
**The PMI Africa IRS (AIRS) Project**

Indoor Residual Spraying (IRS 2) Task Order Six

# AIRS LIBERIA: ENTOMOLOGICAL MONITORING FINAL REPORT

NOVEMBER 1, 2015 – NOVEMBER 30, 2016

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## ACRONYMS

| | |
|---|---|
| **AIRS** | Africa Indoor Residual Spraying |
| **CDC** | Centers for Disease Control and Prevention |
| **gCHV** | general Community Health Volunteers |
| **EHT** | **Environmental Health Technician** |
| **EVD** | Ebola Virus Disease |
| **F&A** | Finance and Administration |
| **HLC** | Human Landing Catch |
| **IR** | Insecticide Resistance |
| **IRS** | Indoor Residual Spraying |
| **LIBR** | Liberian Institute of Biomedical Research |
| **LLIN** | Long-Lasting Insecticidal Nets |
| **LMIS** | Liberia Malaria Indicator Survey |
| **NMCP** | National Malaria Control Program |
| **PMI** | President's Malaria Initiative |
| **PSC** | Pyrethrum Spray Collection |
| **USAID** | United States Agency for International Development |
| **WHO** | World Health Organization |

# EXECUTIVE SUMMARY


**Background**

Malaria vector monitoring has been implemented in selected counties in Liberia by the PMI/AIRS project in collaboration with the National Malaria Control Program (NMCP).

**Methods**

This report covers the period November 2015 to November 2016 where monthly malaria vector mosquito collections were carried out using pyrethrum spray catches and CDC light trap collections in two sentinels sites: Tomato Camp (Bong county) and Frank Town (Montserrado county). From May 2016 to November 2016, two new sentinel sites were prospected as requested by NMCP: Jeneta (Margibi county) and Bokay Town (Grand Bassa county). HLC techniques were also implemented every two months in two locations per sentinel site in order to assess the vector biting behavior.

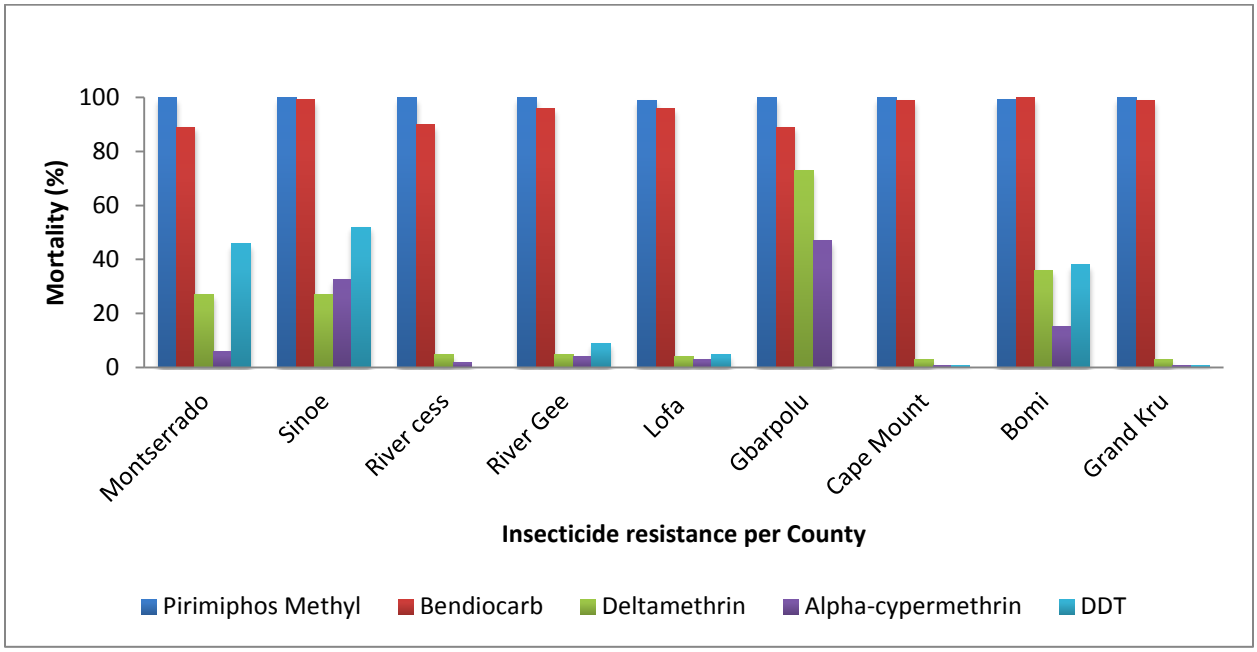
The objective of these activities was to monitor vectors species composition, seasonality, and resting and biting behavior in the four sites. Insecticide resistance bioassays were also implemented in counties of Liberia where they had not been carried out in the previous years in order to complete the mapping of *Anopheles gambiae* s.l. susceptibility to five insecticides recommended for public health use by the World Health Organization (WHO). For resistance molecular mechanism identification and species composition, mosquito samples were preserved on silica gel until lab processing.

**Results**

The peak of biting activity was observed after 12:00AM for indoor and outdoor collections. The indoor biting rates from November 2015 to November 2016 were higher in Tomato Camp (indoor: 2.42 bites/person/night; outdoor: 1.81 bites/person/night) than in Frank Town (indoor: 1.88 bites/person/night; outdoor: 3.15 bites/person/night).

Data collected in Tomato Camp using HLC showed that there is a statistically significant difference (p = 0.042) on the proportion of endophagy (0.5714) and exophagy (0.428). In Frank Town, the proportions of endophagy and exophagy were 0.443 and 0.556, respectively. The difference was not statistically significant (p = 0.179). Among the two new sites, the biting rate was higher in Jeneta (0.73 bites/person/night for indoor and 1.40 bites for outdoor) than in Bokay Town (0.17 bites/person/night for indoor, 0.17 bites for outdoor). In Jeneta, the proportion of endophagy (0.343) and exophagy (0.657) were statistically significant (0.0015).

**FIGURE 1: WHO INSECTICIDE SUSCEPTIBILITY TEST RESULTS ON *AN. GAMBIAE* S.L.: MORTALITY / INSECTICIDE /SITE**




From December 2015 to November 2016, insecticide resistance tests were done in 9 counties across the country to assess the susceptibility status of the local *Anopheles gambiae* s.l. population to pyrethroids (deltamethrin and alpha-cypermethrin), organophosphates (pirimiphos methyl), carbamates (bendiocarb) and organochlorines (DDT). In all counties visited, insecticide susceptibility tests showed that *An. gambiae* s.l. was susceptible to pirimiphos-methyl. Full susceptibility and probable resistance have been observed for bendiocarb. The vector was resistant to alphacypermethrin, deltamethrin and DDT in all sites prospected (Figure 1).

## Conclusion

Vector abundance and biting rate were higher in Tomato Camp, Frank Town and Jeneta but very low in Bokay Town. Abundant rainfall can wash out breeding sites. In all sites, higher densities were not observed during the months where rainfall is high. In Liberia, Bokay Town is near the sea coast where the rainfall is abundant due to the monsoon and also located on a slope, adjacent to a hill which may explain its lower rates among the four sites. In perspective, new sentinel sites will be selected based on a transect study which will be conducted in May, 2017. Resistance to pyrethroids and DDT was widespread.

# 1. INTRODUCTION

Malaria is endemic in Liberia. IRS was conducted between 2009 and 2013. Entomological monitoring and evaluation were conducted along with IRS during those years and have continued on after the last season of IRS activities ended in Bong County in 2013.

In 2014, surveillance continued with two of the sites from 2013: Tomato Camp, Bong County, which was initially sampled in 2013 and Frank Town, Montserrado County, last sprayed in 2012 and included in 2013 as a previously sprayed site.

In 2016, two additional sentinel sites were enrolled as suggested by the NMCP: Bokay Town in Grand Bassa county, (which had been sampled in 2012 as both a pre and post spray site but not sampled since) and Jeneta in Margibi county which has neither been sprayed during the previous campaigns or served as a sample site.

The main objective of the entomological monitoring conducted in sentinel sites was to assess malaria vector species composition, density, seasonality, behavior and susceptibility to insecticides for evidence-based vector control. In the four sentinel sites, *Anopheles* mosquitoes were sampled using human landing catches, pyrethrum spray catches and CDC light trap collections. Insecticide resistance monitoring was carried out in 9 counties from December 2015 to November 2016. Collections were done by the in-country entomologist and NMCP vector control unit staff.

# 2. OBJECTIVES

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

# 3. MATERIALS AND METHODS

## 3.1. STUDY SITES

The village of Tomato Camp (278m, 471806, 778849, 29N, Kpaai District) is located at a higher altitude comparatively to Jeneta (105m, 363952, 736945, 29N), Frank Town (41m, 326593, 709423, 29N, Careysburg District) and Bokay Town (54m, 359226, 686737, 29N, District One). IRS was implemented in Tomato Camp and Frank Town sites in the past.

The four sites are rural areas with farming as the main activity of inhabitants. Potential mosquito breeding sites are present in and around these villages mainly during the rainy season. The vegetation is mainly characterized with scanty canopy and extensive clearing of the forests. Human settlement has modified the environment with the presence of many human made breeding sites like borrow pits and brick pits where mosquitoes could breed.

In these areas suitable breeding sites of *Anopheles gambiae* s.l. mosquitoes are rain dependent, shallow, transient water pools that disappear in the dry season (December to March). House walls are made with mud and the roofs with corrugated iron sheets or grasses.

## 3.2. ADULT MOSQUITO COLLECTIONS

Three mosquito collection methods were used to collect the adult mosquitoes in the sentinel sites. Collection effort was increased in May 2016 in order to get more mosquitoes. PSC collections were done in 20 houses per study site during two consecutive days instead of 10 houses per site in the previous years.

PSC was used to collect indoor resting mosquitoes between 6:30 AM and 8:00 AM at each study site. A commercial insecticide spray (pyrethroids, solvents, and propellant) named *"Bayonet Aerosol Insecticide (BSC301)",* manufactured by "*Foshan Gaoming Jiali Daily Chemicals Co., Ltd. China*", was used for space spray in selected houses that had white cloth/sheet lined on the floor wall to wall. The sprayer tube was used to deliver a fine mist of insecticide in the house after windows and door had been closed. A ten minute knock down period was allowed and the sheets were collected, and mosquitoes were identified and recorded from each house.

From May 2016, CDC collections also were done on two consecutive nights per month using 8 traps, instead of a one-night collection done in the past. CDC light traps were used to capture mosquitoes from 6:00 PM to 6:00 AM both indoors and outdoors in each study village. Eight CDC light traps (four inside and another four outside) were set up in selected houses that had people sleeping under a mosquito net. The consent of the household head was acquired. The traps were placed toward the sleepers legs and hung approximately 150 cm above the ground, depending whether the person slept on the bed or the floor. The outdoor CDC light traps

were up near the same houses with indoor traps but away from the main door. The outdoor traps were not baited.

Human landing catches (HLCs), performed indoors and outdoors, were conducted in two locations per village, in one night, every two months. HLCs were used to collect mosquitoes landing on human baits between 6:00PM and 6:00AM. With legs exposed to attract host-seeking mosquitoes, two human baits sat indoors and another pair sat outdoors. The pairs then exchanged between outdoors and indoors on an hourly basis. The collectors used flashlights and a tubing aspirator to collect mosquitoes once they landed on their legs before they could bite.

### 3.3. INSECTICIDE SUSCEPTIBILITY TESTS

From December 2015 to November 2016, insecticide susceptibility tests were conducted by the NMCP staff and AIRS TME (Technical Manager Entomologist) in 9 counties (Figure 3) using WHO bioassay tubes.
Insecticide resistance tests using adult *An. gambiae* s.l. mosquitoes reared from larvae collected in the areas were conducted in the field. In this reporting period, our efforts were limited on counties where insecticide resistance assays were not carried out (or not completed) during the previous years.

The main goal of the tests is to monitor the susceptibility of *An. gambiae* s.l. mosquitoes to different types of insecticide approved by WHO, used in public health for malaria control, in order to have data from all counties in Liberia. The results will help to plan insecticide use for malaria vector control and to map insecticide resistance status across the country.

**FIGURE 1: AIRS LIBERIA SENTINEL SITES AND INSECTICIDE RESISTANCE MONITORING SITES, 2015 – 2016.**


In all the sites visited, *An. gambiae* s.l. larvae were collected in rice fields, brick pits and water pools along the main roads by the team and gCHVs from 10AM to 02PM. *Anopheles* larvae were reared in a field insectary where relative humidity and temperature are monitored to keep mosquitoes in suitable conditions for the tests. Bioassay tests were done using 3- to 5-day-old adult females emerged from pupae. Tests were done using WHO procedures. Observed knockdown rates were recorded at 10, 15, 20, 30, 40, 50, 60, and 80 minutes, and mortality was recorded after 24h.

After the one hour exposure period, mosquitoes were transferred in holding tubes for 24h observation. Control tubes containing paper impregnated with oil were used to validate the tests. If 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. Observed percentage mortality is equal to the number of dead mosquitoes after 24h divided by the number of exposed mosquitoes multiplied by 100.

### 3.4. TRAINING OF GENERAL COMMUNITY HEALTH VOLUNTEERS (GCHVS)

The gCHVs were selected by Town Chiefs or the District Health Officers in the respective districts visited by the team (Table 1). They were practically trained on how to conduct PSCs, HLCs and setting up of CDC light traps, the three methods used to collect adult mosquitoes in the study sites. The training was conducted by AIRS Abt Technical Manager and one NMCP staff. The training also covered insecticide resistance tests for those involved in larva collection (gCHVs and EHTs (Environmental Health Technicians)). The gCHVs went through a hands-on field training to identify *Anopheles* and *Culex* mosquitoes, both the adult and larval stages. They were able to see mosquitoes under a dissecting microscope for better recognition of their differences. In the field, we explained to gCHVs the major characteristics of *An. gambiae* s.l., potential breeding sites and how to rear larvae in the field. They were also allowed to practice insecticide resistance tests under the supervision of the team members.

**TABLE 1: 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 |
| IR Mapping | | | |
| Larvae collection and susceptibility mapping | 2 | 4 | 1 |
| Insectary Maintenance | | | |
| Container insectary maintenance | 1 | - | 1 |

## 4. RESULTS AND DISCUSSION

### 4.1. ANOPHELINE INDOOR RESTING DENSITIES

From November 2015 to November 2016, 595 female *An. gambiae* s.l. were collected in Frank Town and 971 samples in Tomato Camp (Table 2) using the pyrethrum spray catch. A statistically significant difference ($p < 0.0001$) was observed between the vector abundance determined using PSC in these two sentinel sites. The peak of vector density was observed in June in Tomato Camp and Frank Town. Vector density was estimated by dividing the number of mosquitoes collected by the number of houses prospected per site.

The decrease of vector abundance in August was due to heavy rainfall recorded during that month. When the rainfall was high, *An. gambiae* s.l. breeding sites were washed out and larval density decreased. Too much rainfall can flush away breeding habitats temporarily and also can affect the development of mosquitoes in the breeding sites. These factors could explain the low density of *An. gambiae* s.l. in the two study sites where the intensity of rain was high in August. Overall, vector density was higher in Tomato Camp than in Frank Town (Table 2).

**TABLE 2: NUMBER OF *AN. GAMBIAE* S.L. MOSQUITOES COLLECTED BY PSC AND INDOOR RESTING DENSITIES IN FRANK TOWN AND TOMATO CAMP NOV-15 TO NOV-16.**

| | Frank Town | | Tomato Camp | |
|---|---|---|---|---|
| **Month** | **Number Collected** | **Vector Density** | **Number Collected** | **Vector Density** |
| Nov-15 | 2 | 0.20 | 138 | 13.80 |
| Dec-15 | 0 | 0.00 | 48 | 4.80 |
| Jan-16 | 10 | 1.00 | 158 | 15.80 |
| Feb-16 | 3 | 0.30 | 109 | 10.90 |
| Mar-16 | 100 | 10.00 | 55 | 5.50 |
| Apr-16 | 76 | 7.60 | 71 | 7.10 |
| *May-16 | 77 | 3.85 | 102 | 5.10 |
| Jun-16 | 220 | 11.00 | 187 | 9.40 |
| Jul-16 | 50 | 2.50 | 68 | 3.40 |
| Aug-16 | 29 | 1.45 | 12 | 0.60 |
| Sep-16 | 10 | 0.50 | 3 | 0.20 |
| Oct-16 | 14 | 0.70 | 13 | 0.70 |
| Nov-16 | 4 | 0.20 | 7 | 0.40 |
| **Total** | **595** | | 971 | |

(*)May 2016 to November 2016, 20 houses prospected/month instead of 10 houses during the previous months.

The difference between the number of mosquitoes collected in Frank Town and Tomato Camp was significant statistically ($p<0.005$). In both sites, the vector abundance peak was not observed during the period when the rainfall is highest.

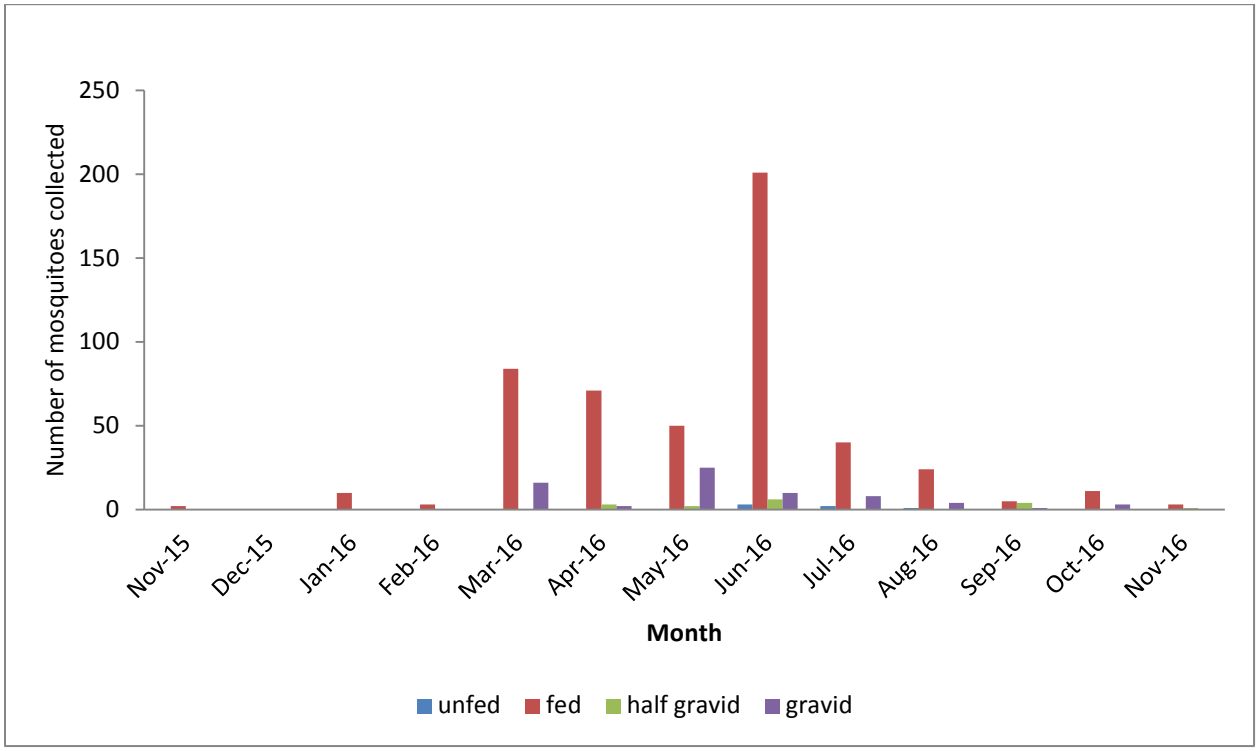
In Tomato Camp, the others species of mosquitoes collected by PSC were: *An. funestus* (3), which is known malaria vector: *An. rufipes* (1), zoophilic; *Culex* species (11), and *Mansonia* species (1). Very few other mosquito species were observed in Frank Town: *Culex* species (1) and *Mansonia* species (1).

**TABLE 3: NUMBER OF AN. GAMBIAE S.L. MOSQUITOES COLLECTED BY PSC AND INDOOR RESTING DENSITIES IN JENETA AND BOKAY TOWN, MAY TO NOV-16.**

| Month | Jeneta | | Bokay Town | |
|---|---|---|---|---|
| | Number Collected | Vector Density | Number Collected | Vector Density |
| May-16 | 106 | 5.30 | 11 | 0.55 |
| Jun-16 | 338 | 16.90 | 72 | 3.60 |
| Jul-16 | 35 | 1.75 | 4 | 0.20 |
| Aug-16 | 9 | 0.45 | 0 | 0.00 |
| Sep-16 | 21 | 1.05 | 0 | 0.00 |
| Oct-16 | 9 | 0.45 | 0 | 0.00 |
| Nov-16 | 24 | 1.20 | 1 | 0.05 |
| **Total** | **542** | | **88** | |

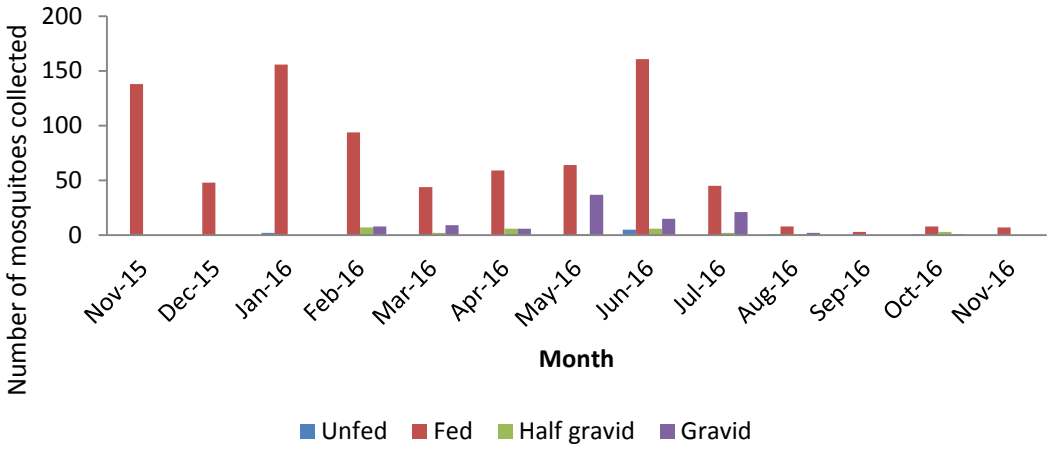
In Jeneta, the number of mosquitoes collected by PSC from May to November 2016, was higher than in Bokay Town ($p<0.001$) and the peak abundance was observed in May – June 2016 (Table 3). The other species of mosquitoes collected in Jeneta and Bokay Town were respectively 8 *Culex* and 10 *Culex*.

**FIGURE 2: *AN. GAMBIAE* S.L. MOSQUITOES COLLECTED USING PSC IN FRANK TOWN BY ABDOMINAL STAGES, NOV-15 TO NOV-16.**



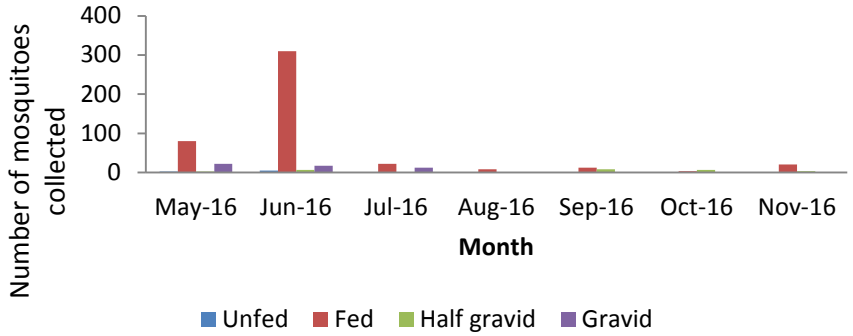
In Frank Town, the percentages according to the abdominal stages (unfed, fed, half gravid, gravid) were respectively 1.01%, 84.71%, 2.69% and 11.60% from November 2015 to November 2016. Most of the mosquitoes collected by PSC were fed (Figure 4). The high number of fed mosquitoes showed that people are exposed to mosquito bites, which could potentially transmit the malaria parasite. The high percentages of fed mosquitoes showed that there is frequent contact between host and vector. People might have been bitten outdoors or indoors if they are not using LLINs regularly.

**FIGURE 3: NUMBER OF AN. GAMBIAE S.L. MOSQUITOES COLLECTED USING PSC IN TOMATO CAMP BY ABDOMINAL STAGES, NOV-15 TO NOV-16.**



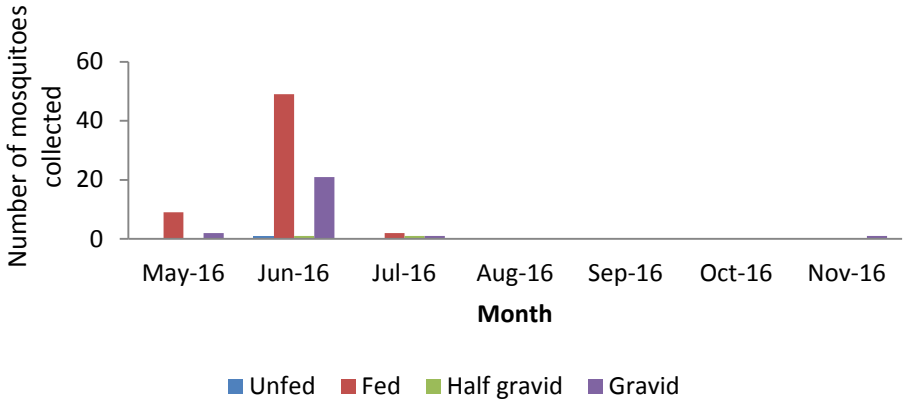

The percentages of the different abdominal stages of *An. gambiae* s.l. mosquitoes collected in Tomato Camp were: unfed (0.9%), fed (86.0%), half gravid (2.9%) and gravid (10.2%). Fed females were highly abundant in November, 2015, January and June 2016 (Figure 5).

**FIGURE 4: NUMBER OF *AN. GAMBIAE* S.L. MOSQUITOES COLLECTED USING PSC IN JENETA BY ABDOMINAL STAGES, MAY-15 TO NOV-16.**



In Jeneta, a total of 542 female *An. gambiae* s.l. and 8 *Culex* mosquitoes were collected using PSC technique. The percentages of the different abdominal stages of *An. gambiae* s.l. mosquitoes collected were: unfed (1.5%), fed (83.9%), half gravid (4.8%) and gravid (9.8%). Fed females were abundant in May and June 2016 before rains started in that area (Figure 6).

**FIGURE 5: NUMBER OF *AN. GAMBIAE* S.L. MOSQUITOES COLLECTED USING PSC IN BOKAY TOWN BY ABDOMINAL STAGES, MAY-15 TO NOV-16.**




A total of 88 *An. gambiae* s.l. mosquitoes were collected in Bokay Twon using PSC technique (Figure 7): unfed (1.1%), fed (68.2%), half gravid (2.3%) and gravid (28.4%). The abundance of others mosquito species was low, since only 10 *Culex* species were collected during indoor spray catches.

## 4.2. HUMAN LANDING COLLECTIONS

**TABLE 4: NUMBER OF *AN. GAMBIAE* S.L. CAPTURED AND BITES/PERSON/NIGHT FROM HLC IN FRANK TOWN, NOV-15 TO NOV-16.**

| Month | Indoor | Indoor bites/person/night | Outdoor | Outdoor bites/person/night | Total |
|---|---|---|---|---|---|
| Nov-15 | 3 | 0.75 | 13 | 3.25 | 16 |
| Jan-16 | 1 | 0.25 | 2 | 0.50 | 3 |
| Mar-16 | 28 | 7.00 | 34 | 8.50 | 62 |
| May-16 | 24 | 6.00 | 22 | 5.50 | 46 |
| Jul-16 | 0 | 0.00 | 5 | 1.25 | 5 |
| Sep-16 | 7 | 1.75 | 3 | 0.75 | 10 |
| Nov-16 | 0 | 0.00 | 0 | 0.00 | 0 |
| **Total** | 63 | -- | 79 | -- | 142 |

In Frank Town, from November 2015 to November 2016, 142 females *An. gambiae* s.l. were collected using human landing catches with two persons indoors and outdoors (Table 4). The bites/person/night were calculated by dividing the total number *An. gambiae* s.l. collected each month by the number of persons involved in the HLC (persons-night). A statistically significant difference was not found between the endophagic (0.44) and exophagic (0.56) rates, p=0.1794.

**TABLE 5: NUMBER OF *AN. GAMBIAE* S.L. CAPTURED AND BITES/PERSON/NIGHT FROM HLC IN TOMATO CAMP, NOV-15 TO NOV-16.**

| Month | Indoor | Indoor bites/person/night | Outdoor | Outdoor bites/person/night | Total |
|---|---|---|---|---|---|
| Nov-15 | 15 | 3.75 | 12 | 3.00 | 27 |
| Jan-16 | 39 | 9.75 | 32 | 8.00 | 71 |
| Mar-16 | 8 | 2.00 | 6 | 1.50 | 14 |
| May-16 | 41 | 10.25 | 35 | 8.75 | 76 |
| Jul-16 | 10 | 2.50 | 0 | 0.00 | 10 |
| Sep-16 | 2 | 0.50 | 1 | 0.25 | 3 |
| Nov-16 | 1 | 0.25 | 1 | 0.25 | 2 |
| **Total** | **116** | -- | **87** | -- | **203** |

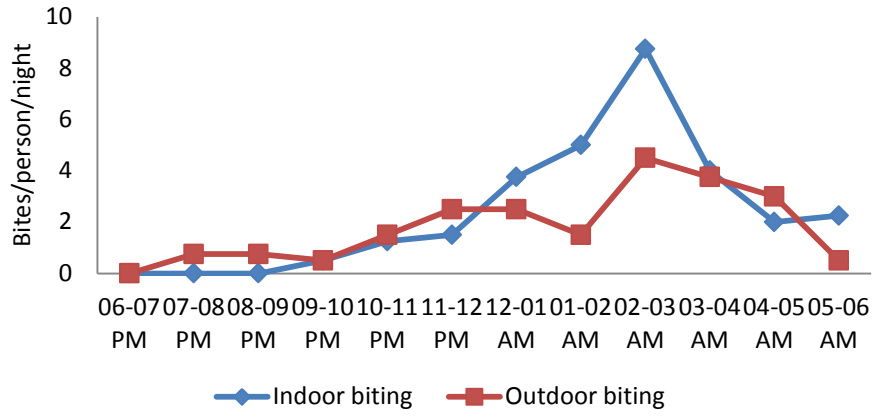
In Tomato Camp, from November 2015 to November 2016, 116 *An. gambiae* s.l. mosquitoes were collected indoors and 87 outdoors using the human landing catch (Table 5).
Data collected in Tomato Camp using HLC showed that, there is a statistically significant difference (p=0.0418) in the proportion of endophagy (0.571) and exophagy (0.429).

**TABLE 6: NUMBER OF AN. GAMBIAE S.L. CAPTURED AND BITES/PERSON/NIGHT FROM HLC IN JENETA AND BOKAY TOWN, NOV-15 TO NOV-16.**

| Site | Jeneta | | | | | Bokay Town | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Month | Number Indoor | Indoor bite /person /night | Number outdoor | Outdoor bite /person /night | Total | Number Indoor | Indoor bite /person /night | Number outdoor | Outdoor bite /person /night | Total |
| May-16 | 34 | 8.50 | 57 | 14.25 | 91 | 2 | 0.50 | 3 | 0.75 | 5 |
| Jul-16 | 1 | 0.25 | 7 | 1.75 | 8 | 4 | 1.00 | 2 | 0.50 | 6 |
| Sep-16 | 0 | 0.00 | 0 | 0.00 | 0 | 2 | 0.50 | 1 | 0.25 | 3 |
| Nov-16 | 0 | 0.00 | 3 | 0.75 | 3 | 0 | 0.00 | 2 | 0.50 | 2 |
| **Total** | **35** | | **67** | | **102** | **8** | | **8** | | **16** |

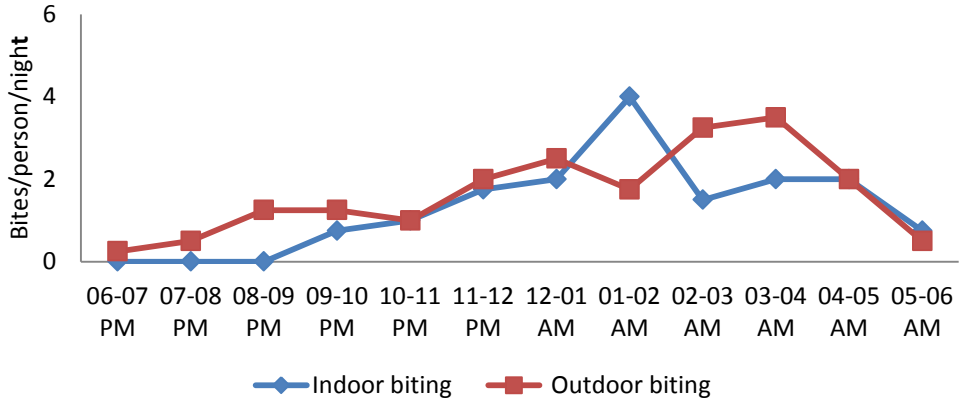
In Jeneta, monthly biting rates were higher than in Bokay Town where very few mosquitoes where collected during HLCs (Table 6). Comparing the indoor and outdoor data from Jeneta, there is a statistically significant difference (p=0.0015) in the proportion of endophagy (0.343) and exophagy (0.657). The abundance of *An. gambiae* s.l. in Bokay Town was low, despite the fact it is a typical rural area with permanent water sources across the year. The reason could be due to proximity of this site to the sea coast where the number of rain days is high and it is located on a slope, adjacent to a hill. Recurrent rains can drain the water, reducing the larvae density in the breeding sites.

**FIGURE 6: HOURLY BITING PER NIGHT IN TOMATO CAMP, NOV-15 TO NOV-16.**



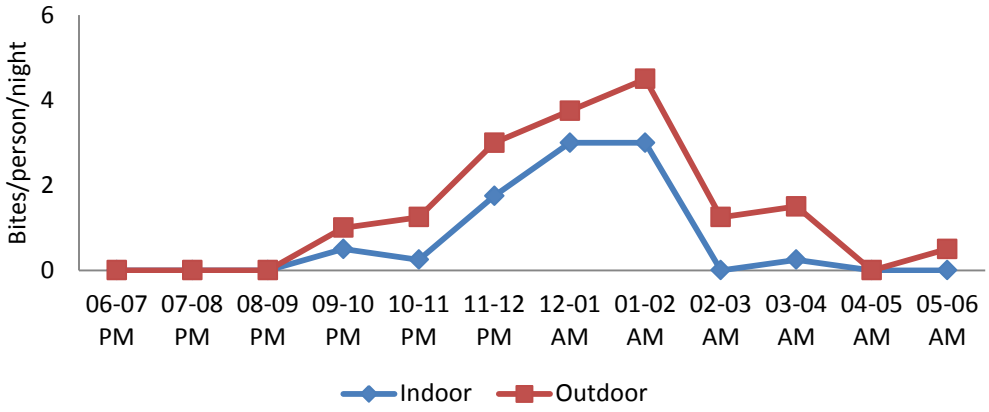

HLC data from Tomato Camp showed that biting activity of *An. gambiae* s.l. is high from 10:00PM to 05:00AM (Figure 8) with the peak around 02-03 AM both indoors and outdoors.

**FIGURE 7: HOURLY BITING PER NIGHT IN FRANK TOWN, NOV-15 TO NOV-16.**



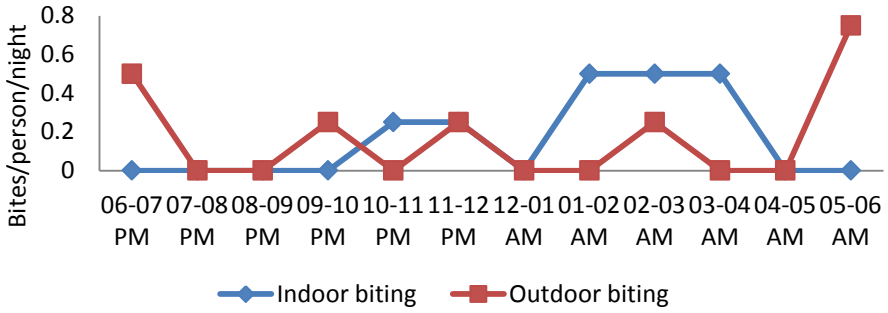

Biting activity in Frank Town started around 08PM (indoors & outdoors) and continued until 06:00AM (Figure 9). A peak of biting was observed from 01:00-04:00AM in the morning.

**FIGURE 8: HOURLY BITING PER NIGHT IN JENETA, NOV-15 TO NOV-16.**




In Jeneta, biting activity started around 08PM (indoors & outdoors) and continued until 06:00AM (Figure 10). A peak of biting was observed from 10:00AM to 03:00AM.

**FIGURE 9: HOURLY BITING PER NIGHT IN BOKAY TOWN MAY TO NOV-16.**




In Bokay Town, biting activity was not regular because of the low vector density in this site (Figure 11). The low number of mosquitoes collected precluded showing a clear biting pattern in this coastal area. We are expecting to continue data collection in the area until May 2017 in order to have a complete year round dataset.

## 4.3. CDC LIGHT TRAP COLLECTIONS

At each sentinel site, eight light traps were used: four traps installed indoors and four traps outdoors, from 06:00PM to 06:00AM. In Frank Town, 252 female *An. gambiae* s.l. were collected during the period November 2015 to November 2016 (Table 7). Since collections were done in 4 houses, the number of collected mosquitoes per month was divided by four in order to estimate the density per site.

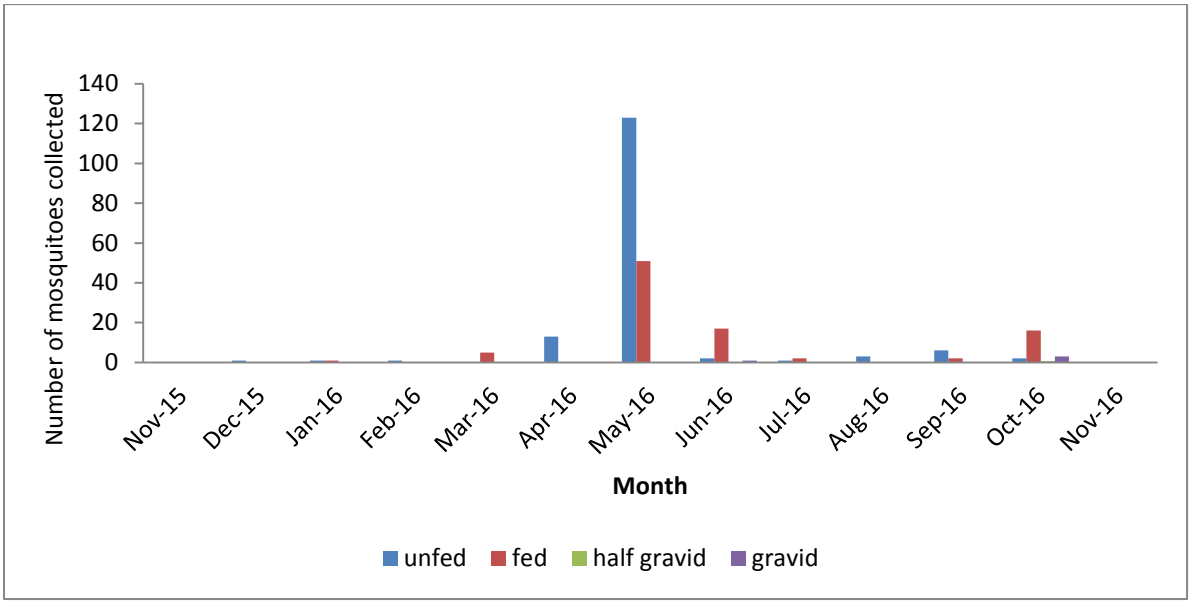
In addition to the *An. gambiae* s.l. collected, 3 *An. rufipes*, 166 *Culex* , 8 *Aedes*, and 3 *Mansonia* species were captured in Frank Town.

**TABLE 7: NUMBER OF *AN. GAMBIAE* S.L. MOSQUITOES COLLECTED BY CDC LIGHT TRAP IN FRANK TOWN, NOVEMBER 2015 TO NOVEMBER 2016.**

| Month | Indoor | Density Indoor | Outdoor | Density outdoor | Total |
|---|---|---|---|---|---|
| Nov-15 | 0 | 0.00 | 0 | 0.00 | 0 |
| Dec-15 | 1 | 0.25 | 0 | 0.00 | 1 |
| Jan-16 | 1 | 0.25 | 1 | 0.25 | 2 |
| Feb-16 | 1 | 0.25 | 0 | 0.00 | 1 |
| Mar-16 | 5 | 1.25 | 0 | 0.00 | 5 |
| Apr-16 | 5 | 1.25 | 8 | 2.00 | 13 |
| May-16 | 151 | 37.75 | 23 | 5.75 | 174 |
| Jun-16 | 9 | 2.25 | 11 | 2.75 | 20 |
| Jul-16 | 0 | 0.00 | 3 | 0.75 | 3 |
| Aug-16 | 2 | 0.50 | 1 | 0.25 | 3 |
| Sep-16 | 5 | 1.25 | 3 | 0.75 | 8 |
| Oct-16 | 16 | 4.00 | 6 | 1.50 | 22 |
| Nov-16 | 0 | 0.00 | 0 | 0.00 | 0 |
| **Total** | **196** | | **56** | | **252** |

The difference was statistically significant ($p < 0.0001$) between the proportion caught indoors (0.778) and outdoors (0.222). Despite, two consecutive nights of collection starting in May 2016, using 8 traps per site, the number of mosquitoes collected were generally low, except in May 2016 where the peak was observed.

**FIGURE 10: *AN. GAMBIAE* S.L. MOSQUITOES COLLECTED USING CDC LIGHT TRAPS BY ABDOMINAL STAGES IN FRANK TOWN, NOV-15 TO NOV-16.**



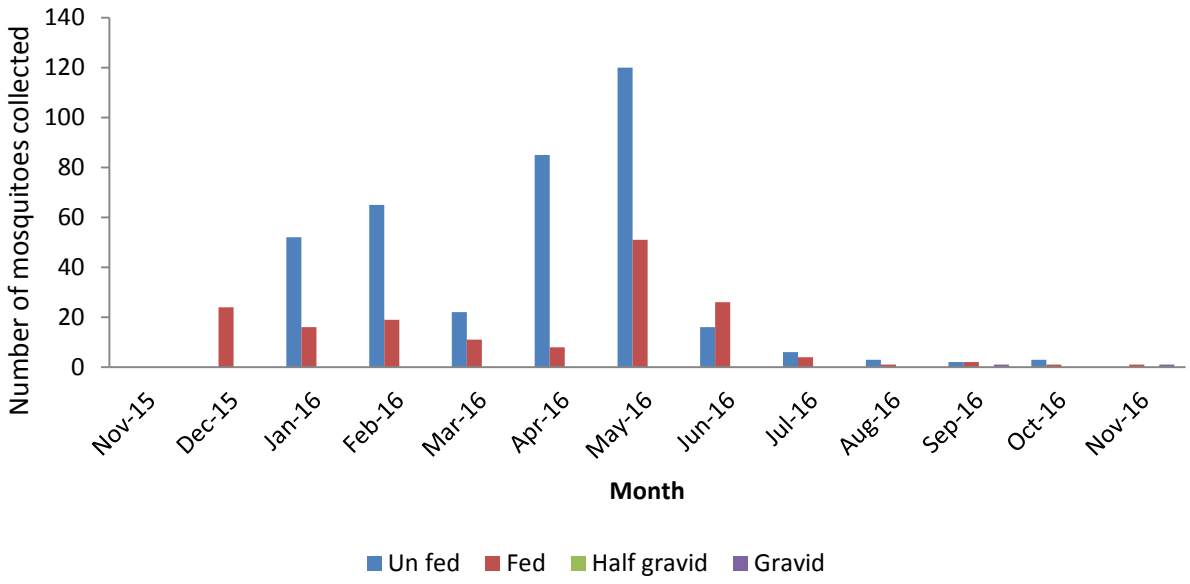

Among *An. gambiae* s.l. mosquitoes collected by CDC light trap in Frank Town, most of them were unfed (Figure 12). The abundance peak was observed in May.

**TABLE 8: NUMBER OF *AN. GAMBIAE* S.L. MOSQUITOES COLLECTED BY CDC LIGHT TRAP IN TOMATO CAMP, NOVEMBER 2015 TO NOVEMBER 2016.**

| CDC | Indoor | Density Indoor | Outdoor | Density outdoor | Total |
|---|---|---|---|---|---|
| Nov-15 | 0 | 0.00 | 0 | 0.00 | 0 |
| Dec-15 | 24 | 6.00 | 0 | 0.00 | 24 |
| Jan-16 | 66 | 16.50 | 2 | 0.50 | 68 |
| Feb-16 | 77 | 19.30 | 7 | 1.80 | 84 |
| Mar-16 | 11 | 2.80 | 22 | 5.50 | 33 |
| Apr-16 | 93 | 23.30 | 0 | 0.00 | 93 |
| May-16 | 148 | 37.00 | 23 | 5.80 | 171 |
| Jun-16 | 34 | 8.50 | 8 | 2.00 | 42 |
| Jul-16 | 10 | 2.50 | 0 | 0.00 | 10 |
| Aug-16 | 3 | 0.80 | 1 | 0.30 | 4 |
| Sep-16 | 5 | 1.30 | 0 | 0.00 | 5 |
| Oct-16 | 3 | 0.80 | 1 | 0.30 | 4 |
| Nov-16 | 1 | 0.30 | 1 | 0.30 | 2 |
| **Total** | **475** | | **65** | | **540** |

In total, 540 *An. gambiae* s.l. mosquitoes were collected in Tomato Camp using CDC light traps (Table 8). The number of mosquitoes captured indoors (475) was higher than the number collected outdoors (65). Data collected using CDC light traps showed that there was a statistically significant difference (p<0001) between the proportion collected indoors (0.879) and outdoors (0.120) in Tomato Camp, Bong County. In addition to *An. gambiae* s.l., other species of mosquitoes were collected in Tomato Camp by light traps: *An. funestus* (2), *An. rufipes* (6), *An. flavicosta* (1), *Culex* (112), *Mansonia* (18) and *Aedes* (1).

**FIGURE 11: *AN. GAMBIAE* S.L. MOSQUITOES COLLECTED USING CDC LIGHT TRAPS IN TOMATO CAMP BY ABDOMINAL STAGES, NOVEMBER 2015 TO NOVEMBER 2016.**



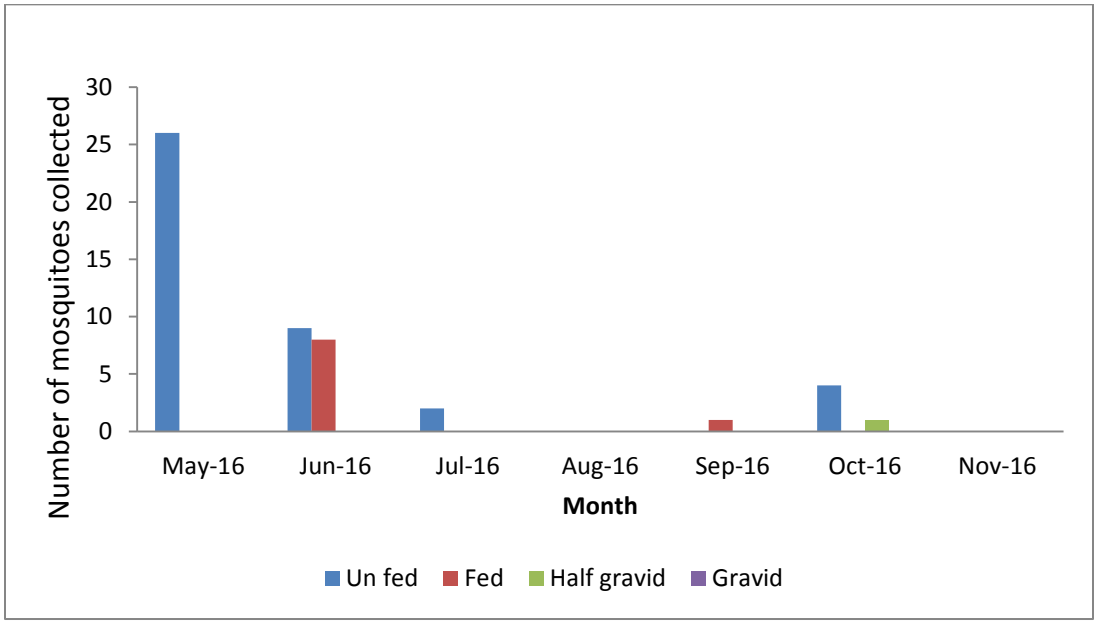

The percentages of unfed, fed and gravid were 69.3%, 30.4% and 0.4%, respectively. In Tomato Camp, the number of unfed *An. gambiae* s.l. mosquitoes was high from January to May 2016 (Figure 13). During the rainy season, the densities decreased drastically because of the drainage of breeding sites which affected larval density.

**TABLE 9: NUMBER OF *AN. GAMBIAE* S.L. MOSQUITOES COLLECTED BY CDC LIGHT TRAP IN JENETA, MAY TO NOVEMBER, 2016.**

| Month | Indoor | Density Indoor | Outdoor | Density outdoor | Total |
|---|---|---|---|---|---|
| May-16 | 20 | 5.00 | 6 | 1.50 | 26 |
| Jun-16 | 12 | 3.00 | 5 | 1.30 | 17 |
| Jul-16 | 2 | 0.50 | 0 | 0.00 | 2 |
| Aug-16 | 0 | 0.00 | 0 | 0.00 | 0 |
| Sep-16 | 1 | 0.30 | 0 | 0.00 | 1 |
| Oct-16 | 3 | 0.80 | 2 | 0.50 | 5 |
| Nov-16 | 0 | 0.00 | 0 | 0.00 | 0 |
| **Total** | **38** | | **13** | | **51** |

In Jeneta, the difference was statistically significant ($p < 0.0001$) between the proportion caught indoors (0.745) and outdoors (0.255). In this site also, despite, two consecutive nights of collections using 8 traps per site, the number of mosquitoes was low, but a maximum of 26 mosquities were collected in May 2016 (Table 9). The number of other mosquitoes collected were: 417 *Culex*, 1 *Aedes*, 1 *Mansonia* and 2 *An. ziemani*.

**FIGURE 12: *AN. GAMBIAE* S.L. MOSQUITOES COLLECTED USING CDC LIGHT TRAPS IN JENETA BY ABDOMINAL STAGES, MAY TO NOVEMBER, 2016.**



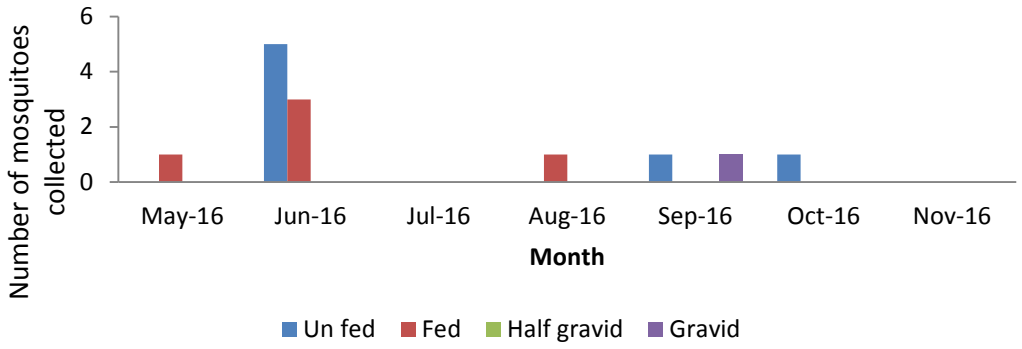

In Jeneta, *An. gambiae* s.l. mosquitoes collected with CDC light trap from May to November 2016 were mainly unfed (Figure 14).

**TABLE 10: NUMBER OF *AN. GAMBIAE* S.L. MOSQUITOES COLLECTED BY CDC LIGHT TRAP IN BOKAY TOWN, MAY TO NOVEMBER 2016.**

| Month | Indoor | Density Indoor | Outdoor | Density outdoor | Total |
|---|---|---|---|---|---|
| May-16 | 1 | 0.30 | 0 | 0.00 | 1 |
| Jun-16 | 6 | 1.50 | 2 | 0.50 | 8 |
| Jul-16 | 0 | 0.00 | 0 | 0.00 | 0 |
| Aug-16 | 1 | 0.30 | 0 | 0.00 | 1 |
| Sep-16 | 1 | 0.30 | 1 | 0.30 | 2 |
| Oct-16 | 1 | 0.30 | 0 | 0.00 | 1 |
| Nov-16 | 0 | 0.00 | 0 | 0.00 | 0 |
| **Total** | **10** | | **3** | | **13** |

In Bokay Town, the difference was not statistically significant (p=0.0522) between the proportion caught indoors (0.769) and outdoors (0.231). After two consecutive nights of collections per month using 8 traps per site, the number of mosquitoes was generally low (Table 10). The number of other mosquitoes collected were: 24 *Culex* and 3 *Aedes*.

**FIGURE 13: *AN. GAMBIAE* S.L. MOSQUITOES COLLECTED USING CDC LIGHT TRAPS IN BOKAY TOWN BY ABDOMINAL STAGES, MAY TO NOVEMBER, 2016.**




Mosquito density was very low in Bokay Town as compared to the other sentinel sites. The low density as described previously could be due to the high rainfall in this area. The rains last longer across the year along the sea coast than in the interior and it is located on a slope. Most of the few *An. gambiae* s.l. mosquitoes collected with CDC light traps were unfed (Figure 14).

## 4.4. INSECTICIDE SUSCEPTIBILITY TESTS

Insecticide susceptibility data collected from 9 sites (one site per county) are shown in Table 10. The number of mosquitoes tested and the percentage mortality after a 24-hour holding period are shown.

**TABLE 11: WHO INSECTICIDE SUSCEPTIBILITY TEST RESULTS BY INSECTICIDE EXPOSED TO *AN. GAMBIAE* S.L. IN 9 COUNTIES OF LIBERIA.**

| County | Sites | Pirimiphos Methyl | | Bendiocarb | | Deltamethrin | | Alpha-cypermethrin | | DDT | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Mortality% | N | Mortality | N | Mortality% | N | Mortality% | N | Mortality% | N |
| Montserrado | Bensonville | 100 | 100 | 89 | 100 | 27 | 100 | 6 | 100 | 46 | 100 |
| Gbarpolu | Bopolu | 100 | 100 | 89 | 100 | 73 | 100 | 47 | 100 | | |
| River cess | Cestos City | 100 | 100 | 90 | 100 | 5 | 100 | 2 | 100 | 0 | 100 |
| River Gee | Fish Town | 100 | 100 | 96 | 100 | 5 | 100 | 4.3 | 100 | 9 | 100 |
| Lofa | Voinjama | 99 | 100 | 96 | 100 | 4 | 100 | 3 | 100 | 5 | 100 |
| Sinoe | Greenville | 100 | 125 | 99.2 | 125 | 27.2 | 125 | 32.8 | 125 | 52 | 125 |
| Cape Mount | Garwula | 100 | 100 | 99 | 100 | 3 | 100 | 1 | 100 | 1 | 100 |
| Bomi | Tubmanburg | 99.2 | 124 | 100 | 100 | 35.87 | 100 | 15.22 | 100 | 38.04 | 100 |
| Grand Kru | Barclayville | 100 | 100 | 99 | 100 | 3 | 100 | 1 | 100 | 1 | 100 |

*Legend: green = susceptible (**S**) ; Red = resistant ( **R**) ; yellow = probable resistance (PR);*

In Bensonville (Montserrado County), the results (July, 2016) indicated that *An. gambiae* s.l. were susceptible to pirimiphos-methyl and resistant to DDT, bendiocarb, deltamethrin and alphacypermethrin. (Table 11). A similar pattern was observed in Gbarpolu (December 2015). The vector was susceptible to pirimiphos-methyl, but resistant to deltamethrin, alphacypermethrin and DDT, with possible resistance to bendiocarb in River Gee (June, 2016), Lofa (August, 2016) and River Cess (September, 2016) sites. *An. gambiae* s.l. mosquitoes tested in Sinoe (May, 2016), Bomi (March, 2016), Cape Mount (October, 2016), and Grand Kru (November 2016) were susceptible to primiphos-methyl (99%-100% mortality) and bendiocarb (99%-100%), but were resistant to DDT, deltamethrin, and alpha-cypermethrin.

During the surveillance in various counties, human activities such as construction were creating good breeding sites for malaria vectors. In Greenville (Sinoe county), larval collections were done essentially in man-made breeding sites (borrow pits & brick pits) located around communities in May, 2016, which is early in the rainy season, before the heavy rains. More larvae were collected there than in the other counties. In Gbarpolu County (December 2015)

most of the potential breeding sites were dry since it was not the rainy season. However, mosquito larvae were collected mainly in sand mining areas and residual swamps. The potential breeding sites in Voinjama (Lofa county) were not swamp areas, but water pools along the road and tire prints in August, 2016 which is the time of heavy rains. Those breeding sites located near houses may expose inhabitants to malaria transmission during the rainy season.

## 5. OBSERVATIONS AND CONCLUSIONS

- NMCP staff members have been trained locally and during regional courses on various aspects of field entomology. The next training will be to learn how to process mosquito samples for molecular analysis locally in collaboration with LIBR;
- A lab colony of *An. gambiae* s.s. (Kisumu) strain was initiated in the insectary;
- Indoor and outdoor collections have shown that vector densities in sentinel sites are higher at the beginning and end of the rainy season than during the rainy season period when breeding sites are flushed by the rains;
- A communication strategy is needed to remind people that they should use LLINs during the dry season to reduce the vector-host contact.

Based on susceptibility test data collected during the study period, *An. gambiae* s.l., the major malaria vector in Liberia, is fully susceptible to primiphos-methyl; it has developed probable resistance to bendiocarb in three out of the nine sites. *An. gambiae* s.l., is resistant to deltamethrin, alpha-cypermethrin and DDT in all sites. Additional assays are needed to test for insecticide resistance intensity in selected sites.

## 6. RECOMMENDATIONS

- The selection of new sentinel sites after the transect study in 2017 may help to better understand the variations of ecological conditions and vector abundance
- For capacity building, the NMCP and Liberian Ministry of Health may need to identify young personnel for masters level training in entomology to support vector surveillance and a teaching program
- The PMI AIRS Project has already set up entomology facilities and activities which are convenient for field surveillance and insectary facilities; however, since the team is growing, a long term plan would be to have permanent facilities where the insectary is isolated from smoke sources/a main road.

## 7. REFERENCES


1. Gillies MT and Coetzee C. 1987. A Supplement to the Anopheline of Africa South of the Sahara. South African Institute for Medical Research. Johannesburg, SA
2. https://malariaworld.org/blog/ivcc-and-syngenta-reach-key-milestone-development-new-insecticide-malaria-mosquito-control (accessed January 06th, 2017).
3. Illustrated Key to *Anopheles* mosquitoes of Liberia. 1966. Stojanovich CJ and Scott HG. U.S department of Health, Education, and Welfare, Public Health Science. Communicable Disease Center, Atlanta, Georgia 30333
4. Common African Mosquitoes and their Medical Importance, J. D Gillett, with 48 Colour Illustrations by Judith G. Smith, William Heinemann Medical Books LTD: London.
5. WHO: Test procedures for insecticide resistance monitoring in malaria vector mosquitoes, World Health Organization 2013. http://www.who.int/malaria