The PMI Africa IRS (AIRS) Project

Indoor Residual Spraying (IRS 2) Task Order Six

AIRS LIBERIA: ENTOMOLOGICAL MONITORING FINAL REPORT

NOVEMBER 1, 2014 – OCTOBER 31, 2015

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ACRONYMS

AIRS	Africa Indoor Residual Spraying
BLAST	Basic Local Alignment Search Tool
CDC	Centers for Disease Control and Prevention
CHV	Community Health Volunteers
EVD	Ebola Virus Disease
F&A	Finance and Administration
HLC	Human Landing Catch
IR	Insecticide Resistance
IRS	Indoor Residual Spraying
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

During the reporting period, monthly (February, 2015 to September 2015) pyrethrum spray catches and CDC light trap collections were carried out as part of the malaria vector monitoring activities in Tomato Camp (Bong County) and in Frank Town (Montesserado County) in Liberia. HLC techniques were used every two months in two locations per village.

For the PSC technique, ten houses were selected randomly per village; eight CDC light traps were used per village (four indoor and four outdoor). Both sentinel sites were sprayed from 2009 to 2012.

The aim of these activities was to assess entomological indicators such as vectors species composition, seasonality, and behavior in the two sites. Insecticide resistance bioassays were implemented in different counties of Liberia in order to map *Anopheles gambiae* s.l. susceptibility to five insecticides recommended by the World Health Organization (WHO). Mosquito samples were preserved for molecular analysis which will allow determining species composition, as well as genes and enzymes involved in insecticide resistance.

Results

The biting rates from March to September 2015 were higher in Frank Town than in Tomato Camp. The peak of biting activity was observed after 12:00AM for indoor and outdoor collections.

FIGURE 1: HOURLY BITING RATES IN ROUTINE SITES, 2015

Figure 1a : Hourly biting rates from March to September 2015, Frank Town.

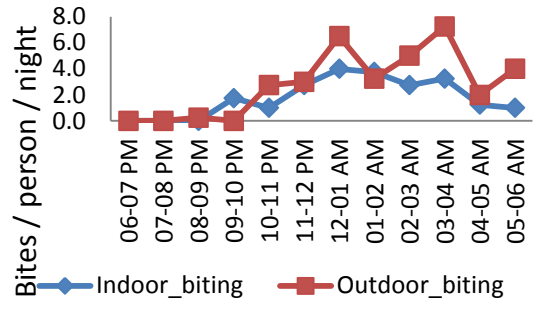
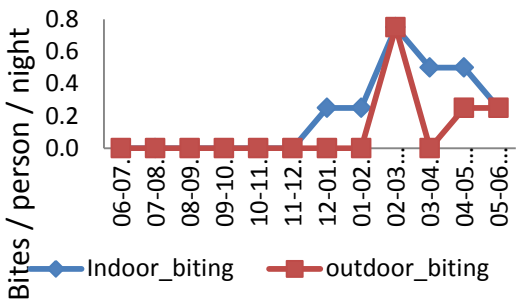


Figure 1b : Hourly biting rates from March to September 2015, Tomato Camp.



Data collected in Tomato Camp using HLC showed that there is no statistical significant difference (p = 0.197) on the proportion of endophagy (0.667) and exophagy (0.333). In Frank Town, the proportions of endophagy and exophagy were 0.387 and 0.612, respectively. The difference was statistically significant (p = 0.0008).

Insecticide Resistance

Data showed that *An. gambiae* s.l. females used during the tests were susceptible to fenitrothion, pirimiphos-methyl and bendiocarb, but were resistant to deltamethrin, alphacypermethrin and DDT (Table 1).

TABLE 1: SUMMARY OF INSECTICIDE SUSCEPTIBILITY TESTS CONDUCTED ON *AN. GAMBIAE* S.L. IN LIBERIA, OCTOBER 2014 – NOVEMBER 2015

Site	Alpha-cypermethrin	Deltamethrin	DDT	Bendiocarb	Pirimiphos-methyl	Fenitrothion
Bong	22% (100)	67% (100)	2% (100)	97% (100)	100% (100)	--
Nimba	9% (100)	30% (100)	6% (100)	94% (100)	100% (100)	--
Grand Gedeh	44.8% (125)	62.5% (100)	26% (100)	100% (100)	--	--
Margibi	5% (100)	22% (100)	49% (100)	97% (100)	100% (100)	--
Grand Bassa	27% (100)	68% (100)	4% (100)	94% (100)	100% (100)	--
Maryland*	--	65% (100)	--	--	--	100% (100)
Maryland**	--	--	29% (100)	98% (100)	--	--
Grand Bassa	--	--	--	99% (100)	--	--

* New Kru; ** Old Kru : tests done in 2014 | Bong, Nimba, Grand Gedeh : tests done in 2015.

Molecular results

Among mosquitoes collected from March to July 2015 and identified morphologically as *An. gambiae* s.l., 150 samples were sent to Notre Dame University, Indiana, USA for sequencing. Using BLAST, nucleotide sequences of *An. gambiae* s.l. samples were compared to sequences from NCBI databases. Based on similarity searches, a total of 145 mosquitoes from Tomato Camp (N=64) and Frank Town (N=81) were identified as *An. gambiae s.s.* Among Frank Town samples, five specimens were identified as *An. coluzzii*.

Conclusion

According to the data, *An. gambiae* s.l. is the major vector of malaria in both sites prospected routinely and for insecticide susceptibility. The biting rate was higher in Frank Town than in Tomato Camp. The same pattern was observed for vector abundance. Insecticide susceptibility tests showed that in all the study sites where the

tests were conducted, *An. gambiae* s.l. was susceptible to pirimiphos-methyl and fenitrothion. Full susceptibility and possible resistance have been observed for bendiocarb. The vector was resistant to alphacypermethrin, deltamethrin and DDT.

1. INTRODUCTION

In Liberia, malaria is endemic across the country; its prevalence is high in the central part of the country, including Bong and Marguibi counties (Liberia Malaria Indicator Survey, 2011). In 2012, Indoor Residual Spraying (IRS) operations were implemented in these areas and in Careysburg District, Montserrado County. In 2015, entomological monitoring and evaluation was conducted in two sentinel areas where IRS was conducted in 2012: Tomato Camp in Bong County and Frank Town in Montserrado County. A former control site, without IRS operations, named SKT (Sergent Kolli Town) located in Bong county area was not covered by entomological surveillance activities in 2015.

The main objective of the entomological monitoring activity was to understand the vector dynamics at the sentinel sites.

This study required sampling of *Anopheles* mosquitoes using human landing catches, pyrethrum spray catches and CDC light trap collections, as well as insecticide resistance monitoring. Unfortunately, entomological monitoring activities were interrupted by the Ebola outbreak which affected the country from 2014 to the first half of 2015. The in-country project entomologist left the country and monitoring activities were adjourned. However, the NMCP vector control team was able to restart collections in March 2015. A new in-country entomologist recruited by AIRS home office, arrived in Liberia in May 2015. Due to these interruptions, there remain gaps of data in entomological data collections.

Despite the Ebola outbreak, data was collected in two sentinel sites to determine changes of entomological indicators. Entomological surveys were conducted to assess the mosquito indoor resting densities and biting behavior. The surveillance involved the collection of adult mosquitoes using three sampling methods, (pyrethrum spray catches, human landing collections and CDC light traps). Data collected from February to September 2015 are analyzed in this report. Insecticide resistance bioassays were conducted to monitor changes on susceptibility of the malaria vectors against insecticides being used for malaria vector control.

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

During this surveillance, two villages Tomato Camp (278m; 29N 0471806; UTM 0778849, Kpaai District) and Frank Town (41m; 29N 0326593; UTM 0709423, Careysburg District) were selected as study sites. Both are rural areas with farming as the main activity. Potential mosquito breeding sites are present in these villages mainly during the rainy season. Altitudinal range of the study sites is 41-239 M above sea level, and sites are mainly characterized with scanty canopy and extensive clearing of the forests for human settlement. The breeding sites exploited by the main malaria vector *Anopheles gambiae* s.l. are rain dependent, shallow, transient water pools that disappear in the dry season (December to March). Most of house walls are made with mud and the roofs with corrugated iron sheets. These types of houses are suitable for mosquitoes resting.

3.2. ADULT MOSQUITO COLLECTIONS

Three mosquito collection methods were used to collect the adult mosquitoes in the sentinel sites. Pyrethrum spray collection (PSC) was used to collect indoor resting mosquitoes between 6:30 AM and 8:00 AM at each study site; ten houses were sampled at each study village one night per month. 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.

The human landing collection (HLC) was used to collect mosquitoes landing on human baits between 6:00PM and 6:00AM, both indoor and outdoor. With legs exposed to attract host seeking mosquitoes, two human baits sat indoor and other pair sat outdoor. The pairs then interchanged between outdoor and indoor on hourly basis. The collectors used flash lights and tubing aspirator to collect mosquitoes once they landed on their legs before they could bite. Two houses were used for HLC in each village one night per month.

CDC light traps were also used to capture mosquitoes starting 6:00 PM to 6:00 AM the next day, both indoor and outdoor in each study village one night per month. 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 bait's leg position while asleep and hanged 0.2 - 0.5m above the ground depending whether the bait slept on the bed or the floor. In April 2015, NMCP had completed mass distribution of bed nets in the country and most houses had people using bed nets already. The outdoor CDC light traps were set outside the same houses with indoor traps. The outdoor traps were unbaited.

3.3. INSECTICIDE SUSCEPTIBILITY TESTS

From October to November 2014, insecticide susceptibility tests were conducted solely by the NMCP staff in Maryland and Grand Bassa counties (Figure 2). In 2015, Bioassays were done in Zwedru, Grand Gedeh (June), Sanniquellie, Nimba and Jorquelle, Bong (August).

The PMI AIRS Entomologist in Liberia and two technicians from NMCP have conducted field trips to conduct insecticide resistance tests using adults *An. gambiae* s.l. mosquitoes emerged from larvae collected in the areas. 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. The outcome data will help to plan insecticide use for malaria vector control.

FIGURE 2: AIRS LIBERIA SENTINEL SITES AND INSECTICIDE RESISTANCE MONITORING SITES, 2015

Anopheles gambiae larvae were collected in rice fields, break pits and water pools along the main roads by the team and community health workers (CHVs) from 10AM to 02PM. *Anopheles* larvae were reared in a field insectary. Bioassay tests were done using 3-5 days old adult females emerged from collected pupae. Tests were done using WHO procedures. Observed knock-down were recorded during 10', 15', 20', 30', 40', 50', 60', 80' and mortality after 24h.

After the one hour exposure period, mosquitoes were transferred in holding tubes for 24h observation. Control tubes were used to validate the tests. If control mortality is between 5-20%, mortality data need to be corrected with Abbott's formula. With more than 20% mortality among control mosquitoes, the tests were discarded. In each table, we mentioned the number of females mosquitoes exposed to insecticide. 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. MOLECULAR IDENTIFICATION

150 *An. gambiae* s.l. samples were sent to Notre Dame University, Indiana, USA for processing. DNA were extracted from head-thorax portions of mosquitoes, then sequenced for non-coding sequences regions of *An. gambiae* s.l. Generated sequences were submitted to NCBI website (http://blast.ncbi.nlm.nih.gov/Blast.cgi) for analysis using sequence comparison tools called the Basic Local Alignment Search Tool (BLAST). It is an online resource developed by NCBI, which can find regions of similarity between a query sequence and sequences stored in databases, and then calculate the statistical significance of matches. It can help to find genes functions or relationship between sequences in terms of evolution. This approach was used to identify *An. gambiae* s.l. samples previously identified morphologically under dissecting microscope.

3.5. TRAINING OF GENERAL COMMUNITY HEALTH VOLUNTEERS (GCHVS)

The gCHVs were selected by the District Health Officers in respective districts. They were practically trained on how to conduct PSC, HLC 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 technician. For insecticide resistance also, CHVs went through a hands-on field training to identify and determine the differences between *Anopheles* and *Culex* for adult and larval stages. They were able to see mosquitoes under 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 on how to rear larvae in the field. They were also allowed to see the different steps of insecticide resistance tests without being involved in the experiments which were conducted by Entomology staff.

TABLE 2: STAFFING FOR ENTOMOLOGICAL SURVEILLANCE ACTIVITIES

Activity	Entomology Technicians per Site	Local Mosquito Collectors (CHVs) 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

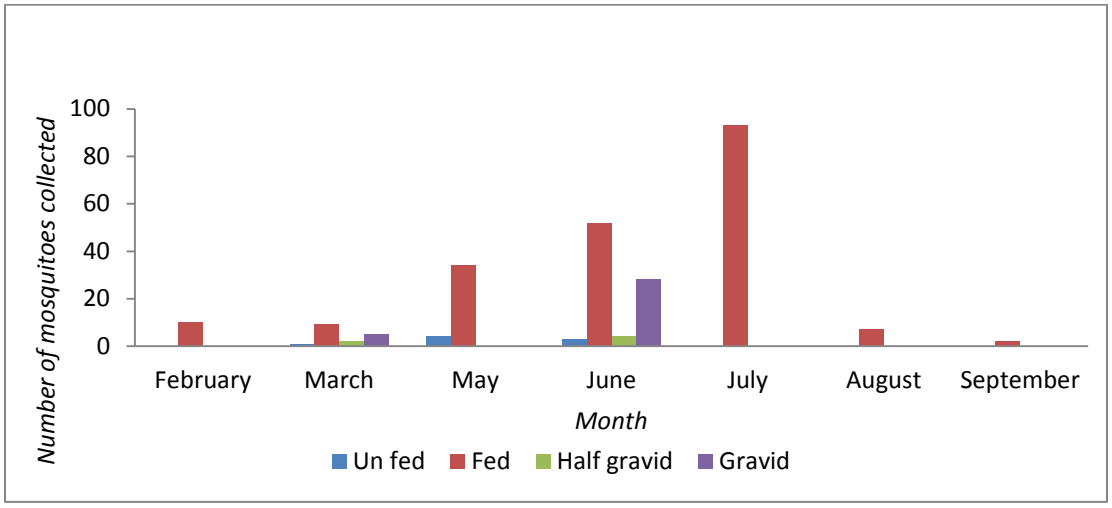
From February to September 2015, 254 female *An. gambiae* s.l. were collected in Frank Town and 170 samples in Tomato Camp (Table 3) using the pyrethrum spray catches. A statistically significant difference ($p < 0.0001$) was observed between the vector abundance determined using PSC in these two sentinel sites. The peak of vector density was observed in June in Tomato Camp and in July for Frank Town. In both sites four samples of *An. rufipes* were collected, and *Culex* and *Aedes* mosquitoes were found in the areas. Vector density was estimated by dividing the number of mosquitoes collected by the number of houses prospected (10 houses per site). The decrease of vector abundance in August was due to heavy rainfall recorded during that month. In fact, when the rainfall is higher, *An. gambiae* s.l. breeding sites are washed out and larval density decreases. 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 Frank Town than in Tomato Camp.

TABLE 3: NUMBER OF *AN. GAMBIAE* S.L. MOSQUITOES COLLECTED BY PSC AND INDOOR RESTING DENSITIES IN STUDY SITES, 2015

Month	Frank Town		Tomato Camp	
	Number Collected	Vector Density	Number Collected	Vector Density
February	10	1	--	--
March	17	1.7	25	2.5
May	38	3.8	25	2.5
June	87	8.7	78	7.8
July	93	9.3	19	1.9
August	7	0.7	17	1.7
September	2	0.6	6	0.2
Total	**254**	**-**	**170**	**-**

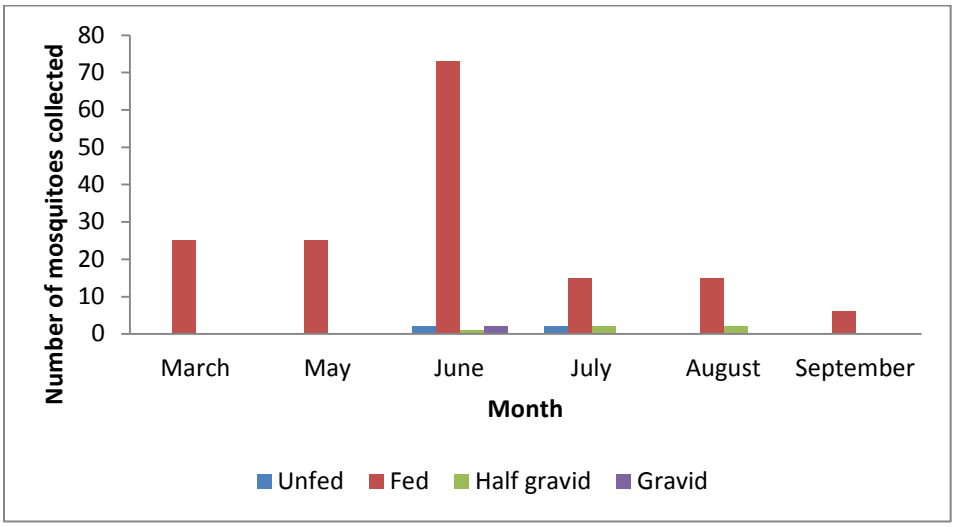
(--) No collection done in February.

FIGURE 3: *AN. GAMBIAE* S.L. MOSQUITOES COLLECTED USING PSC IN FRANK TOWN, 2015



In Frank Town, the percentages according to the blood digestion stages (Unfed, Fed, Half gravid, Gravid) are respectively 3.1%, 81.5%, 2.4% and 13.0% from February to September, 2015. Most of mosquitoes collected by the PSC were fed (Figure 3). Fed mosquitoes will potentially be able to transmit the malaria parasite if their blood meal was infected. The high percentages of fed mosquitoes showed that there is frequent contact between host and vector.

FIGURE 4: NUMBER OF *AN. GAMBIAE* S.L. MOSQUITOES COLLECTED USING PSC IN TOMATO CAMP, 2015



The percentages of the different abdominal stages of *An. gambiae* s.l. mosquitoes collected in Tomato Camp are: Unfed (2.4%), Fed (93.3%), Half gravid (3.0%) and Gravid (1.2%). Fed females are highly abundant mainly in June (Figure 4).

4.2. HUMAN LANDING COLLECTIONS

From February to September, 2015, 221 females *Anopheles gambiae* s.l. were collected from Frank Town using human landing catches with two persons indoor and outdoor. Among these, 86 *An. gambiae* s.l. (38.5%) were collected indoor (Table 4) and the highest number was observed in July 2015. Since two persons collected mosquitoes, the number of mosquitoes collected was divided by the number of persons to determine the human biting rate. With regard to outdoor collections, the highest biting rate was also observed in July 2015 (Table 4 and Figure 5). In February and March, human biting rate was very low, because of the dry weather condition where most the breeding sites have no water to allow mosquito larvae to bread and reach adult stage.

TABLE 4: NUMBER OF *AN. GAMBIAE* S.L. CAPTURED AND BITES/PERSON/NIGHT FROM HLC IN FRANK TOWN, 2015

Month	Indoor	Indoor bites/person/night	Outdoor	Outdoor bites/person/night	Total
February	3	1.5	0	0	3
March	0	0	0	0	0
May	16	8	27	13.5	43
July	66	33	109	54.5	175
September	1	0.5	0	0	1
Total	**86**	-	**136**	-	**221**

In February 2015, mosquitoes were only found indoor; because of the cold weather mosquito's activity might be limited outdoor (Table 4). The bites /person/night was calculated by dividing the total number *Anopheles gambiae* s.l. collected each month by the number of persons involved in the HLC. A statistically significant difference was found between the endophagic (0.387) and exophagic (0.612) rates (p=0.0008).

FIGURE 5: NUMBER OF *AN. GAMBIAE* S.L. MOSQUITOES COLLECTED BY HLC IN FRANK TOWN, 2015

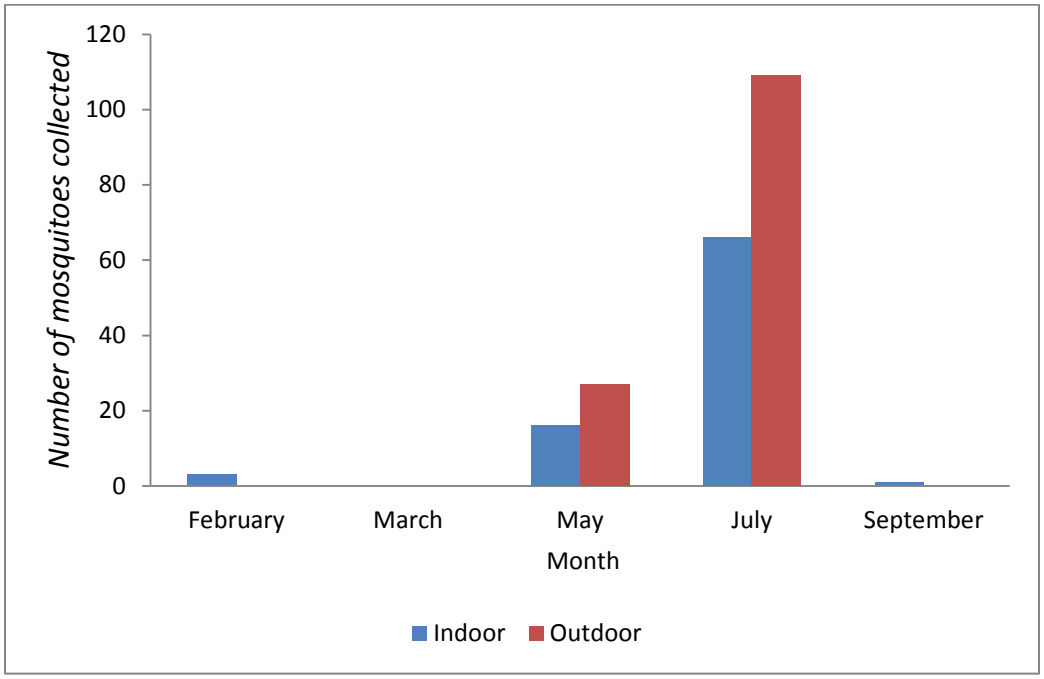
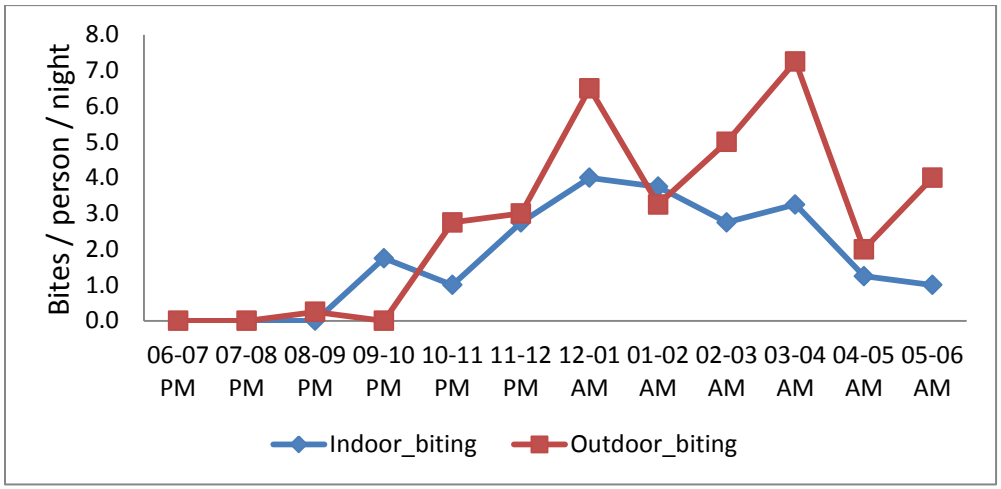


Figure 5 shows that the abundance of *An. gambiae* s.l. collected in Frank Town was high in July 2015. February to March corresponds to the dry season where most of breeding sites disappeared and the vector densities decreased.

FIGURE 6: HOURLY BITING PER NIGHT IN FRANK TOWN, 2015



The data showed that biting activity is high from 10:00PM to 05:00AM (Figure 6). *Anopheles gambiae* s.l. females are very active in terms of host seeking for blood meals during this period.

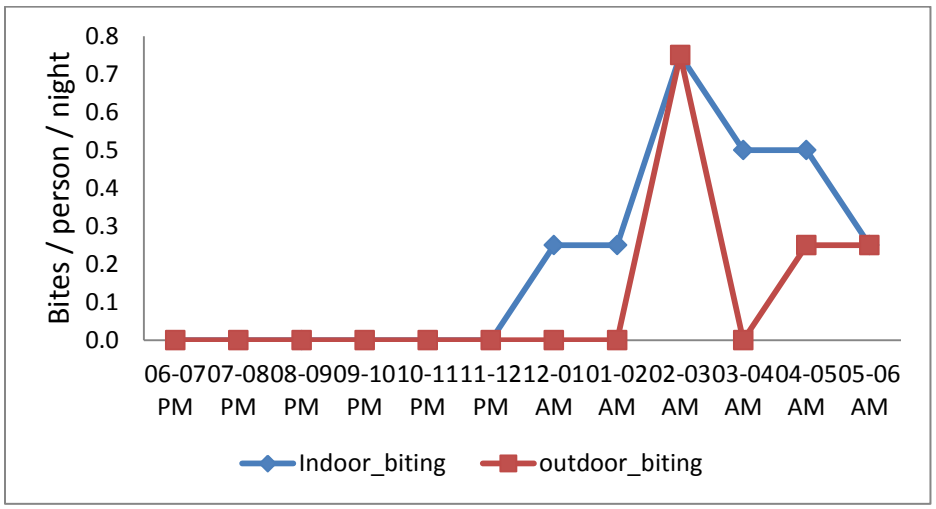
TABLE 5: NUMBER OF *AN. GAMBIAE* S.L. CAPTURED AND BITES/PERSON/NIGHT FROM HLC IN TOMATO CAMP, 2015

Month	Indoor	Indoor bites/person/night	Outdoor	Outdoor bites/person/night	Total
March	0	0	0	0	0
May	0	0	0	0	0
July	6	3	2	1	8
September	4	2	3	1.5	7
Total	**10**	-	**5**	-	**15**

In Tomato Camp, the mosquito biting rate was very low during all collection periods. From March to September, only ten *An. gambiae* s.l. mosquitoes were collected indoor and five outdoor using the human landing catches (Table 5). In contrast to Frank Town, the vector biting rate was generally low in Tomato Camp. During the rainy season, in HLC nights, there was rain in both sites.

Data collected in Tomato Camp using HLC showed that, there is no statistically significant difference (p = 0.197) on the proportion of endophagy (0.667) and exophagy (0.333).

FIGURE 7: HOURLY BITING PER NIGHT IN TOMATO CAMP, 2015



Biting activity in Tomato Camp started around 12PM and continued to 06:00AM (Figure 7). Most of *An. gambiae* s.l. mosquitoes were collected indoor. A peak of biting was observed from 02:00-03:00AM, however the abundance was low.

4.3. CDC LIGHT TRAP COLLECTIONS

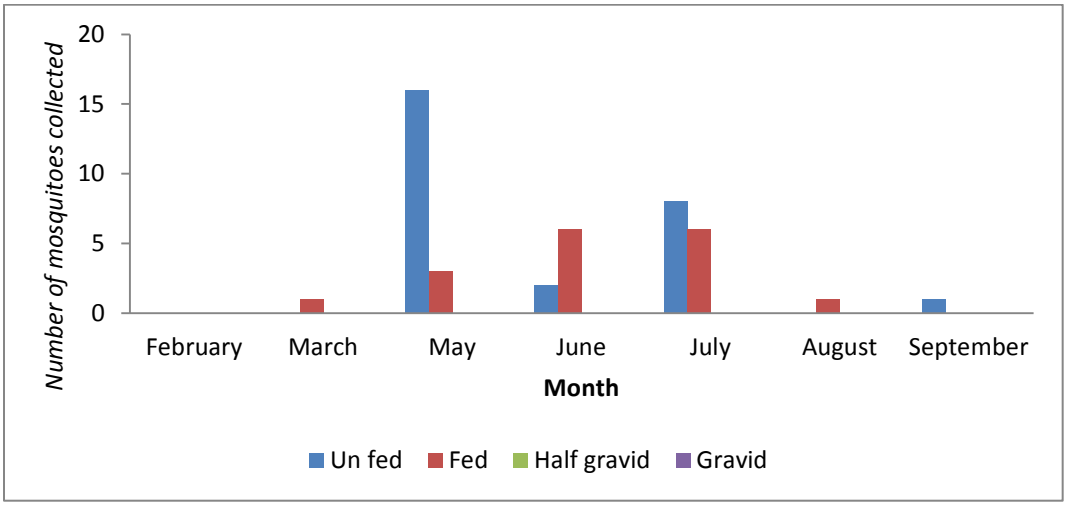
At each sentinel site, eight light traps were used: four traps installed indoor and four traps outdoor, from 06:00PM to 06:00AM. In Frank Town, 44 females *An. gambiae* s.l. were collected during the period February to September 2015 (Table 6). Since collections were done in 4 sites, the number of collected mosquitoes per month was divided by four in order to estimate the density per site. In addition to the *An. gambiae* s.l. collected, one *An. funestus* and two *An. rufipes* and *Culex* species as well were captured in Frank Tow in August. For the overall period of collection, more *An. gambiae* s.l. were sampled indoor than in outdoor.

TABLE 6: NUMBER OF *AN. GAMBIAE* S.L. MOSQUITOES COLLECTED BY CDC LIGHT TRAP IN FRANK TOWN, 2015

Month	Indoor	Density Indoor	Outdoor	Density outdoor	Total
February	0	0	0	0	0
March	1	0.25	0	0	1
May	15	3.75	4	1	19
June	8	2	0	0	8
July	5	1.25	9	2.25	14
August	1	0.25	0	0	1
September	1	0.25	0	0	1
Total	**31**	-	**13**	-	**44**

The difference was statistically significant ($p = 0.010$) between the proportion caught indoor (0.705) and outdoor (0.296).

FIGURE 8: *AN. GAMBIAE* S.L. MOSQUITOES COLLECTED USING CDC LIGHT TRAPS IN FRANK TOWN, 2015



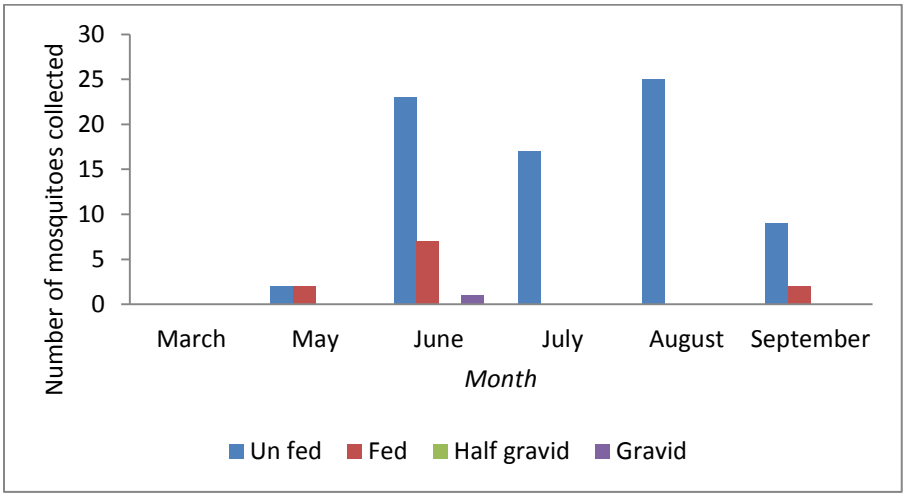
Among *An. gambiae* s.l. mosquitoes collected with CDC light trap in Frank Town, most of them were unfed (Figure 8). The peak was observed in May, not during the rainy season period.

TABLE 7: NUMBER OF *AN. GAMBIAE* S.L. MOSQUITOES COLLECTED BY CDC LIGHT TRAP IN TOMATO CAMP, 2015

CDC	Indoor	Density Indoor	Outdoor	Density outdoor	Total
March	0	0	0	0	0
May	0	0	4	1	4
June	28	7	3	0.8	31
July	9	2.3	8	2	17
August	0	0	25	6.25	25
September	9	2.25	2	0.5	11
Total	**46**	**-**	**42**	**-**	**88**

In total, 88 *An. gambiae* s.l. mosquitoes were collected in Tomato Camp using CDC light traps (Table 7). The number of mosquitoes captured (indoor and outdoor) are almost similar: 46 and 42 females for indoor and outdoor, respectively. In August, more mosquitoes were collected outdoor. Observed densities were calculated using the same method described earlier: number of mosquitoes / number of collection sites. Data collected using CDC light trap showed that, there is no statistically significant difference ($p = 0.670$) on the proportion collected indoor (0.523) and outdoor (0.477) in Tomato Camp, Bong County. Similar tendency was observed on this site using data collected from the human landing catch (HLC).

FIGURE 9: *AN. GAMBIAE* S.L. MOSQUITOES COLLECTED USING CDC LIGHT TRAPS IN TOMATO CAMP, 2015



The percentages of unfed, fed and gravid were 87.0%, 12.50% and 1.14%, respectively. In Tomato Camp, the number of unfed *An. gambiae* s.l. mosquitoes was higher from June to September 2015 (Figure 9).

4.4. MOLECULAR IDENTIFICATION RESULTS

Among mosquitoes collected from March to July 2015 (using CDC, PSC, HLC) and identified morphologically as *An. gambiae* s.l., 150 samples were sent to Notre Dame University, Indiana, USA for sequencing. Using BLAST, nucleotide sequences of *An. gambiae* s.l. samples were compared to sequences from NCBI databases. Based on similarity search, 145 mosquitoes from Tomato Camp (N=64) and Frank Town (N=81) were identified as *An. gambiae* s.s. Among Frank Town samples, five specimens were identified as *An. coluzzii*.

4.5. INSECTICIDE SUSCEPTIBILITY TESTS

TABLE 8: WHO INSECTICIDE SUSCEPTIBILITY TEST RESULTS BY INSECTICIDE EXPOSED TO *AN. GAMBIAE* S.L. IN ZWEDRU, GRAND GEDEH COUNTY, LIBERIA, JUNE 2015

Insecticide Classes	Insecticide	Number of Mosquitos' exposed	Mortality% (24 hrs)	Status
Pyrethroid	Alpha-cypermethrin 0.05	125	44.8	R
Organochlorine	DDT 4%	100	26	R
Pyrethroid	Deltamethrin 0.05 %	100	62.5	R
Carbamate	Bendiocarb 0.1 %	100	100	S

In Zewedru (Grand Gedeh County), the results indicated that *An. gambiae* s.l. mosquitoes were susceptible (**S**) to bendiocarb and resistant (**R**) to DDT, deltamethrin and alphacypermethrin insecticides (Table 8).

TABLE 9: WHO INSECTICIDE SUSCEPTIBILITY TEST RESULTS BY INSECTICIDE EXPOSED TO *AN. GAMBIAE* S.L. IN SANNIQUELLIE, NIMBA COUNTY, LIBERIA, AUGUST 2015

Class	Insecticide	Number of mosquitoes exposed	Mortality%	Status
Pyrethroid	Alpha-cypermethrin 0.05	100	9	R
Organochlorine	DDT 4%	100	6	R
Pyrethroid	Deltamethrin 0.05 %	100	30	R
Carbamate	Bendiocarb 0.1 %	100	94	PR
Organophosphate	Primiphos Methyl 0.1%	100	100	S

In August 2015, *An. gambiae* s.l. mosquitoes from Nimba and Bong were also exposed to an organophosphate (primiphos-methyl) and to the other four insecticides mentioned for the tests in Zwedru and Grand Gedeh. The mortality rate after 24 hours was 100% for primiphos-methyl against mosquitoes used from Sanniquellie (Table 9). However, a probable resistance (PR) to bendiocarb was observed (94%). In this site, the resistance level was very high for DDT and alpha-cypermethrin.

TABLE 10: WHO INSECTICIDE SUSCEPTIBILITY TEST RESULTS BY INSECTICIDE EXPOSED TO *AN. GAMBIAE* S.L. IN JORGELLE DISTRICT, BONG COUNTY, LIBERIA, AUGUST 2015.

Class	Insecticide	Number of mosquitoes exposed	Mortality%	Status
Pyrethroid	Alpha-cypermethrin 0.05%	100	22	R
Organochlorine	DDT 4%	100	2	R
Pyrethroid	Deltamethrin 0.05 %	100	67	R
Carbamate	Bendiocarb 0.1 %	100	97	PR
Organophosphate	Primiphos Methyl 0.1%	100	100	S

In Jorquellie, Bong County, *An. gambiae* s.l. mosquitoes were susceptible to primiphos-methyl with 100% of mortality after 24 hours. Tested mosquitoes were less susceptible (PR) to bendiocarb (97%) and resistant to DDT (2%), alpha-cypermethrin (22%), and deltamethrin (67%) (Table 10).

TABLE 11: WHO INSECTICIDE SUSCEPTIBILITY TEST RESULTS BY INSECTICIDE EXPOSED TO *AN. GAMBIAE* S.L. IN KAKATA, MARGIBI COUNTY, LIBERIA, OCTOBER 2015

Class	Insecticide	Number of mosquitoes exposed	Mortality%	Status
Pyrethroid	Alpha-cypermethrin 0.05%	100	5	R
Organochlorine	DDT 4%	100	49	R
Pyrethroid	Deltamethrin 0.05 %	100	22	R
Carbamate	Bendiocarb 0.1 %	100	97	PR
Organophosphate	Primiphos Methyl 0.1%	100	100	S

In October 2015, larvae collections were done in Margibi County for insecticide susceptibility tests. Tested *An. gambiae* s.l. mosquitoes were susceptible to primiphos-methyl with 100% mortality after 24 hours. They were resistant to the other insecticides (Table 11).

TABLE 12 : WHO INSECTICIDE SUSCEPTIBILITY TEST RESULTS BY INSECTICIDE EXPOSED TO *AN. GAMBIAE* S.L. IN BUCHANAN, GRAND BASSA COUNTY, LIBERIA, NOVEMBER 2015

Class	Insecticide	Number of mosquitoes exposed	Mortality%	Status
Pyrethroid	Alpha-cypermethrin 0.05%	100	27	R
Organochlorine	DDT 4%	100	4	R
Pyrethroid	Deltamethrin 0.05 %	100	68	R
Carbamate	Bendiocarb 0.1 %	100	94	R
Organophosphate	Primiphos Methyl 0.1%	100	100	S

Tested mosquitoes in Grand Bassa County were susceptible to primiphos-methyl (100%), but they were resistant to the other insecticides tested, including DDT (4%), (Table 12).

TABLE 13: WHO INSECTICIDE SUSCEPTIBILITY TEST RESULTS BY INSECTICIDE EXPOSED TO *AN. GAMBIAE* S.L. IN MARYLAND AND GRAND BASSA COUNTIES, LIBERIA, OCTOBER TO NOVEMBER 2014

Location	Class	Insecticide	Number of mosquitoes exposed	Mortality% (24h)	Status
Maryland New Kru Town	Pyrethroid	Deltamethrin 0.05 %	100	65	R
	Organophosphate	Fenithrothion 1%	100	100	S
Maryland Old Kru Town	Carbamate	Bendiocarb 0.1 %	100	98	S
	Organochlorine	DDT 4%	100	29	R
Grand Bassa Bokay	Carbamate	Bendiocarb 0.1 %	100	99	S

In 2014, the NMCP team conducted insecticide resistance tests in Grand Bassa and Maryland counties (Table 11). *An. gambaie* s.l. mosquitoes used for the tests were susceptible to fenithrothion and bendiocarb, but resistant to DDT and deltamethrin.

4.6. CONCLUSION

- *An. gambiae* s.l. is fully susceptible to primiphos-methyl in study sites;
- *An. gambiae* s.l. has developed probable resistance to bendiocarb;
- *An. gambiae* s.l. is resistant to deltamethrin and alpha-cypermethrin in all the sites;
- *An. gambiae* s.l. is highly resistant to DDT throughout the tested sites.

4.7. RECOMMENDATION

- The insecticide resistance tests should continue until all fifteen counties are covered in order to have country wide data for mapping insecticide resistance in the country;
- Instead of doing insecticide resistance during the rainy season, it is better to extend it to the dry season if potential larval breeding are available in areas.

5. OBSERVATIONS AND CONCLUSIONS

Despite the interruption of activities due to the Ebola outbreak, the main objectives of entomological monitoring were accomplished. NMCP staff members have been trained to conduct all field activities and some of them have benefited from training outside the country. They were able to learn different aspects of malaria vector monitoring such as:

- Morphological identification of main malaria vectors in Liberia;
- World Health Organization (WHO) tube bioassays;
- Larval sampling and rearing in the field;
- Female mosquito ovary dissection; and
- Sample preservation.

Indoor and outdoor collections have shown that after IRS withdrawal, vector densities in sentinel sites are not high. This might be due to intense rainfall during collection periods and breeding sites being flushed. In general, *An. gambiae* s.l. is the major malaria vector in the areas studied.

According to bioassay data, *An. gambiae* s.l. mosquitoes are susceptible to organophosphate and carbamate insecticides; however, they are resistant to organochlorines and pyrethroids. Additional assays are needed in order to map insecticide resistance across the country.

6. RECOMMENDATIONS

- Additional resources are required to supply the insectary with a permanent source of electricity.
- The expansion of sentinel sites may help to understand better malaria transmission in Liberia and to generate data for improving vector control based on ecological conditions.
- For capacity building, NMCP and Liberian Ministry of Health may need to identify young personnel for training in entomology. The PMI AIRS Project has already set up entomology facilities and activities which are convenient for field surveillance and insectary facilities where fellow trainees could improve their skills and then take the leadership in the future.

7. REFERENCES

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