PMI VECTORLINK LIBERIA PROJECT

PMI VECTORLINK LIBERIA

ENTOMOLOGICAL MONITORING

FINAL REPORT DRAFT

DECEMBER 1, 2017–SEPTEMBER 30, 2018

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CONTENTS

ACRONYMS

CDC	Centers for Disease Control and Prevention
ELISA	Enzyme-Linked Immunosorbent Assay
gCHVs	General Community Health Volunteers
HBR	Human Biting Rate
HLC	Human Landing Catch
IRS	Indoor Residual Spraying
kdr	Knock-Down Resistance
LLIN	Long-Lasting Insecticidal Net
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 Homozygous
RS	Mutant Heterozygous
SS	Susceptible Homozygous
WHO	World Health Organization

EXECUTIVE SUMMARY

Entomological monitoring of malaria vectors was conducted in Liberia from December 2017 to September 2018 in three sentinel sites—Tomato Camp, Jeneta, and Frank Town located in Bong, Margibi, and Montserrado counties, respectively. The adult mosquito samples were collected using three different methods: human landing catch (HLC), pyrethrum spray catch (PSC), and Centers for Disease Control and Prevention (CDC) light traps (LT). The entomological parameters assessed were vector composition, distribution, behavior, and infection status of malaria vectors.

Insecticide susceptibility, intensity of the resistance and synergist assays with piperonyl butoxide (PBO) were completed using CDC bottle assays in seven sites to monitor the resistance status of *Anopheles gambiae* s.l. to pyrethroids used for long-lasting insecticidal net (LLIN) impregnation.

In May 2018, malaria vectors were sampled in 10 sites along a transect from Camp 4 in Nimba County to Kollieman Town in Montserrado County. Previously, in May and October 2017, a similar sampling was done in the same 10 sites to monitor entomological parameters listed above using PSC and CDC LT methods of collection.

The results showed that, *An. gambiae* s.l. was the predominant (90%) malaria vector in all three sentinel sites, with *An. funestus* comprising the remaining 10 percent. CDC light trap collections show that the peak densities of *An. gambiae* s.l. were from April to June with greater numbers of mosquitoes collected indoors (84%) than outdoors. However, unlike the traps installed indoors, the outdoor traps were not baited. Human biting rates (HBRs) were calculated from the HLC collections. Frank Town reported the highest *An. gambiae* s. l. mean HBR among the sites (HBR= 9.3/bites/person/night). Mean HBR was higher outdoors (12.1 bites/person/night) than indoors (6.5 bite/person/night). The majority (74%) of indoor resting *An. gambiae* s.l. were blood fed.

During the transect collection in May 2018, *An. gambiae* s.l. was the predominant malaria vector collected in the 10 sites. *Anopheles funestus* was found at most of the sites, with the highest numbers found at Jenepleta, Koyah, Zeanzue, and Zolowee. Based on the abundance of *An. funestus*, Koyah in Bong County has been selected as sentinel site for 2018–2019 activities.

Insecticide susceptibility tests revealed that *An. gambiae* s.l. is resistant to deltamethrin and alpha-cypermethrin at all sites with mortality rates ranging from eight percent to 80 percent after 30 minutes of exposure. With deltamethrin, resistance (28% to 85% mortality) was found at 10 times the diagnostic dose at Saint John and Big Fantim Town in Grand Bassa County, Tubmanburg in Bomi County, Jackson Farm in Margibi, Harper in Maryland County, and Camp 3 in Nimba County.

For synergist assays, pre-exposure to PBO did not restore *An. gambiae* s.l. susceptibility to alpha-cypermethrin and deltamethrin with mortality ranging from 10 to 63 percent without PBO and mortality ranging from 36 percent to 89 percent with pre-exposure to PBO across all the sites. These data suggest there may be multiple mechanisms of pyrethroid resistance and PBO-treated LLINs may not be more effective than standard LLINs in Liberia.

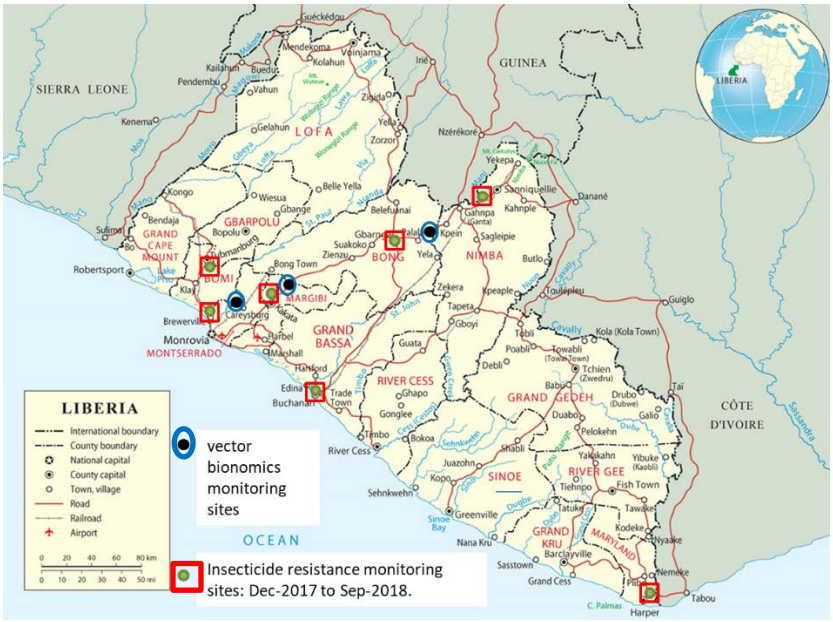
1. INTRODUCTION

The prevalence of malaria among children (6-59 months) was about 45 percent in Liberia, according to 2016 Malaria Indicator Survey (MIS). Malaria case management and distribution and use of long-lasting insecticidal nets (LLINs) have contributed to the reduction in prevalence of malaria from 2009 to 2016. However, drug-resistance and the resistance of vectors to insecticides could affect the ongoing efforts to control malaria in Africa in general and in Liberia specifically, in addition to the challenge of appropriate insecticide formulations to use for indoor residual spraying (IRS) and LLIN impregnation.

From 1945 to 1962, synthetic insecticide was used in IRS on a large scale for malaria control in Monrovia, the capital city of Liberia, and from 2009 to 2013 in PMI-supported IRS in three counties (Bong, Grand Bassa and Margibi) within the country. Since 2014, insecticide resistance and vector bionomics monitoring has been conducted in selected sentinel sites in the country. Continuous assessment of the spread of insecticide resistance among the malaria vector will help increase understanding of the evolution of the resistance within the country and inform decision making for selecting insecticides for IRS or LLINs. Next generation dual-insecticide LLINs or those that incorporate a synergist such as PBO to enhance susceptibility to pyrethroids provide alternatives to standard pyrethroid LLINs and have the potential to mitigate the development of resistance in malaria vector populations.

In 2017 and 2018, VectorLink conducted routine entomological surveillance in three sites in three counties and insecticide resistance monitoring in seven sites in seven counties to inform evidence-based decisions for malaria vector control in the country (Figure 1, Annex 1). Monthly entomological surveillance was conducted using HLCs, PSCs, and CDC light trap collections to assess the variation of vector density, composition, and behavior. In the seven sites selected for insecticide resistance activities, VectorLink assessed *An. gambiae* s.l. susceptibility to select insecticides at different intensities and whether susceptibility to pyrethroids was restored following exposure to a synergist.

FIGURE 1: VECTORLINK LIBERIA INSECTICIDE RESISTANCE AND ENTOMOLOGICAL MONITORING SITES, DECEMBER 2017–SEPTEMBER 2018



VectorLink completed data collection for a three-point transect study across 10 selected villages in four counties (Nimba, Bong, Margibi and Montserrado) to provide additional information on the species composition and abundance of malaria vectors in Liberia. VectorLink collected data in May 2017, October 2017, and May 2018. Data were used to guide selection of sentinel sites in 2019, specifically those sites with high densities of *An. funestus*.

The main objectives of VectorLink Liberia entomological monitoring activities are to:

- Determine insecticide susceptibility of *An. gambiae* s.l., the primary local malaria vector, in seven sites in seven counties.
- Assess *Anopheles* vector bionomics, including species composition, density, and behavior in three sites in three counties.
- Determine the sporozoite infection rates of vector mosquitoes collected during routine surveillance.
- Maintain and support a functional insectary.
- Build local capacity in entomological surveillance methods and techniques through formal and informal training.

2. MATERIALS AND METHODS

2.1 ROUTINE ENTOMOLOGICAL MONITORING (VECTOR BIONOMICS)

Entomological monitoring activities were conducted at three sites: Tomato Camp (Kpaai District, Bong County), Jeneta (Five District, Margibi County), and Frank Town (Careysburg District, Montserrado County). All the sites are located in hot and humid rural areas where the vegetation is scant and there is open canopy due to extensive forest clearing. Tomato Camp is located at a higher altitude than Jeneta and Frank Town, and Frank Town is more coastal and experiences more rainfall than the two other sites. Both Tomato Camp and Frank Town are in districts where IRS was implemented from 2009 to 2013; no IRS has been conducted in Jeneta. House walls are made of mud, roofs are formed mainly from corrugated iron sheets or grass, and farming was the main activity of the residents. Malaria prevalence is similar in the three sites, ranging from 52 percent in the region around Jeneta and Frank Town (South Central) to 62 percent in the region around Tomato Camp (North Central) (LMIS, 2016).

Potential mosquito breeding sites were present in and around villages which allowed mosquito development through the year, but mainly during the rainy season (May to October). Although the majority of naturally-occurring breeding sites disappear in the dry season (December to March), the human-made mosquito breeding sites such as borrow pits, brick pits, and gardening wells allow mosquito larvae to grow during the dry season. Wells are exposed to sunlight and are not deep since groundwater is abundant in Liberia.

2.1.1 ADULT MOSQUITO COLLECTIONS

Three methods were used to collect adult mosquitoes in the three sentinel sites: PSC, HLC, and CDC LT. See Table 1 for a summary of the methods.

TABLE 1: LONGITUDINAL MONITORING ADULT MOSQUITO COLLECTION METHODS

Collection method	Time	Frequency	Sample
PSC	6:00 am to 8:00 am	Two days per site per month	20 houses per site (10 houses each day for two days)
HLC	6:00 pm to 6:00 am	One night every two months	Two houses per site
CDC LT	6:00 pm to 6:30 am	Two nights per site per month	Eight houses per site (four houses per night), using eight CDC LTs—four indoor, four outdoor

For PSC, white cloth/sheets were placed on the floor from wall to wall in sampled houses. The teams sprayed the commercial pyrethroid insecticide, Hewelon[1] in the house after closing windows, doors, and any other open spaces around the house. All food and drinking water were covered or removed from the house before spraying. After a 10-minute knock-down period, the sheets were collected.

CDC light traps were set up both outdoors (un-baited) and indoors in selected houses where people slept under a mosquito net. The consent of the head of each household was acquired beforehand. The indoor traps were placed approximately 150 cm above the sleeper's legs. The outdoor CDC LTs were

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

set up at least four meters from the same houses with the indoor traps. The outdoor traps were not baited. Mosquitoes were collected from the traps once—in the morning, following the evening shift.

HLCs were performed indoors and outdoors to collect adult mosquitoes landing on human baits. With legs exposed to attract host-seeking mosquitoes, two human baits were seated indoors and another pair outdoors. The collectors switched between indoors and outdoors on an hourly basis. The collectors used flashlights and a tubing aspirator to collect mosquitoes that landed on their legs before the mosquitoes could bite. The teams transferred the mosquitoes hourly to paper cups covered with mosquito net. During the collection the temperature and the relative humidity were recorded hourly.

The following indicators were calculated based on the number of mosquitoes collected through each collection method:

Collection Method	Indicator	Definition
PSC	Indoor Resting Density	# mosquitoes / house / day
CDC LT	Indoor Density	# mosquitoes collected indoors / trap / night
	Outdoor Density	# mosquitoes collected outdoor / trap / night
HLC	Human Biting Rate	# bites / person / night

All mosquitoes collected through each method were identified morphologically under a dissection microscope, in the field, by the team of well-trained technicians from the University of Liberia, the National Malaria Control Program (NMCP), and VectorLink. The *Anopheles* species identified (Gillies and Coetzee 1987) were preserved on silica gel for laboratory processing to identify infection status by Enzyme-Linked Immunosorbent Assay (ELISA). The laboratory analysis began at the Liberia Institute for Biomedical Research (LIBR) after a training held there in April 2018.

2.2 TRANSECT STUDY

In May 2018, a team comprised of three NMCP staff and the VectorLink Liberia entomologist completed a study in 10 villages across a transect from Camp 4 in Nimba County to Kollieman Town in Montserrado County (Figure 2) to inform selection of new sites for routine vector monitoring and insecticide resistance surveillance in the country. Coordinates of these sites are available in Annex 2. The May 2018 collections followed the same protocols as those used during previous collections in May 2017 and October 2017. Mosquitoes were collected using PSC and CDC LT (Table 2). HLC was not used due to time and resource limitations.

FIGURE 2: LOCATION OF PMI VECTORLINK LIBERIA TRANSECT STUDY SITES

TABLE 2: TRANSECT STUDY ADULT MOSQUITO COLLECTION METHODS

Collection Method	Time	Frequency	Sample
PSC	6:00 am to 8:00 am	One day per site in May	20 houses per site in one day
CDC LT	6:00 pm to 6:30 am	One night per site in May	Four houses per site per night, using eight CDC LTs—four indoor, four outdoor

All mosquitoes were identified morphologically, in the field. The *Anopheles* species identified (Gillies and Coetzee 1987) were preserved on silica gel for laboratory processing to identify infection status by ELISA.

2.3 INSECTICIDE SUSCEPTIBILITY TESTS

Insecticide susceptibility tests were done across seven sites. Resistance intensity and synergist assays were conducted for select insecticides and in select sites (Table 3). To gather adult mosquitoes for susceptibility tests, VectorLink, NMCP, and general community health volunteers (gCHVs) collected *An. gambiae* s.l. larvae in rice fields, brick pits, and water pools close to the sentinel site. The collected larvae were reared to adult mosquitoes in a field insectary where the field team monitored relative humidity and temperature to keep mosquitoes in suitable conditions for the tests. When field testing is not possible, tests were conducted in the container box insectary located in Monrovia. The team tested Pirimiphos methyl and bendiocarb while it was at the site since Harper is an especially difficult area to access due to poor road conditions.

TABLE 3: SUMMARY OF INSECTICIDE SUSCEPTIBILITY TEST

Insecticide	Test	Method	Sites
Deltamethrin Alpha-cypermethrin	• Susceptibility and resistance testing (1X, 2X, 5X and 10X diagnostic dose) • Synergistic Assay	CDC Bottle Assay	All seven sites
Permethrin	Susceptibility and resistance testing (1X, 2X, 5X and 10X diagnostic dose)	CDC Bottle Assay	Jackson Farm/Margibi
Bendiocarb	Susceptibility	World Health Organization (WHO) Method	Harper, Maryland
Pirimiphos methyl	Susceptibility	WHO Method	Harper, Maryland

Synergist-testing done on both deltamethrin and alpha-cypermethrin only if enough larvae collected.

For each test, the team preserved a subsample of dead mosquitoes and all the survivors in labeled Eppendorf tubes for further laboratory analysis.

2.3.1 Susceptibility Tests for Bendiocarb and Pirimiphos Methyl Using the WHO Method

Filter papers impregnated with bendiocarb and pirimiphos methyl received from Malaysia were used to monitor the susceptibility status of *An. gambiae* s.l. to those insecticides for which a probable resistance was observed during the previous years. Four tubes were used each for exposure and holding while two additional sets of tubes with olive oil impregnated papers were used as control to validate the tests. Two-to five-day old female *An. gambiae* s.l. mosquitoes were transferred to the holding tubes for one hour before transferring them to the exposure tubes. The knockdown was recorded every 15 minutes for one hour. After one hour of exposure, mosquitoes were transferred back in the holding tubes and fed with a 10 percent sugar solution. The mortality was recorded 24 hours after the test.

2.3.2 Susceptibility Tests and Intensity Assays for Pyrethroids Using the CDC Bottle Method

For the susceptibility and resistance intensity assays for pyrethroids (deltamethrin, permethrin and alpha-cypermethrin), CDC provided the vials containing the technical grade insecticide concentrations. The diagnostic dose 1X (12.5 µg/bottle), 2X (25 µg/ bottle), 5X (62.5 µg/bottle), and 10X (125 µg/bottle) of each insecticide were tested at each site but with different replicates.

The dilution was done with 50 ml acetone per tube. Four bottles were coated with one ml of different concentrations of the prepared insecticide solutions. The control bottles were coated with one ml acetone only. All the bottles were kept overnight to dry before use the next day. Twenty-five females *An. gambiae* s.l. two-to five-days old were transferred into each of the tubes, for a total of 125 mosquitoes. The mortality was recorded every 15 minutes up to two hours.

2.3.3 Synergist Assay Using the CDC Bottle Method

Pre-exposure to a synergist (PBO) was done before introducing mosquitoes to alpha-cypermethrin and deltamethrin to see if susceptibility could be restored. A concentration (100 µg/bottle) of PBO was diluted with 50 ml acetone solution. One bottle was coated with one ml of PBO solution to be used as the synergist-exposure bottle. A second bottle was coated with one 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. was introduced into the synergist-exposure bottle for one hour. Another 125 females *An. gambiae* s.l. were introduced for one hour into the synergist-control bottle coated with acetone only.

After one hour, the mosquitoes were transferred 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, 25 females were introduced using a mouth aspirator and mortality was recorded every 15 minutes, up to two hours. When control mortality was higher than five percent and less than 20 percent, mortality data were corrected with Abbott's formula. With more than 20 percent mortality among control mosquitoes, the tests were discarded.

2.4 LABORATORY ANALYSES

Analysis of samples collected from routine entomological surveillance from 2015 and beyond commenced following a training on ELISA-CSP in April 2018. The head and thorax portions of each mosquito were removed for use in ELISAs, with the remainder being preserved for polymerase chain reaction (PCR) assays to identify molecular species. As sporozoite rate was only completed on samples collected from 2015-2017, no results will be presented in this report. Analysis on 2018 results is ongoing. Results will be presented as an addendum to the respective annual reports. Molecular species identifications will be conducted in 2019 following a planned training on PCR methods.

3. RESULTS AND DISCUSSION

3.1 ROUTINE ENTOMOLOGICAL COLLECTIONS

Across all collection methods and sites, the most abundant mosquito collected among *Anopheles* species was *An. gambiae* s.l., followed by *An. funestus,* which is a secondary malaria vector in Liberia (Figure 3, Table 4). A total of 381 *An. funestus* were caught at the three sites with 38 percent from Frank Town, 33 percent from Jeneta, and 29 percent from Tomato Camp. *Culex*, *Aedes*, and *Mansonia* species were also collected.

FIGURE 3: SPECIES COMPOSITION OF *ANOPHELES* MOSQUITOES COLLECTED BY PSC, CDC LT AND HLC FROM THREE SENTINEL SITES, DECEMBER 2017–SEPTEMBER 2018

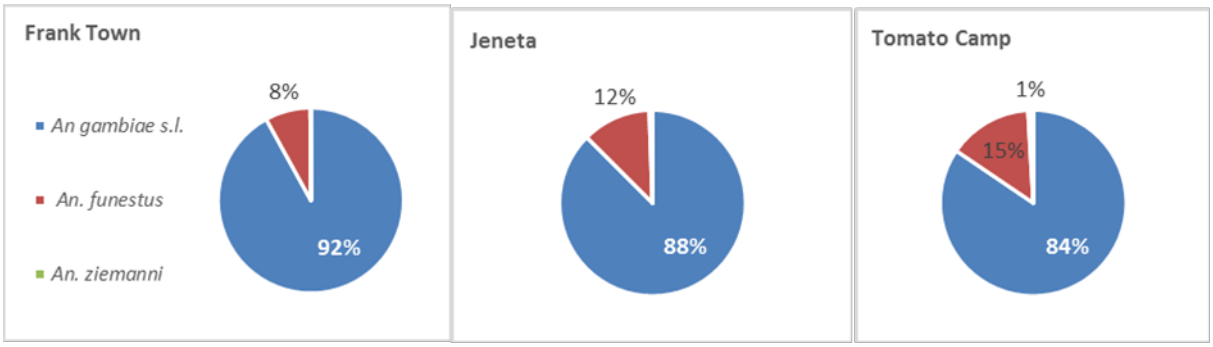


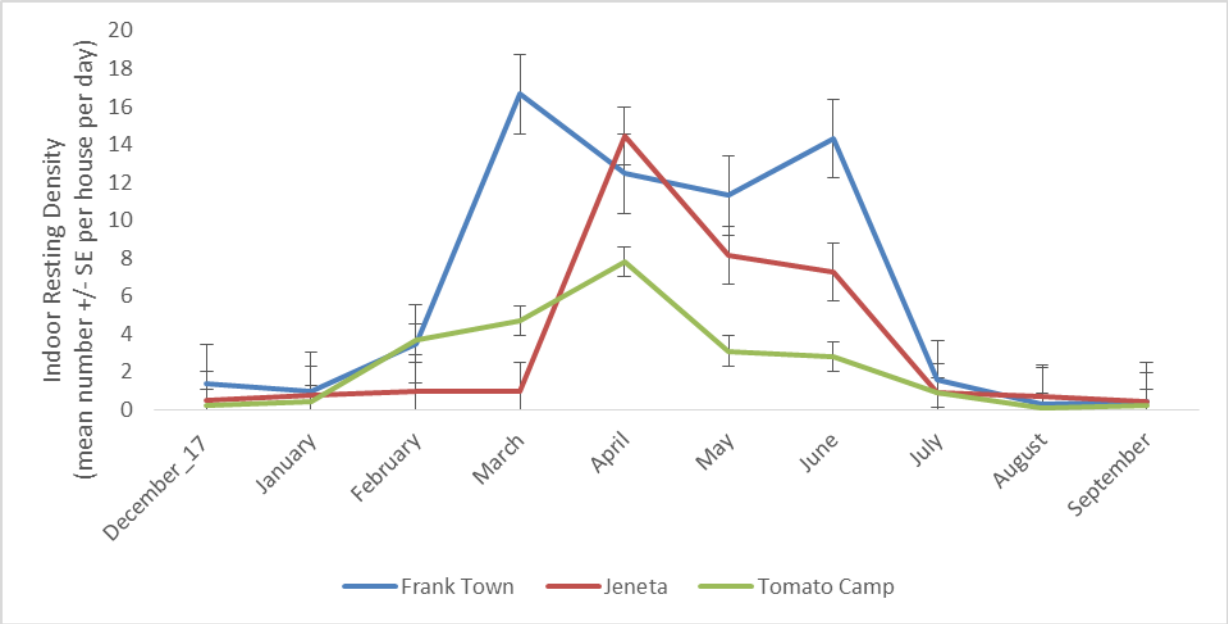
TABLE 4: ABUNDANCE OF ANOPHELES AND MOSQUITOES SPECIES COLLECTED IN THE THREE SENTINEL SITES, DECEMBER 2017–SEPTEMBER 2018

Site	Method	*An. gambiae*	*An. funestus*	*An. ziemanni*	*An. rufipes*	*An. nili*	*An. hancocki*	*Culex*	*Aedes*	*Mansonia*
Frank Town	PSC	1,256	2	0	0	0	0	10	2	0
	CDC LT	248	0	1	1	0	0	522	13	12
	HLC	186	143	0	0	0	0	3	0	0
	Total 1	*1,690*	*145*	*1*	*1*	*0*	*0*	*535*	*15*	*12*
Jeneta	PSC	701	62	0	0	0	0	53	2	1
	CDC LT	67	13	3	0	1	1	736	2	0
	HLC	148	50	1	0	0	0	20	0	0
	Total 2	*916*	*125*	*4*	*0*	*1*	*1*	*809*	*4*	*1*
Tomato Camp	PSC	481	87	0	0	0	0	35	3	1
	CDC LT	135	22	3	4	0	0	300	9	20
	HLC	27	2	0	0	0	0	40	0	0
	Total 3	*643*	*111*	*3*	*4*	*0*	*0*	*375*	*12*	*21*
Total		**3,249**	**381**	**8**	**5**	**1**	**1**	**1,719**	**31**	**34**

3.1.1 INDOOR RESTING DENSITY

From December 2017 through September 2018, 2,438 female *An. gambiae* s.l. (including 1,256 in Frank Town, 701 in Jeneta, and 481 in Tomato Camp) were collected using PSC. Additionally, a total of 151 *An. funestus* females were collected across all three sites. In general, indoor resting densities of the vectors peaked from April through June in all three sentinel sites, with the exception of Frank Town, which recorded peak densities in March (Figure 4). This coincided with the early start of rains in Frank Town, which began in March. The densities of *An. gambiae* s.l. were lower from July to September during the latter half of the rainy season when many of the breeding sites of mosquitoes get washed out.

FIGURE 4: INDOOR RESTING DENSITIES OF *AN. GAMBIAE* S.L. AS COLLECTED BY PSC, IN FRANK TOWN, JENETA AND TOMATO CAMP SENTINEL SITES, DECEMBER 2017–SEPTEMBER 2018



In the field, the *An. gambiae* s.l. mosquitoes were recorded based on their blood-digestion stage. The majority of *An. gambiae* s.l. collected by PSC were blood-fed, comprising nearly 75 percent of mosquitoes collected in all three sentinel sites (Figures 5-7).

FIGURE 5: ANNUAL DISTRIBUTION OF *AN. GAMBIAE* S.L. MOSQUITOES BY ABDOMINAL STAGE, COLLECTED BY PSC IN FRANK TOWN, DECEMBER 2017–SEPTEMBER 2018

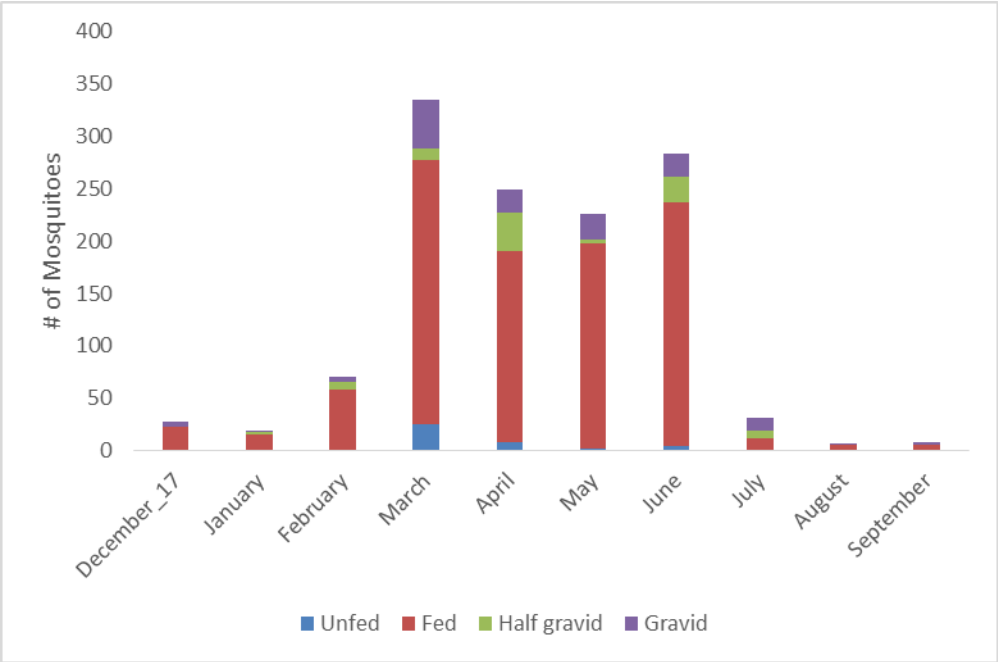


FIGURE 6: ANNUAL DISTRIBUTION OF *AN. GAMBIAE* S.L. MOSQUITOES BY ABDOMINAL STAGE, COLLECTED BY PSC IN TOMATO CAMP, DECEMBER 2017–SEPTEMBER 2018

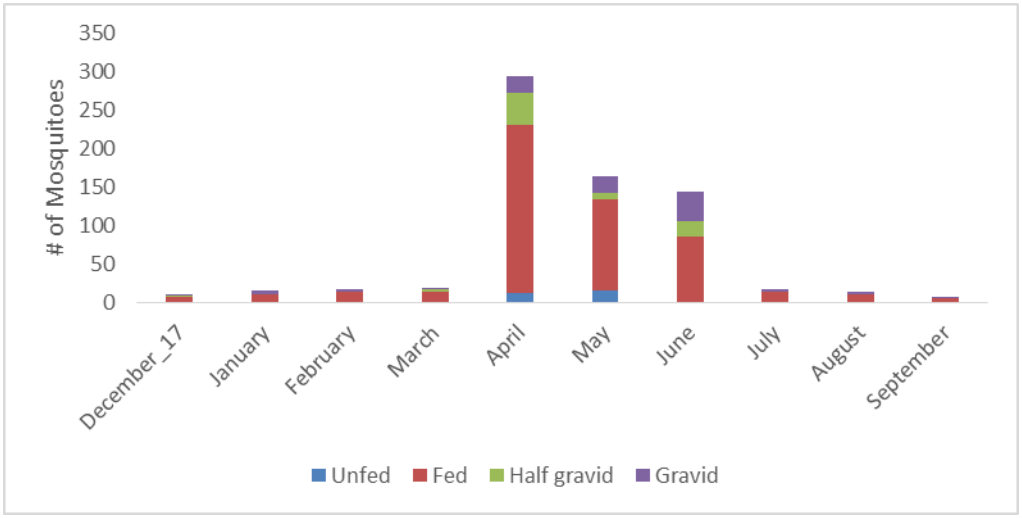
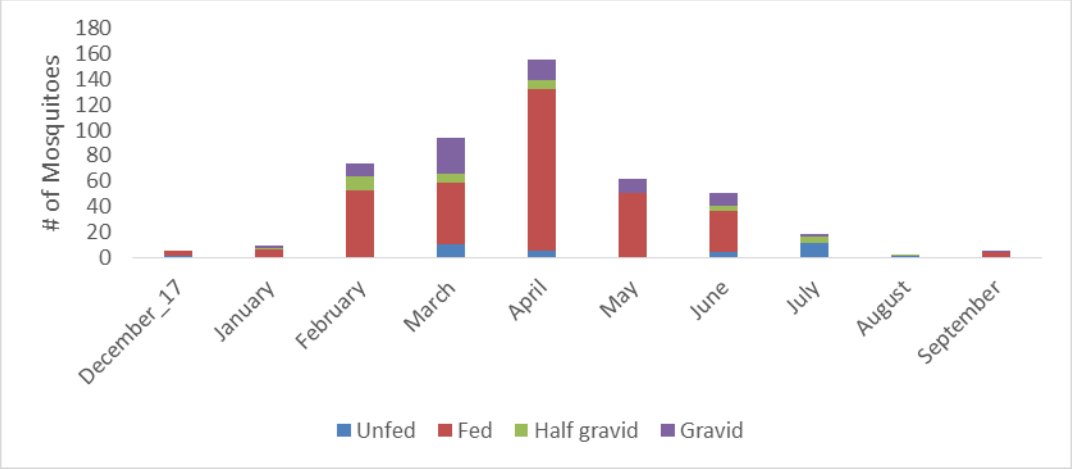


FIGURE 7: ANNUAL DISTRIBUTION OF *AN. GAMBIAE* S.L. MOSQUITOES BY ABDOMINAL STAGES COLLECTED BY PSC IN JENETA, DECEMBER 2017–SEPTEMBER 2018



3.1.2 HUMAN LANDING COLLECTIONS

A total of 361 *An. gambiae* s.l. were collected in the HLC performed bi-monthly at five collection points across the three sentinel sites from January through September 2018. This number is lower than the number collected using CDC LTs because the HLC was done in only two houses per village for one night and bimonthly. The CDC LT collections were made from 20 houses each month. Analysis of total *An. gambiae* s.l. collection from January through September revealed a preference for outdoor biting in both Frank Town and Jeneta. The preference was not apparent in Tomato Camp because of the very low number collected (Table 5). The mean human biting rate (bites/person/night) was highest in Frank Town (9.3 bites/person/night) and lowest in Tomato Camp (1.4 bites/person/night).

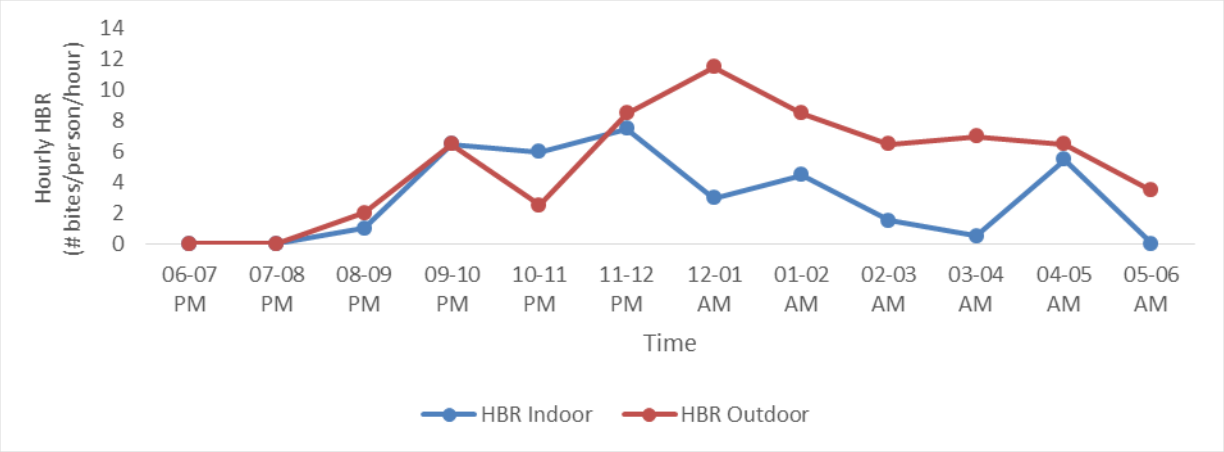
In addition to the *An. gambiae* s.l. mosquitoes, 195 *An. funestus* were collected through HLC across the three sentinel sites: Frank Town (73%), Jeneta (26%), and Tomato Camp (1%). There was no significant difference between the number of *An. funestus* mosquitoes collected indoor and outdoor. The other mosquito species caught were 24 *Culex* and one *An. ziemanni*.

TABLE 5: AVERAGE HBR OF *AN. GAMBIAE* S.L. AND *AN. FUNESTUS* IN TOMATO CAMP, FRANK TOWN, AND JENETA, AS COLLECTED BY HLC, JANUARY–SEPTEMBER 2018

Site	*An. gambiae* s.l.			*An. funestus*		
	Indoor	Outdoor	*Total*	Indoor	Outdoor	*Total*
Frank Town	6.5	12.1	*9.3*	6.0	8.3	*7.2*
Jeneta	4.0	10.8	*7.4*	2.7	2.3	*2.5*
Tomato Camp	1.6	1.1	*1.4*	0.2	0	*0.1*

The highest *An. gambiae* s.l. hourly biting rate was observed in Frank Town where the peak indoor biting time was 11:00 pm-12:00 pm with 7.5 bites per person per hour and outdoor biting peaking at 12:00–1:00 pm with 11.5 bites per person per hour (Figure 8). The data showed that biting activity in Frank Town was higher after 10:00 pm through 5:00 am. Despite the low number of mosquitoes collected, *An. gambiae* s.l. females were very active after midnight in seeking hosts for blood meals.

FIGURE 8: INDOOR AND OUTDOOR *AN. GAMBIAE* S.L. HOURLY MEAN BITING RATE USING HLC AT FRANK TOWN, DECEMBER 2017–SEPTEMBER 2018



In Jeneta, the outdoor biting was higher than the indoor biting, although both remained fairly constant throughout the night (Figure 9). The biting rate of *An. gambiae* s.l. was very low in Tomato Camp with the HBR ranging from 0 to 2 for both indoor and outdoor biting (Figure 10).

FIGURE 9: INDOOR AND OUTDOOR *AN. GAMBIAE* S.L. HOURLY MEAN BITING RATE USING HLC AT JENETA, DECEMBER 2017–SEPTEMBER 2018

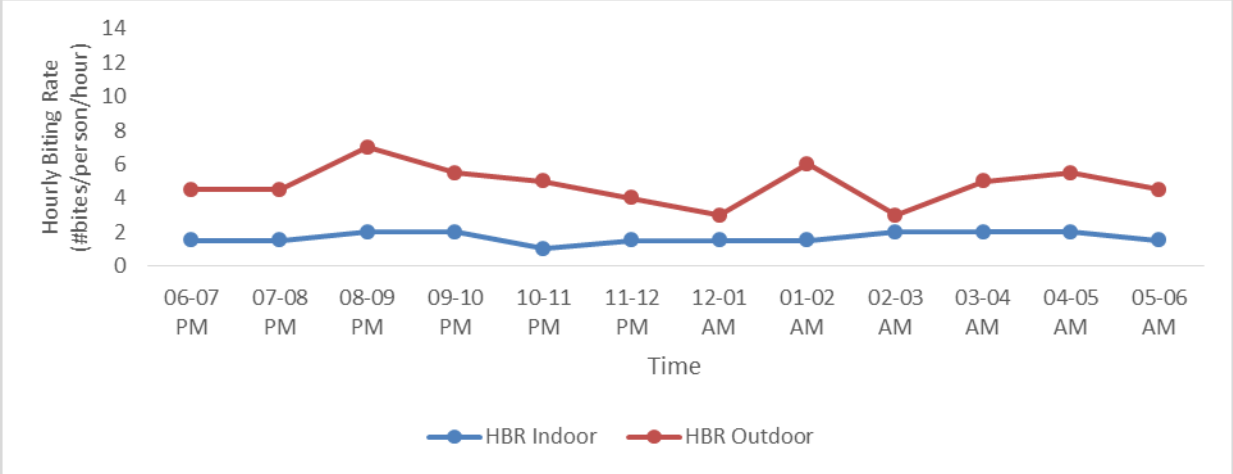
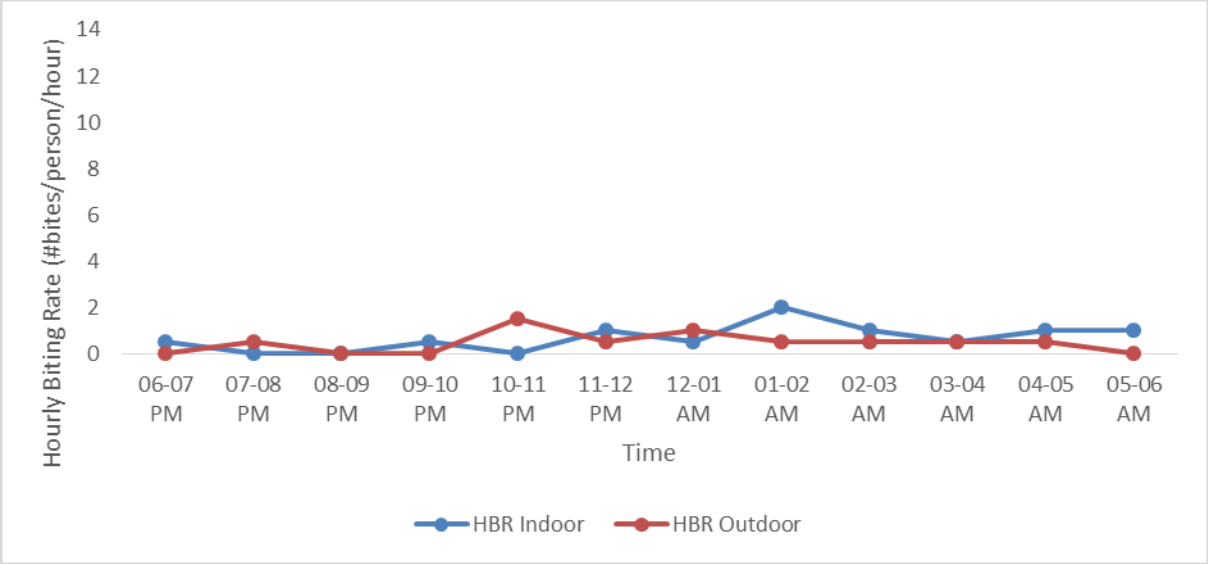
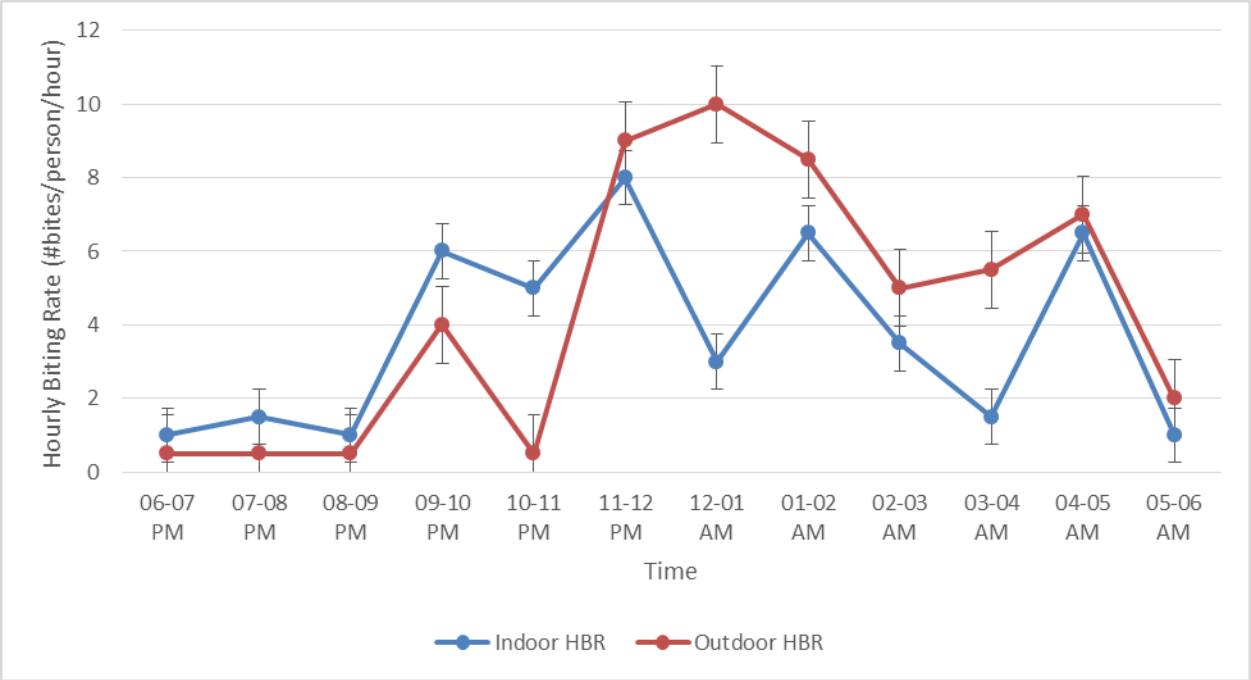


FIGURE 10: INDOOR AND OUTDOOR *AN. GAMBIAE* S.L. HOURLY MEAN BITING RATE USING HLC AT TOMATO CAMP, DECEMBER 2017–SEPTEMBER 2018



A total of 143 *An. funestus* were collected during the reporting period. The outdoor hourly biting rate was highest between 11:00 pm and 2:00 am (Figure 11). The indoor biting rate varied throughout the night.

FIGURE 11: INDOOR AND OUTDOOR *AN. FUNESTUS* S.L. HOURLY MEAN BITING RATE USING HLC AT FRANK TOWN, DECEMBER 2017–SEPTEMBER 2018



3.1.3 CDC Light Trap Collections

In total, 450 *An. gambiae* s.l. were collected using CDC LTs from December 2017 to September 2018 with 55 percent collected at Frank Town, 15 percent collected at Jeneta, and 30 percent collected at Tomato Camp. The majority (84%) were collected indoors (Figure 12). The highest indoor densities of

An. gambiae s.l. were observed in March in Frank Town and in May in Jeneta and Tomato Camp. Across all three sites, the number of mosquitoes collected outdoors were low (Figure 13). Since the outdoor traps were not baited while indoor traps were, indoor and outdoor collections are not comparable.

FIGURE 12: MEAN *AN. GAMBIAE* S.L. COLLECTED INDOORS PER TRAP PER NIGHT BY CDC LT AT FRANK TOWN, TOMATO CAMP AND JENETA, DECEMBER 2017–SEPTEMBER 2018

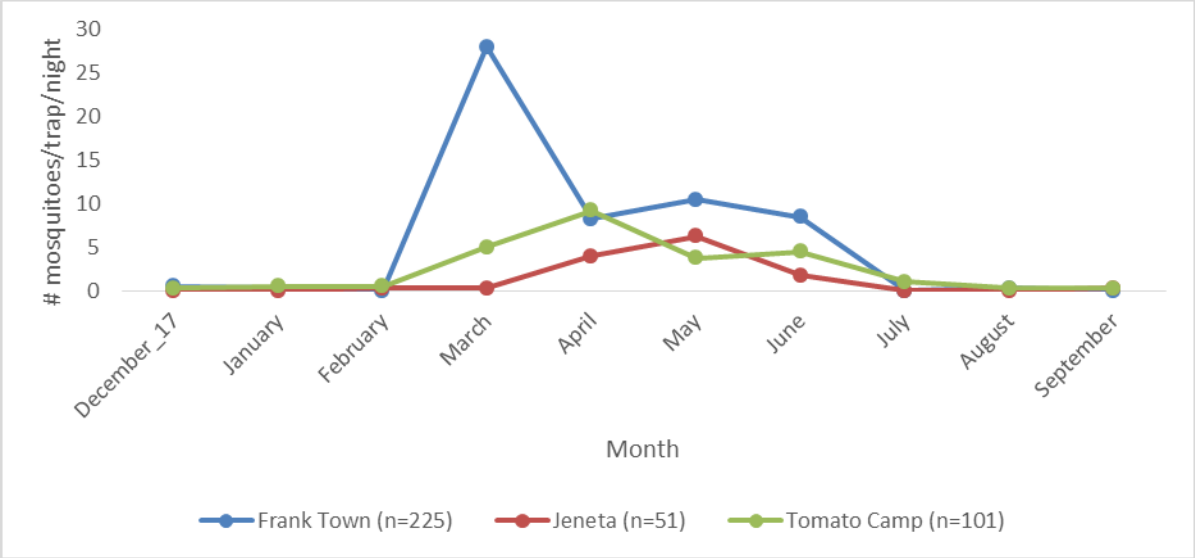
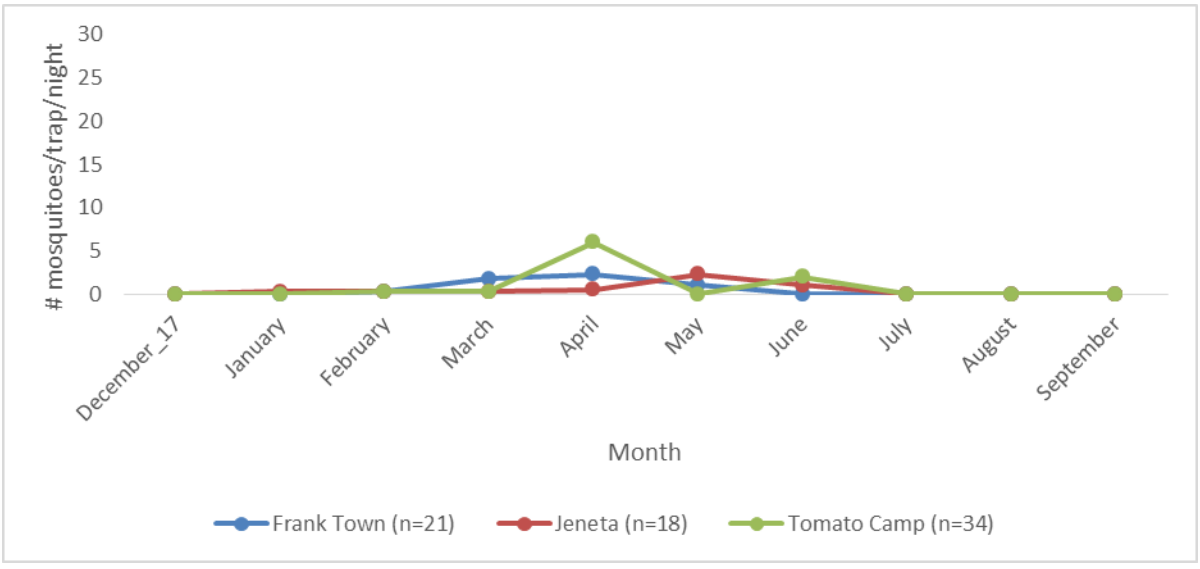


FIGURE 13: MEAN *AN. GAMBIAE* S.L. COLLECTED OUTDOORS PER TRAP PER NIGHT BY CDC LT AT FRANK TOWN, TOMATO CAMP AND JENETA, DECEMBER 2017–SEPTEMBER 2018

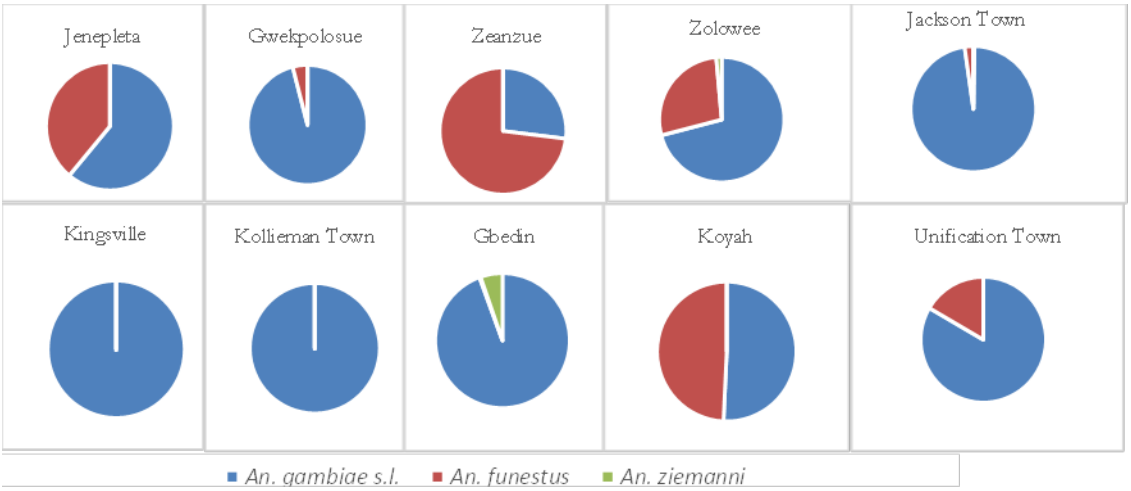


3.2 TRANSECT STUDY

3.2.1 PYRETHRUM SPRAY CATCHES AND CDC LIGHT TRAP COLLECTIONS

The most abundant vector collected across both methods in May 2018 was *An. gambiae* s.l. (1,381) with the highest densities collected in the rice growing areas (Gbedin and Jackson Town). This was followed by 356 *An. funestus*, 23 *An. ziemanni*, 150 *Culex*, and 2 *Aedes* mosquitoes collected. A higher relative abundance of *An. funestus* was recorded in Zeanzue, Koyah, Zolowee and Jenepleta (Figure 14). VectorLink selected Koyah in Bong County as a sentinel site for vector monitoring and Zolowee in Nimba County for insecticide surveillance in 2019 to learn more about *An. funestus* and its role in malaria transmission in Liberia.

FIGURE 14: SPECIES COMPOSITION OF *ANOPHELES* MOSQUITOES COLLECTED BY PSC AND CDC LT FROM THE TRANSECT SITES, MAY 2018



The highest number of *An. gambiae* s.l. was observed in Jackson Town in Margibi County. Indoor resting density was highest in Jackson Town (9.35/house) and in Gbedin (7.85/house) (Figure 15). Among mosquitoes collected using CDC LTs, the highest density of *An. gambiae* s.l. was also observed in Gbedin (45.25/trap) and Jackson Town (20.4/trap) (Figure 16). These are both rice-growing areas which allow for breeding site availability year round. Too few *An. funestus* (total 25 indoor and 16 outdoor) were collected using CDC LTs to show density.

FIGURE 15: INDOOR RESTING DENSITIES OF *AN. GAMBIAE* S.L. AND *AN. FUNESTUS* FROM THE TRANSECT SITES, MAY 2018 (PSC COLLECTIONS)

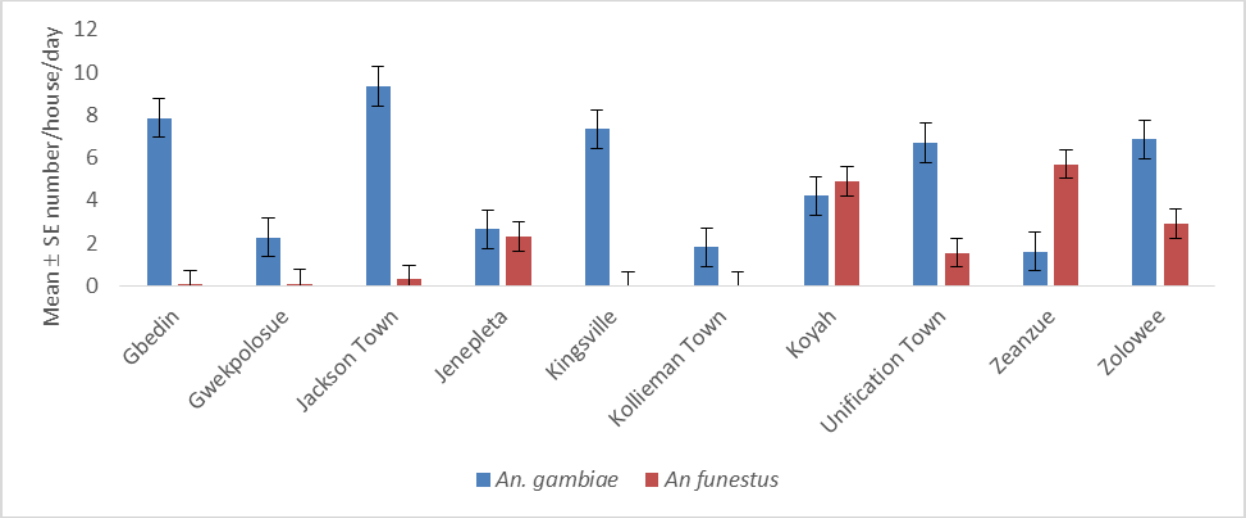
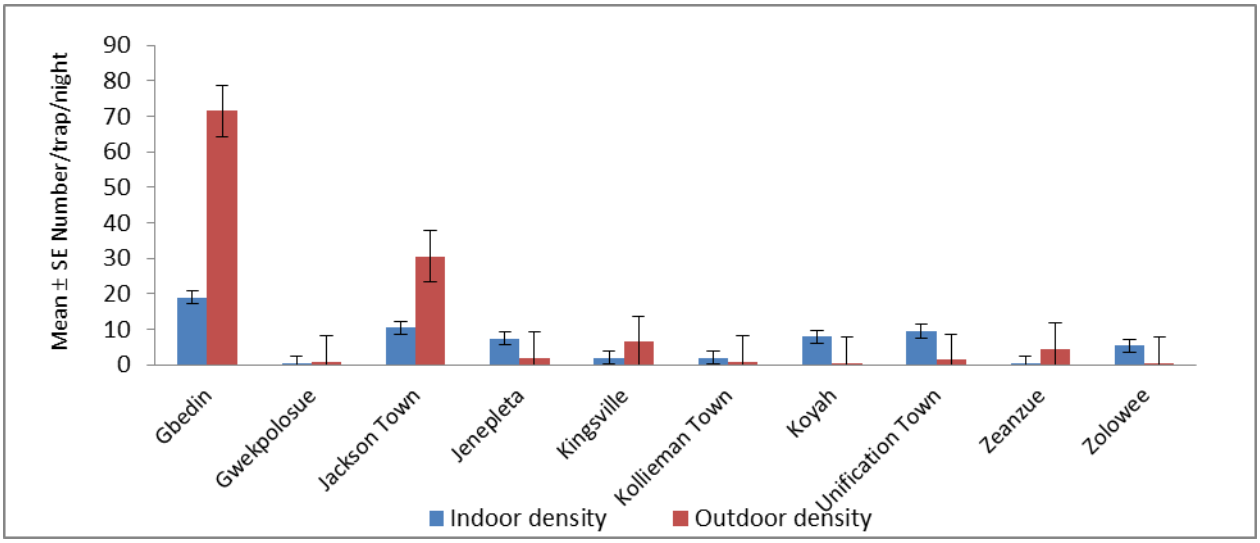


FIGURE 16: *AN. GAMBIAE* S.L. COLLECTED USING CDC LT (INDOOR + OUTDOOR) FROM THE TRANSECT SITES, MAY 2018

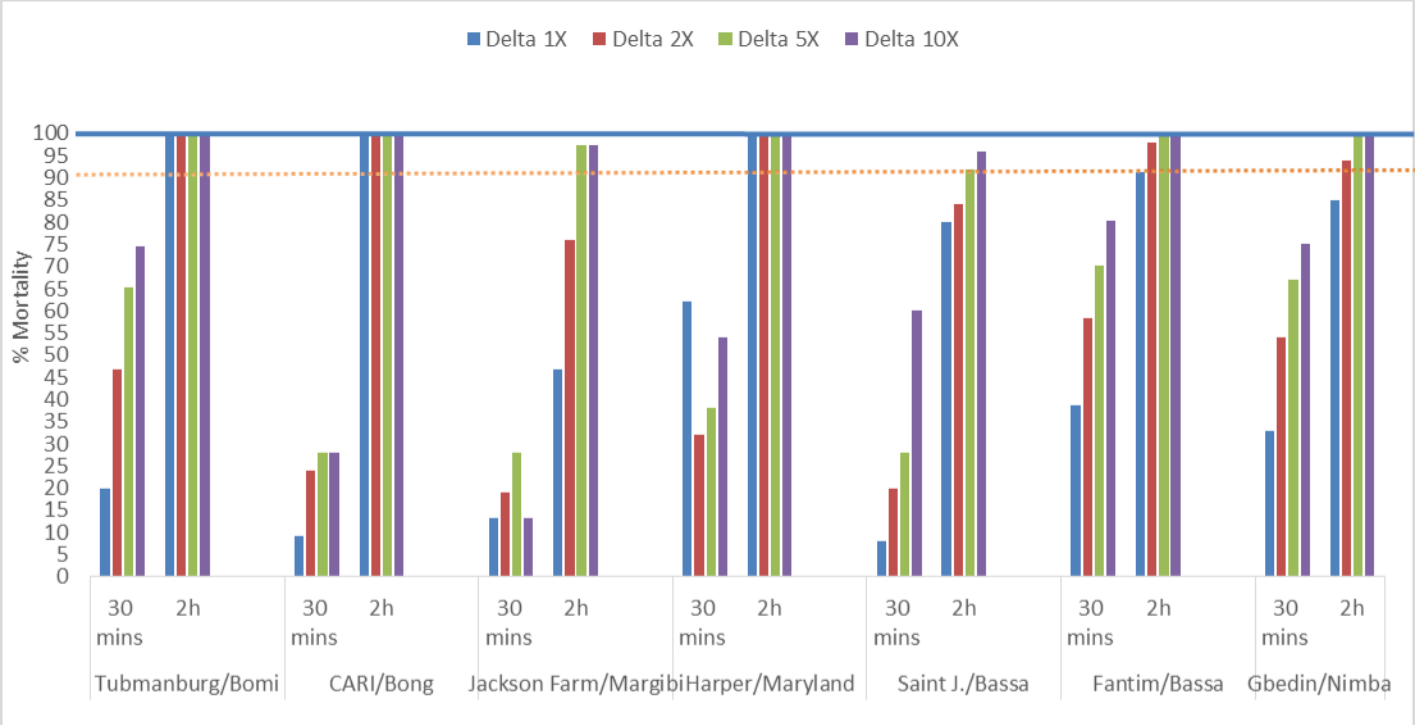


3.3 INSECTICIDE RESISTANCE MONITORING

3.3.1 INSECTICIDE SUSCEPTIBILITY AT DIAGNOSTIC DOSAGES AND INTENSITY ASSAYS

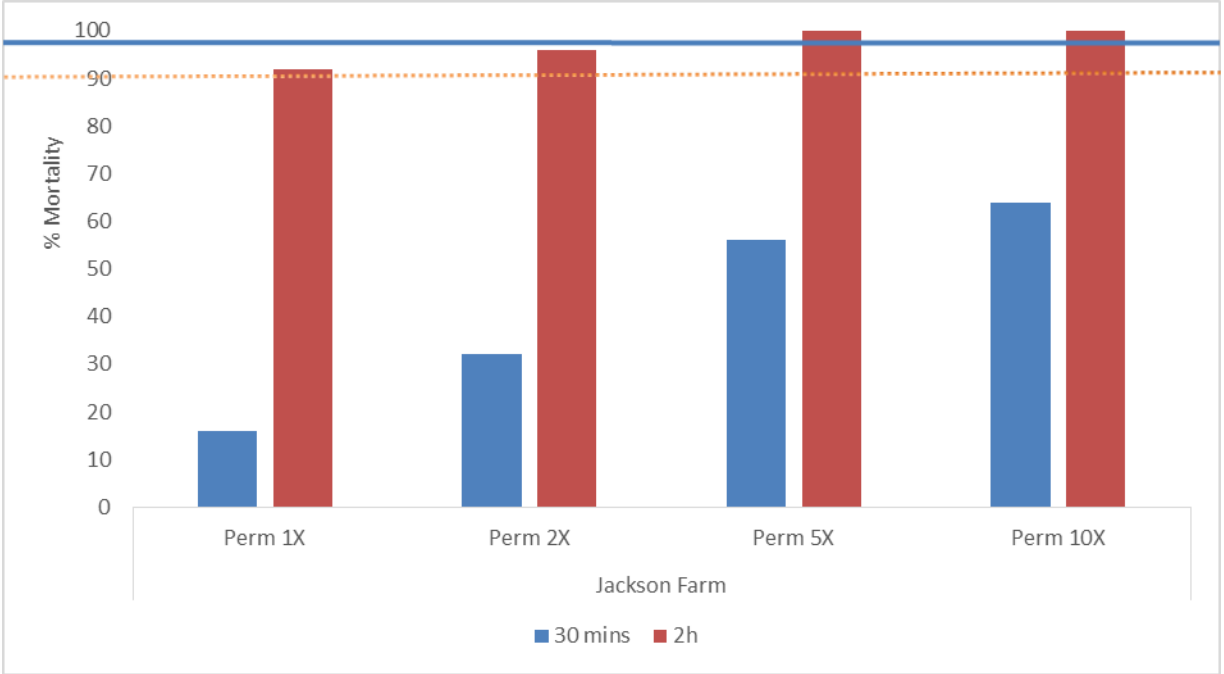
In all sites, *An. gambiae* s.l. mosquitoes were resistant to 1X, 2X, 5X and 10X the diagnostic dose of deltamethrin, after 30 minutes of exposure (Figure 17). The mortality rates for deltamethrin 10X ranged from 13.8 percent to 89 percent. After two-hour exposure, the mortality rates for all four concentrations were 100 percent in three of the seven sites—Tubmanburg, CARI, and Harper.

FIGURE 17: PERCENT MORTALITY OF *AN. GAMBIAE* S.L. FROM DIFFERENT SITES IN LIBERIA EXPOSED TO 1X, 2X, 5X, 10X DELTAMETHRIN USING CDC BOTTLE TESTS, DECEMBER 2017–SEPTEMBER 2018



Jackson Farm was the only site in which sufficient larvae were collected to conduct resistance intensity testing for all three pyrethroids (Figure 18). Mosquitoes were resistant to permethrin at the four concentrations (1X, 2X, 5X, 10X). The trend is similar to what was observed with deltamethrin and alpha-cypermethrin tested in the other sites.

FIGURE 18: PERCENT MORTALITY OF *AN. GAMBIAE* S.L. FROM JACKSON FARM, MARGIBI COUNTY, EXPOSED TO 1X, 2X, 5X, AND 10X PERMETHRIN, USING CDC BOTTLE TESTS, APRIL 2018



3.3.2 SYNERGISTS ASSAYS USING THE CDC BOTTLE ASSAY

The CDC bottle assay method was used to evaluate the synergist effect of PBO on deltamethrin and alpha-cypermethrin resistant *An. gambiae* s. l. Overall, the data showed that PBO did not restore full susceptibility to deltamethrin and alpha-cypermethrin in *An. gambiae* s.l. populations at the diagnostic time (30 minutes). This suggests the existence of mechanisms of resistance other than oxidases.

At Jackson Farm (Margibi County), PBO did not fully restore susceptibility (≥98% mortality) to deltamethrin or alpha-cypermethrin during either test in January 2018 or April 2018 (Figures 19 and 20). However, mortalities with PBO were greater in April 2018 than in January 2018.

FIGURE 19: MORTALITY OF *AN. GAMBIAE* S.L. FROM JACKSON FARM, MARGIBI COUNTY, EXPOSED TO DELTAMETHRIN AND ALPHA-CYPERMETHRIN WITH OR WITHOUT PRE-EXPOSURE TO PBO, USING CDC BOTTLE ASSAY, JANUARY 2018

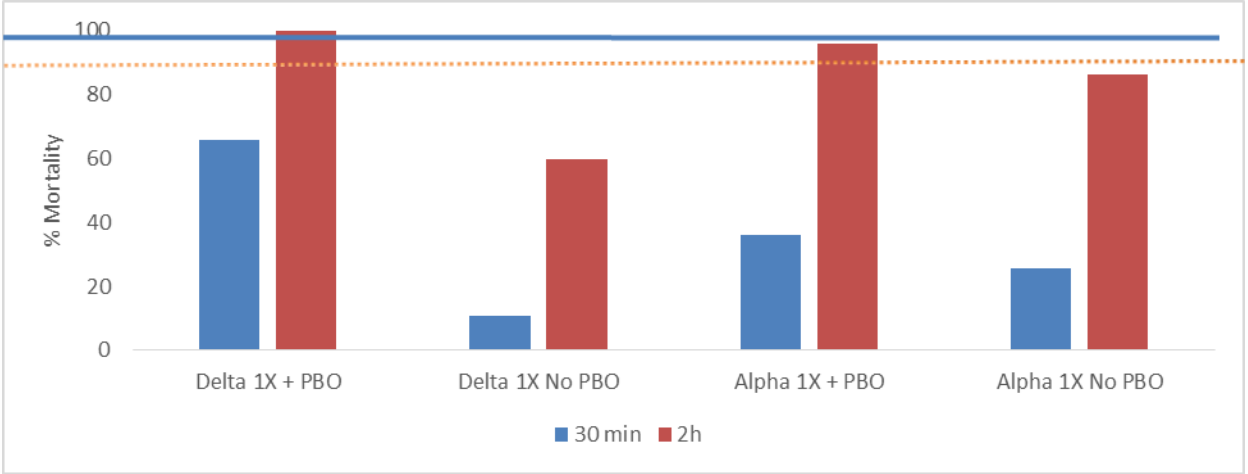
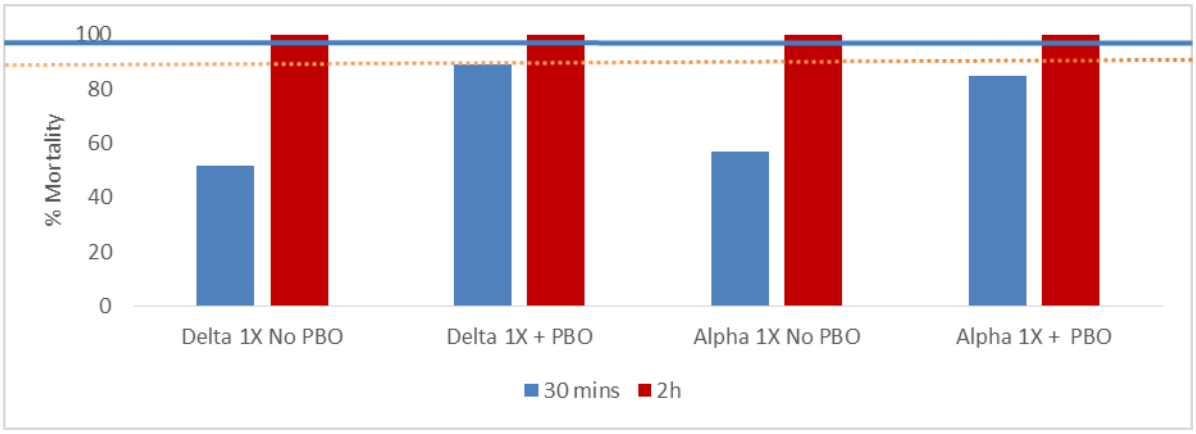


FIGURE 20: MORTALITY OF *AN. GAMBIAE* S.L. FROM JACKSON FARM, MARGIBI COUNTY, EXPOSED TO DELTAMETHRIN AND ALPHA-CYPERMETHRIN WITH OR WITHOUT PRE-EXPOSURE TO PBO USING CDC BOTTLE ASSAY, APRIL 2018



For the tests done in 15th Gate in Monteserrado County (Figure 21), Tubmanburg in Bomi County (Figure 22), Big Fantim in Grand Bassa County (Figure 23), and in Gbedin Camp 3 in Nimba County (Figure 24), pre-exposure with PBO did not restore susceptibility to alpha-cypermethrin or deltamethrin after 30 minutes.

FIGURE 21: MORTALITY OF *AN. GAMBIAE* S.L. FROM 15TH GATE, MONTSERRADO COUNTY, EXPOSED TO DELTAMETHRIN AND DELTAMETHRIN PLUS PBO USING CDC BOTTLE ASSAY, FEBRUARY 2018

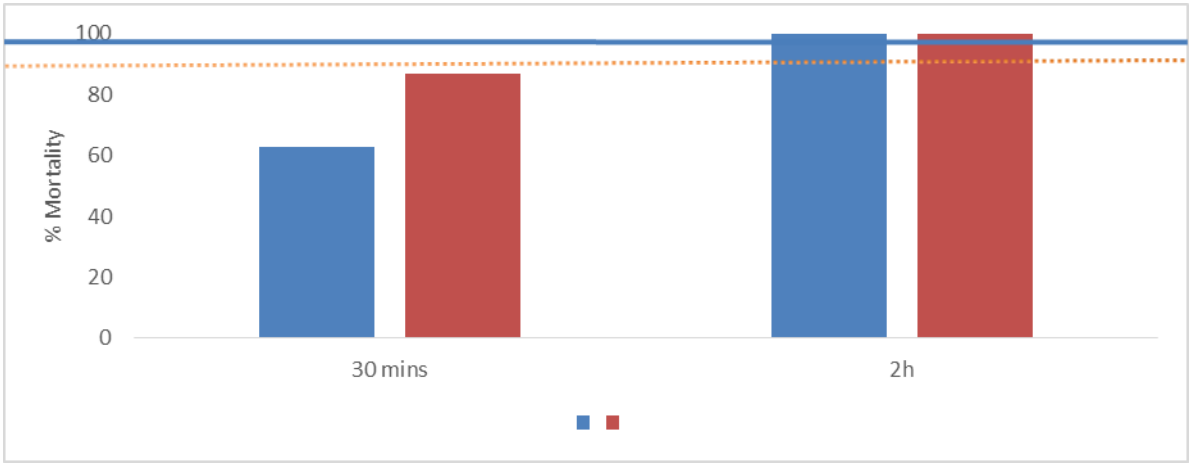


FIGURE 22: MORTALITY OF *AN. GAMBIAE* S.L. FROM TUBMANBURG, BOMI COUNTY, EXPOSED TO DELTAMETHRIN AND DELTAMETHRIN PLUS PBO USING CDC BOTTLE ASSAY, JULY 2018

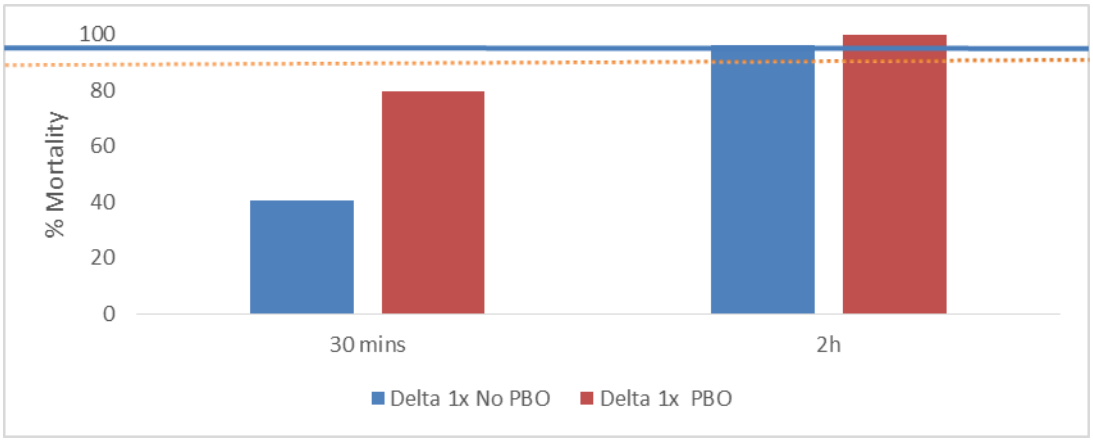


FIGURE 23: MORTALITY OF *AN. GAMBIAE* S.L. FROM BIG FANTIM TOWN, GRAND BASSA COUNTY, EXPOSED TO DELTAMETHRIN AND DELTAMETHRIN PLUS PBO USING CDC BOTTLE ASSAY, AUGUST 2018

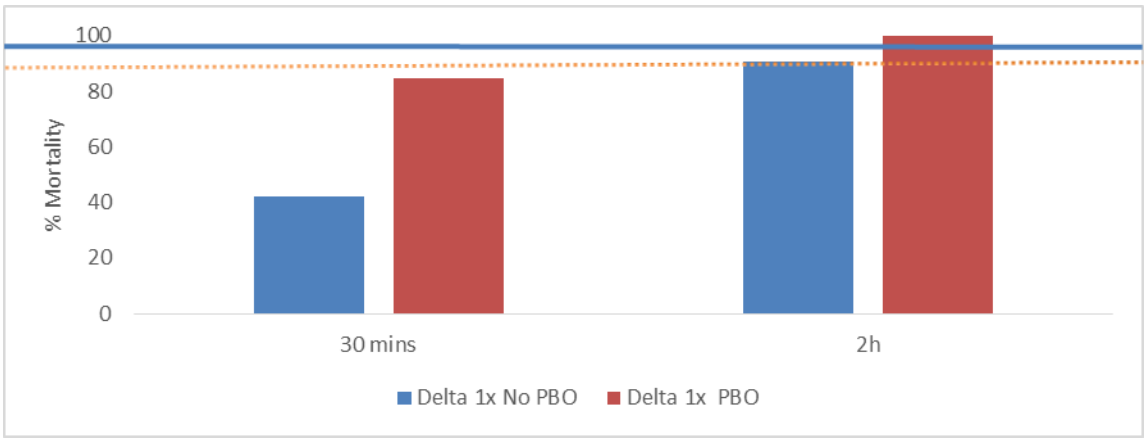
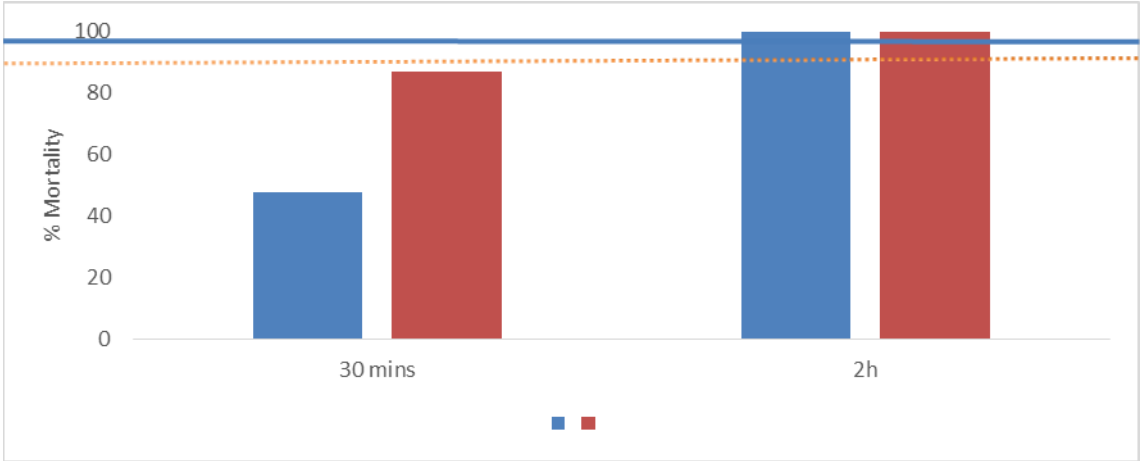
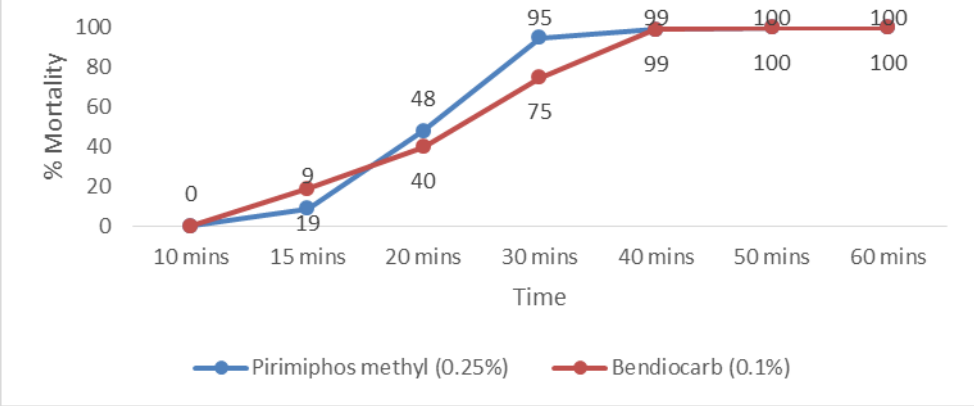


FIGURE 24: MORTALITY OF *AN. GAMBIAE* S.L. FROM GBEDIN CAMP 3, NIMBA COUNTY EXPOSED TO DELTAMETHRIN 1X AND DELTAMETHRIN PLUS PBO USING CDC BOTTLE ASSAY, SEPTEMBER 2018



The results of WHO tube tests conducted in Harper, Maryland County revealed that *An. gambiae* s.l. was susceptible to bendiocarb and pirimiphos methyl with 100 percent mortality after 60 minutes post-exposure (Figure 25).

FIGURE 25: MORTALITY OF AN. GAMBIAE FROM HARPER, MARYLAND COUNTY EXPOSED TO PIROMIPHOS METHYL AND BENDIOCARB USING WHO TUBE TEST, JUNE 2018.



3.4 CAPACITY BUILDING

VectorLink conducted field-based training on insecticide resistance testing, adult mosquito collection methods, morphological mosquito identification, and LLIN bioassays (Table 6). VectorLink trained five staff from the NMCP vector control team on the CDC bottle assay techniques to assess the intensity of insecticide resistance. A total of 28 gCHVs were trained on insecticide resistance testing in the seven sites where insecticide resistance tests were conducted from December 2017 through September 2018. Training covered larval collection methods, mosquito rearing techniques, and insecticide susceptibility testing methods. Insecticide resistance tests were only done under supervision by VectorLink or NMCP.

In the three sentinel sites where entomological monitoring was conducted, 12 gCHVs participated in refresher training on adult mosquito collection methods (PSC, HLC and CDC LT). In all sites, the

training sessions included basic training on morphological identification of mosquito species under a dissecting microscope as well as the major characteristics of *An. gambiae* s.l., breeding sites and how to rear larvae in the field. A briefing was provided on the importance of insecticide resistance surveillance and vector monitoring.

One entomologist from the University of Liberia received training on the field collection methods (HLC, PSC, and CDC LT) as well as mosquito identification and data reporting. A discussion with the Department Sciences at University of Liberia is ongoing on how to extend the collaboration by involving students in VectorLink activity implementation.

In February 2018, four NMCP staff received a field training on LLIN bioassay using cone method. One of them attended a training in June 2018 on LLINs durability held in Monrovia by Johns Hopkins University. In April 2018, CDC facilitated a training for six people (four LIBR staffs and two NMCP staffs) on ELISA-CSP technique for sporozoite rate assessment.

TABLE 6: SUMMARY OF TRAININGS

Training	NMCP	LIBR	gCHVs	University of Liberia
Insecticide Resistance Testing	5		28	
Adult mosquito collection methods refresher trainings			12	1
Field Morphological ID			40	1
ELISA-CSP*	2	4		
LLIN bioassays	4			

**Conducted by CDC*

In 2019, a training on PCR testing will be held in collaboration with CDC for NMCP, LIBR and UL staffs to build research capacity on molecular identification of *Anopheles* mosquitoes.

To support insecticide susceptibility tests, a container insectary was established on the NMCP compound for rearing of *Anopheles gambiae* Kisumu strain which known as susceptible to insecticide. The team maintained the colony from 2016 to present, despite the fluctuations of temperature and relative humidity in the facility due to lack of constant electricity supply. A humidifier installed in the insectary allows for control of the relative humidity (88%) while an air-conditioner set at 28 degrees is regulating the temperature. Larval and pupal samples collected in the field are also reared at the container insectary in Monrovia in order to conduct tests.

VectorLink hired and trained two technicians who are working with the NMCP to implement the field activities and to maintain the insectary. The insectary technician monitors the temperature and relative humidity daily to ensure that mosquitoes are in appropriate conditions.

4. OBSERVATIONS AND CONCLUSIONS

As observed through monthly collections in the three sentinel sites, indoor and outdoor vector densities peaked from April through June and declined from August through September. Trends observed are similar to past years. The peak biting coincided with the start of the rainy season, while the decline coincided with the heaviest rains which wash out breeding sites. The only IRS currently implemented in-country is done by private companies trying to protect workers against malaria. It is important to communicate that IRS would be most effective in April when densities begin to increase.

More sensitization campaign is recommended to encourage the communities to use LLINs throughout the year, especially during peak vector density. Given the consistently high proportion of blood-fed *An. gambiae* s.l. collected by PSC, there is a high risk of infected vectors biting human hosts.

Even with the washing out of naturally occurring breeding sites during the rainy season, breeding sites resulting from human activities (construction, tires, garden well, etc.) were observed in the communities. Targeted communications could help reduce the presence of these sites.

The HLC data recorded higher biting rates during the second part of the night. However, with the low densities, it is challenging to compare indoor and outdoor collections. The number of mosquitoes collected indoors using the CDC LTs was higher than non-baited, outdoor traps. The use of CO_2 source in 2019 collections will allow a comparison between indoor and outdoor collections. CDC LTs might be a convenient way to sample the host-seeking population of malaria vectors.

The transect study showed a high abundance of *An. funestus*, a secondary vector of malaria in Liberia, in select sites. In 2019, its contribution in malaria transmission will be assessed in Koyah as a new sentinel site while susceptibility of *An. funestus* to insecticides will be assessed in Zolowee (Nimba County) where insecticide resistance tests will be conducted.

The data on insecticide susceptibility and resistance intensity using CDC bottle bioassays conducted in seven sites confirmed a high intensity of pyrethroid resistance. Although the susceptibility of *An. gambiae* s.l. after exposure to PBO was not fully restored for alpha-cypermethrin or deltamethrin, data did show that monooxygenase enzymes may be partially involved in the insecticide resistance. More investigations are needed to monitor all mechanisms of resistance. These data should be considered when making vector control decisions, particularly those relevant to LLIN procurement and distribution in Liberia.

The mosquito samples collected in 2018 will be processed this year at LIBR for sporozoite rate determination, molecular species identification and insecticide resistance mechanisms. Once available, this data will be shared as an addendum to this report.

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6. ANNEXES

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

County	IR Site (District)	Larval Collection Site for IR (WHO & CDC Bottle)	Status
Montserrado	Careysburg	Fifteen Gate	February 2018
Bong	Suakoko	CARI	December 2017
Margibi	Kakata	Kakata / Jackson Town	January and April 2018
Grand Bassa	Buchanan	Buchanan / Big Fantim	August 2018
Nimba	Gbedin	Gbedin Camp3	September 2018
Bomi	Tubmanburg	Tubmanburg	July 2018
Maryland	Harper	Harper	June 2018

ANNEX 2: GEOGRAPHIC COORDINATES OF TRANSECT STUDY SITES

County	District	Site	Longitude	Latitude
Nimba	Yah-Meh	Unification Town	7°32.627'N	8°33.834' W
Nimba	Saniquellie Mah	Zolowee	7°26.066'N	8°37.912' W
Nimba	Saniquellie Mah	Gbedin Camp 3	7°16.813'N	8°49.521'W
Margibi	District 5	Jackson Town	6°36.193'N	10°16.679'W
Margibi	District 4	Gwekpolosue	6°37.761'N	10°21.070'W
Bong	Jorquelleh	Koyah	7°02.958'N	9°28.115'W
Bong	Jorquelleh	Jenepleta	7°00.549'N	9°21.050'W
Bong	Suakoko	Zeanzue	6°52.841'N	9°45.620'W
Montserrado	Careysburg	Kollieman Town	6°25.345'N	10°36.540'W
Montserrado	Careysburg	Kingsville	6°24.198'N	10°29.087'W