PMI VECTORLINK LIBERIA PROJECT

PMI VECTORLINK LIBERIA ENTOMOLOGICAL MONITORING FINAL REPORT

OCTOBER 1, 2019–SEPTEMBER 30, 2020

Recommended Citation: *The PMI VectorLink Project. Annual Report: October 1, 2019 - September 30, 2020.* Rockville, Maryland. The PMI VectorLink Project, Abt Associates Inc.

Contract: AID-OAA-I-17-00008

Task Order: AID-OAA-TO-17-00027

Submitted to: United States Agency for International Development/PMI

Submitted: February 2, 2021

Approved: June 14, 2021

Abt Associates 1 6130 Executive Blvd1
1 Rockville, MD 20852 1 T. 301.347.5000 1 F. 301.913.9061
1 www.abtassociates.com

CONTENTS

LIST OF TABLES

LIST OF FIGURES

ACRONYMS

CDC	Centers for Disease Control and Prevention
CHW	Community health worker
ELISA	Enzyme-linked immunosorbent assay
HLC	Human landing catch
IRS	Indoor Residual Spray
ITNs	Insecticide-Treated Nets
LIBR	Liberia Institute for Biomedical Research
LISGIS	Liberia Institute of Statistics and Geo-Information Services
LT	Light trap
MOH	Ministry of Health
NMCP	National Malaria Control Program
PBO	Piperonyl Butoxide
PCR	Polymerase chain reaction
PMI	President's Malaria Initiative
PSC	Pyrethrum spray catch
SBC	Social and behavior change
SOP	Standard Operation Procedure
UL	University of Liberia

EXECUTIVE SUMMARY

Malaria vector monitoring and insecticide resistance testing were the main activities performed by VectorLink Liberia in collaboration with National Malaria Control Program (NMCP) from October 2019 to September 2020. Monthly longitudinal entomological monitoring (vector bionomics) data were collected from four sites between October 2019 and March 2020: Fissebu (Lofa County), Koryah (Bong County), Madina (Grand Cape Mount County) and Saint John (Grand Bassa County). From January to March (2020), the team performed vector bionomics monitoring collections at four new sites: Gbedin (Nimba County), Jackson Farm (Margibi County), Koryah (Bong County) and Suehn Town (Bomi County).

The number of sites was increased to expand the entomological indicator coverage in relation to the planned distribution and performance of new generation Interceptor® G2 nets. This net contains a pyrethroid (alpha-cypermethrin) and chlorfenapyr which is an insecticide that disrupts the insect's ability to produce energy and results in mortality. The Interceptor® G2 net is planned for mass distribution in Liberia around June, 2021. Vector bionomics monitoring data included vector species composition, distribution, behavior, and sporozoite rate

In the field, three different methods of collection were used: pyrethrum spray catch (PSC), human landing catch (HLC), and Centers for Disease Control and Prevention light traps (CDC-LTs). Activities were suspended at all eight sites from April to July 2020 due to travel restrictions related to the COVID-19 pandemic. Collections resumed in August 2020 and data were collected for August and September.

Insecticide resistance tests were conducted in seven sentinel sites across the country. The susceptibility status of *Anopheles gambiae* s.l. to pyrethroids was assessed using the CDC bottle bioassay method. Synergist tests were conducted with pyrethroids after pre-exposure to piperonyl butoxide (PBO) and pyrethroids only without PBO to assess the role of a synergist in enhancing the vector population susceptibility to pyrethroids.

In the eight sentinel sites, using the three methods of collection (PSC, HLC, and CDC LTs), 5,157 *An. gambiae* s.l. and 742 *Anopheles funestus* s.l. were collected in eight months (87.4% and 12.6%, respectively). The predominant vector was *An. gambiae* s.l. in six out of eight sites: Koryah (60.2%), Madina (100%), Saint John (99.1%), Jackson Farm (98.7%), and Gbedin (96.8%). *Anopheles funestus* s.l. was more prevalent in Zeanzue (70.2%) and Fissebu (66.8%). Due to the interruption of field collection due to COVID-19, it was not possible to observe the variation in seasonal trends in vector abundance in relation to the rainy and dry seasons.

Among the *Anopheles* mosquitoes collected using PSC, the highest indoor residual density of *An. gambiae* s.l. was observed in: Gbedin (13.6 mosquitoes per house per day in September 2020), Suehn Town (8.2 mosquitoes per house per day in March 2020), and Jackson Farm (7.8 mosquitoes per house per day in January 2020). For CDC-LTs collections, traps were installed only indoors in the eight sites, and the highest numbers of *An. gambiae* s.l. were recorded in Gbedin (34.5 mosquitoes per trap per night in September 2020 and 18.8 mosquitoes per trap per night in August 2020). HLCs were performed indoors and outdoors at all eight sites and the highest indoor human biting rate was 1.00 bite per person per hour in Suehn Town and 0.84 bite per person per hour in Jackson Farm. With outdoor collections, the rates were 1.11 and 1.06 bite per person per hour in Suehn Town and Jackson Farm, respectively. The vector was resistant to deltamethrin and permethrin, mortality rates

ranging between 37 and 57% across all sites. Pre-exposure of *An. gambiae* s.l. samples to the synergist PBO increased susceptibility to both deltamethrin and permethrin. For deltamethrin, susceptibility was fully restored (98% mortality) in three of the six sites. At the Jackson Farm site, for permethrin, after pre-exposure to PBO mortality increased from 37% to 96%. Chlorfenapyr was tested using *An. gambiae* s.l. mosquitoes, and after 24 hours up to 72 hours holding period, 100% mortality was recorded in all the sites from October 2019 to September 2020.

I. INTRODUCTION

Indoor residual spraying (IRS) was implemented in Liberia by the President's Malaria Initiative (PMI) in collaboration with the NMCP from 2009 to 2013. Insecticide resistance monitoring during this time indicated that *Anopheles gambiae* s.l., the major vector of malaria in Liberia was resistant to pyrethroids. Therefore, in 2013, after presenting empirical evidence on vector resistance to pyrethroids and evidence of susceptibility to pirimiphos-methyl, the NMCP and IRS Task Force switched from using pyrethroids to pirimiphos-methyl for IRS in 2013. After the 2013 campaign, IRS was stopped due to the increased costs of non-pyrethroid insecticide.

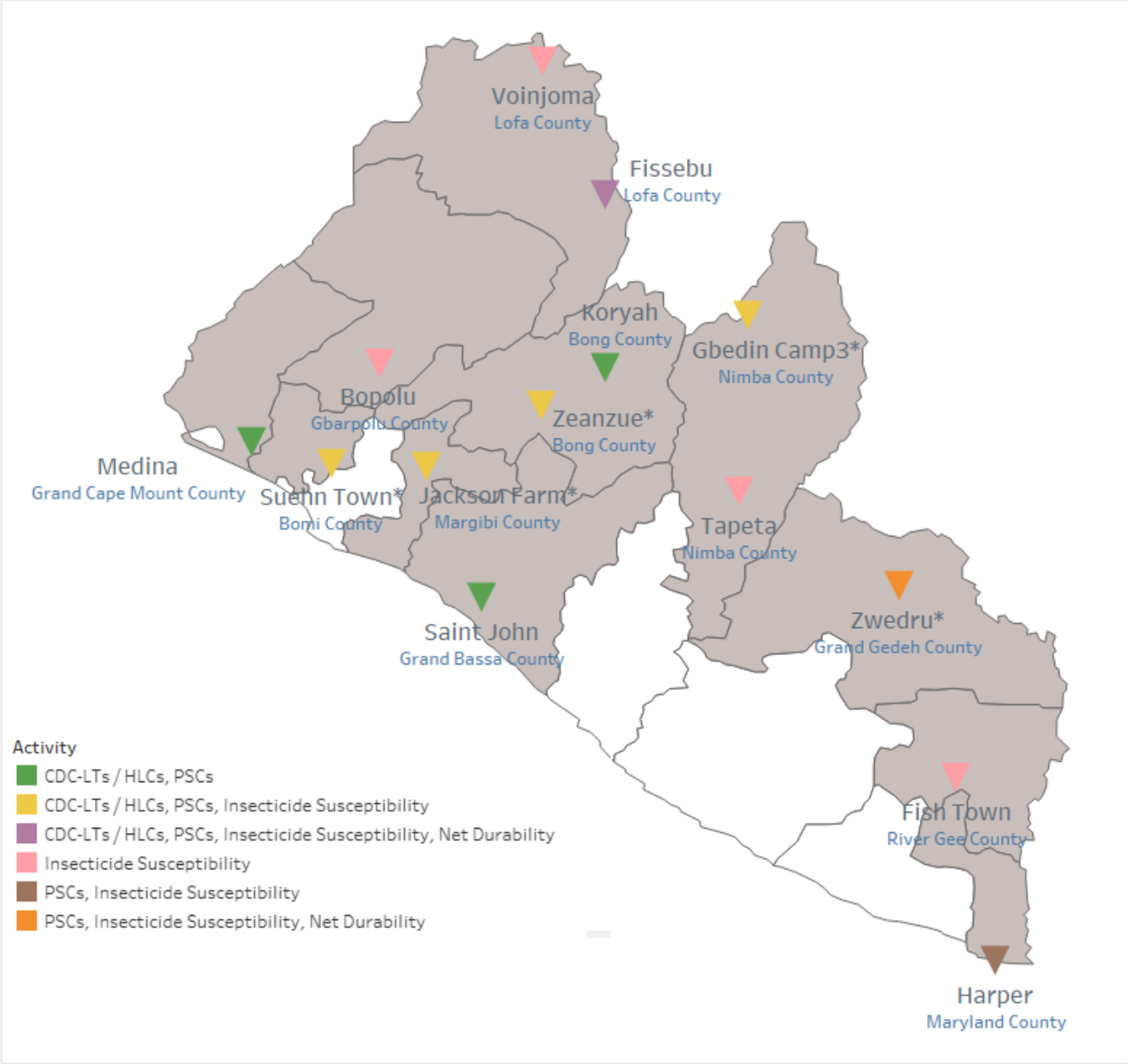
After the withdrawal of IRS in 2014, PMI and NMCP continued monthly routine entomological monitoring to generate data on vector bionomics including species composition, distribution, and behavior. In addition, a container insectary was established on the NMCP compound for rearing *An. gambiae* for insecticide susceptibility tests and to perform morphological species identification of field-collected mosquitoes.

Data collection on insecticide resistance among the malaria vectors is used to better understand the evolution of the resistance and to inform the selection of vector control interventions, mainly ITNs.

From 2016 through September 2018, monthly routine entomological monitoring was conducted in three sites: Frank Town (Montserrado County), Jeneta (Margibi County) and Tomato Camp (Bong County), to replace these three sites above, starting in October 2018, VectorLink began monthly routine entomological monitoring at four sentinel sites: Fissebu (Lofa County), Koryah (Bong County), Madina (Grand Cape Mount County) and Saint John (Grand Bassa County), (Figure 1). Starting in January 2020, four new sites were added to collect baseline vector bionomics data before the planned mass distribution of Interceptor® G2 ITNs in 2021. The four added sites were: Gbedin (Nimba County), Jackson Farm (Margibi County), Koryah (Bong County) and Suehn Town (Bomi County) for a total of eight sites. Pyrethrum Spray Catches (PSCs), Human Landing Catches (HLCs), and Centers for Disease Control light traps (CDC-LTs) were used to collect mosquitoes and allow for monitoring seasonal vector density, species composition, and behavior.

Insecticide resistance testing activities were conducted in seven sites, where VectorLink assessed *An. gambiae* s.l. susceptibility to selected insecticides, along with the synergist PBO.

FIGURE 1: VECTORLINK LIBERIA ENTOMOLOGICAL MONITORING, INSECTICIDE RESISTANCE AND DURABILITY MONITORING SITES, OCTOBER 2019 – SEPTEMBER 2020



The main objectives of VectorLink Liberia entomological monitoring activities are as follows:

- Assess *Anopheles* vector bionomics, including species composition, density, and behavior, in eight sites across seven counties.
- Conduct molecular analyses to determine the species, sporozoite infection rates, and blood-meal sources of mosquitoes collected during routine collection, as well as identify potential mutations contributing to insecticide resistance.
- Determine insecticide susceptibility of *An. gambiae* s.l., the primary local malaria vector, to pyrethroids

(with pre-exposure to PBO) and to chlorfenapyr in ten sites from nine counties.

- Determine insecticide susceptibility of *An. funestus* s.l. in sites where it is the dominant vector.
- 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 SAMPLING SITES AND COLLECTION METHODS

2.1.1 SAMPLING SITES

VectorLink, worked with the NMCP and the University of Liberia (UL), to conduct monthly vector monitoring activities in eight sites and insecticide resistance testing in seven sites (Table 1). Originally, the plan was to conduct resistance testing for chlorfenapyr and pyrethroids in ten sites from nine counties, but testing could not be conducted in three of the sites from three counties due to COVID-19. They are now included to be done in Year 4. Three methods were used to collect adult mosquitoes in the routine monitoring sites: PSC, HLC, and indoor CDC-LTs (Table 2).

TABLE 1: SUMMARY OF SITES PLANNED FOR ENTOMOLOGICAL SURVEILLANCE AND INSECTICIDE RESISTANCE MONITORING ACTIVITIES

Province/ Region	County	Site	PSC / CDC-LT / HLCs	Insecticide Susceptibility	ITN Durability Monitoring
North Central	Lofa	Fissebu	X		X
	Bong	Koryah	X		
		Zeanzue*	X	X	
	Bomi	Tubmanburg		ND	
		Suehn Town*	X	X	
	Nimba	Gbedin Camp 3*	X	X	
South Central	Grand Bassa	LAC Plantation^		X	
		Saint John	X		
	Margibi	Jackson Farm*	X	X	
	Monteserrado	15 Gate		X	
North Western	Grand Cape Mount	Medina	X		
South Eastern A	Grand Gedeh	Zwedru			X
South Eastern B	Maryland	Harper		ND	
	River Gee	Fish Town		ND	
	River Cess	Cestos City		X	

**New sites added in January 2020 to increase entomological data before mass campaign distribution of new nets (IG2s) planned for May 2021.*
^= LAC Plantation was replaced by Compound 3 and 15 Gate by Number 7, the nearest sites where larvae were available.
ND = Not done due to COVID-19 restrictions but will be conducted in Year 4.

2.1.2 PYRETHRUM SPRAY CATCHES

In each site, PSCs were performed for two consecutive days sampling a total of 25 houses. The teams sprayed the commercial pyrethroid insecticide, Kwik,[1] according to VectorLink SOP (SOP03/01) following PMI guideline for COVID-19 mitigation measures.

1 Kwik ingredients: Transfluthrin 0.05% ; Tetramethrin 0.20% ; B-Cyfluthrin 0.20% ; PBO 0.50% ; Solvent & Propellant 99.55%, ASI Al Sharhan Industries, Kuwait.

2.1.3 CDC Light Trap Collection

CDC-LTs were used from September 2018 to December 2019 as a proxy for HLC and were installed indoor and outdoor. From January 2020, CDC-LTs were set-up only indoors at selected houses and performed according to VectorLink protocols (SOP01/01).

2.1.4 Human Landing Collection

HLCs were conducted indoors and outdoors, starting in January 2020. HLCs were conducted both indoors and outdoors. In January and February 2020, HLCs were done using a mouth aspirator, but in March 2020, according to COVID-19 mitigation guidance, glass tubes were used for collections.

TABLE 2: ADULT MOSQUITO COLLECTION METHODS USED FOR LONGITUDINAL MONITORING

Collection method	Time	Frequency	Sample*	Indicator
PSC	5:00 am to 8:00 am	Monthly	25 houses per site over 2 consecutive days	Indoor resting density: # mosquitoes collected per room
CDC LT (Indoors only)	6:00 pm to 6:00 am	Monthly	Eight houses per site (four houses per night), using four baited, indoor CDC LTs	Indoor trap density: # mosquitoes collected per trap per night
HLC	6:00 pm to 6:00 am	Monthly	Two houses per site for two consecutive nights using two persons - one indoor and one outdoor - per house	Indoor and outdoor human biting rate: # bites per person per hour and per night

*All houses were randomly selected, and the same houses were visited each month, to the greatest extent possible.

A morphological identification of all mosquitoes collected through each method was done at each site under a dissection microscope by the technical team of VectorLink, NMCP, and UL. The counting of *An. gambiae* s.l. mosquitoes based on their blood-digestion stage are more accurate when samples are fresh. All *Anopheles* species were identified according to Coetzee et al., 2000. *Anopheles* samples were preserved on silica gel for further processing of the head-thorax portions in the laboratory at Liberia Institute for Biomedical Research (LIBR) using enzyme-linked immunosorbent assay (ELISA) for sporozoite rate assessment. Samples will also be processed at LIBR by polymerase chain reaction (PCR) technique for molecular identification of vector species and blood meal source determination.

2.2 Insecticide Resistance Monitoring

VectorLink, the NMCP, and Community Health Workers (CHWs) conducted insecticide resistance monitoring in seven sites (Table 3). Due to COVID-19 travel restrictions, it was not possible to cover all sites selected in the Year 3 work plan. Larvae were collected in the field and transported to the main insectary in Monrovia for rearing and testing of adult females according to SOP14/01. Rearing was done in a second room of the insectary to avoid contamination of the susceptible laboratory colony, used as control.

TABLE 3: SUMMARY OF INSECTICIDE SUSCEPTIBILITY AND SYNERGIST ASSAYS PERFORMED

Region	County	Site	Insecticide Tested for Susceptibility
North Central	Bong	Zeanzue	Deltamethrin ±PBO/ Chlorfenapyr
	Bomi	Tubmanburg	Not done due to COVID-19
		Suehn Town	Deltamethrin ±PBO
	Nimba	Gbedin Camp3	Chlorfenapyr
South Central	Grand Bassa	LAC Plantation	Deltamethrin ±PBO/ Chlorfenapyr
	Margibi	Jackson Farm	Permethrin ±PBO / Deltamethrin ±PBO
	Monteserrado	Gate 15	Deltamethrin ±PBO/ Chlorfenapyr
South Eastern B	Maryland	Harper	Not done due to COVID-19
	River Gee	Fish Town	Not done due to COVID-19
	River Cess	Cestos City	Deltamethrin ±PBO/ Chlorfenapyr

*Chlorfenapyr and deltamethrin were prioritized for testing field collected mosquitoes. Permethrin testing was conducted if there were enough mosquitoes reared.

For each test, the team preserved a subsample of dead mosquitoes as well as all the survivors, which were stored in labeled Eppendorf tubes for further molecular laboratory analysis.

2.2.1 INSECTICIDE SUSCEPTIBILITY TEST FOR PYRETHROIDS– CDC BOTTLE ASSAY METHOD

The susceptibility assays of *An. gambiae* s.l. to the pyrethroids (deltamethrin and permethrin) were performed using technical grade insecticide concentrations provided by CDC Atlanta according to SOP04/01. In Year 3, Liberia VectorLink tested 1x concentration (12.5µg per bottle) of deltamethrin and 1x concentration (21.5 µg per bottle) of permethrin.

2.2.2 INSECTICIDE SUSCEPTIBILITY TEST FOR CHLORFENAPYR – CDC BOTTLE ASSAY METHOD

Chlorfenapyr is an insecticide with a slow mechanism of action used in combination with alpha-cypermethrin in the Interceptor® G2 ITNs. It was tested based on larvae availability and was conducted in fewer sites than planned due to the travel restriction related to COVID-19.

An. gambiae s.l. populations tested at the seven sites were susceptible to chlorfenapyr (Figure 15). In Jackson Farm and Zeanzue, 100% percent mortality was observed by 24 hours post-exposure (Figure 15), which corresponds to the mortality of lab colony mosquitoes tested using chlorfenapyr. Two sites – Gbedin and Compound 3 (first test) did not reach 100% mortality until 72 hours. However, the second test at Compound 3, reached 100% mortality at 48 hours.

The dilution process was done following the CDC bottle bioassay protocol. Each insecticide grade was diluted with 50 ml acetone per tube. Four bottles replicate were coated with one ml of different concentrations of the prepared insecticide solutions. One ml acetone only was used to coat the control bottles. All the bottles were kept covered overnight to dry before using the next day. Twenty-five female *An. gambiae* s.l. two to five days old were transferred into each of the bottles. The mortality was recorded every 15 minutes for up to two hours. *An. gambiae* s.l. was also tested for susceptibility to chlorfenapyr using the CDC bottle assay method as per the VectorLink SOP (SOP04/01). Acetone was used as a solvent to dilute chlorfenapyr to a concentration of 100 µg per bottle. Four bottles were coated with diluted chlorfenapyr. One bottle was coated with acetone only and

used as a control. A batch of 20-25 female *An. gambiae* s.l. mosquitoes (two to five-day-old) were transferred into each of the exposure and control bottles. Every 5 minutes the number of knockdowns was recorded up to one-hour exposure. Then, mosquitoes were transferred into cups and fed with a 10% sugar solution for holding up to 72 hours. Mortality was recorded every 24 hours for up to 72 hours or until 100% mortality was recorded, whichever came first.

2.2.3 SYNERGIST ASSAYS - CDC BOTTLE ASSAY METHOD

Pre-exposure to a synergist, PBO, was done before introducing mosquitoes to the diagnostic doses of deltamethrin and permethrin to see whether susceptibility could be restored. A concentration (100µg per 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 female *An. gambiae* s.l. were introduced into the synergist-exposure bottle for one hour. Another 125 female *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, using 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.

CDC bioassay tests are discarded if control mortality was > 10%. Abbott's formula was used to correct results if the mortality in the control bottle was between 3% and 10%.

3. Results And Discussion

3.1 Vector Bionomics

Below are the results from PSC, HLC, and CDC-LT collections (mosquito collections were paused from April to July 2020 due to COVID-19). The team was not able to perform mosquito collections in Jackson Farm and Saint John in March 2020 due to COVID-19. Collections did not start in the additional ITN monitoring sites until January 2020, following the work plan approval in December 2019.

3.1.1 Vector Abundance and Species Composition

A total of 7,339 mosquitoes were collected using PSC, HLC, and CDC-LTs. Of this, 81% were *Anopheles* and the remaining 19% were *Culicine* mosquitoes (*Culex, Mansonia,* and *Aedes). Anopheles gambiae* s.l. was most dominant among the *Anopheles* mosquitoes collected: 87.1% *An. gambiae* s.l., 12.5% *An. funestus* s.l., 0.27% *An. ziemanni*, and less than 1% *An. rufipes* (Table 4). The high abundance of *Anopheles* mosquitoes in Gbedin, Suehn Town, and Jackson Farm is likely due to the presence of rice field breeding habitats in these rural areas (Table 4).

Table 4: Total Mosquitoes Collected from Eight Sentinel Sites, October 2019 – March 2020 and August – September 2020 (All Collection Methods)

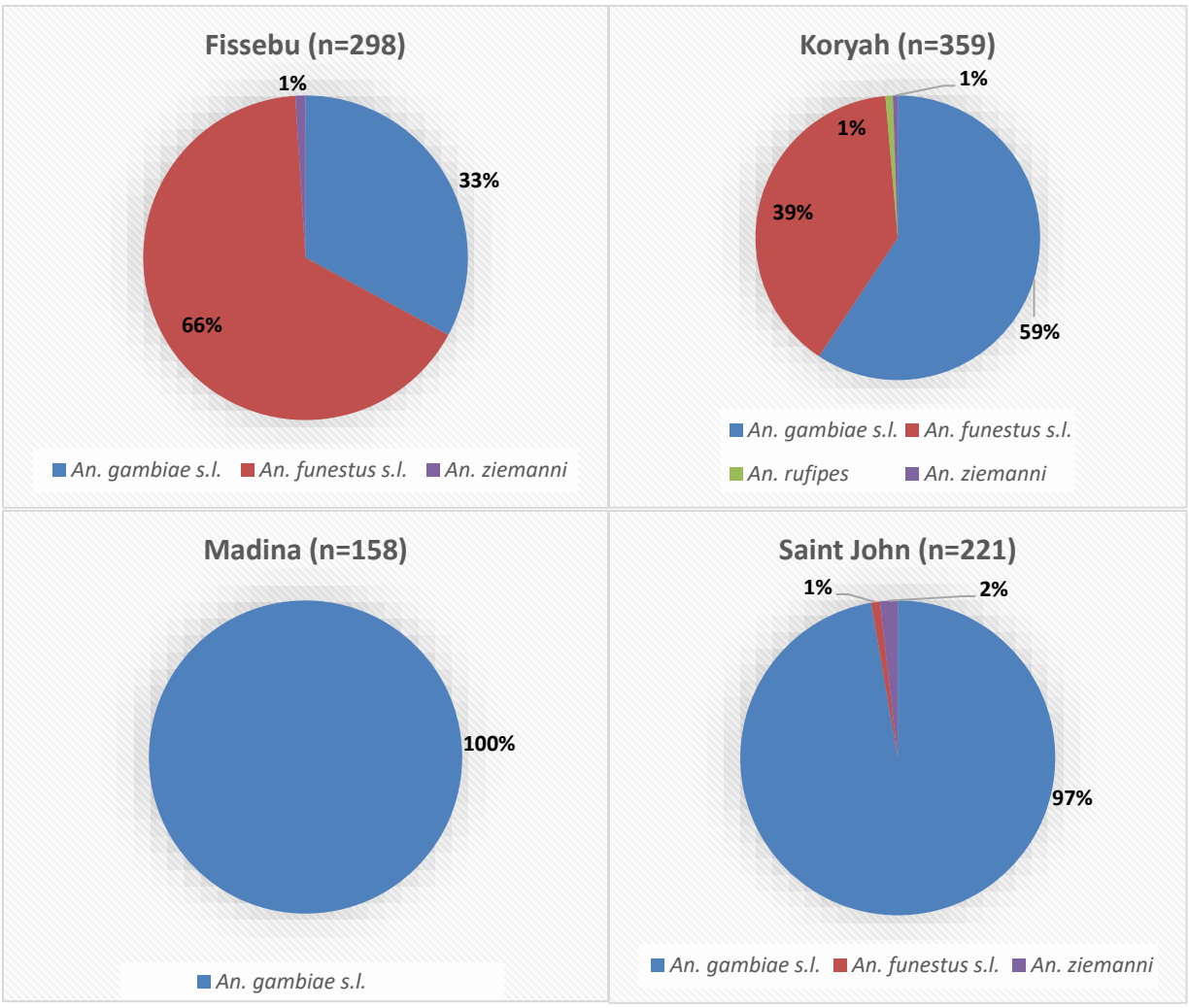
Site	*An gambiae* s.l.		*An funestus* s.l.		*An Rufipes*		*An. ziemanni*		*Total Anopheles*		*Culex*	*Aedes*	*Mansonia*
	n	*(%)*	*n*	*(%)*	*n*	*(%)*	*n*	*(%)*	*N*	*(%)*			
Fissebu	98	(33)	197	(66)	0	(0)	3	(1)	298	(5)	136	0	1
Koryah	213	(59)	141	(39)	3	(1)	2	(1)	359	(6)	300	0	23
Madina	158	(100)	0	(0)	0	(0)	0	(0)	158	(3)	439	0	1
Saint John	215	(97)	2	(1)	0	(0)	4	(2)	221	(4)	109	0	0
Gbedin	1,622	(96)	59	(4)	0	(0)	1	(0)	1,682	(28)	66	0	2
Suehn Town	1,833	(99)	23	(1)	0	(0)	2	(0)	1,858	(31)	56	0	7
Jackson Farm	887	(99)	12	(1)	0	(0)	0	(0)	899	(15)	109	0	0
Zeanzue	131	(30)	308	(70)	0	(0)	4	(1)	443	(7)	171	1	0
Total	5,157	(87)	742	(13)	3	(0)	16	(0)	5,918	(100)	1,386	1	34

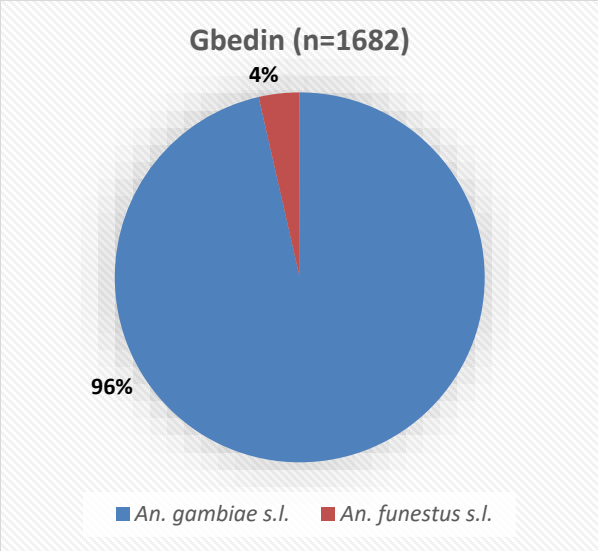
Figure 2 below shows that while *An. gambiae* s.l. is the most abundant malaria vector in six out of the eight sentinel sites, *An. funestus* s.l. is predominant in two sites out of the eight sites (Zeanzue and Fissebu) and was also represented 39% of the *Anopheles* collected. These sites are observed to have water bodies, including swamps, known to be preferred for breeding by *An. funestus* s.l.

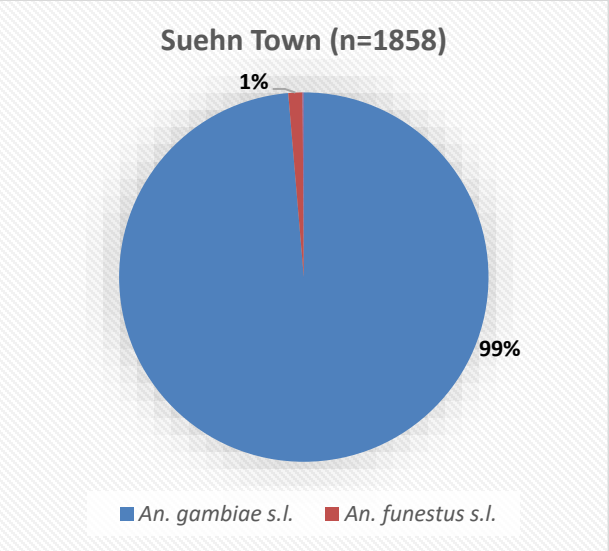
Anopheles funestus s.l. is known as one of the most efficient malaria vectors in Africa. However, very little is known about the contribution of *An. funestus* s.l. to malaria transmission in Liberia. The Liberia VectorLink project plans to characterize this vector through PCR identification to the species level, assess its infectivity and

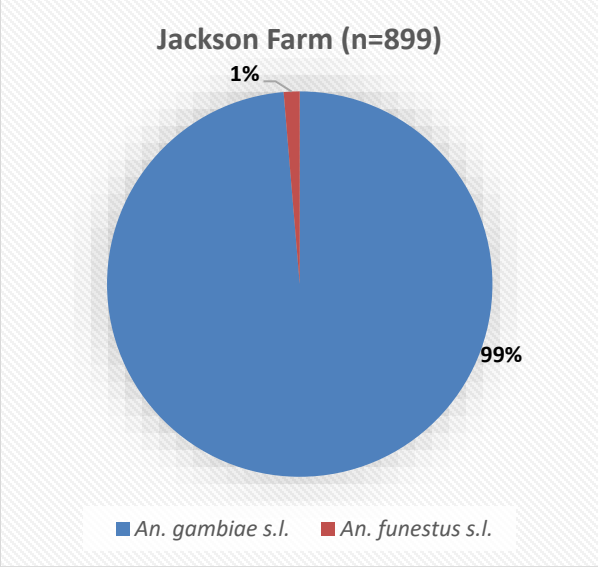
susceptibility to insecticides. Information might be useful to understand its contribution to malaria transmission in the country and for planning its control.

FIGURE 2: SPECIES COMPOSITION OF *ANOPHELES* MOSQUITOES COLLECTED BY PSC, HLC AND CDC-LT FROM EIGHT SENTINEL SITES, OCT 2019 – MARCH 2020 AND AUGUST – SEPTEMBER 2020




Gbedin (n=1682)
4%
96%
An. gambiae s.l.
An. funestus s.l.


Suehn Town (n=1858)
1%
99%
An. gambiae s.l.
An. funestus s.l.


Jackson Farm (n=899)
1%
99%
An. gambiae s.l.
An. funestus s.l.

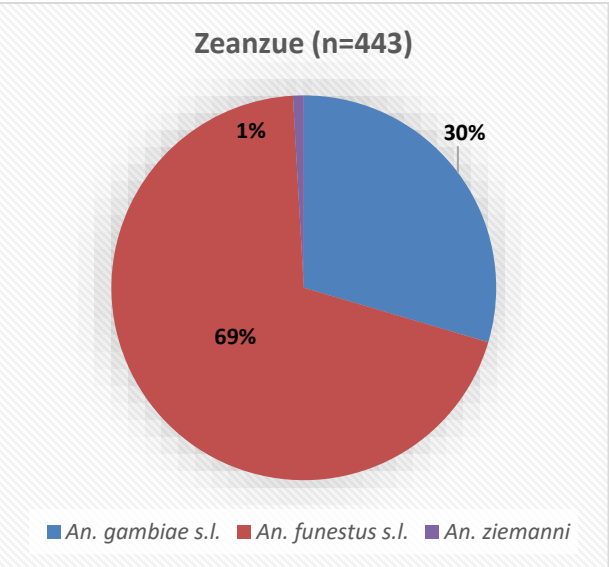

Zeanzue (n=443)
1%
30%
69%
An. gambiae s.l.
An. funestus s.l.
An. ziemanni

Table 5 below shows the total number of mosquitoes collected by method and site. The CDC-LT collections were only conducted indoors while the HLC was performed indoors and outdoors. Overall, the HLC method seems to be more productive for *An. gambiae* s.l. collection than the other methods.

TABLE 5: *ANOPHELES* AND OTHER MOSQUITOES SPECIES COLLECTED IN EIGHT SENTINEL SITES, OCTOBER 2019 - MARCH 2020 AND AUGUST – SEPTEMBER 2020.

Site	Method	*An. gambiae* s.l.		*An. funestus* s.l.		*An. rufipes*		*An. ziemanni*		*Culex*		*Aedes*		*Mansonia*	
		n	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%
Fissebu	PSC	29	30	91	46	0	0	0	0	12	9	0	0	0	0
	CDC-LT	26	27	73	37	0	0	3	100	99	73	0	0	1	100
	HLC	43	44	33	17	0	0	0	0	25	18	0	0	0	0
	Total	**98**	100	**197**	100	**0**	0	**3**	100	**136**	100	**0**	0	**1**	100
Koryah	PSC	107	50	68	48	3	100	0	0	13	4	0	0	0	0
	CDC-LT	41	19	47	33	0	0	1	50	280	93	0	0	23	100
	HLC	65	31	26	18	0	0	1	50	7	2	0	0	0	0
	Total	**213**	100	**141**	100	**3**	100	**2**	100	**300**	100	**0**	**0**	**23**	100
Madina	PSC	133	84	0	0	0	0	0	0	94	21	0	0	0	0
	CDC-LT	8	5	0	0	0	0	0	0	337	77	0	0	0	0
	HLC	17	11	0	0	0	0	0	0	8	2	0	0	1	100
	Total	**158**	100	**0**	**0**	**0**	**0**	**0**	**0**	**439**	100	**0**	0	**1**	100
Saint John	PSC	134	62	1	50	0	0	0	0	23	21	0	0	0	0
	CDC-LT	28	13	1	50	0	0	4	100	84	77	0	0	0	0
	HLC	53	25	0	0	0	0	0	0	2	2	0	0	0	0
	Total	**215**	100	**2**	100	0	0	**4**	100	**109**	100	**0**	0	**0**	0
Gbedin	PSC	531	33	6	10	0	0	1	100	32	48	0	0	0	0
	CDC-LT	613	38	15	25	0	0	0	0	28	42	0	0	0	0
	HLC	478	29	38	64	0	0	0	0	6	9	0	0	2	100
	Total	**1,622**	100	**59**	100	**0**	0	**1**	100	**66**	100	**0**	0	**2**	100
Suehn Town	PSC	534	29	0	0	0	0	0	0	3	5	0	0	0	0
	CDC-LT	175	10	22	96	0	0	0	0	21	38	0	0	0	0
	HLC	1,124	61	1	4	0	0	2	100	32	57	0	0	7	100
	Total	**1,833**	100	**23**	100	**0**	0	**2**	100	**56**	100	**0**	0	**7**	100
Jackson Farm	PSC	420	47	2	17	0	0	0	0	2	2	0	0	0	0
	CDC-LT	132	15	4	33	0	0	0	0	95	87	0	0	0	0
	HLC	335	38	6	50	0	0	0	0	12	11	0	0	0	0
	Total	**887**	100	**12**	100	**0**	0	**0**	0	**109**	100	**0**	0	**0**	0
Zeanzue	PSC	92	70	170	55	0	0	0	0	11	6	0	0	0	0
	CDC-LT	12	9	61	20	0	0	0	0	111	65	0	0	0	0
	HLC	27	21	77	25	0	0	4	0	49	29	1	100	0	0
	Total	131	100	308	100	0	0	4	0	171	100	1	100	0	0
Total	PSC	1,980	38	338	46	3	100	1	6.3	190	14	0	0	0	0
	CDC-LT	1,035	20	223	30	0	0	8	50	1,055	76	0	0	24	71
	HLC	2,142	42	181	24	0	0	7	44	141	10	1	100	10	29
	Total	**5,157**	100	**742**	100	**3**	100	**16**	100	**1,386**	100	**1**	100	**34**	100

3.1.2 INDOOR RESTING DENSITY

Indoor resting densities were high in Gbedin (13.6 mosquitoes per house per day) in September, Suehn Town (10.2 mosquitoes per house per day) in March, followed by Jackson Farm (8.8 mosquitoes per house per day) in February. These sites are all rice-growing areas where the densities are comparatively higher than the other sites where mosquitoes per house per day is between 0 and 2 in Fissebu, Koryah, and Saint John. (Table 6).

TABLE 6: INDOOR DENSITIES OF *AN. GAMBIAE* S.L. COLLECTED BY PSC, OCTOBER 2019–MARCH 2020 AND AUGUST-SEPTEMBER 2020.

Year-Month	Original Monitoring Sites				ITN Monitoring Sites			
	Numbers per House per Day							
	Fissebu	Koryah	Madina	Saint John	Gbedin	Suehn Town	Jackson Farm	Zeanzue
19-Oct	0.12	0.2	0.36	1.56	NA	NA	NA	NA
19-Nov	0	0.08	0.28	0.24	NA	NA	NA	NA
19-Dec	0	0.16	0.24	0.2	NA	NA	NA	NA
20-Jan	0	0.2	0.08	0.24	2.2	10.16	7.84	0.36
20-Feb	0	0.64	0	0.24	1.28	2.8	8.8	0.56
20-Mar	0.04	1.96	0.08	NA	0.76	8.24	NA	1.6
20-Aug	0.24	0	0.16	0.96	3.36	0	0.16	0.68
20-Sep	0.76	1.04	4.12	1.92	13.64	0.16	0	0.48

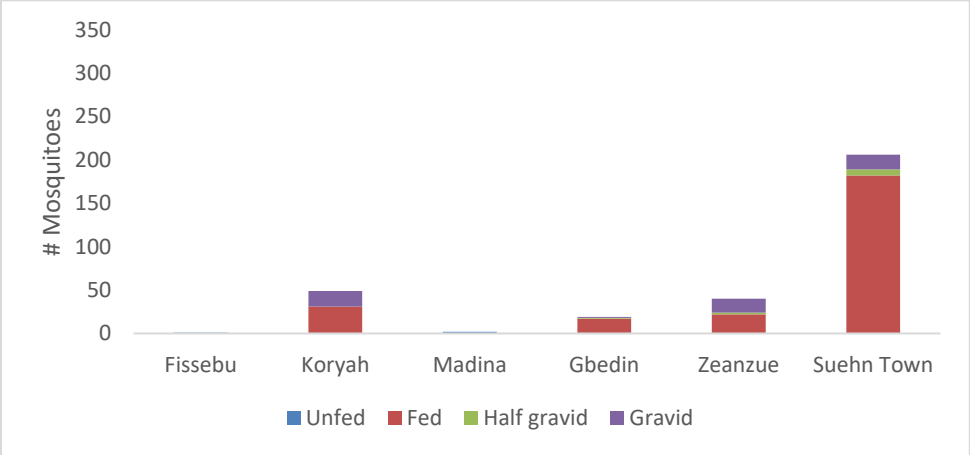
NA: collection was delayed in these sites for three months due to workplan approval process.

3.1.3 ABDOMINAL STAGE

High proportions of fresh blood-fed *An. gambiae* s.l. were collected from all sites, indicating that a considerable number of the vectors were able to bite humans or animals. During this reporting period, no test was performed to determine the source of the blood. This will be conducted at LIBR upon training. However, as the mosquitoes were collected from sleeping rooms using PSC, it is highly likely they had fed on humans. More blood-fed females in the collections could mean there was more human-vector contact and potential exposure to malaria transmission despite the ITN distribution. As these mosquitoes were collected resting inside sleeping rooms, the high number of fed mosquitoes could mean they are still getting access to bite people despite the expectations that people would use their nets and get protected from bites. Most of *An. gambiae* s.l. mosquitoes collected in March 2020 using PSC were blood-fed; in Suehn Town where vector indoor resting density is high (Figure 3).

The number of mosquitoes collected from Suehn Town in August dropped to zero due to the frequent rains which affected the larval sources. More mosquitoes were collected in August from Gbedin where breeding sites are mainly rice fields (Figure 4).

FIGURE 3: DISTRIBUTION OF *AN. GAMBIAE* S.L. MOSQUITOES BY ABDOMINAL STAGE, COLLECTED BY PSC IN SIX SITES, MARCH 2020



In March 2020, due to travel restrictions, the team was not able to perform collections in Saint John and Jackson Farm.

FIGURE 4: DISTRIBUTION OF *AN. GAMBIAE* S.L. MOSQUITOES BY ABDOMINAL STAGE, COLLECTED BY PSC IN EIGHT SITES, AUGUST 2020

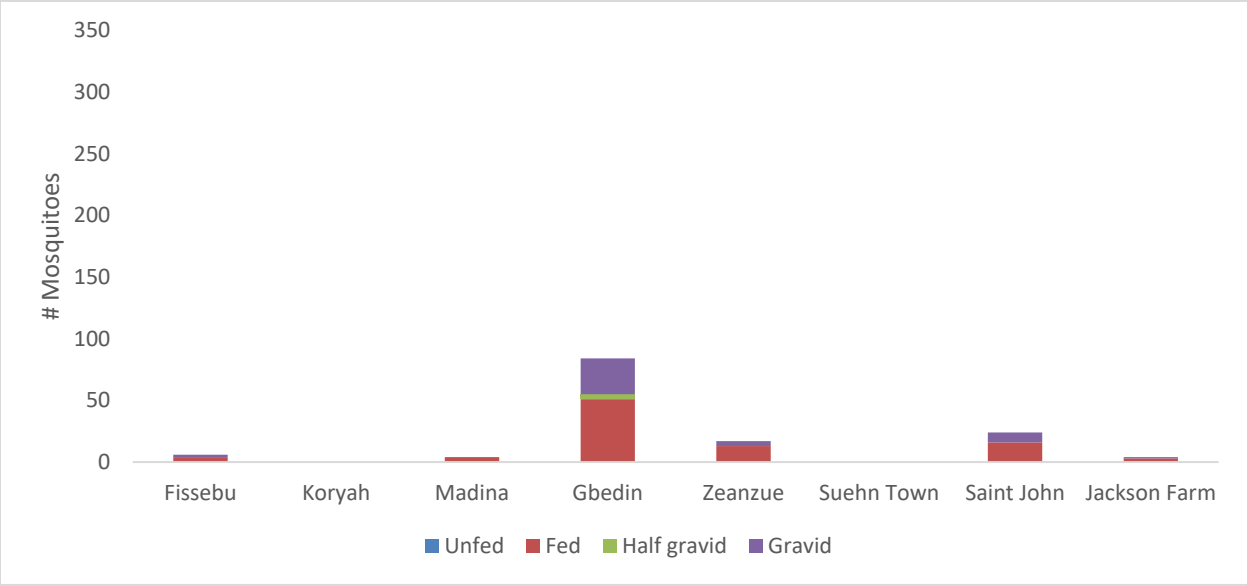
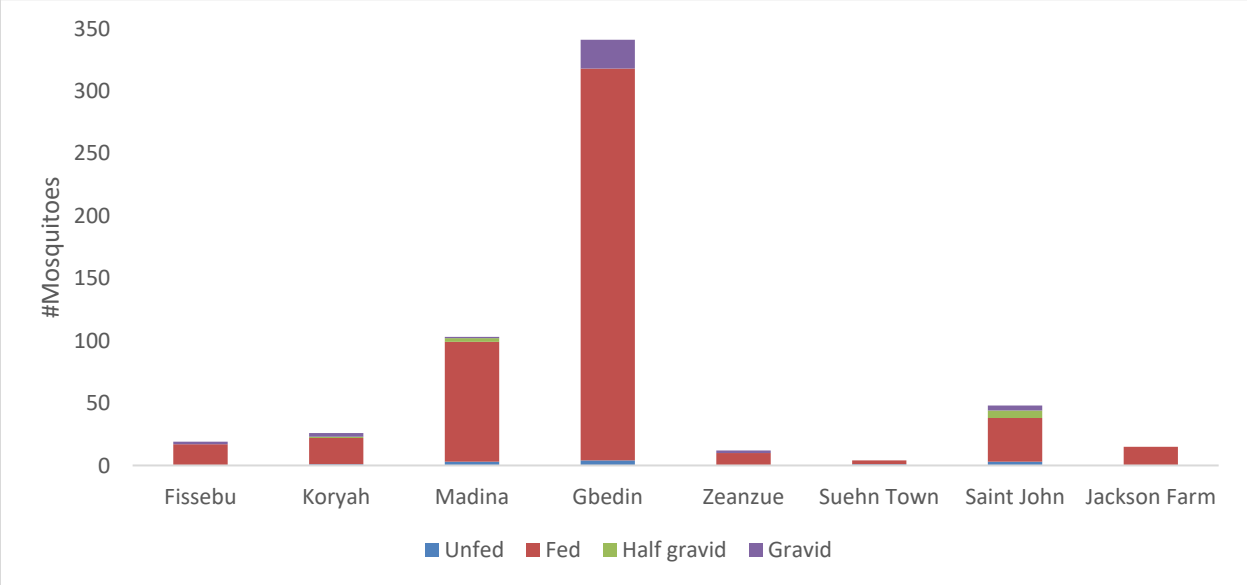


FIGURE 5: DISTRIBUTION OF *AN. GAMBIAE* S.L. MOSQUITOES BY ABDOMINAL STAGE, COLLECTED BY PSC IN EIGHT SITES, SEPTEMBER 2020



3.1.4 CDC Light Trap Collections

A total of 1,023 *An. gambiae* s.l. mosquitoes were collected indoors by CDC LT during the reporting period (Table 7).

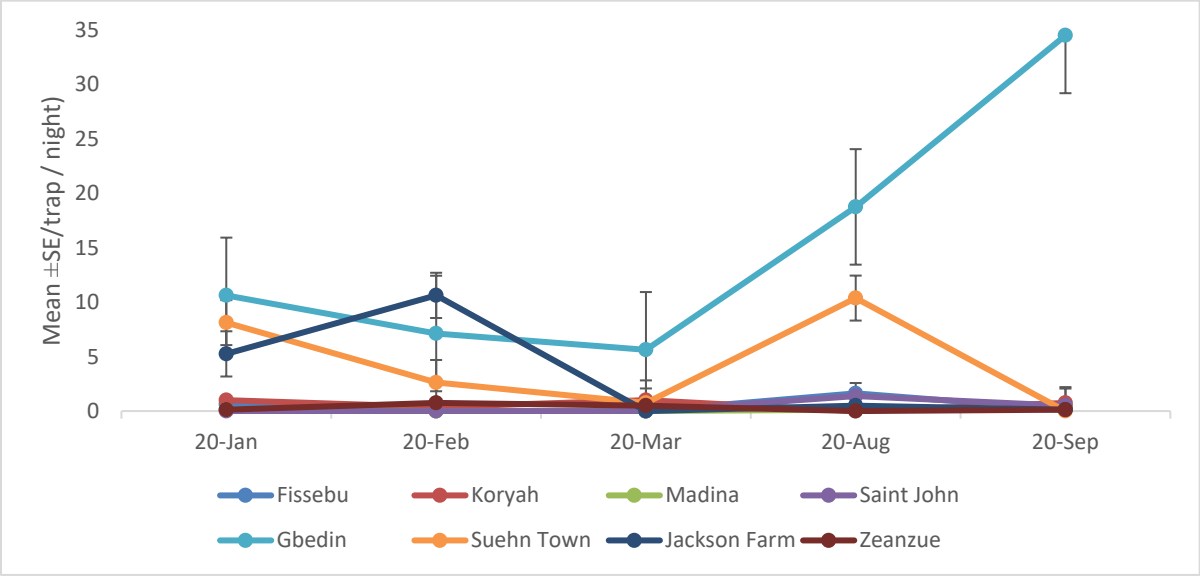
TABLE 7: *AN. GAMBIAE* S.L. COLLECTED BY CDC LT, INDOOR, OCTOBER 2019–MARCH 2020 AND AUGUST - SEPTEMBER 2020

	Original Monitoring Sites				ITN Monitoring Sites				Total
Month-Year	Fissebu	Koryah	Madina	Saint John	Gbedin	Suehn Town	Jackson Farm	Zeanzue	
October-19	0	1	0	8	NA	NA	NA	NA	9
November-19	1	1	0	0	NA	NA	NA	NA	2
December-19	4	10	1	1	NA	NA	NA	NA	16
January-20	5	8	1	0	85	65	42	1	207
February-20	0	3	0	0	57	21	85	6	172
March-20	0	8	0	NA	45	6	NA	4	63
August-20	13	0	1	11	150	83	4	0	262
September-20	1	6	3	4	276	0	1	1	292
Total	**24**	**37**	**6**	**24**	**613**	**175**	**132**	**12**	**1,023**

NA: The monthly entomological monitoring was not performed due to the travel restriction decided by health authorities in March. These sites were the last sites to be sampled in March 2020.

When looking at data across the eight sites collected from January to September 2020, the highest density was observed in Gbedin followed by Suehn Town and Jackson Farm (Figure 6). The mosquito population peaked in September for Gbedin, August for Suehn Town, and February for Jackson Farm.

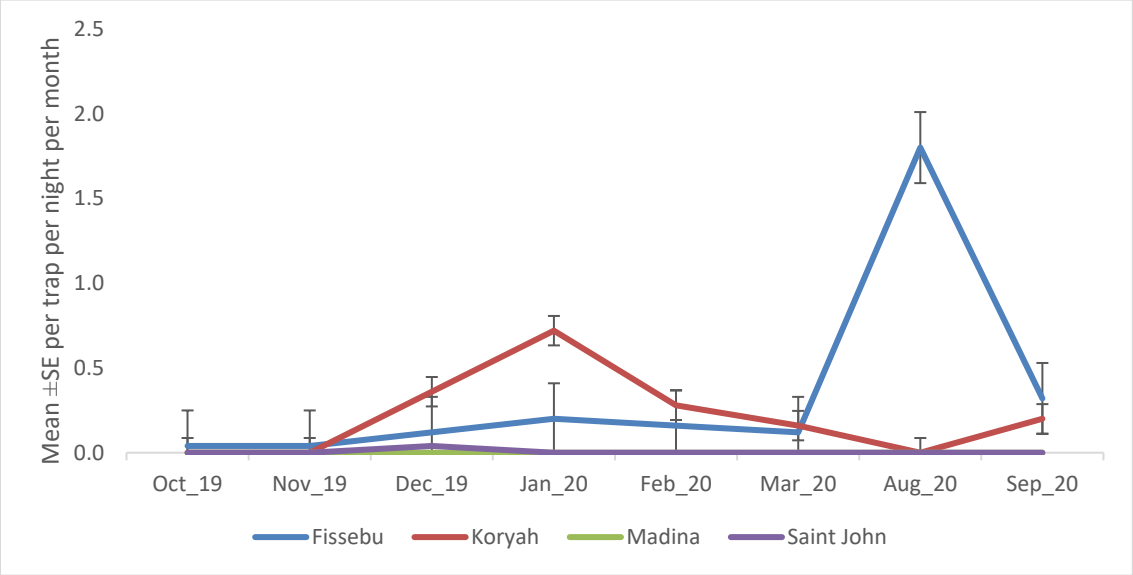
FIGURE 6: MEAN DENSITIES PER TRAP PER NIGHT OF *AN. GAMBIAE* S.L. SAMPLES COLLECTED BY CDC LT, INDOOR, OCTOBER 2019–MARCH 2020 AND AUGUST-SEPTEMBER 2020.



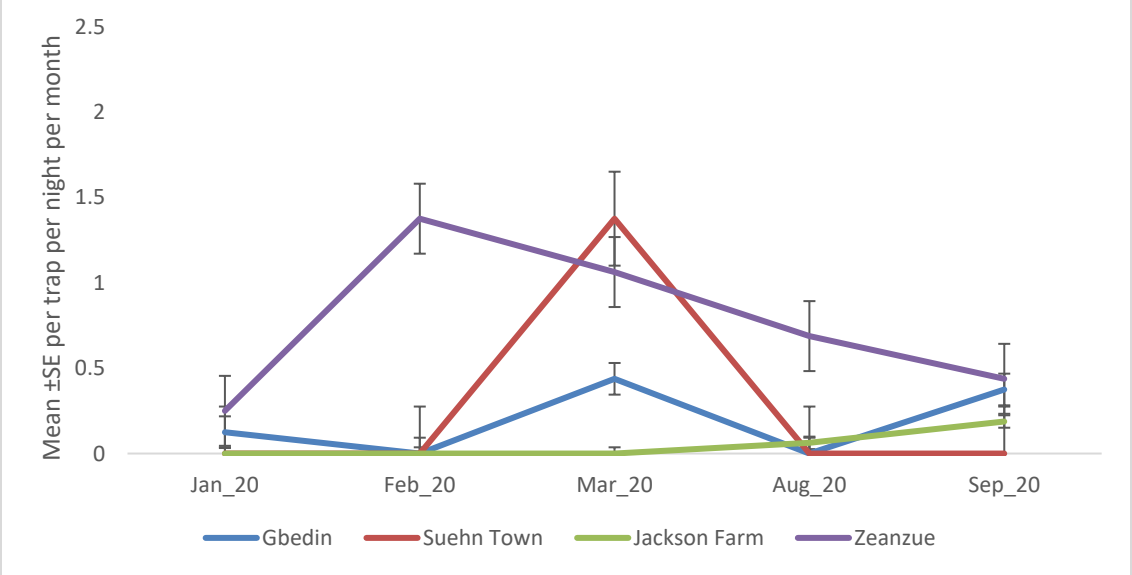
Using CDC LTs, the highest number of *An. funestus* s.l. mosquitoes were collected from Fissebu in August (1.8 females per trap per night) (Figure 7). *An. funestus* s.l was collected at all sites, except Madina. No clear trend in seasonal population variability could be presented because of the four months interruption of collections due to COVID-19 restrictions and a low number of mosquitoes collected. There are also only four months of data for the newly added sites. However, it seems that even in sites where *An. funsestus* s.l is dominant over *An. gambiae* s.l. (Zeanzue and Fissebu) the numbers collected per trap were still low.

FIGURE 7: *ANOPHELES FUNESTUS* S.L NUMBERS COLLECTED BY CDC-LT INDOORS, PER TRAP PER NIGHT, OCTOBER 2019–MARCH 2020 AND AUGUST – SEPTEMBER 2020.

A) ORIGINAL MONITORING SITES



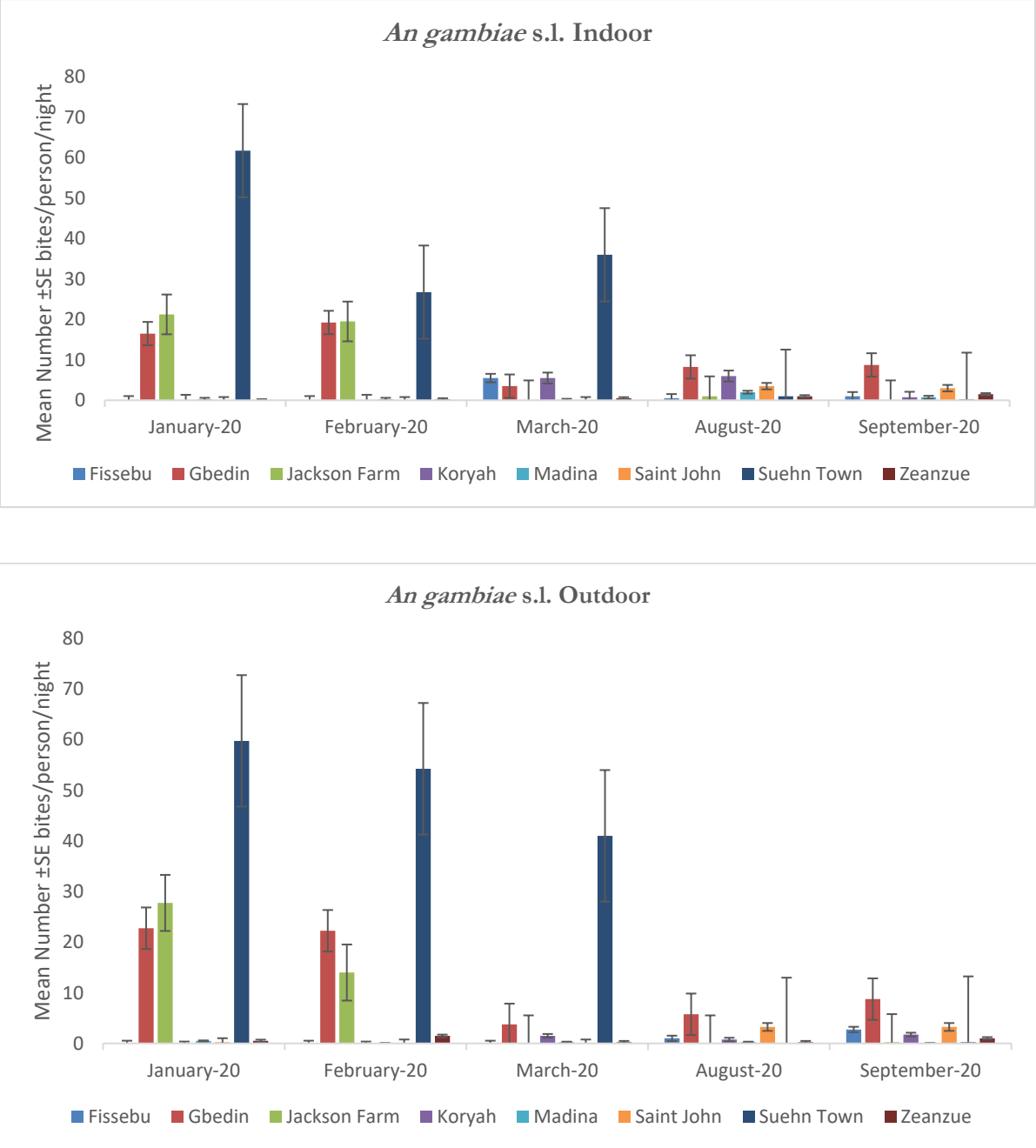
B) ITN MONITORING SITES



3.1.5 HUMAN BITING RATE AND PATTERN

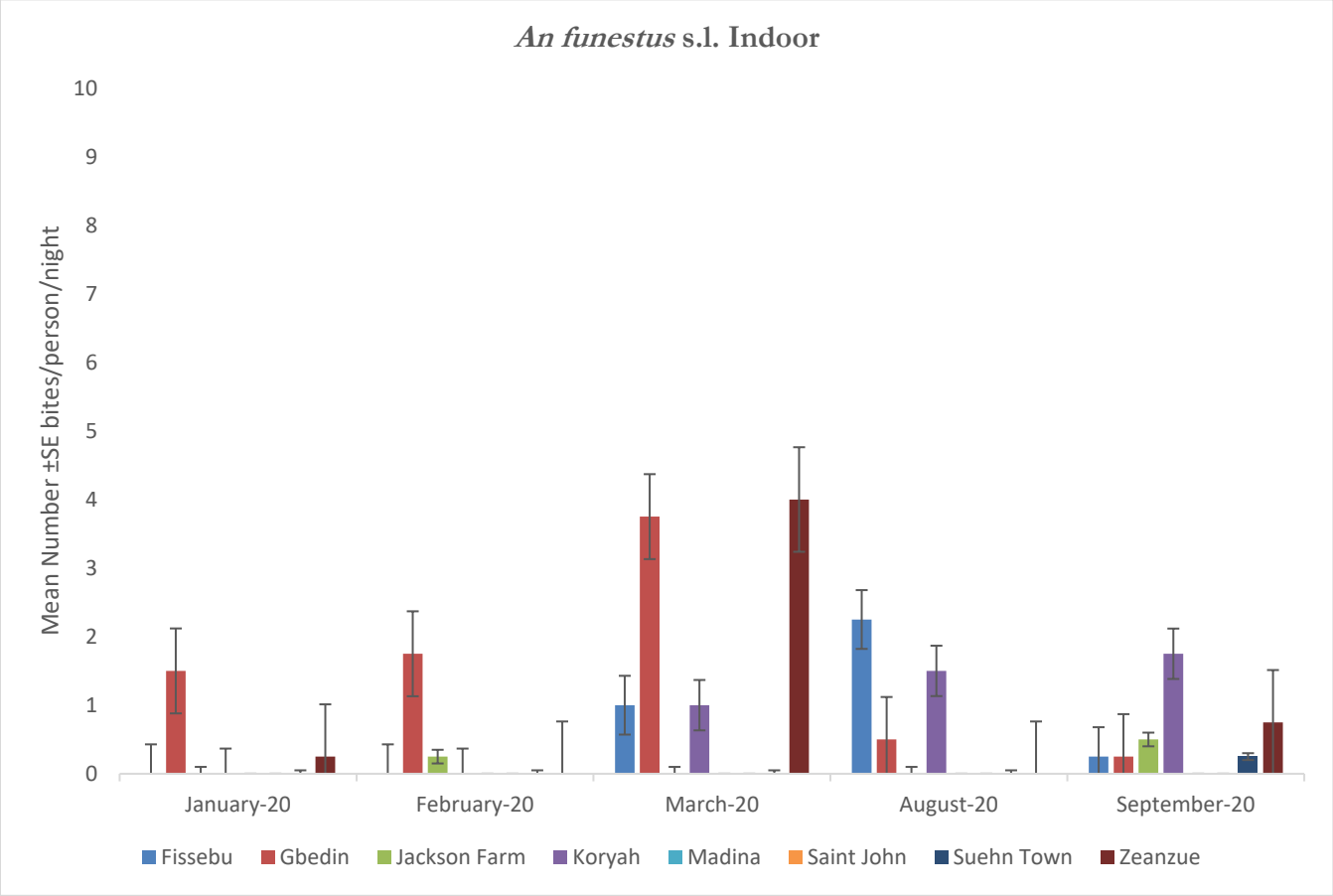
The highest indoor and outdoor biting rate of *An. gambiae* s.l. was observed in Suehn Town in January 2020 at 61.8 bites per person per night and 59.8 bites per person per night, respectively (Figure 8). HLC collections did not start until January 2020 in all sites, following approval of the work plan in December 2019.

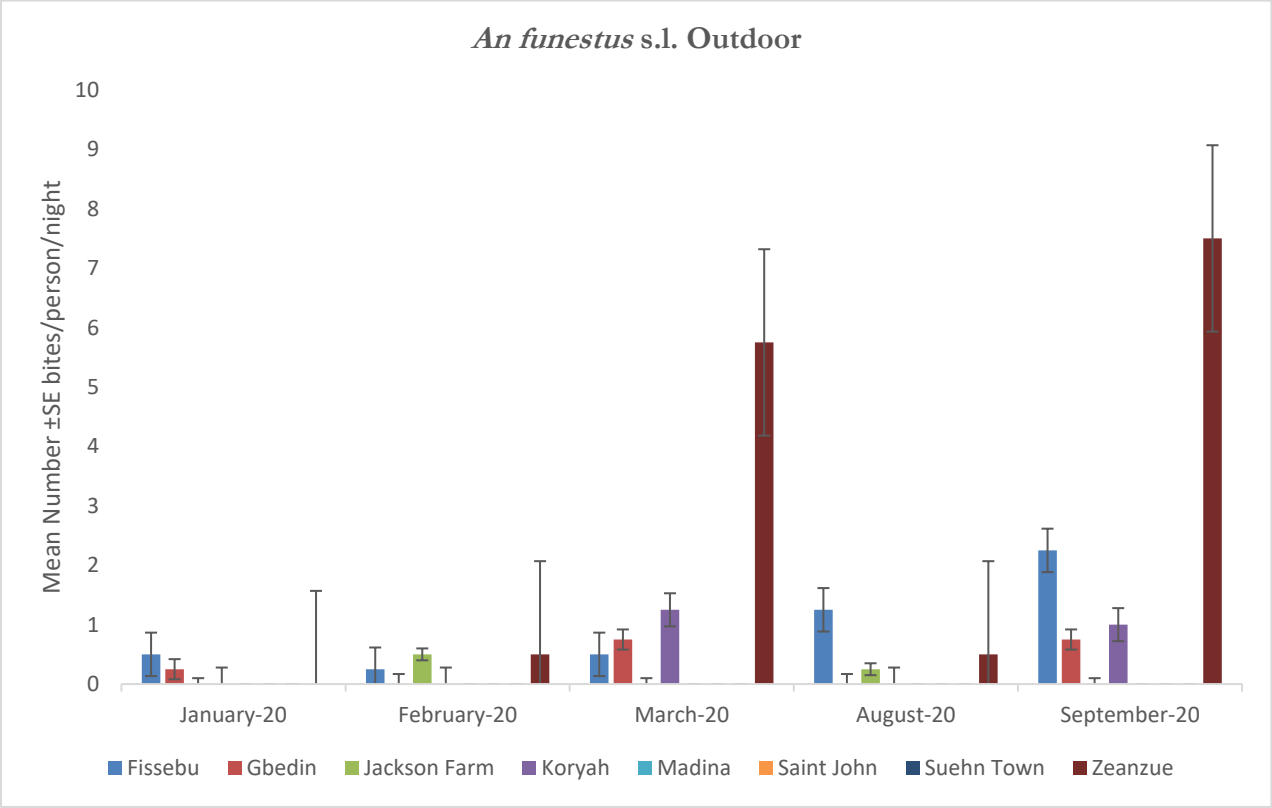
FIGURE 8: NIGHTLY HUMAN BITING RATE OF *AN. GAMBIAE* S.L. COLLECTED FROM INDOOR AND OUTDOOR HLC, JANUARY – MARCH 2020 AND AUGUST - SEPTEMBER 2020.



The biting rate by *An. funestus* s.l. was overall lower than *An. gambiae* s.l. with the highest indoor biting rates occurring at Zeanzue and Gbedin in March 2020 (4.0 and 3.8 bites per person per night, respectively) and the highest outdoor biting rates at Zeanzue site in September 2019 and March 2020 (5.8 bites and 7.5 bites per person per night) (Figure 9).

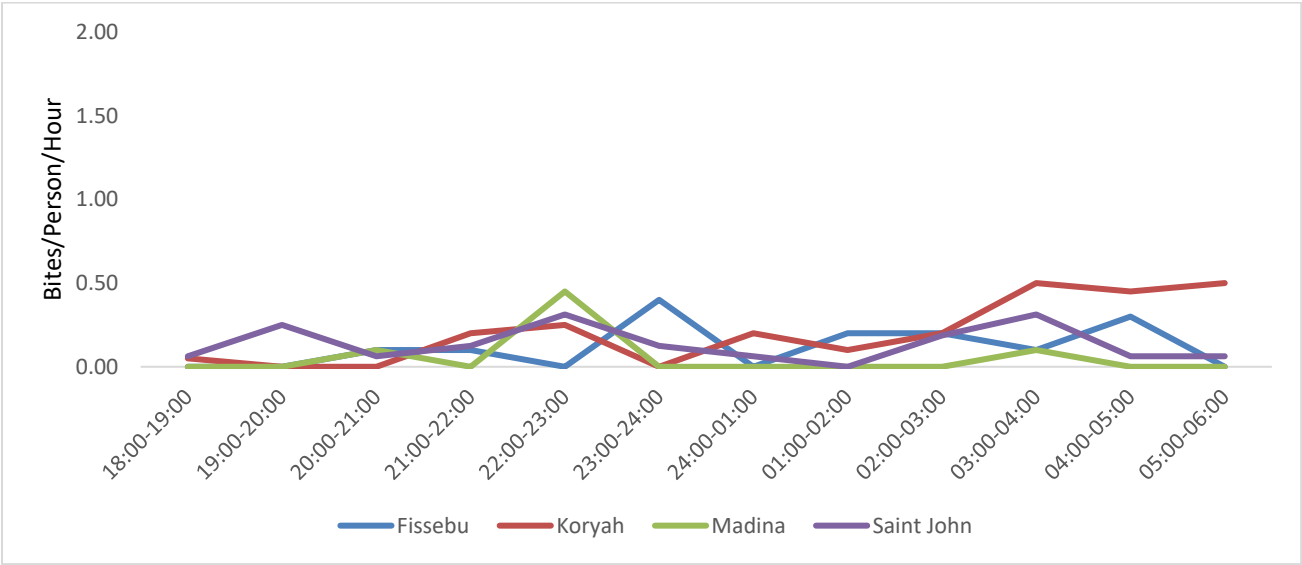
FIGURE 9: NIGHTLY HUMAN BITING RATE OF *AN. FUNESTUS* S.L. COLLECTED FROM INDOOR AND OUTDOOR HLC, JANUARY–MARCH 2020 AND AUGUST - SEPTEMBER 2020.





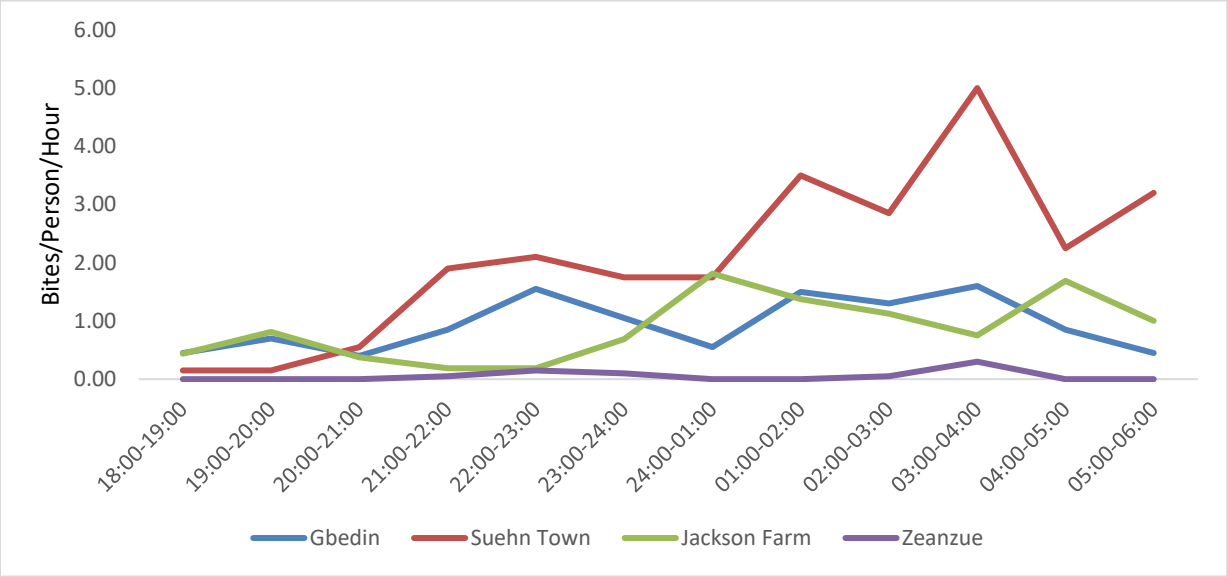
The hourly rates in those sites with data collection from January 2020 are less than 1.0 bites per person per hour (Figure 10). There is no clear trend in hourly biting activity except some minor peaks at 11:00 PM to 12:00 AM as well as toward early morning (5:00 AM to 6:00 AM).

FIGURE 10: HOURLY HUMAN BITING RATE OF *AN. GAMBIAE* S.L. COLLECTED FROM INDOOR HLC IN ORIGINAL MONITORING SITES, JANUARY –MARCH 2020 AND AUGUST - SEPTEMBER 2020.



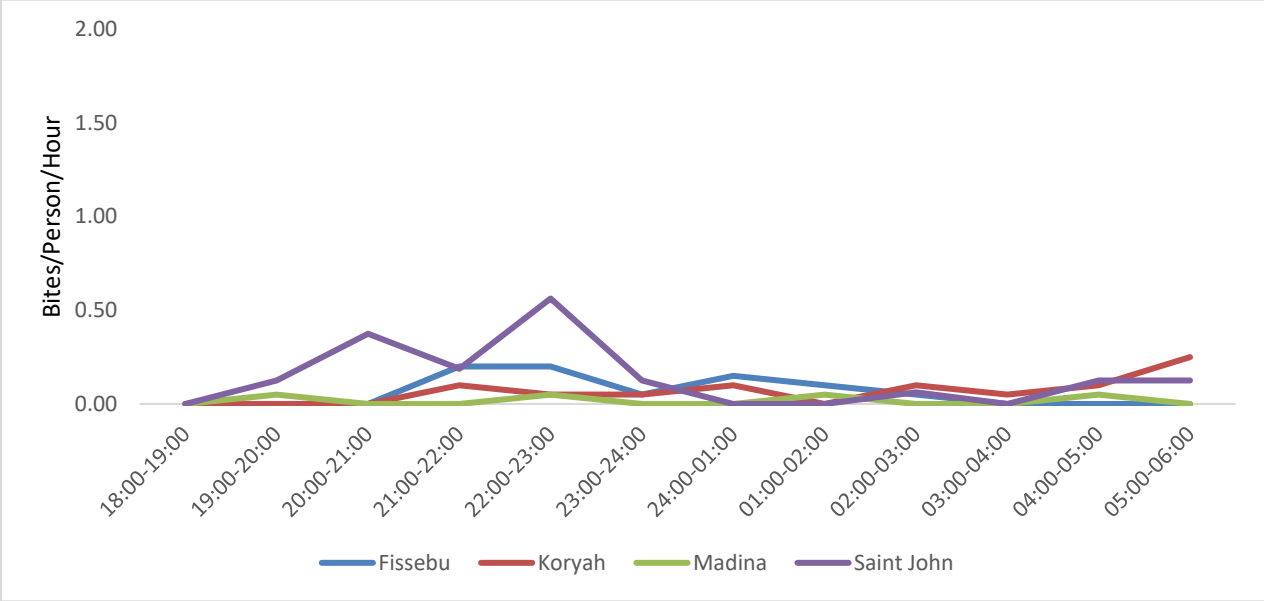
In the sites added in January 2020, the hourly indoor biting rate for *An. gambiae* s.l. was higher in Suehn Town than in the other sites. The biting activity started to peak after 9:00 PM and was higher towards the end of the night (5:00 AM to 6:00 AM). The biting rate was low in Zeanzue (Figure 11).

FIGURE 11: HOURLY HUMAN BITING RATE OF *AN. GAMBIAE* S.L. COLLECTED FROM INDOOR HLC IN FOUR ITN MONITORING SITES, JANUARY – MARCH 2020 AND AUGUST - SEPTEMBER 2020.



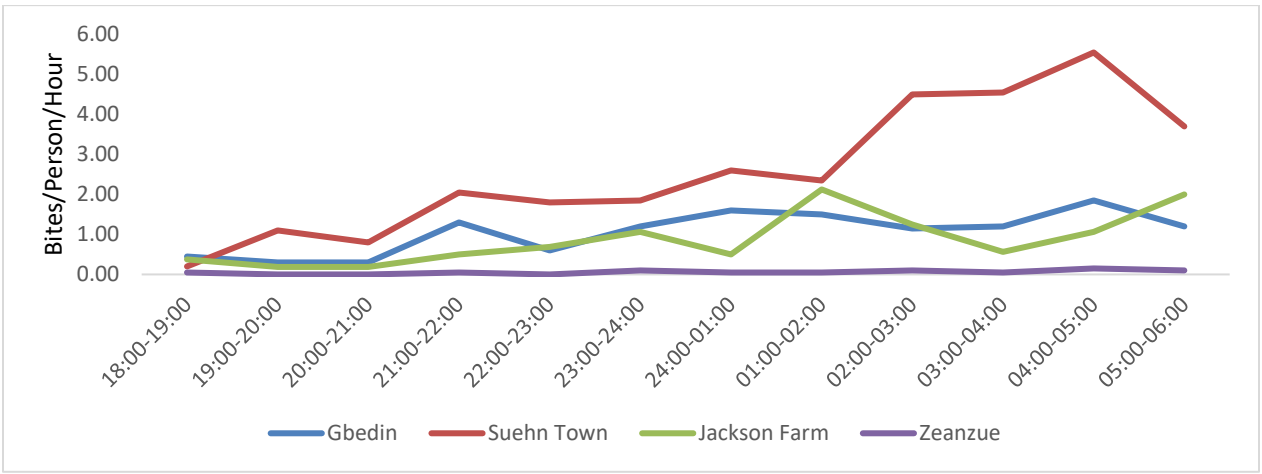
Similar to the indoor biting hourly rates across the four sites with collections reported from January 2020, the outdoor hourly rates are less than 1 bites per person per hour (Figure 12). There is no clear trend in hourly biting activity except a minor peak at Saint John around 11:00 PM.

FIGURE 12: HOURLY HUMAN BITING RATE OF *AN. GAMBIAE* S.L. COLLECTED FROM OUTDOOR HLC IN ORIGINAL MONITORING SITES, JANUARY – MARCH 2020 AND AUGUST - SEPTEMBER 2020.



In the sites added January 2020, the hourly outdoor biting rate for *An. gambiae* s.l. was highest in Suehn Town (Figure 13). Biting activity at this site started to peak after 7:00 PM, progressively increasing throughout the night, and was distinctly higher towards the end of the night, after 2:00 AM. The biting rate was relatively low and flat in the other three sites, so no clear trend in hourly biting could be observed.

FIGURE 13: HOURLY HUMAN BITING RATE OF *AN. GAMBIAE* S.L. COLLECTED FROM OUTDOOR HLC IN FOUR ITN MONITORING SITES, JANUARY – MARCH 2020 AND AUGUST - SEPTEMBER 2020.



3.2 INSECTICIDE RESISTANCE MONITORING

3.2.1 INSECTICIDE SUSCEPTIBILITY TEST FOR PYRETHROIDS

The data collected in Year 2 showed that *An. gambiae* s.l. populations were resistant to all doses of pyrethroids (1x, 2x, 5x, and 10x the diagnostic dose) after 30 minutes of exposure. In Year 3, only 1x the diagnostic dose

of deltamethrin and permethrin was tested in seven sites. The vector was resistant to deltamethrin and permethrin, mortality rates ranging between 37 and 57% across all sites (Figure 14).

3.2.2 Insecticide Susceptibility Testing of Chlorfenapyr

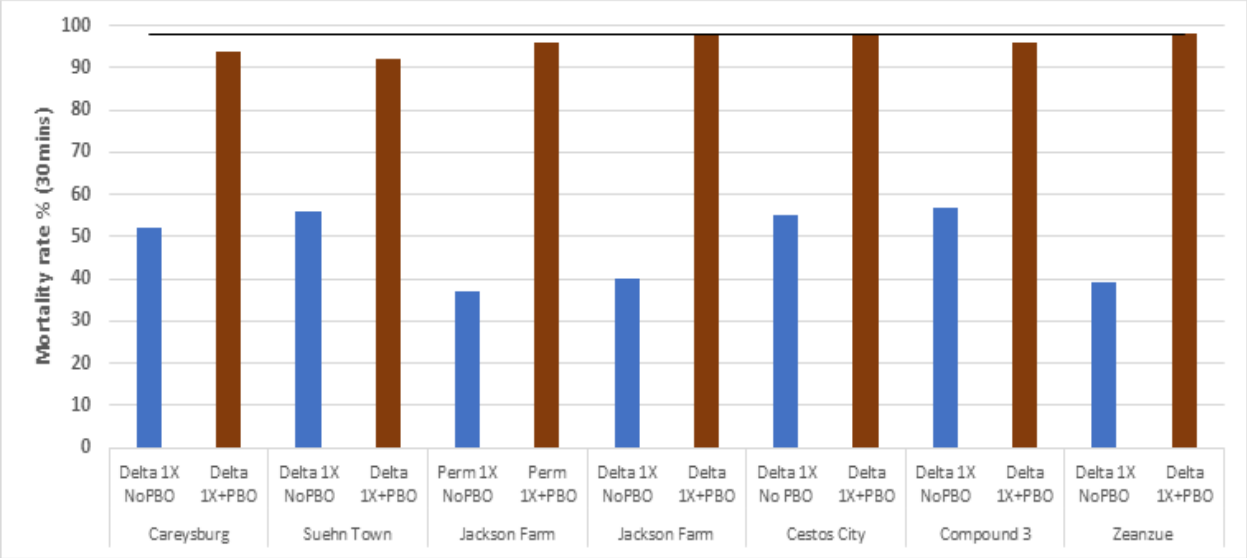
Chlorfenapyr is an insecticide with a slow mechanism of action used in combination with alpha-cypermethrin in the Interceptor® G2 ITNs. It was tested based on larvae availability and was conducted in fewer sites than planned due to the travel restriction related to COVID-19.

An. gambiae s.l. populations tested at the seven sites were susceptible to chlorfenapyr (Figure 15). In Jackson Farm and Zeanzue, 100% percent mortality was observed by 24 hours post-exposure (Figure 15), which corresponds to the mortality of lab colony mosquitoes tested using chlorfenapyr. Two sites – Gbedin and Compound 3 (first test) did not reach 100% mortality until 72 hours. However, the second test at Compound 3, reached 100% mortality at 48 hours.

3.2.3 Synergist Assays - CDC bottle assay Method

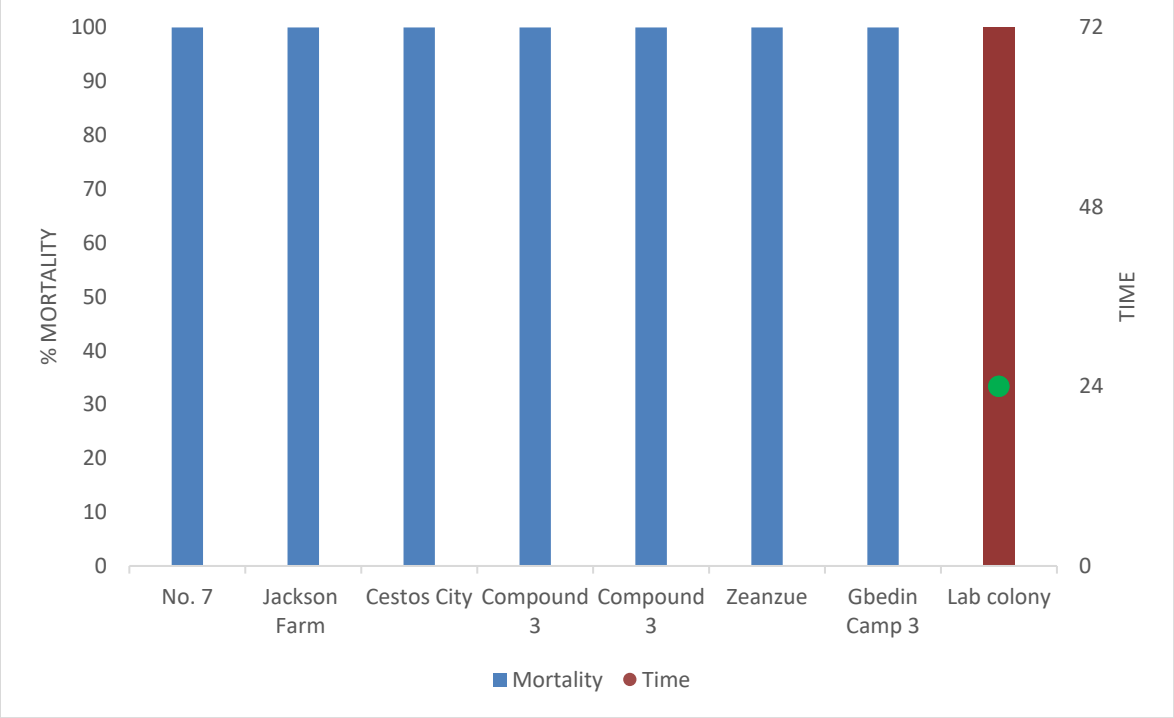
In Year 3, deltamethrin and permethrin with or without PBO was tested in seven sites. Consistent with historic data, VectorLink observed an increase in the mortality rate among mosquitoes pre-exposed to PBO before being tested with deltamethrin and permethrin. However, the PBO did not restore full susceptibility to pyrethroids at the 30 minutes diagnostic time (Figure 14). It seems that mechanisms of resistance other than oxidases may be involved in the high phenotypic resistance to pyrethroids observed in Liberia. There are ongoing efforts in collaboration with CDC Atlanta to build molecular diagnostic capacity at LIBR to further identify possible mechanisms of resistance.

FIGURE 14: PERCENTAGE MORTALITY OF *AN. GAMBIAE* S.L. FROM DIFFERENT SITES IN LIBERIA EXPOSED TO 1X DELTAMETHRIN DIAGNOSTIC DOSE AND TO PBO USING CDC BOTTLE ASSAYS, FEBRUARY– SEPTEMBER 2020



Note: Solid black line indicates 98% cut-off point for susceptibility

FIGURE 15: MORTALITY RATE OF *AN. GAMBIAE* S.L. PER EXPOSURE TO CHLORFENAPYR USING CDC BOTTLE ASSAY



*Red line indicates time (hours) after exposure it to attain 100% mortality, by site.

**Lab colony is a susceptible strain of *An. Coluzzii*.

TABLE 8: MORTALITY RATE OF *AN. GAMBIAE* S.L. PER EXPOSURE TO CHLORFENAPYR USING CDC BOTTLE ASSAY AND TIME POST EXPOSURE

County	Site	Number tested	Mortality after 24hours (%)	Mortality after 48hours (%)	Mortality after 72hours (%)	Month
Monteserrado	Number 7	80	76.25	100		February
Margibi	Jackson Farm	80	100			March/April
River Cess	Cestos City	80	92.50	100		June
Grand Bassa	Compound 3	160	76.25	96.25	100	July
Grand Bassa	Compound 3	160	87.5	100		July
Bong	Zeanzue Town	160	100			September
Nimba	Gbedin Camp 3	160	76	99.4	100	September
	Lab colony	80	100			July

3.3 LABORATORY ANALYSES

Laboratory analysis activities are being conducted by LIBR staff. Due to COVID-19, these analysis activities are delayed as LIBR staff had to focus on processing COVID-19 samples. In July 2020, the LIBR staff were able to resume focus on mosquito sample processing, which is currently ongoing at LIBR. Year 3 samples are expected to be processed by May 2021. A new ELISA reader purchased in May 2020 by PMI is being used by LIBR staff for the project sample processing. In Year 3, VectorLink planned to conduct a PCR training with LIBR staff, but due to COVID-19, the training has been pushed to Year 4.

3.4 COVID-19 Prevention Measures

One of the main challenges this year was the suspension of activities due to COVID-19 and subsequent resumption of activities under strict COVID-19 prevention protocols. Per PMI's guidance, VectorLink and NMCP worked to identify measures for COVID-19 mitigation. For instance, instead of disinfecting the bedsheets for each PSC, additional bedsheets were purchased to avoid using the same set of bedsheets in different houses. For HLC, instead of using the mouth aspirators, the CHWs were provided with 5ml glass tubes to catch mosquitoes landing on their legs. All CHWs and VectorLink staff wore N95 masks and used hand sanitizer while performing entomological activities. In all the sites where the team was working, there were no reported COVID-19 cases. VectorLink resumed activities in August 2020 at all sites without any incident.

3.5 Capacity Building Activities

VectorLink worked to build the capacity of the NMCP, CHWs, and UL staff through training and on-site practical experience (Table 9). VectorLink, UL, and the NMCP led training on the basic morphology of adult and larval mosquitoes for the four CHWs and two supervisors at each site. In February, VectorLink trained NMCP staff on new data collection forms and VectorLink SOPs for entomological monitoring. In July, VectorLink provided remote training to the NMCP staff on ITN durability monitoring. In August, the same NMCP staff were involved in the supervision of the 24-month ITN durability monitoring survey (held at 29 months due to COVID-19 associated delays) of ITNs in Lofa and Grand Gedeh counties. NMCP staff were trained on ITN durability monitoring bioassays conducted in-country this year. VectorLink Liberia supported NMCP to build capacity which included training of NMCP and UL staff on vector monitoring and insecticide resistance assays performed respectively in sentinel sites and selected counties.

TABLE 9: SUMMARY OF CAPACITY BUILDING ACTIVITIES

	Numbers Trained				
Activity	NMCP	CHWs	Field Supervisors	University of Liberia	Total
Insecticide resistance testing	4	28			32
Adult and larval mosquito collection methods refresher training	2	32	16	1	51
Field morphological ID	4	32	16	1	51
ITN durability monitoring bioassay training	2				2

Due to COVID-19-, all international training sessions were canceled. One NMCP staff attended November 2019 ASTMH in Washington, DC.

4. OBSERVATIONS AND CONCLUSIONS

Overall, identification of entomological and seasonal trends in the sentinel sites was challenging due to the intermittent collection periods resulting from COVID-19 travel restrictions. However, the data collected shows several patterns that reflect vector abundance, e.g., higher mosquito abundance in the rice-growing sites like Suehn Town, Gbedin, and Jackson Farm than in other sites.

Anopheles gambiae s.l. was the main vector at all sites except Fissebu and Zeanzue where *An. funestus* s.l. was found in higher densities. Sites like Gbedin, Jackson Farm and Suehn Town are typical remote villages with higher vector densities. As Madina displayed low density, it may not be the best option for entomological monitoring regarding the monitoring of the Interceptor® G2 ITNs.

The tests performed on *An. gambiae* s.l. populations in the seven sites revealed that *An. gambiae* s.l. was highly resistant to pyrethroids (deltamethrin and permethrin). Among mosquitoes not pre-exposed to PBO, the mortality ranged between 37% to 57%. The pre-exposure of mosquitoes to PBO increased deltamethrin susceptibility to 98% in three out of the six sites tested and to 92 to 96% in other sites indicating that the enzymatic mechanism involving monooxygenase is likely involved. More investigation is needed on the molecular resistance mechanisms.

Four sites were added in Year 3 to expand entomological monitoring for baseline data collection before Interceptor® G2 ITN distribution planned for May 2021. It is recommended only those sites with higher mosquito density continue to be monitored into Year 4 (Jackson Farm, Suehn Town, Gbedin, and Zeanzue). An assessment of the entomological indicators in these sites will help evaluate the variations of vector density as well as sporozoite rate changes in the lifetime of the ITNs. Bioassay results confirmed that chlorfenapyr is effective against *An. gambiae* s.l. and supports the decision to distribute Interceptor® G2 ITNs treated with chlorfenapyr and alpha-cypermethrin for the 2021 mass campaign.

Overall, hourly biting behavior showed an increase of activity after midnight. Biting rates were higher during the second part of the night (12:00 AM to 5:00 AM). The outdoor biting rate was higher than the indoor biting rate at all sites. Due to the interruption of collections for COVID-19 precaution, the data is not complete to show seasonal trends. However, the ongoing monthly collection will allow a better assessment of seasonal variation in the biting rate.

The lab analysis using ELISA-CSP for sporozoite rate assessment is ongoing at LIBR with priority to the processing of backlog samples. PCR training will be held in Year 4 which will allow LIBR to process samples for blood meal origin, species identification, and identification of mechanisms of insecticide resistance.

The high proportion of blood-fed mosquitoes from sleeping rooms may indicate frequent contact between host and vector probably due to inconsistent use of ITNs used by communities. The NMCP/SBC team should

scale up communication efforts for behavior change to improve ITNs use in rural communities, and this could be addressed by more engagement of the community health workers.

5. REFERENCES

1. Braack L, Gouveia de Almeida AP, Cornel AJ, Swanepoel R, de Jager C. Mosquito-borne arboviruses of African origin: review of key viruses and vectors. *Parasite Vectors*. 2018; 11(1):29. Published 2018 Jan 9. doi:10.1186/s13071-017-2559-9

2. Coetzee, M. Key to the females of Afrotropical *Anopheles* mosquitoes (Diptera: Culicidae). *Malar J* 19, 70 (2020). https://doi.org/10.1186/s12936-020-3144-9 National Malaria Control Program (NMCP) [Liberia], Ministry of Health (MOH), Liberia Institute of Statistics and Geo-Information Services (LISGIS), and ICF. 2017. Liberia Malaria Indicator Survey 2016. Monrovia, Liberia: MOH, LISGIS, and ICF.

3. https://malariaworld.org/blog/ivcc-and-syngenta-reach-key-milestone-development-new-insecticide-malaria-mosquito-control (accessed January 6, 2017).

4. Stojanovich CJ and Scott HG. 1966. *Illustrated Key to Anopheles Mosquitoes of Liberia*. Atlanta, Georgia: U.S Department of Health, Education, and Welfare, Public Health Science. Communicable Disease Center.

5. Gillett, JD. Common African Mosquitoes and Their Medical Importance, with 48 Colour Illustrations. London: William Heinemann Medical Books Ltd.

6. World Health Organization. 2013. *Test procedures for insecticide resistance monitoring in malaria vector mosquitoes*. http://www.who.int/malaria: December 31, 2019.

6. ANNEXES

ANNEX 1: *ANOPHELES GAMBIAE* S.L. COLLECTED BY PSC, OCTOBER 2019 – MARCH 2020 AND AUGUST-SEPTEMBER 2020

Month-Year	Original monitoring sites				ITN monitoring sites				Total
	Fissebu	Koryah	Madina	Saint John	Gbedin	Suehn Town	Jackson Farm	Zeanzue	
October-19	3	5	9	39	NA	NA	NA	NA	56
November-19	0	2	7	6	NA	NA	NA	NA	15
December-19	0	4	6	5	NA	NA	NA	NA	15
January-20	0	5	2	6	55	254	196	9	527
February-20	0	16	0	6	32	70	220	14	358
March-20	1	49	2	NA*	19	206	NA*	40	317
August-20	6	0	4	24	84	0	4	17	139
September-20	19	26	103	48	341	4	0	12	553
Total	29	107	133	134	531	534	420	92	1,980

ANNEX 2: *ANOPHELES FUNESTUS S.L.* COLLECTED BY PSC, OCTOBER 2019–MARCH 2020

Month-Year	Original monitoring sites				ITN monitoring sites				Total
	Fissebu	Koryah	Madina	Saint John	Gbedin	Jackson Farm	Suehn Town	Zeanzue	
October-19	22	4	0	0	NA	NA	NA	NA	26
November-19	30	1	0	0	NA	NA	NA	NA	31
December-19	12	2	0	1	NA	NA	NA	NA	15
January-20	7	45	0	0	4	0	0	26	82
February-20	10	8	0	0	1	0	0	56	75
March-20	10	8	0	NA*	1	NA*	2	88	109
Total	91	68	0	1	6	0	2	170	338

NA= Collections were delayed in these four sites selection in 2019 due workplan approval delay.
NA= Pause of activities March-July 2020 due to COVID-19.*

ANNEX 3: *ANOPHELES GAMBIAE* S.L. COLLECTED BY CDC LT, INDOOR, OCTOBER 2019–MARCH 2020 AND AUGUST-SEPTEMBER 2020

	Original monitoring sites				ITN monitoring sites				Total
Month-Year	Fissebu	Koryah	Madina	Saint John	Gbedin	Suehn Town	Jackson Farm	Zeanzue	
October-19	0	1	0	8	NA	NA	NA	NA	9
November-19	1	1	0	0	NA	NA	NA	NA	2
December-19	4	10	1	1	NA	NA	NA	NA	16
January-20	5	8	1	0	85	65	42	1	207
February-20	0	3	0	0	57	21	85	6	172
March-20	0	8	0	NA	45	6	NA	4	63
August-20	13	0	1	11	150	83	4	0	262
September-20	1	6	3	4	276	0	1	1	292
Total	24	37	6	24	613	175	132	12	1,023

ANNEX 4: *ANOPHELES FUNESTUS* S.L. COLLECTED BY CDC LT, INDOOR, OCTOBER 2019–MARCH 2020 AND AUGUST - SEPTEMBER 2020

Month	Original monitoring sites				ITN monitoring sites				
	Fissebu	Koryah	Madina	Saint John	Gbedin	Suehn	Jackson Farm	Zeanzue	Total
October-19	1	0	0	0	NA	NA	NA	NA	1
November-19	1	0	0	0	NA	NA	NA	NA	1
December-19	3	9	0	1	NA	NA	NA	NA	13
January-20	5	18	0	0	2	0	0	4	29
February-20	4	7	0	0	0	0	0	22	33
March-20	3	4	0	NA	7	22	NA	17	53
August-20	45	0	0	0	0	0	1	11	57
September-20	8	5	0	0	6	0	3	7	29
Total	70	43	0	1	15	22	4	61	216

ANNEX 5: *ANOPHELES GAMBIAE* S.L. COLLECTED BY HLC, INDOOR, JANUARY–MARCH 2020 AND AUGUST - SEPTEMBER 2020

Month	Fissebu	Gbedin	Jackson Farm	Koryah	Madina	Saint John	Suehn Town	Zeanzue	Total
January_20	0	66	85	0	1	0	247	0	399
February_20	0	77	78	0	1	0	107	1	264
March_20	22	14	NA	22	0	NA	144	2	204
August_20	2	33	4	24	8	14	4	4	93
September_20	4	35	0	3	3	12	1	6	64

Total	28	225	167	49	13	26	503	13	1024

ANNEX 6: *ANOPHELES GAMBIAE* S.L. COLLECTED BY HLC, OUTDOOR, JANUARY–MARCH 2020 AND AUGUST - SEPTEMBER 2020

Month	Fissebu	Gbedin	Jackson Farm	Koryah	Madina	Saint John	Suehn Town	Zeanzue	Total
January_20	0	91	111	0	2	1	239	2	446
February_20	0	89	56	0	0	0	217	6	368
March_20	0	15	NA	6	1	NA	164	1	187
August_20	4	23	0	3	1	13	0	1	45
September_20	11	35	1	7	0	13	1	4	72
Total	15	253	168	16	4	27	621	14	1118

ANNEX 7: *ANOPHELES FUNESTUS* S.L. COLLECTED BY HLC, INDOOR, JANUARY–MARCH 2020 AND AUGUST - SEPTEMBER 2020

Month	Fissebu	Gbedin	Jackson Farm	Koryah	Madina	Saint John	Suehn Town	Zeanzue	Total
January_20	0	6	0	0	0	0	0	1	7
February_20	0	7	1	0	0	0	0	0	8
March_20	4	15	NA	4	0	NA	0	16	39
August_20	9	2	0	6	0	0	0	0	17
September_20	1	1	2	7	0	0	1	3	15
Total	14	31	3	17	0	0	1	20	86

ANNEX 8: *ANOPHELES FUNESTUS* S.L. COLLECTED BY HLC, OUTDOOR, JANUARY–MARCH 2020 AND AUGUST - SEPTEMBER, 2020

Month	Fissebu	Gbedin	Jackson Farm	Koryah	Madina	Saint John	Suehn Town	Zeanzue	Total
January_20	2	1	0	0	0	0	0	0	3
February_20	1	0	2	0	0	0	0	2	5
March_20	2	3	NA	5	0	NA	0	23	33
August_20	5	0	1	0	0	0	0	2	8
September_20	9	3	0	4	0	0	0	30	46
Total	19	7	3	9	0	0	0	57	95

ANNEX 9: *ANOPHELES GAMBIAE* S.L. COLLECTED BY HLC, INDOOR AND OUTDOOR, JANUARY–MARCH 2020 AND AUGUST - SEPTEMBER 2020

Location	Fissebu	Gbedin	Jackson Farm	Koryah	Madina	Saint John	Suehn Town	Zeanzue	Total
Indoor	28	225	167	49	13	26	503	13	1024
Outdoor	15	253	168	16	4	27	621	14	1118
Total	43	478	335	65	17	53	1124	27	2142
Endophagic Index (a)	0.7	0.5	0.5	0.8	0.8	0.5	0.4	0.5	0.5
Exophagic Index (b)	0.3	0.5	0.5	0.2	0.2	0.5	0.6	0.5	0.5
Ratio (a/b)	0.3	-0.1	0.0	0.5	0.5	0.0	-0.1	0.0	0.0
Chi^2 (p-value)	0.047*	0.2	0.9	0.0004*	0.029	0.8907	0.0004*	0.8474	0.0422*

*difference statistically significant