total	HBR Indoor (bites/person/night)	HBR Outdoor (bites/person/night)
Tomato Camp	13 (0.65)	7 (0.35)	20	0.30	0.16
Frank Town	43(0.37)	74 (0.63)	117	0.98	1.68
Jeneta	23 (0.46)	27 (0.54)	50	0.52	0.61
Bokay Town**	3 (0.50)	3 (0.50)	6	0.13	0.07
Total	**82**	**111**	**193**		

Note: (*) Indexes for endophagy and exophagy. Values in parentheses represent proportions of endophagy and exophagy.
(**) only calculated for collections from January to June 2017.

The highest HBR for both indoor and outdoor was observed in Frank Town where the indoor HBR was 0.98 bites per person per night and the outdoor HBR was 1.68 bites per person per night. Note that in Bokay Town, HLC was only done for six nights for the period from January to June 2017 due to low numbers of mosquitoes collected.

4.3 CDC LIGHT TRAP COLLECTIONS

From December 2016 through November 2017, 657 *An. gambiae* s.l. were collected in CDC light traps at Frank Town, Tomato Camp, and Jeneta. Few mosquitoes (13 *An. gambiae* s.l.) were collected in Bokay Town compared with the other sentinel sites, and were an insufficient number to include the site in subsequent analyses. The team collected the greatest number of *An. gambiae* s.l. in CDC light traps in May at Jeneta and in June at both Frank Town and Tomato Camp (Figure 8 and 9). For the latter two sites, this differs from the peak indoor resting density we observed with PSCs, which was in May (Figure 3). Few other mosquito species were collected, although small numbers of *An. funestus*, *An. rufipes*, and *An. ziemanni*were also collected.

FIGURE 8. NUMBER OF *AN. GAMBIAE* S.L. COLLECTED INDOORS PER TRAP PER NIGHT IN CDC LIGHT TRAP COLLECTIONS AT FRANK TOWN, TOMATO CAMP AND JENETA, DECEMBER 2016 – NOVEMBER 2017.

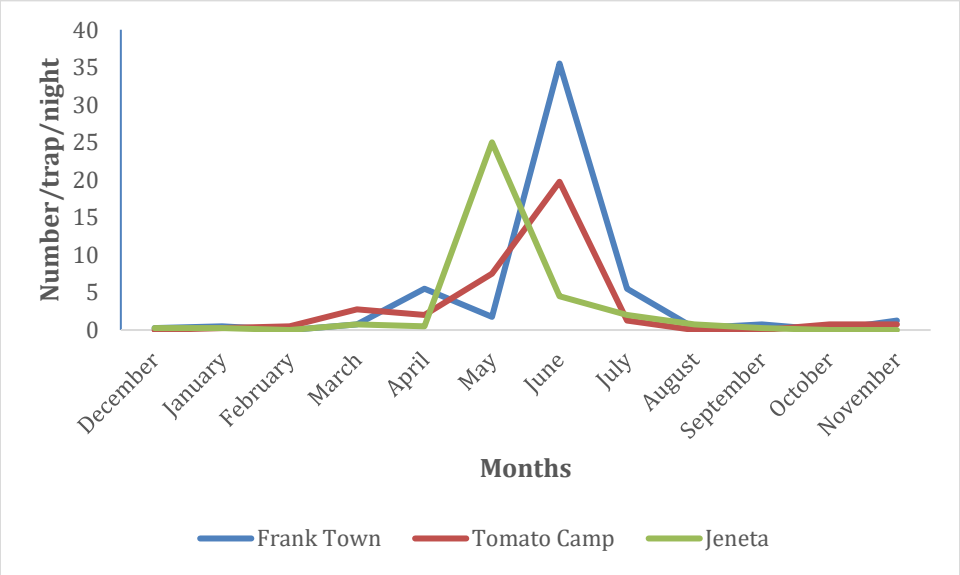
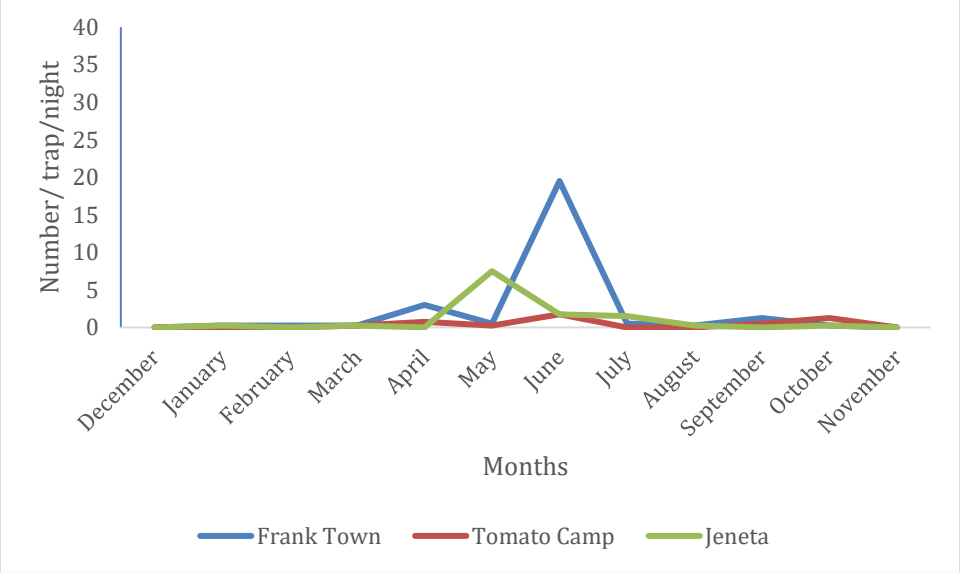


FIGURE 9. NUMBER OF *AN. GAMBIAE* S.L. COLLECTED OUTDOORS PER TRAP PER NIGHT IN CDC LIGHT TRAP COLLECTIONS AT FRANK TOWN, TOMATO CAMP AND JENETA, DECEMBER 2016 – NOVEMBER 2017.



4.4 RESULTS OF TRANSECT STUDY

4.4.1 PYRETHRUM SPRAY CATCHES AND CDC LIGHT TRAP COLLECTIONS

The data collected during the transect study showed that *An. gambiae* s.l. is the most abundant vector, with *An. funestus,* a secondary vector of malaria in Liberia, was also found in nearly all sites (see Annex 3 for summary of species composition by site).

During the study using the PSC method, a total of 1883 mosquitoes were collected (1,772 female *An. gambiae* s.l., 99 *An. funestus*, 8 *Culex*, 3 *Aedes,* and 1 *Mansonia).* Out of the total *An. gambiae* s.l. collected, 1,268 were bloodfed (72%). The highest number of *An. gambiae* s.l. was observed in Jackson Town in Margibi County. Table 2 shows the indoor resting density as determined by PSC, the HBR at each site, and the abdominal stages of mosquitoes collected in the areas.

The HBR was highest in Jackson Town (Margibi County), Kingville (Montserrado County), and Gwekpolosue (Margibi County). All these sites are rice-growing areas where widespread breeding sites are available for *An. gambiae* s.l. (Table 2).

TABLE 2: ABDOMINAL STAGES, INDOOR RESTING DENSITY, AND HBR OF *AN. GAMBIAE* S.L. MOSQUITOES COLLECTED BY PSC IN SITES VISITED DURING THE TRANSECT STUDY, MAY 11-21, 2017

Site	Number of people in house	Abdominal Stages					Indoor Resting Density (number per house per day)	HBR (bites per person/night)
		Unfed	Fed	Half gravid	Gravid	Total		
Gbedin Camp3	50	96	85	1	29	211	10.55	1.70
Gwekpolosue	64	5	216	0	55	276	13.8	3.38
mJackson Town	56	16	332	4	63	415	20.75	5.93
Jenepleta	33	1	47	0	7	55	2.75	1.42
Kingville	57	21	245	0	67	333	16.65	4.30
Kollieman Town	63	0	122	0	12	134	6.7	1.94
Koyah	41	0	89	0	22	111	5.55	2.17
Unification Township	31	31	77	4	10	122	6.1	2.48
Zeanzue	47	0	36	0	15	51	2.55	0.77
Zolowee	32	12	19	6	27	64	3.2	0.59
Total	**474**	**182**	**1,268**	**15**	**307**	**1,772**		

Among mosquitoes collected using the CDC light trap method, the vector composition showed a high density per trap per night for *An. gambiae* s.l. (44.63/trap/night). For this method, the densities of *An. funestus,* a secondary vector of malaria in Liberia, were highest in Zolowee and Unification Township in Nimba County, and Jenepleta and Koyah in Bong County (Table 3).

TABLE 3: DISTRIBUTION OF TYPE OF MOSQUITOES COLLECTED USING CDC LIGHT TRAP IN 10 SITES VISITED DURING THE TRANSECT STUDY, MAY 11-21, 2017

Site	*An. gambiae s.l.*	*An. funestus*	*An. nili*	*An. ziemanni*	*An. rufipes*	*Culex*	*Aedes*	*Mansonia*	*Toxorhynchites*
Gbedin Camp3	357	3	2	0	0	46	0	2	0
Gwekpolosue	8	0	0	1	0	14	3	0	0
Jackson Town	119	0	0	0	1	54	0	3	0
Jenepleta	4	15	2	0	1	21	0	1	0
Kingville	176	0	0	0	0	22	1	1	0
Kollieman Town	4	0	0	0	0	23	0	0	0
Koyah	12	7	0	0	0	19	0	0	0
Unification Township	7	15	0	0	0	24	0	0	0
Zeanzue	8	0	0	0	0	46	0	0	0
Zolowee	45	22	0	0	4	10	0	1	1
Total	**740**	**62**	**4**	**1**	**6**	**279**	**4**	**8**	**1**

In October 2017, collections were done again in the 10 sites investigated in May to compare the entomological parameters for the two periods (Tables 4 and 5).

TABLE 4: ABDOMINAL STAGES, DENSITY, AND HBR OF *AN. GAMBIAE* S.L. MOSQUITOES IN SITES VISITED DURING THE TRANSECT STUDY BY PSC, OCTOBER 14-24, 2017

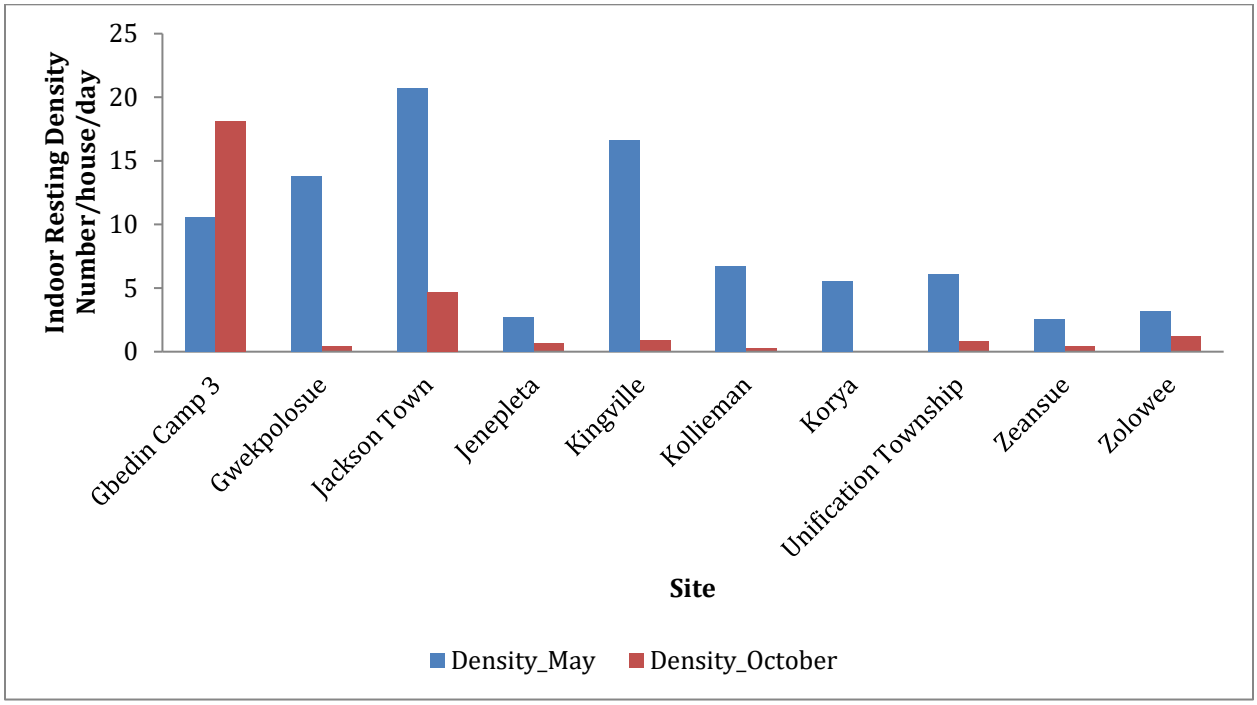
Site	Number People in house	Abdominal Stages					Indoor resting density (number per house per day)	HBR (bites per person/night)
		Unfed	Fed	Half gravid	Gravid	Total		
Gbedin Camp 3	56	42	274	0	47	363	18.15	4.89
Gwekpolosue	59	0	6	0	2	8	0.40	0.10
Jackson Town	61	0	70	0	23	93	4.65	1.15
Jenepleta	59	1	9	0	4	14	0.70	0.15
Kingville	51	0	12	1	5	18	0.90	0.24
Kollieman	55	0	4	0	1	5	0.25	0.07
Koyah	52	0	1	0	0	1	0.05	0.02
Unification Township	50	1	6	5	4	16	0.80	0.12
Zeanzue	66	0	7	0	2	9	0.45	0.11
Zolowee	54	2	13	0	9	24	1.20	0.24
Total	**563**	**46**	**402**	**6**	**97**	**551**		

TABLE 5: DISTRIBUTION OF MOSQUITOES COLLECTED USING CDC LIGHT TRAP IN 10 SITES VISITED DURING THE TRANSECT STUDY, OCTOBER 14-24, 2017

Sites	*An. gambiae s.l.*	*An. Funestus*	*An. rufipes*	*An. ziemanni*	*Culex*	*Aedes*	*Mansonia*
Gbedin Camp 3	323	0	6	0	4	0	11
Gwekpolosue	5	0	1	6	53	5	1
Jackson Town	0	0	0	0	20	0	0
Jenepleta	2	0	1	0	4	0	0
Kingsville	0	0	0	0	41	0	0
Kollieman	0	0	0	0	46	0	0
Koyah	0	1	0	0	39	0	0
Unification Town	0	0	0	0	10	0	0
Zeanzue	6	12	0	0	8	0	0
Zolowee	10	9	0	1	28	0	0
Total	**346**	**22**	**8**	**7**	**253**	**5**	**12**

In October, the indoor resting density of *An. gambiae* s.l. was lower than in May (Figure 10) but the species compositions were similar (Table 5 and 7). The next step will be determining the sporozoite infection rates for the two seasons to estimate the seasonal entomologic inoculation rates along the transect. This will be done in 2018 as part of lab processing by ELISA.

FIGURE 10: INDOOR RESTING DENSITY PER HOUSE OF *AN. GAMBIAE* S.L. COLLECTED BY PSC IN 10 TRANSECT STUDY SITES IN LIBERIA, MAY AND OCTOBER 2017



The number of *An. funestus* collected using PSC and CDC is indicated in Table 6. Indoor resting density as determined by PSC is summarized as well. Jenepleta, Koyah and Zeanzue were the sites with the highest number of *An. funestus* collected. Overall, the difference between the number collected in May and October was statistically significant (p=0.00027) using the Chi-square test.

TABLE 6: NUMBER OF *ANOPHELES FUNESTUS* MOSQUITOES COLLECTED USING PSC AND CDC LIGHT TRAP IN 10 SITES VISITED DURING THE TRANSECT STUDY, MAY AND OCTOBER, 2017.

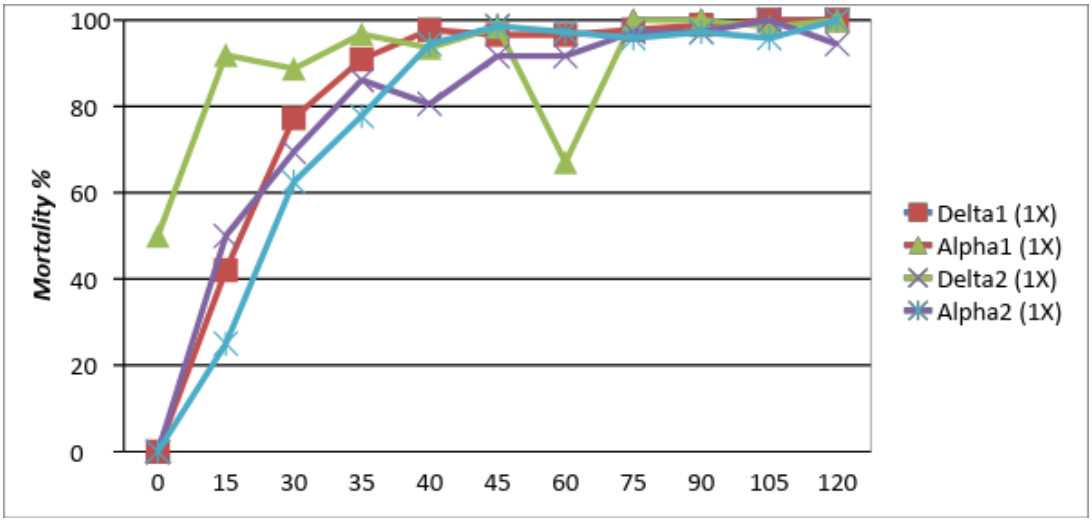
Site	PSC					*CDC Light Trap*			TOTAL
	May, 2017	**October, 2017**	***Sub Total***	**Density May**	**Density October**	**May, 2017**	**Oct, 2017**	***Sub Total***	
Gbadin Camp3	3	0	*3*	0.15	0	3	0	*3*	**6**
Gwekpolosue	11	1	*12*	0.55	0.05	0	0	*0*	**12**
Jackson Town	0	0	*0*	0	0	0	0	*0*	**0**
Jenepleta	19	20	*39*	0.95	1	15	0	*15*	**54**
Kingville	1	0	*1*	0.05	0	0	0	*0*	**1**
Kollieman Town	0	0	*0*	0	0	0	0	*0*	**0**
Koyah	35	5	*40*	1.75	0.25	7	1	*8*	**48**
Unification Township	1	1	*2*	0.05	0.05	15	0	*15*	**17**
Zeanzue	27	24	*51*	1.35	1.2	0	12	*12*	**63**
Zolowee	2	3	*5*	0.1	0.15	22	9	*31*	**36**
Total	**99**	**54**	***153***			**62**	**22**	***84***	**237**
chi-square :13.24 ; p-value: 0.00027									

4.5 INSECTICIDE RESISTANCE TESTS

4.5.1 INSECTICIDE RESISTANCE AT DIAGNOSTIC DOSAGES AND RESISTANCE INTENSITY ASSAYS

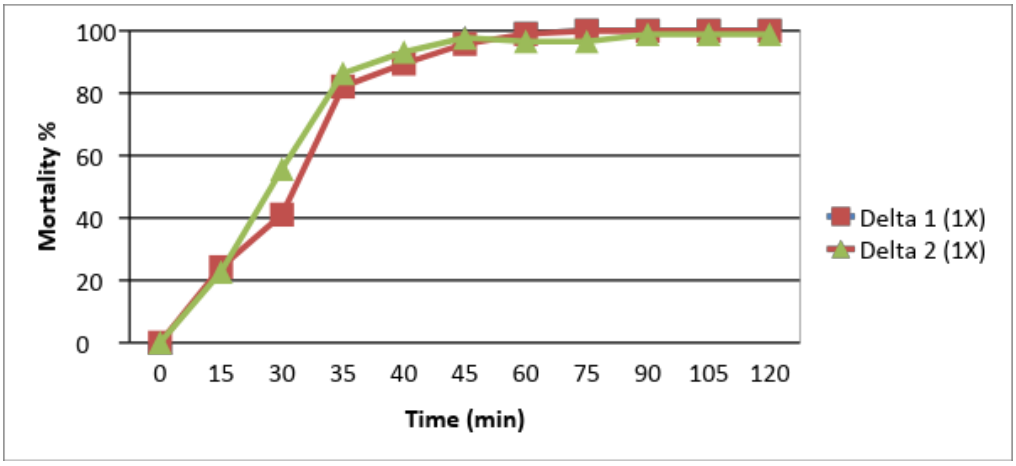
Figure 11 shows that the observed mortality rates for deltamethrin 1X were 77% at test 1 and 69% at test 2. The observed mortality rates for alpha-cypermethrin 1X were 89% at test 1 and 63% at test 2. The populations of *An. gambiae* s.l. were resistant to both pyrethroids.

FIGURE 11: TIME TO MORTALITY FOR *AN. GAMBIAE* S.L. FROM BENTOR, MONTESERRADO COUNTY, EXPOSED TO 1X DELTAMETHRIN AND 1X ALPHA-CYPERMETHRI USING CDC BOTTLE TESTS, MAY 2017



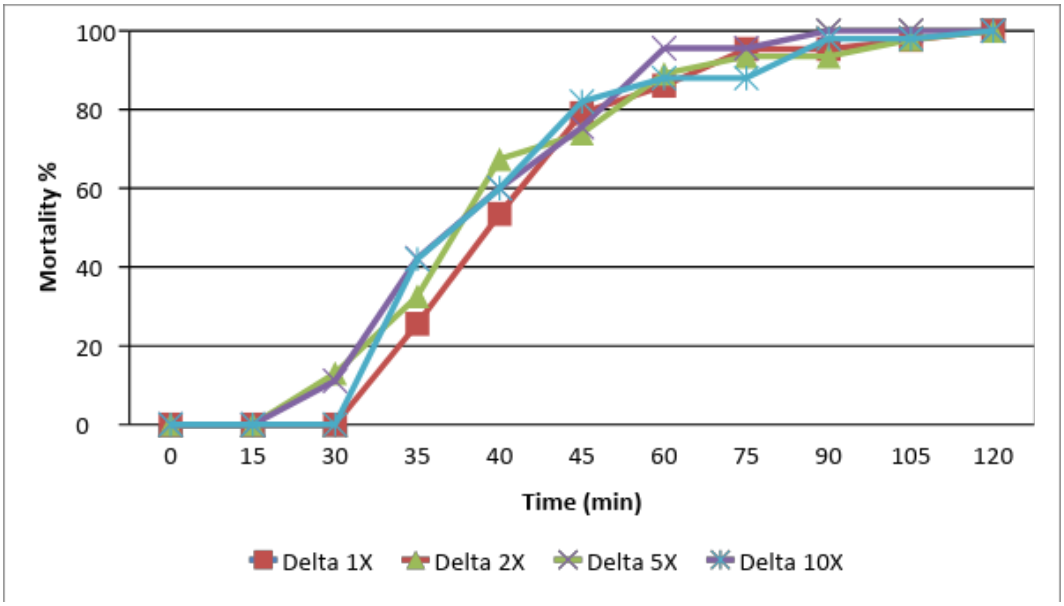
In Sergent Kolly, this test was performed to assess the susceptibility of *An. gambiae* s.l. at the diagnostic doses of deltamethrin. In Sergent Kolly Town (Bong County), the mortality rate at 30 minutes diagnostic time was in the range of 40% to 60% using deltamethrin 1X (Figure 12).

FIGURE 12: TIME TO MORTALITY FOR *AN. GAMBIAE* S.L. FROM SERGENT KOLLY TOWN, BONG COUNTY, EXPOSED TO 1X DELTAMETHRIN, USING CDC BOTTLE TESTS, MAY 2017



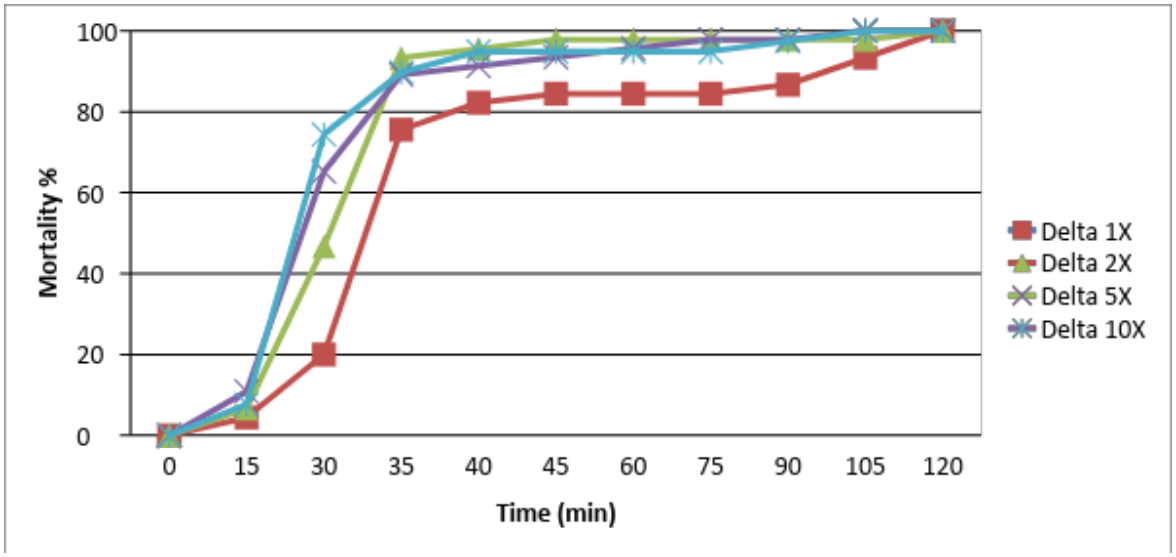
In Saint John of Grand Bassa County, results indicated a high level of resistance intensity up to the 10X level (Figure 13).

FIGURE 13: INTENSITY RESISTANCE TESTS OF *AN. GAMBIAE* S.L. FROM SAINT JOHN, GRAND BASSA COUNTY, EXPOSED TO DIFFERENT CONCENTRATIONS OF DELTAMETHRIN (1X, 2X, 5X, 10X), USING CDC BOTTLE TESTS, MAY 2017



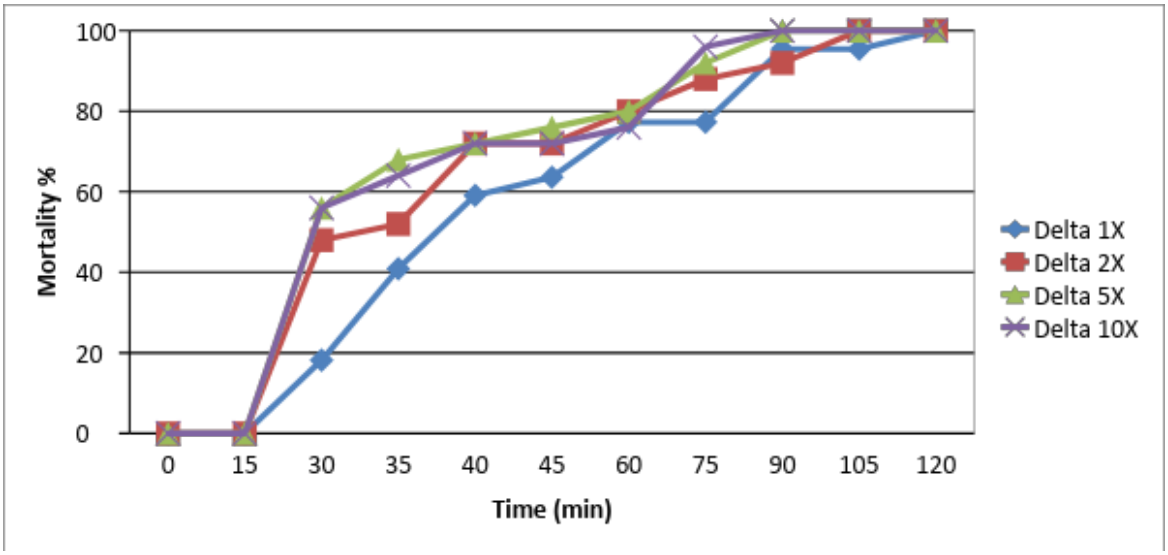
In Bomi County, the intensity of deltamethrin resistance was high at the 10X level (Figure 14).

FIGURE 14: INTENSITY RESISTANCE TESTS OF *AN. GAMBIAE* S.L. FROM TUBMANBURG, BOMI COUNTY, EXPOSED TO DIFFERENT CONCENTRATIONS DELTAMETHRIN (1X, 2X, 5X, 10X), USING CDC BOTTLE TESTS, SEPTEMBER 2017



The data collected in CARI, Bong County, in December 2017 also showed high intensity deltamethrin resistance in the area (Figure 15).

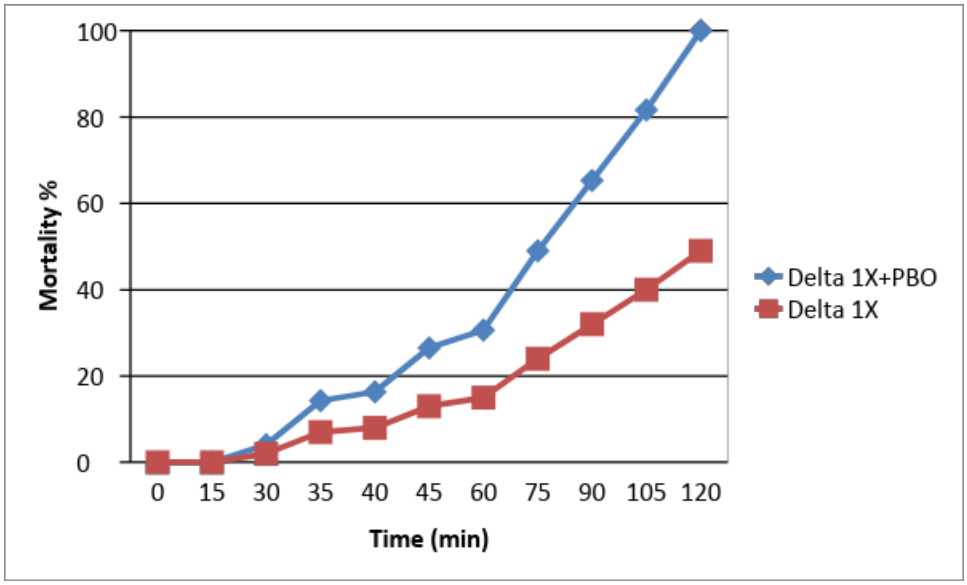
FIGURE 15: INTENSITY RESISTANCE TESTS OF *AN. GAMBIAE* S.L. FROM CARI, BONG COUNTY, EXPOSED TO DIFFERENT CONCENTRATIONS OF DELTAMETHRIN (1X, 2X, 5X, 10X) USING CDC BOTTLE TESTS, DECEMBER 2017



4.5.2 SYNERGISTS ASSAYS USING THE CDC BOTTLE ASSAY AND WHO TUBE TEST METHODS

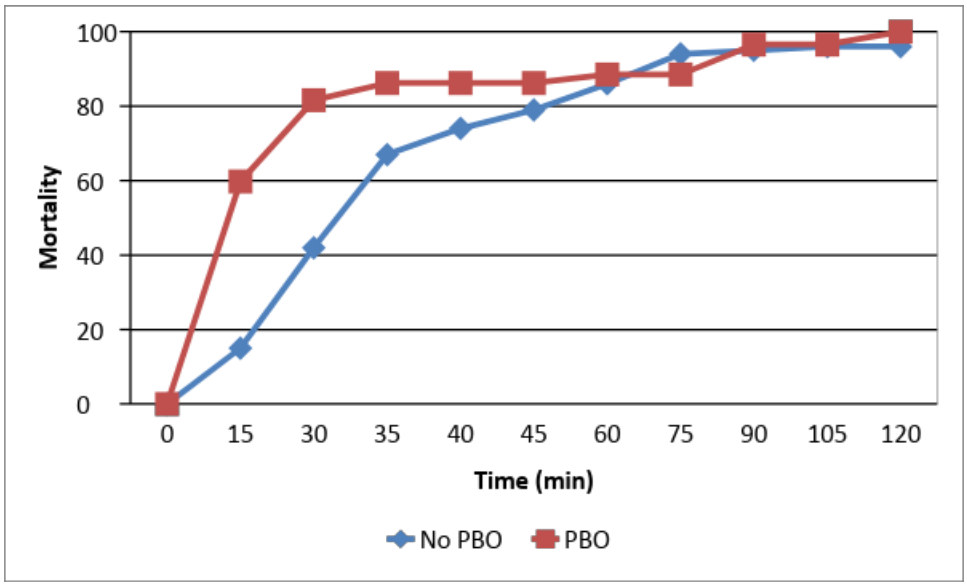
The entomology team used the CDC bottle assay method on mosquitoes from Gbedin Camp 3 in Nimba County to assess the synergist effect of PBO on deltamethrin resistant *An. gambiae* s.l. The results indicated that PBO did not restore full susceptibility to deltamethrin in the population at the diagnostic time, suggesting the existence of mechanisms of resistance other than oxidases (Figure 16). Testing at other sites will be completed as part of the 2018 entomological surveillance activities.

FIGURE 16: TIME TO MORTALITY FOR *AN. GAMBIAE* S.L. FROM GBEDIN CAMP 3, NIMBA COUNTY, EXPOSED TO DELTAMETHRIN 1X AND DELTAMETHRIN PLUS PBO WITH CDC BOTTLE ASSAY, NOVEMBER 2017.



In February 2018, mosquitoes collected in 15th Gate, Montserrado County were used for synergist tests based on the CDC method. In this case also, the synergist (PBO) did not totally restore susceptibility, indicating other mechanisms of resistance may also be involved (Figure 17).

FIGURE 17: TIME TO MORTALITY FOR *AN. GAMBIAE* S.L. FROM 15TH GATE, MONTSERRADO COUNTY, EXPOSED TO DELTAMETHRIN 1X AND DELTAMETHRIN PLUS PBO WITH CDC BOTTLE ASSAY, FEBRUARY, 2018



The results on synergist assays from Sergent Kolly Town, Bong County, on deltamethrin and alphacypermethrin resistant *An. gambiae* s.l. using the WHO tube test method, are in Table 7. For

deltamethrin, the mortality rate among mosquitoes pre-exposed to PBO (62%) was higher than in the mosquitoes without pre-exposure to PBO (20%). The difference was statistically significant ($p<0.05$). Similarly for alpha-cypermethrin, the percentage mortality of mosquitoes pre-exposed to PBO was 90%, an increase from 33% when the mosquitoes were exposed to alpha-cypermethrin alone. The results indicated that PBO partially abolished resistance to both deltamethrin and alpha-cypermethrin, indicating the involvement of oxidases, but also other mechanisms of resistance in the area.

TABLE 7: WHO INSECTICIDE SUSCEPTIBILITY TEST RESULTS BY INSECTICIDE EXPOSED WITH OR WITHOUT PBO TO *AN. GAMBIAE* S.L. FROM BONG COUNTY, AUGUST 2017

	No PBO		With PBO		Chi^2
Insecticide	**Tested**	**Mortality %**	**Tested**	**Mortality %**	**p-value**
Deltamethrin 0.05%	100	20	100	62	$p<0.05$
Alpha-cypermethrin 0.05%	100	33	100	90	$p<0.05$

4.6 LABORATORY ANALYSES ON 2015-2016 SAMPLES

4.6.1 SPECIES COMPOSITION

The University of Witwatersrand, South Africa, analyzed a sample of 200 mosquitos we collected during the 2015-2016 season for molecular identification of *An. gambiae* s.l. complex species. In total, 30 samples did not amplify, and the university could not obtain PCR results, which may the result of DNA degradation. The university identified all the remaining samples as *An. gambiae* (n=170) and further analyzed them to differentiate between *An. gambiae* (n=32) and *An. coluzzii* (n=122).

4.6.2 MOLECULAR DIAGNOSTIC OF INSECTICIDE RESISTANCE MECHANISM

Using the sample described above, the university used hydrolysis probes for *kdr*-mutation to analyze a subsample of *An. gambiae* s.l. that was exposed to pyrethroids and analyzed another subsample exposed to organophosphate or carbamate insecticides for Ace-1^R mutation. A summary of data is in Table 8. There are three genotypes represented by letters: SS for susceptible homozygote, RS for mutant heterozygote, and RR as mutant homozygote. The frequencies of *kdr* mutation genotypes, *kdr*-W and *kdr*-E (W=West Africa and E=East Africa) were calculated based on observed numbers following this formula: the percentage of RR = RR / (SS + RS + RR) × 100%.

For the 49 *An. gambiae* s.l. exposed to pyrethroids, including dead and alive samples, for *kdr*-W genotypes there were: 24 RR (49%), 24 RS (49%), and 1 SS (2%). For the *kdr*-E, only SS genotypes were reported: 51 SS (100%).

TABLE 8: SUMMARY DATA OF KDR MUTATIONS AND ACE-IR GENES AMONG *AN. GAMBIAE* AND *AN. COLUZZII* EXPOSED TO INSECTICIDES BY THE WHO TUBE METHOD*

Species	Insecticide	Phenotype	Kdr-W	Kdr-E
An. gambiae s.s	Deltamethrin (10)	Survive (6)	5RR,1RS	6SS
		Dead (4)	3RR, 1 No ID	3SS, 1 No ID
An. coluzzii	Deltamethrin (44)	Survive (30)	13RR; 14RS; 3 No ID	27SS; 3 No ID
		Dead (14)	2RR; 9RS; 3No ID	13SS; 1No ID
	Alpha-cypermethrin (2)	Survive (0)	N/A	N/A
		Dead (2)	1RR;1SS	2SS
Species	**Insecticide**	**Phenotype**	**ACE-1R**	
An. gambiae s.s	Bendiocarb (10)	Survive (8)	2RS; 5SS;1 No ID	
		Dead (2)	2SS	
	Pirimiphos Methyl (12)	Survive (1)	1SS	
		Dead (11)	1RS;9SS; 1 No ID	
An. coluzzii	Bendiocarb (53)	Survive (30)	1RR; 4RS; 17SS; 8 No ID	
		Dead (23)	20SS; 3 No ID	
	Pirimiphos Methyl (23)	Survive (4)	4SS	
		Dead (19)	15 SS; 4 No ID	

(): The numbers in parenthesis represent the subsample processed.*

4.7 CAPACITY BUILDING

From December 2016 through November 2017, with AIRS's/PMI support a total of ## staff were trained to support Liberia NMCP to strengthen malaria vector control. The staff trained included four new gCHVs (one in Tomato Camp, two in Jeneta, and one in Frank Town), ## benefited refresher training, , In addition, one staffer from the University of Liberia received training on the field collection methods (HLC, PSC, and CDC light trap) and on mosquito identification and data reporting. A total of five staff from the NMCP vector control team received training on the CDC bottle assay technique to assess the intensity of insecticide resistance. Staff trained by activity and technical areas are listed in Table 9. The practical training covered how to perform adult mosquito collection methods (PSC, HLC and CDC light traps) and how to distinguish *Anopheles* from the other mosquitoes' genera based on morphological characteristics. The training covered insecticide resistance tests for those involved in larval collections. The gCHVs went through a hands-on field training to identify *Anopheles* and *Culex* mosquitoes at larval stages. They were able to see mosquitoes under a dissecting microscope for better recognition of their differences. In the field, training facilitators explained to gCHVs the major characteristics of *An. gambiae* s.l., potential breeding sites, and how to rear larvae in the field. After a briefing on the importance of insecticide resistance surveillance, participants were able to practice insecticide resistance tests under the supervision of the team members.

TABLE 9: STAFFING FOR ENTOMOLOGICAL SURVEILLANCE ACTIVITIES

Activity	Entomology Technicians per Site	Local Mosquito Collectors (gCHVs) per Site	AIRS Liberia Project Staff
PSC	3	4	1
HLC	2	4	1
CDC light traps	2	4	1
Insecticide Resistance Mapping			
Larvae collection and insecticide resistance	2	4	1
Insectary Maintenance			
Container insectary maintenance	1	-	1

By the end of November 2017, NMCP has the capacity to carry on a number of vector control activities with limited supervisión. In 2018, AIRS will continue its' technical support to mentor NMCP vector control staff and train additional staff to scale up and monitor malaria vector resistance and behavior feeding behavior in multiple sites, increase capacity for ELISA testing in country, start PCR testing in Country and strengthen routine vector control monitoring.

5. OBSERVATIONS AND CONCLUSIONS

Indoor and outdoor collections demonstrated that peak vector densities in sentinel sites occur in May and June, which coincides with the beginning but not the peak of the rainy season (August-September). This has important implications for vector control activities in Liberia. If implemented again in the future, IRS should begin before vector densities begin to increase, most likely in April. It is also important to provide appropriate messaging and to sensitize the community to use Long-lasting Insecticide-treated Nets (LLINs) during the peak transmission season when human-vector contact is most frequent.

The high proportion of *An. coluzzii* compared with *An. gambiae* collected during the 2015-2016 season suggests that the distribution of this vector may be expanding. We didn't study the determinants of such potential expansion, however, potential reasons might likely include the environmental changes stemming from irrigation, construction, and deforestation activities, which are increasing the number of suitable breeding sites. *An. coluzzii* adapts well to small water pools and irrigated zones. It tolerates more dry conditions than *An. gambiae* and could maintain transmission during the dry season. The high number of blood-fed mosquitoes from the PSCs might indicate frequent contact between host and vector, which may increase the risk of malaria transmission

The collection in Bokay Town was canceled due to the low mosquito density in the area and surveillance activities will not continue there in 2018 due to the

The HLC data suggested there is a tendency toward outdoor biting in Frank Town. Outside of this, it is difficult to establish trends in the other sites due to low biting rates and the subsequent small number of mosquitoes collected through HLC. Therefore, HLC may not be the right option for mosquito collection. Instead, CDC light traps might be the best way to sample the host-seeking population of malaria vectors in Liberia. The number of mosquitoes the CDC light traps collected was three times greater than the number HLC collected. The team also will assess the utility of baited CDC-light traps outdoor for future regular monthly collections as a replacement for HLC in Liberia.

In contrast to the sentinel sites, the transect study revealed a diverse vector population and identified sites where *An. funestus* abundance is relatively high. This species is known as a secondary vector in Liberia and could maintain malaria transmission when the densities of *An. gambiae* s.l. decrease. Given this possibility, future studies should expand beyond the sites in the transect study to gain a better understanding of the vector species composition. If findings indicate widespread geographic distribution of *An. funestus* in the country, it will be important to assess the vector's resting and feeding behavior along with susceptibility to insecticides to determine the suitability of existing vector control interventions to this species in the country.

The preliminary data on insecticide susceptibility and resistance intensity using CDC bottle bioassays showed high intensity of pyrethroid resistance at 10 times diagnostic dosages at all sites. It is important to establish the extent of pyrethroid resistance intensity through surveillance in wider geographic areas. The first data on the synergist assay of resistance have shown that monooxygenase enzymes are involved as mechanism of insecticide resistance but are not the only mechanism of resistance. The restoration of susceptibility after PBO was more pronounced for the alphacypermethrin- than deltamethrin-resistant *An. gambiae* s.l. This has practical relevance in the decision-making process of selecting LLIN products for malaria vector control in the country. It is important to collect more data to

determine the underlying mechanisms of resistance in most of the sites in Liberia to inform the decision-making process on the types of new generation LLINs to mitigate pyrethroid resistance in Liberia. We suggest geographical expansion of insecticide monitoring activities, including synergist assays, to determine the extent and mechanisms of pyrethroid resistance nationwide. As next generation LLINs are expected to become available over the next couple of years, future insecticide susceptibility testing activities should prioritize new insecticides, namely chlorfenapyr.

All mosquito samples collected during entomological monitoring, insecticide resistance and transect activities are stored until they can be processed for molecular species identification, insecticide resistance mechanisms, and determination of sporozoite rates. This could not be done in-country due to space and staff capacity limitations. However, in 2018 VectorLink is focusing on building capacity and establishing space for processing. The team has since procured necessary supplies for ELISA processing and identified space for laboratory work at Liberian Institute for Biomedical Research (LIBR). As part of a CDC on-site training, two technicians--one from the NMCP and one from LIBR--have received training in ELISA CSP processing. They currently are running analyses on samples collected in 2015-2016. Recommended next steps are to support a second CDC technical assistance site visit to establish and provide training on all molecular techniques. We expect to complete analysis of samples collected in 2016–2017 in 2018.

6. BIBLIOGRAPHY

1. Gillies MT and Coetzee C. 1987. *A Supplement to the Anopheline of Africa South of the Sahara*. Johannesburg: South African Institute for Medical Research.
2. National Malaria Control Program (NMCP) [Liberia], Ministry of Health (MOH), Liberia Institute of Statistics and Geo-Information Services (LISGIS), and ICF. 2017. Liberia Malaria Indicator Survey 2016. Monrovia, Liberia: MOH, LISGIS, and ICF.
3. https://malariaworld.org/blog/ivcc-and-syngenta-reach-key-milestone-development-new-insecticide-malaria-mosquito-control (accessed January 6, 2017).
4. Stojanovich CJ and Scott HG. 1966. *Illustrated Key to Anopheles mosquitoes of Liberia*. Atlanta, Georgia: U.S Department of Health, Education, and Welfare, Public Health Science. Communicable Disease Center.
5. Gillett, JD. *Common African Mosquitoes and their Medical Importance, with 48 Colour Illustrations*. London: William Heinemann Medical Books LTD.
6. World Health Organization. 2013. *Test procedures for insecticide resistance monitoring in malaria vector mosquitoes*. http://www.who.int/malaria

7. ANNEX

ANNEX 1: LOCATIONS OF INSECTICIDE RESISTANCE MONITORING SITES IN LIBERIA FROM DECEMBER 2016 TO NOVEMBER 2017

County	IR Site (District)	Larval Collection Site for IR (WHO & CDC Bottle)	Routine Monitoring Sentinel Site	Site For Cone Testing of LLINs	Status
Montserrado	Careysburg	Fifteen Gate / Bentor	Fifteen Gate	Fifteen Gate	May 2017
Bong	Suakoko	Sergeant Kollie Town	CARI	CARI	August 2017
Margibi	Kakata	Kakata / Jackson Town	Jackson Town	Jackson Town	To be completed in 2018
Grand Bassa	Buchanan	Buchanan / Saint John	Buchanan	Buchanan	October 2017
Nimba	Gbedin	Gbedin Camp3	Gbedin Camp3	Gbedin Camp3	November 2017
Bomi	Tubmanburg	Tubmanburg	Tubmanburg	Tubmanburg	September 2017

ANNEX 2: RELATIVE ABUNDANCE OF OTHER MOSQUITOES COLLECTED IN THE THREE SENTINEL SITES, DECEMBER 2016 TO NOVEMBER 2017.

Site	*An. funestus*	*An. ziemanni*	*An. rufipes*	*Culex*	*Aedes*	*Mansonia*
Frank Town	5	0	14	515	16	17
Tomato Camp	15	1	5	457	0	15
Jeneta	12	1	8	899	5	5
Bokay Town	0	0	6	102	4	0
Total	**32**	**2**	**33**	**1,973**	**25**	**37**

ANNEX 3: SUMMARY OF SPECIES COMPOSITION FROM PSC AND CDC LIGHT TRAP COLLECTIONS IN TRANSECT STUDY, MAY 2017 AND OCTOBER 2017

TABLE A3.1: SUMMARY OF SPECIES COMPOSITION FROM PSC AND CDC LIGHT TRAP COLLECTIONS IN TRANSECT STUDY, MAY 2017 AND OCTOBER 2017

Site	*An. gambiae* s.l.	*An. funestus*	*An. nili*	*An. ziemanni*	*An. rufipes*	*Culex*	*Aedes*	*Mansonia*	*Toxorhyn-chites*
Gbadin Camp3	568	6	2	0	0	46	0	2	0
Gwekpolosue	284	11	0	1	0	15	3	0	0
Jackson Town	534	0	0	0	1	56	1	4	0
Jenepleta	59	34	2	0	1	21	0	1	0
Kingville	509	1	0	0	0	22	2	1	0
Kollieman Town	138	0	0	0	0	24	1	0	0
Koyah	123	42	0	0	0	19	0	0	0
Unification Township	129	16	0	0	0	26	0	0	0
Zeanzue	59	27	0	0	0	47	1	0	0
Zolowee	109	24	0	0	4	29	0	1	1
Total	**2,512**	**161**	**4**	**1**	**6**	**305**	**8**	**9**	**1**

TABLE A3.2: SUMMARY OF SPECIES COMPOSITION FROM PSC AND CDC LIGHT TRAP COLLECTIONS IN TRANSECT STUDY, MAY 2017 AND OCTOBER 2017

Sites	*An. gambiae* s.l.	*An. funestus*	*An. nili*	*An. ziemanni*	*An. rufipes*	*Culex*	*Aedes*	*Mansonia*	*Toxorhyn-chites*
Gbadin Camp3	686	0	0	0	6	4	0	11	0
Gwekpolosue	13	1	0	6	1	53	5	1	0
Jackson Town	93	0	0	0	0	23	0	1	0
Jenepleta	16	20	0	0	1	4	0	0	0
Kingville	18	0	0	0	0	86	0	0	0
Kollieman Town	5	0	0	0	0	65	0	0	0
Koyah	1	6	0	0	0	39	0	0	0
Unification Township	16	1	0	0	0	10	0	0	0
Zeanzue	15	36	0	0	0	8	0	0	0
Zolowee	34	12	0	1	0	33	0	0	0
Total	**897**	**76**	**0**	**7**	**8**	**325**	**5**	**13**	**0**

ANNEX A3.3: NUMBER OF *ANOPHELES FUNESTUS* AND OTHER AMOSQUITOES COLLECTED USING PSC IN 10 SITES VISITED, MAY AND OCTOBER, 2017.

Site	May , 2017, PSC					October , 2017, PSC		
	An. funestus	***Culex***	***Aedes***	***Mansonia***		***An. funestus***	***Culex***	***Mansonia***
Gbadin Camp3	3	0	0	0		0	0	0
Gwekpolosue	11	1	0	0		1	0	0
Jackson Town	0	2	1	1		0	3	1
Jenepleta	19	0	0	0		20	0	0
Kingville	1	0	1	0		0	45	0
Kollieman Town	0	1	1	0		0	19	0
Koyah	35	0	0	0		5	0	0
Unification Township	1	2	0	0		1	0	0
Zeanzue	27	1	1	0		24	0	0
Zolowee	2	19	0	0		3	5	0
Total	**99**	**26**	**4**	**1**		**54**	**72**	**1**

ANNEX 4: GEOGRAPHIC COORDINATES OF TRANSECT STUDY SITES

County	District	Site	Longitude	Latitude
Nimba	Yah-Meh	Unification Town	7º32.627'N	8º33.834' W
Nimba	Saniquellie Mah	Zolowee	7º26.066'N	8º37.912' W
Nimba	Saniquellie Mah	Gbedin Camp 3	7º16.813'N	8º49.521'W
Margibi	District #5	Jackson Town	6º36.193'N	10º16.679'W
Margibi	District 4	Gwekpolosue	6º37.761'N	10º21.070'W
Bong	Jorquelleh	Koyah	7º02.958'N	9º28.115'W
Bong	Jorquelleh	Jenepleta	7º00.549'N	9º21.050'W
Bong	Suakoko	Zeanzue	6º52.841'N	9º45.620'W
Montserrado	Careysburg	Kollieman Town	6º25.345'N	10º36.540'W
Montserrado	Careysburg	Kingsville	6º24.198'N	10º29.087'W