U.S. PRESIDENT'S MALARIA INITIATIVE

THE PMI VECTORLINK SIERRA LEONE

ANNUAL ENTOMOLOGICAL MONITORING ADDENDUM REPORT

MARCH 1, 2020 – FEBRUARY 28, 2021

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SIERRA LEONE
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ACRONYMS

Ace-1	Acetylcholinesterase 1
CDC	U.S. Centers for Disease Control and Prevention
CRID	Centre for Research in Infectious Diseases
EIR	Entomological Inoculation Rate
ELISA	Enzyme-linked Immunosorbent Assay
HBR	Human Biting Rate
HLC	Human Landing Catch
IRS	Indoor Residual Spraying
ITN	Insecticide-treated Net
kdr	Knockdown Resistance
NMCP	National Malaria Control Program
PCR	Polymerase Chain Reaction
PMI	President's Malaria Initiative
PSC	Pyrethrum Spray Catch
VGSC	Voltage-Gated Sodium Channel Gene
WHO	World Health Organization

I. Introduction

This report is an addendum to the Entomological Monitoring Annual Report, March 2020–February 2021, submitted by the President's Malaria Initiative (PMI) VectorLink Sierra Leone project in May 2021. This addendum presents laboratory data on *Plasmodium* infection rates in *Anopheles gambiae* s.l. samples collected during routine monitoring in two indoor residual spraying (IRS) districts (Bo and Bombali) and three non-IRS districts (Port Loko, Kono and Western Area rural). However, the data was collected before the IRS campaign in May 2021. The report also presents insecticide resistance mechanisms associated with pyrethroid resistance, including voltage gated sodium channel (*Vgsc*) allele frequency for *Anopheles* mosquitoes that were collected in 2020 before the IRS campaign in May 2021. Specifically, it describes the *Plasmodium* infection rate of *An. gambiae* s.l. and *An. funestus* s.l. samples that were collected monthly in the five districts. We also determined frequency of *Vgsc*-1014F (formerly *kdr*-west), - *Vgsc* 1014S (formerly *kdr*-east) alleles and N1575Y mutation (resistance mechanisms linked to pyrethroid resistance) and Acetylcholinesterase (*Ace-1)* mutation (linked to carbamate and organophosphate resistance) in *An. gambiae* s.l., that were exposed to insecticides during susceptibility tests in the five districts in Sierra Leone.

These data were not available when the annual report was submitted due to unforeseen delays in the laboratory processing of mosquito samples. The team also investigated the frequency of *Kdr-e/w* in susceptible laboratory colony (Kisumu), originally established from eggs obtained from Rwanda and Ghana, at the Makeni insectary.

2. METHODOLOGY

2.1 PROTOCOLS FOR MOLECULAR ANALYSIS

All molecular analyses were conducted at the Centre for Research in Infectious Diseases (CRID), Cameroon. The mosquito samples collected from the sentinel sites were stored individually in Eppendorf tubes with silica gel and sent to CRID for molecular analysis. Technicians analyzed the samples under supervision from senior scientists at the institute. The protocols are described in detail in the methods section in the Entomological Monitoring Annual Report, March 2020–February 2021but are briefly outlined in table 1 below.

Table 1: Protocols Used for Laboratory Analyses of *Anopheles* Malaria Vectors, 2020

Molecular Analysis	Protocol	Output
Polymerase chain reaction (PCR)	Santolamazza et al. (2008)	Species identification: Identified *An. gambiae* complex sibling species including *An. coluzzii*, *An. gambiae* s.s., *An. melas* and *An. arabiensis.*
	Koekemoer et al. (2002)	Species identification: Identified *An. funestus* complex sibling species including *An. funestus* s.s., *An. leesoni,* and *An. parensis.*
	Bass et al. (2007 and 2010)	*Vgsc*-L1014F; *Vgsc*-L1014S, N1575Y & *Ace*-1 allele frequency: Monitored pyrethroid target site resistance mechanism frequency and carbamate/organophosphate resistance
Enzyme-linked immunosorbent assay (ELISA)	Wirtz et al (2010)	*Sporozoite detection:* Determined the presence of *Plasmodium falciparum* sporozoites in *An. gambiae* s.l. and *An. funestus* s.l.

The following parameters were estimated:

- Human Biting Rate (HBR) per night (reported as bites per person per night)
  - = the total number of vectors collected/number of collectors/number of nights of capture
- Sporozoite rate = the proportion of *Anopheles* found positive for *Plasmodium* infection
- Daily EIR = sporozoite rate x HBR
- Monthly EIR = daily EIR x number of days in the month
- Seasonal EIR = Σ monthly EIR for months of either wet (May-October) or dry (November to February and March 2020) seasons.
- Annual EIR = Σ Monthly EIRs

2.2 SAMPLES PROCESSED FOR MOLECULAR ANALYSIS

For each of the tests described above, a subset of samples were sent to CRID for molecular analysis (Table 2).

Table 2: Number of Samples Processed for Molecular Analysis

#	Tests	Source	No. Processed	Total Collected
1	PCR to separate *An. gambiae* s.s into *An. gambiae* & *An. coluzzii*	HLC/ PSC/ CDC	4,034	22,431
5	PCR ID; *An funestus* s.l.	HLC/ PSC/ CDC	322	1,482
6	PCR *kdr (kdr- west; kdr- east,* and Ace- 1)	Insecticide Resistance	455	4,649
7	PCR N1575Y	Insecticide Resistance	155	4,649
8	ELISA for *detection of Plasmodium falciparum infection*	Mainly HLC	5,911	19,136
9	PCR for blood meal analysis	PSC/CDC	996	4,777
10	PCR for species confirmation on *An. gambiae* -Kisumu strain	Insectary colony	200	NA
11	PCR *kdr (kdr- west; kdr- east,* N1575Y and Ace- 1)	Insectary colony	100	NA
12	ELISA for detection of *Plasmodium falciparum infection*	*An. coustani* sampled in the same period	304	322

3. RESULTS

3.1 LABORATORY ANALYSIS FOR INFECTION DETECTION AND INSECTICIDE RESISTANCE MUTATION

A subset of samples collected using pyrethrum spray catch (PSC), Centers for Disease Control-miniature light trap (CDC-LT) and human landing catch (HLC) were sent to CRID in Cameroon for molecular analysis to identify sibling species, screen for sporozoite infection, insecticide resistance mutation (*Kdr-east/west* and *Ace-1*) and identify source of blood meal for the fed mosquitoes. The results of species ID and blood meal source were reported in the annual report. This addendum report contains the *Plasmodium* infection rates and prevalence of *Kdr-east/west* and *Ace-1* mutations in malaria vectors collected and tested between March 2020 and February 2021.

3.1.1 BITING LOCATION OF *AN. GAMBIAE S.L.* AND *AN. FUNESTUS S.L.* BY SIBLING SPECIES

A total of 4,034 *An. gambiae* s.l. and 234 *An. funestus* s.l. mosquitoes from the routine HLC in surveillance sites were successfully identified to sibling species (Table 3). The biting location of these mosquitoes was analyzed using tests of proportion and generalized linear model. In general, there was no significant difference in biting location (indoor/outdoor) of sibling species apart from rural site of Sand Sand Water (Western Area District) (p = 0.015 for *An. coluzzii* and p<0.001 for *An. gambiae* s.s. and peri-urban site of Masongbo (Bombali District); p < 0.001 for *An. coluzzii* and p =0.02 for *An. gambiae* s.s. in Bombali District) (Figure 1). The outdoor/indoor biting rate for each species was analyzed using a generalized linear model while controlling for site. There was no preference for indoor/outdoor biting for both *An. gambiae* s.s. and *An. coluzzii* apart from Masongbo (peri-urban) in Bombali District and Sand Sand Water (rural) in Western Area District where mosquitoes were likely to be biting indoor (Figure 1). In peri-urban site of Gerihun (Bo District), mosquitoes were also likely to be biting outdoor (Figure 1). However, *An. funestus* s.l. was more likely to be found biting indoor than outdoor when compared to both *An. coluzzii* and *An. gambiae* s.s. (Table 4).

Table 3: Molecular Species Distribution of *An. gambiae* s.l. and *An. funestus* s.l. Sampled in all Districts, March 2021-February 2021.

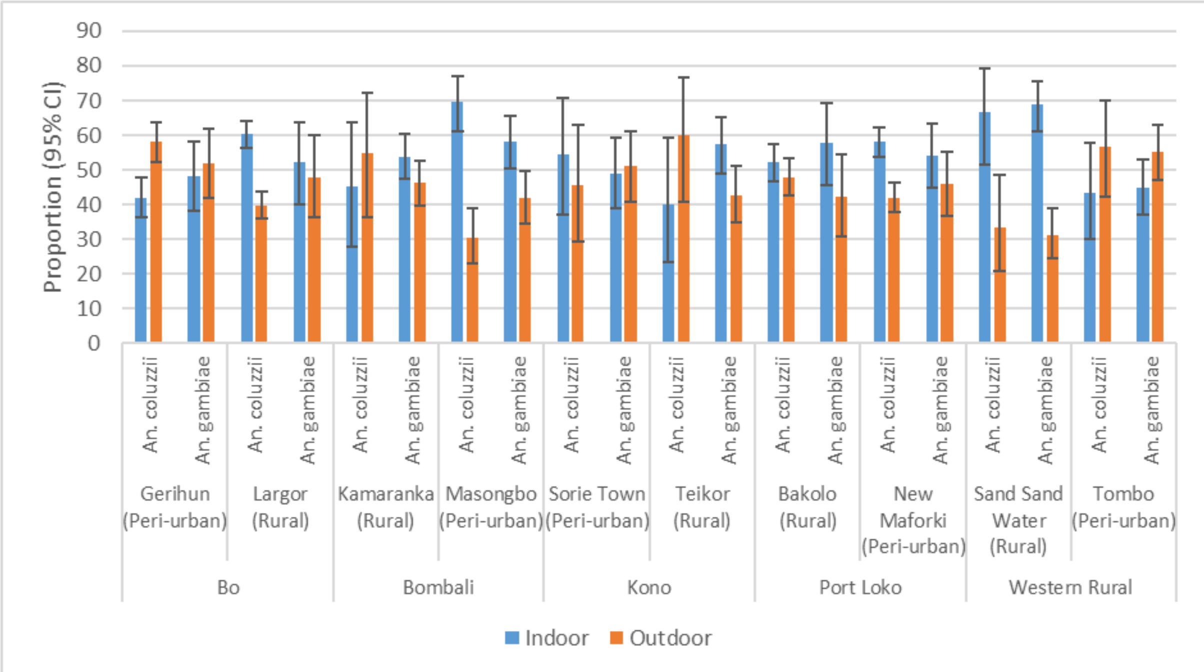
			An. gambiae s.l.					*An. funestus* s.l.			
Description	District	Site	*An. arabiensis*	*An. coluzzii*	*An. gambiae*	*An. melas*	*Total*	*An. funestus*	*An. funestus/An. leesoni*	*An. leesoni*	Total
IRS Sites	Bo	Gerihun (peri-urban)		364	125		489	137	1	8	146
		Largor (rural)		698	95		793	38		3	41
	Bombali	Kamaranka (rural)		47	304		351	2			2
		Masongbo (peri-urban)	1	158	221		380	2			2
Non-IRS Sites	Kono	Sorie Town (peri-urban)		40	105		145	16			16
		Teikor (rural)	1	39	166		206	24		1	25
	Port Loko	Bakolo (rural)		375	71	33	479				
		New Maforki (peri-urban)		565	128		693	4			4
	Western Rural	Sand Sand Water (rural)		59	198	4	261				
		Tombo (peri-urban)		56	171	10	237				
Total			2	2,401	1,584	47	4,034	223	1	12	236

Table 4: Generalized Linear Model Investigating the Effect of Species on Outdoor/ Indoor Biting Behavior of *Anopheles* Mosquitoes

		Estimate	Std. Error	z Value	Pr(>|z|)	*p*
Factor	(Intercept)	1.13	0.11	1.06	0.29	0.289
Species	*An. gambiae*	ref				
	An. melas	**0.33**	**0.17**	**-6.50**	**0.00**	**<0.001**
	An. coluzzii	1.07	0.07	0.98	0.32	0.325
	An. funestus	**2.66**	**0.20**	**5.00**	**0.00**	**<0.001**
	An. leesoni	2.36	0.71	1.20	0.23	0.229
	Hybrid (*An. coluzzii/An. gambiae)*	**0.08**	**1.05**	**-2.39**	**0.02**	**<0.05**

Notes: Estimates are odds ratios indicating likelihood of mosquitoes being sampled biting either indoor or outdoor. Value above 1 indicates more likely to be sampled indoor than outdoor while value below 1 indicate likelihood of being sampled outdoor. Bold values are those with significant odds ratios.

Figure 1: Biting Location of *An. coluzzii* and *An. gambiae* s.s. by Site and District, March 2020-February 2021

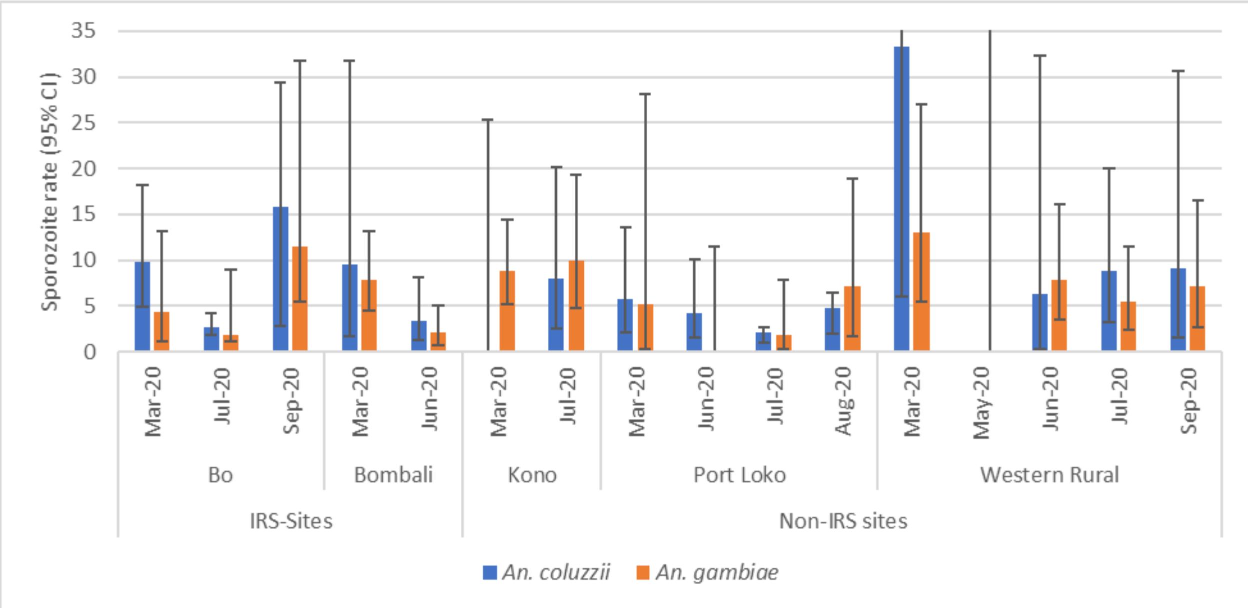


3.1.2 *PLASMODIUM* INFECTION

A total of 5,589 *An. gambiae* s.l. and 322 *An. funestus* s.l. were tested for *Plasmodium* infection, and 282/5,911 (4.77 %) were found to be positive. *Plasmodium* infection was detected in 4.94% (276/5,589) of *An. gambiae* s.l. and 1.86% (6/322) of *An. funestus* s.l. The majority of *An. gambiae* s.l. screened for *Plasmodium* sporozoite infection were *An. coluzzii* followed by *An. gambiae* s.s. (Annex B) and agrees with overall molecular species identification results in the annual report. Of the mosquitoes screened for *Plasmodium* sporozoite, 48% were identified to sibling species using PCR. *Plasmodium* infection in *An. gambiae* s.s. was the highest at 6.0% (75/1,250) followed by *An. coluzzii* 4.66% (65/1,394) and *An. melas* 2.94% (1/34) (Annex B). There were only six hybrids of *An. gambiae* and *An. coluzzii* and none were infected with *Plasmodium* parasites. Only six out of 322 (1.86%) *An. funestus* s.l. were positive for *Plasmodium* sporozoites (Annex B). All six sporozoite positive *An. funestus* s.l. were collected in Bo (four in Gerihun and two in Largor) where four were collected indoor while two outdoor. A sub-sample of 304 *An. coustani* were also screened for *Plasmodium,* 10 of which were found to be infected. However, the results were discarded because the laboratory failed to boil the mixture during their processing. Failure to boil have been associated with false positivity in secondary malaria vectors. This error has now been corrected in the SOP for CSP-ELISA.

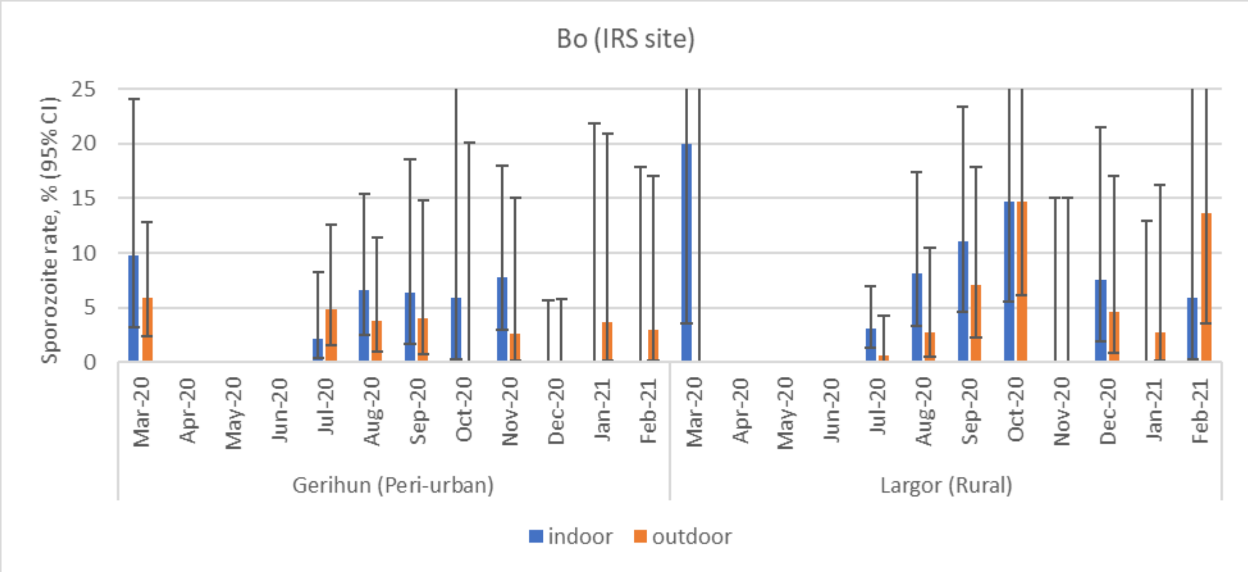
The sporozoite rate, defined as number of mosquitoes that tested positive divided by the total mosquitoes screened for *Plasmodium* sporozoites, varied between sites, species, and months (Figures 2 and 3). Sporozoite rate was highest in *An. gambiae* s.l. in Western Rural District in March 2020 (Figure 2). Two districts, Bo and Bombali, were selected to receive IRS with a clothianidin-based insecticide in May 2021. These districts were selected because they had malaria prevalence of 38% to 40% and had routine entomological data from longitudinal monitoring (Malaria Indicator Survey 2016)[1]. The data presented in this report, however, was collected before IRS was implemented in May 2021.

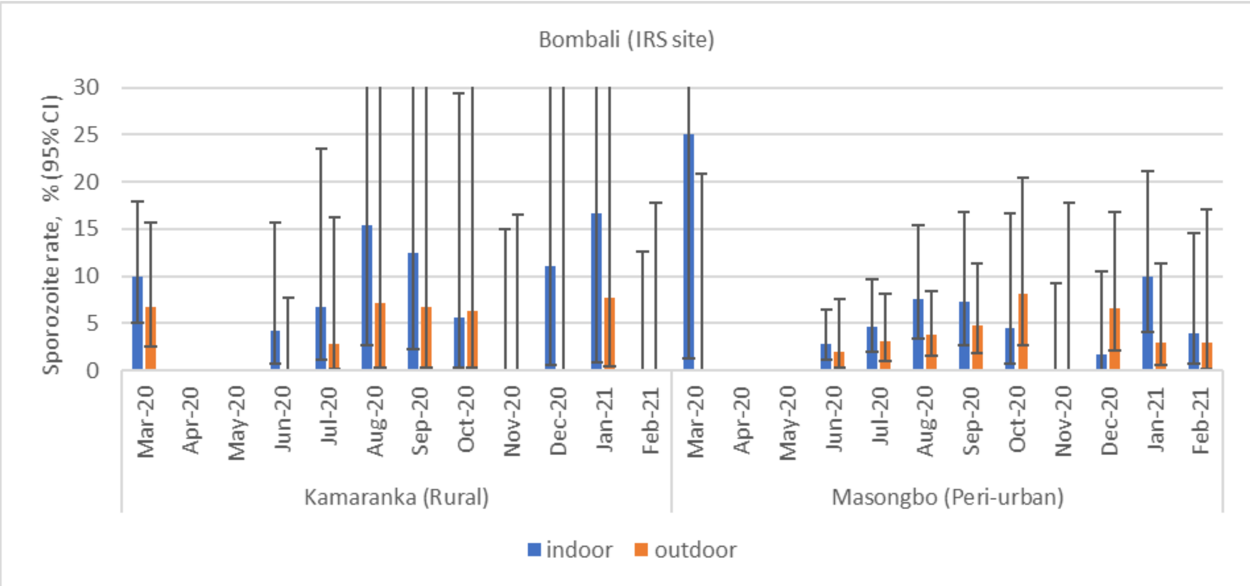
Figure 2: Sporozoite Rates of *An. coluzzii* and *An. gambiae* Collected Indoor and Outdoor by Human Landing Catch by Month and Site

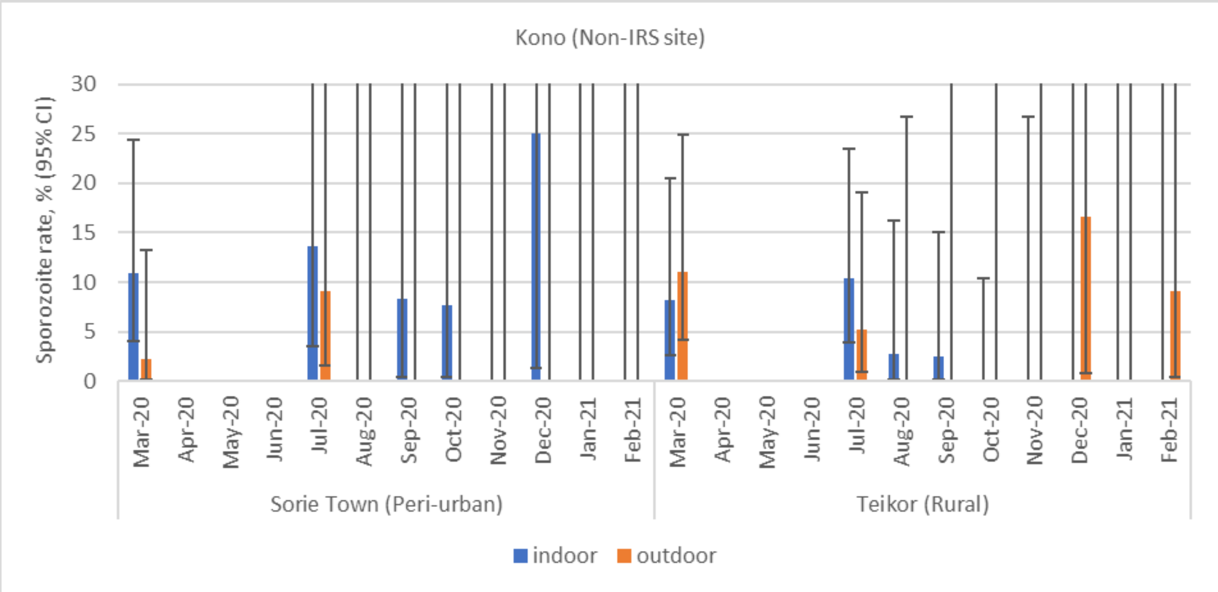


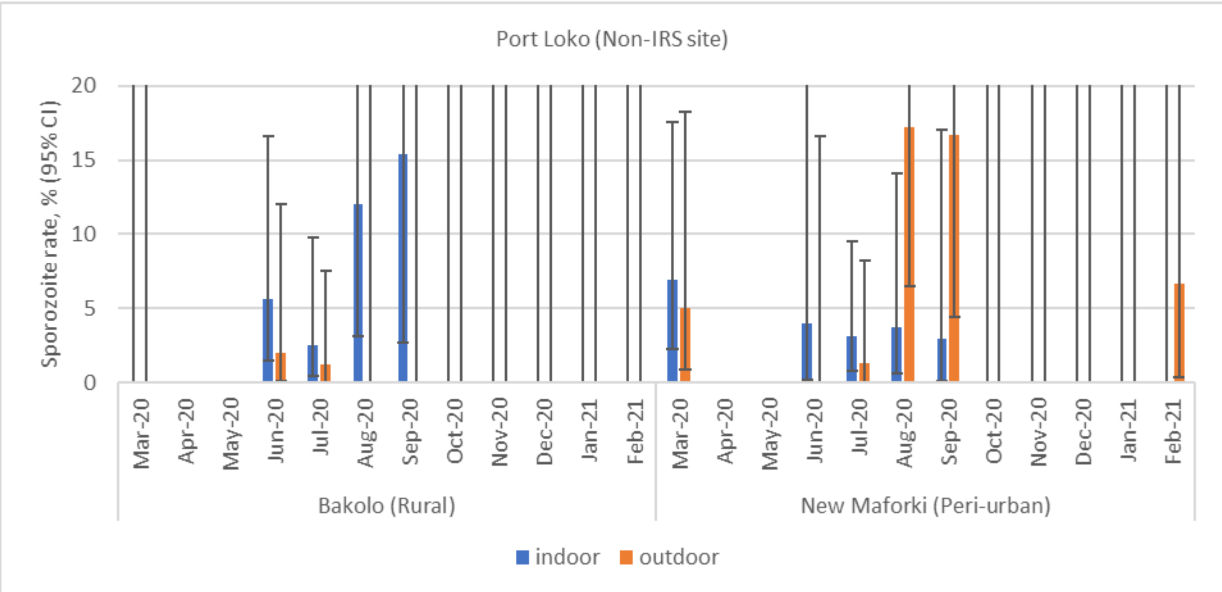
[1] National Malaria Control Programme (NMCP) [Sierra Leone], Statistics Sierra Leone (SSL), University of Sierra Leone, Catholic Relief Services (CRF), ICF. 2016. *Sierra Leone Malaria Indicator Survey 2016*. Freetown, Sierra Leone: NMCP, SSL, CRS, ICF.

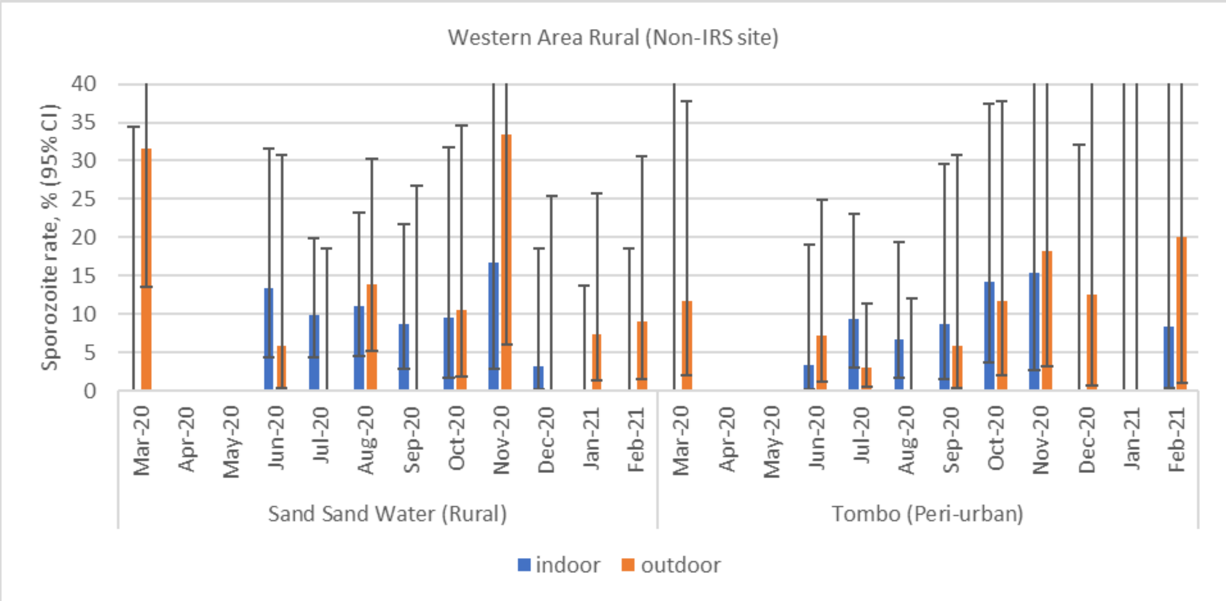
Figure 3: Sporozoite Rates in *An. gambiae* s.l. Collected Indoor and Outdoor by Human Landing Catch in IRS Intervention Sites and Non-IRS Sites between March 2020 and February 2021






Kono (Non-IRS site)
Sporozoite rate, % (95% CI)
30
25
20
15
10
5
0
Mar-20
Apr-20
May-20
Jun-20
Jul-20
Aug-20
Sep-20
Oct-20
Nov-20
Dec-20
Jan-21
Feb-21
Sorie Town (Peri-urban)
Teikor (Rural)
indoor
outdoor


Port Loko (Non-IRS site)
Sporozoite rate, % (95% CI)
20
15
10
5
0
Mar-20
Apr-20
May-20
Jun-20
Jul-20
Aug-20
Sep-20
Oct-20
Nov-20
Dec-20
Jan-21
Feb-21
Bakolo (Rural)
New Maforki (Peri-urban)
indoor
outdoor



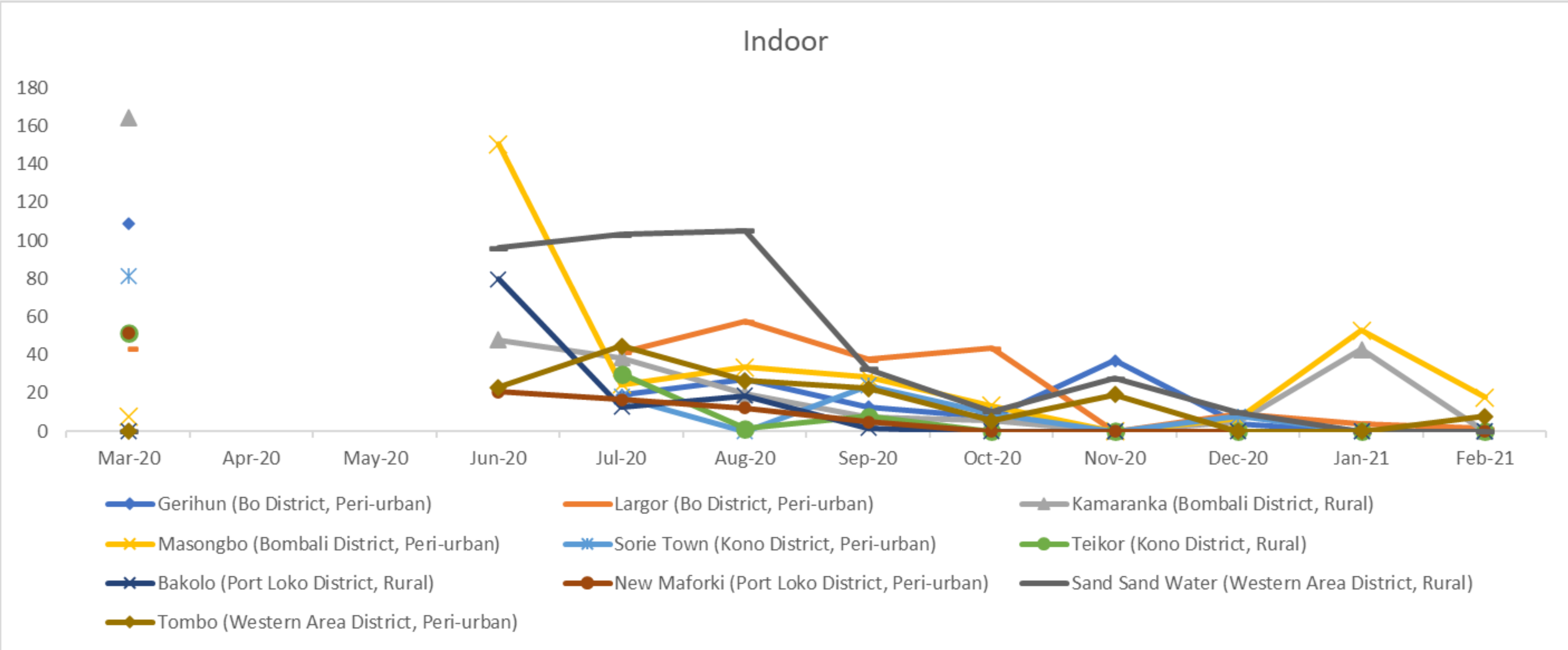
Notes: There were no collections in April, May, and June 2020 due to Covid-19 apart from Bakolo, New Maforki, Sand Sand Water and Tombo where collections were done in June. Detailed number of samples screened for *Plasmodium* are provided in annexes B – K).

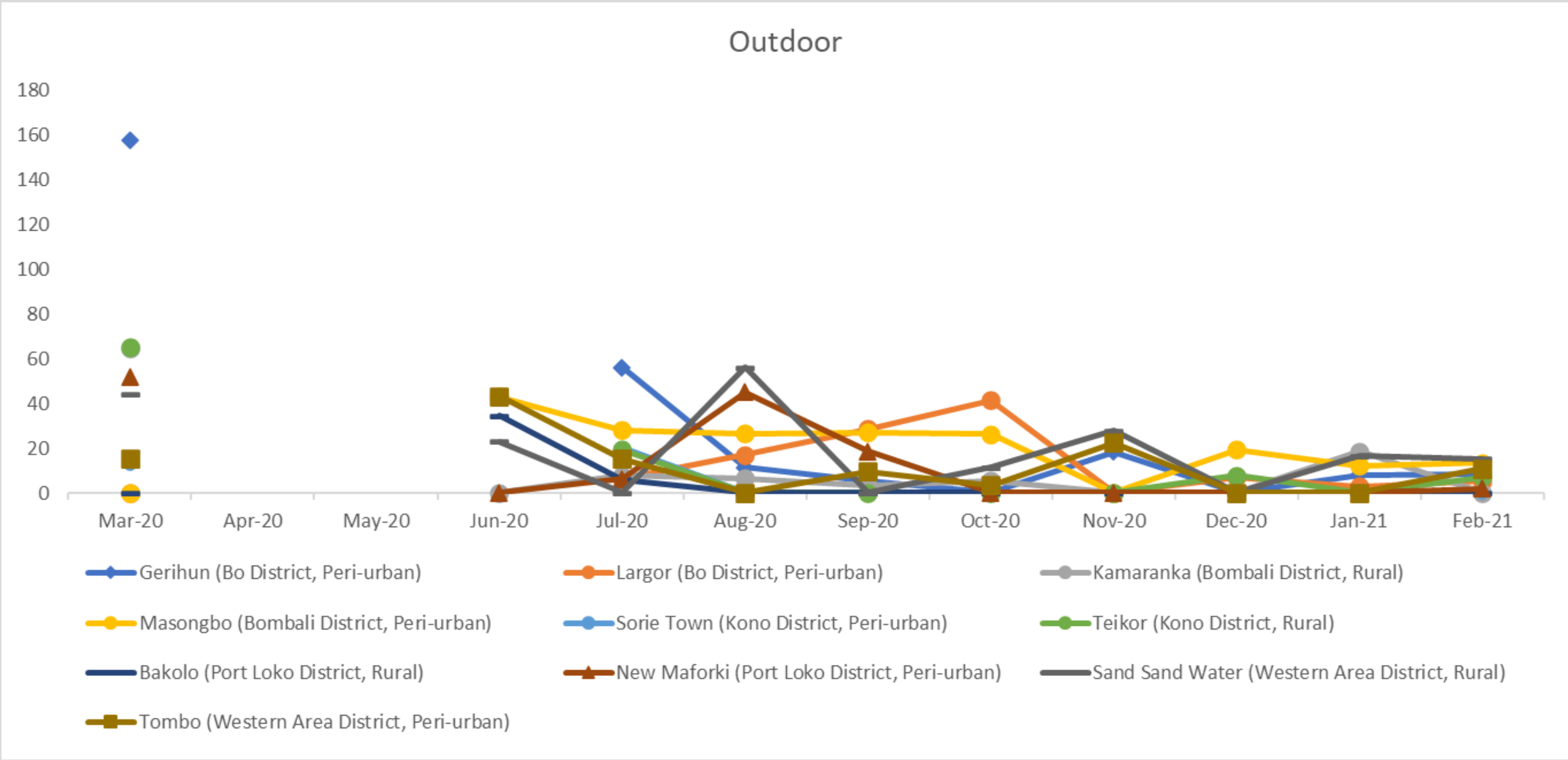
3.1.3 Entomological Inoculation Rate

The Entomological Inoculation Rate (EIR), expressed as the number of infective bites per person per unit time, either month (i/b/p/m), season (i/b/p/s) or year (i/b/p/y) was estimated for each site during the sampling period of March 2020 - February 2021. Due to Covid-19, there were no collections between April and June 2020, apart from Bombali District (Kamaranka and Masongbo), Port Loko District (Bakolo and New Maforki) and Western Area Rural (Sand Sand Water and Tombo) where collections were done in June. Seasonal EIR was estimated with wet season EIR covering between June-October 2020, while dry season covering from November 2020 to February 2021 and March 2020.The annual EIR estimation has been calculated using the months during which actual sampling took place, between March 2020 and February 2021. Thus, aEIR is the sum of March EIR, wet EIR and dry EIR.

The highest annual EIR (aEIR) and wet season EIR (sEIR) was recorded in Sand Sand Water (rural) in Western Area District (384.4 i/b/p/y and 347.15 i/b/p/s) followed by Masongbo (peri-urban) village in Bombali District (337.6 i/b/p/y and 252.3 i/b/p/s) (Figure 4, Annexes B-K). Sand Sand Water is a rural site in the West Coast region that has persistent mosquito breeding habitats while Masongbo is a peri-urban site that is closer to irrigation swamps that contributes to high mosquito density. There was no clear pattern between the dry and wet seasons in the EIR in the sentinel sites. The data indicates that people are potentially getting infective bites during the wet and dry seasons and both indoors and outdoors, though overall EIRs are slightly higher in the wet months than the dry season (Figure 4).

Figure 4: Monthly Entomological Inoculation Rates (mEIR) by Site, March 2020-February 2021




Outdoor
180
160
140
120
100
80
60
40
20
0
Mar-20
Apr-20
May-20
Jun-20
Jul-20
Aug-20
Sep-20
Oct-20
Nov-20
Dec-20
Jan-21
Feb-21
Gerihun (Bo District, Peri-urban)
Largor (Bo District, Peri-urban)
Kamaranka (Bombali District, Rural)
Masongbo (Bombali District, Peri-urban)
Sorie Town (Kono District, Peri-urban)
Teikor (Kono District, Rural)
Bakolo (Port Loko District, Rural)
New Maforki (Port Loko District, Peri-urban)
Sand Sand Water (Western Area District, Rural)
Tombo (Western Area District, Peri-urban)

The monthly EIR (mEIR) for all sites combined peaked in June (for those that sampling was done) and decreased progressively into the dry season till February (Figure 5). Indoor mEIR seemed to be higher compared to outdoor mEIR (Figure 5), and when data was aggregated by IRS intervention and non-IRS sites (Figure 6). The mEIR for each site per district varied by month and site (Figures 7 and 8). The IRS districts of Bo and Bombali recorded the highest mEIR, indicating the intense transmission in those districts. The highest mEIR was recorded indoor in Kamaranka, Bombali District in March 2020 with 164.4 i/b/p/m (Figure 7). This was followed by outdoor mEIR of 157.7 i/b/p/m in Gerihun in March (Figure 7). In all but Tombo, Sand Sand Water, Bakolo and Masongbo sites, mEIR was always highest in March 2020 (Figures 7 and 8). There was no clear relationship between mEIR estimates within sites and rainfall pattern in Sierra Leone probably because there was no sampling in April-June 2020. In addition, there was a night-time curfew that limited the entomology team to do supervision during the human landing catches that could have impacted the sampling exercises.

Figure 5: Monthly Mean Indoor and Outdoor Entomological Inoculation Rate (mEIR) of *An. gambiae* s.l. in All Sites, March 2020–February 2021

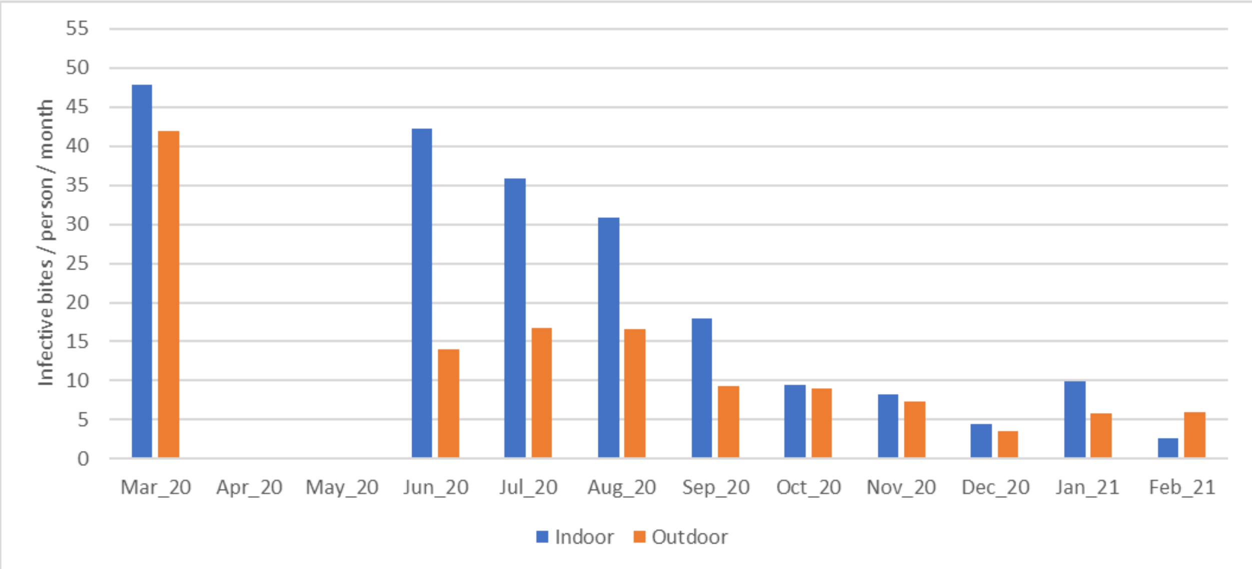


Figure 6: Monthly Indoor and Outdoor Entomological Inoculation Rate (mEIR) of *An. gambiae* s.l. in Indoor Residual Spraying (IRS) and Non-IRS Districts by Month, March 2020–February 2021.

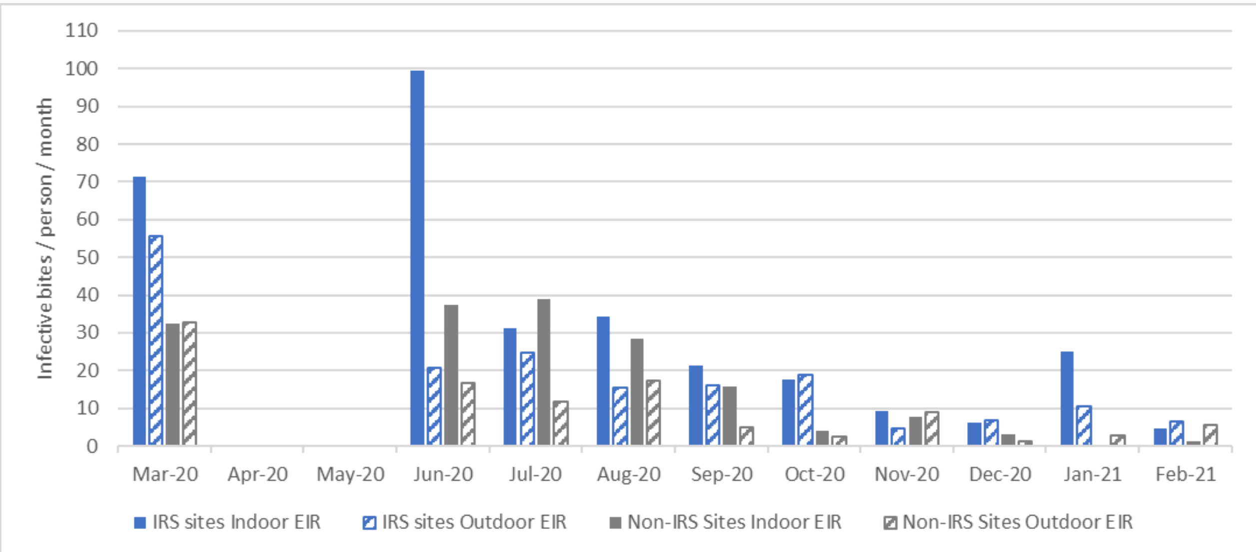
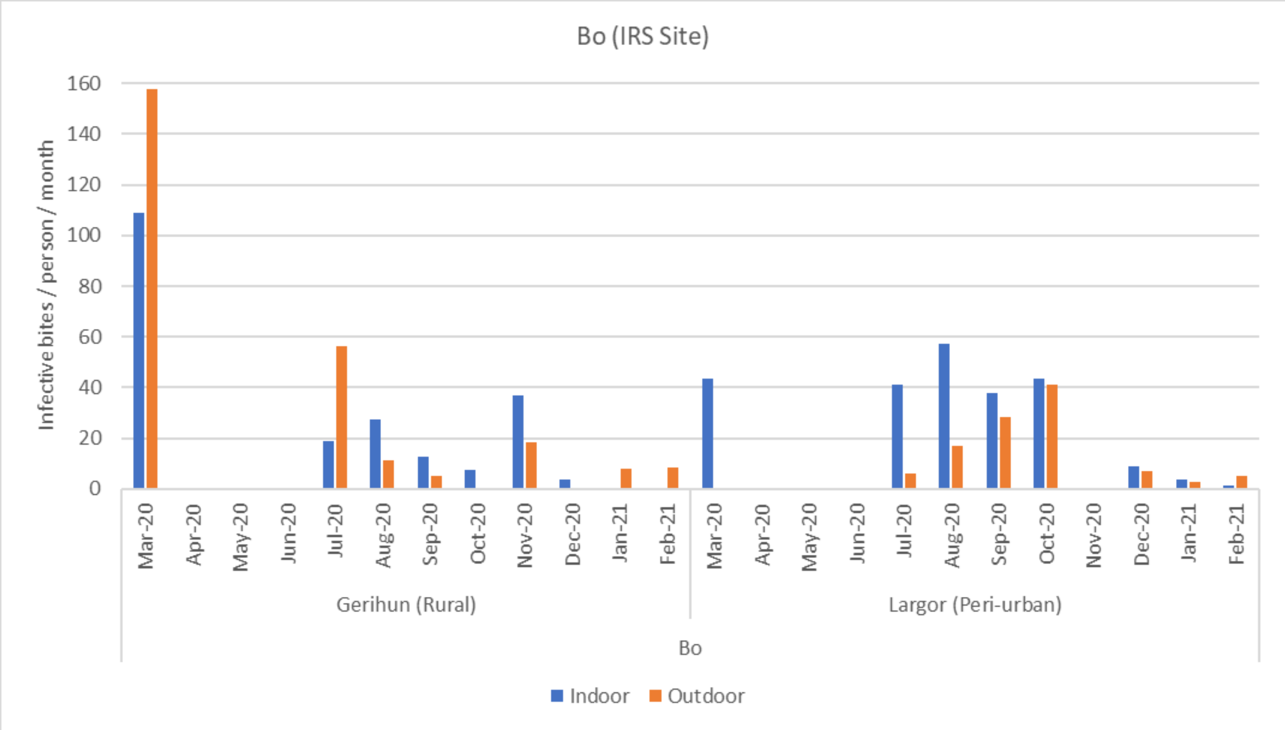


Figure 7: Monthly Indoor and Outdoor Entomological Inoculation Rate (mEIR) of *An. gambiae* s.l. in Indoor Residual Spraying (IRS) Districts by Month and Site, March 2020–February 2021.



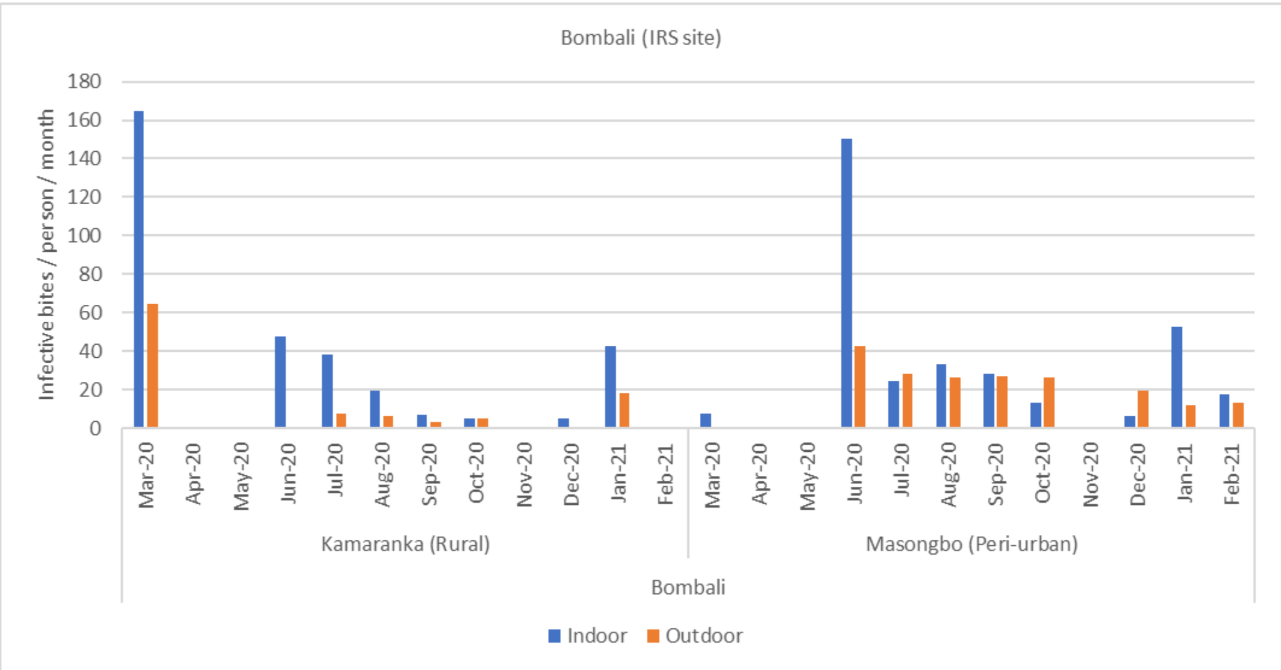
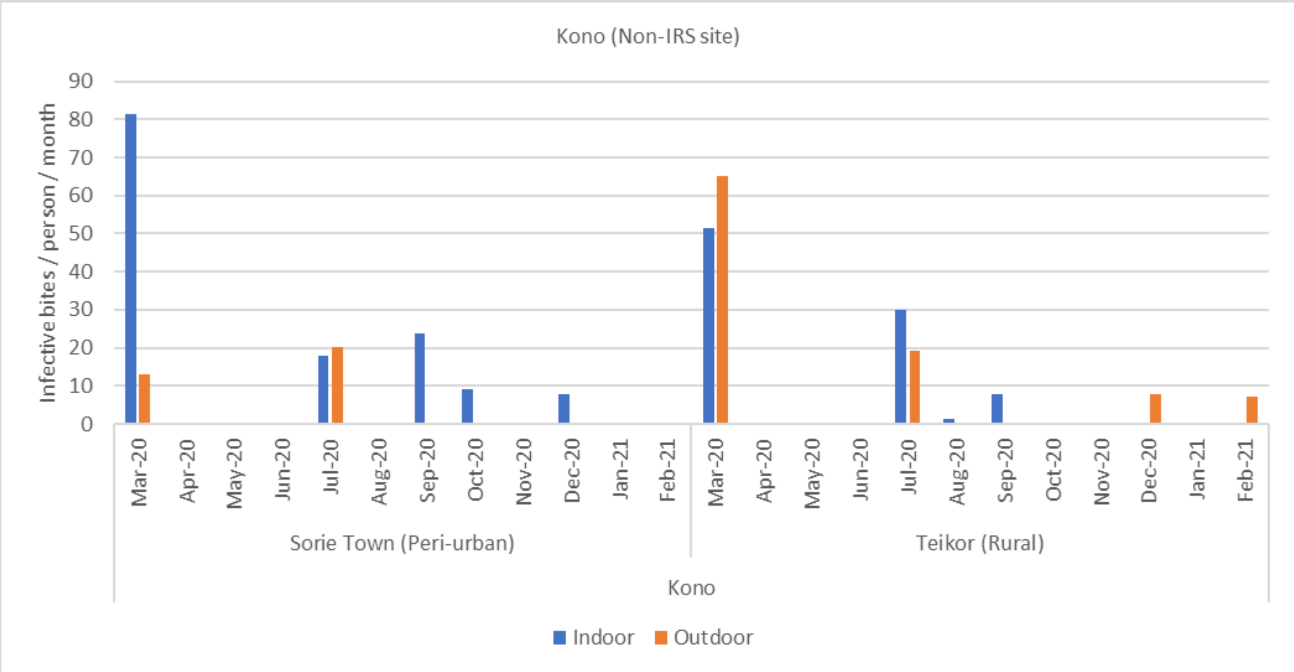
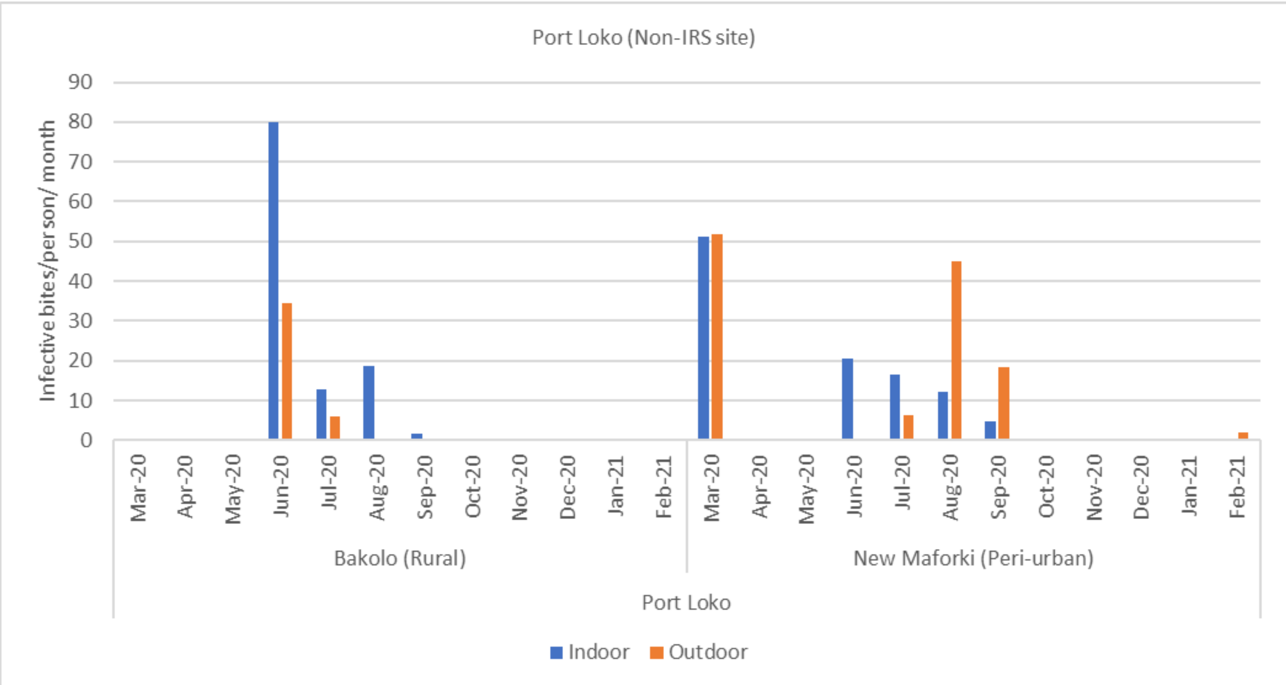
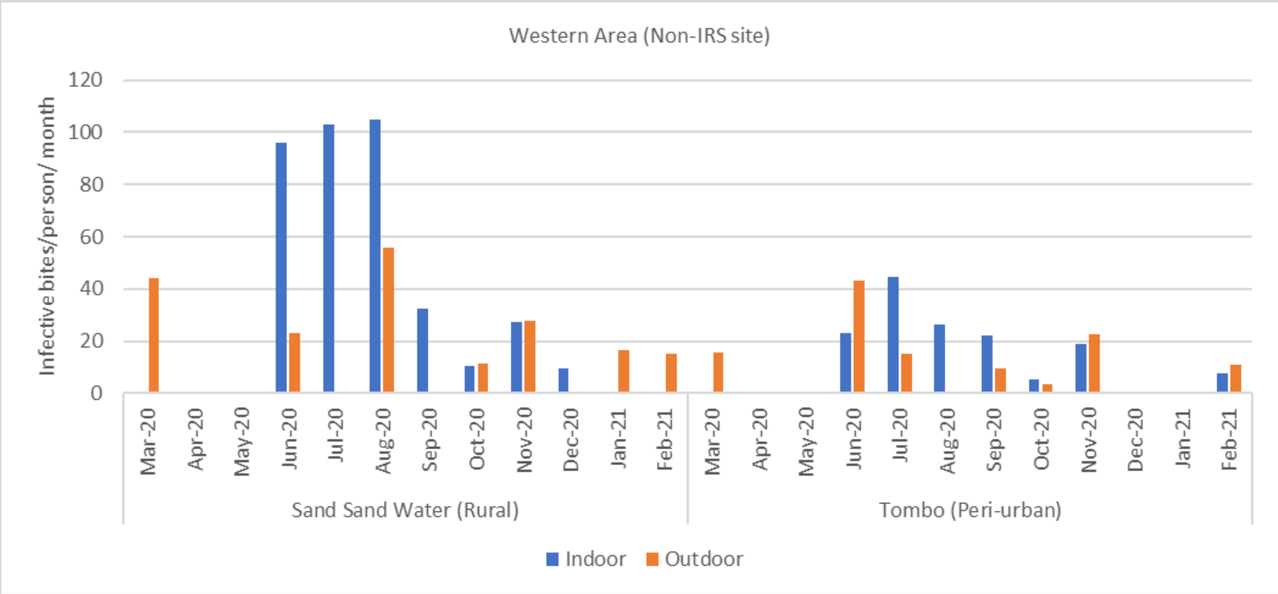


Figure 8: Monthly Indoor and Outdoor Entomological Inoculation Rate (mEIR) of *An. gambiae* s.l. in Non-Indoor Residual Spraying (non-IRS) Districts by Month and Site, March 2020–February 2021.







3.1.4 Frequency of the *Vgsc*-1014F, 1014S, N1575Y and G119S Alleles; Mechanism of Insecticide Resistance

In 2020, a sub sample of mosquitoes from the susceptibility tests were screened for the presence of *kdr-w* (L1014F), *kdr-e* (L1014S), and *ace-1*(G119S). A total of 455 mosquitoes out of 4,649 were assayed for molecular species identification and screened for resistance mutations. Of these, 14 were not identified to species level. Of the remaining 441 that were successfully identified, 85.9% were *An. gambiae* s.s. while 14.0% were *An. coluzzii*. This distribution is different from the species identification on adults collected via HLC which showed *An. coluzzii* as predominant. This is probably because close family lines may be sampled during larvae collection particularly when there are limited breeding habitats.

In all sites, in both species, *kdr-w* mutation was fixed in the sampled mosquitoes indicating high level of insecticide resistance in Sierra Leone (Table 5). The frequency of *kdr-e* mutation was low in all sites in both species (Table 4). The *ace-1*mutation that confers resistance to carbamates/organophosphates is present in Sierra Leone although at low frequency (Table 5). The highest frequency of *ace-1*was in *An. gambiae* s.s. in Kono and Western Area rural districts (Table 5). This could mean that the use of carbamates/organophosphates pesticides in agriculture could have an impact, because the National Malaria Control Program (NMCP) does not use carbamates/organophosphates for malaria control. Regular monitoring of the *Ace-1* distribution would be important in making decisions in the selection of insecticides for vector control. To better understand the frequency distribution, additional samples would need to be sampled to cover a wider geographical area. Only 155 mosquitoes from Bo and Bombali districts were screened for presence of N1575Y mutation. Two of these were homozygous resistant while 12 were heterozygous resistant (Table 6).

Table 5: Distribution of Insecticide Resistance Mutation to Pyrethroids (*Kdr-w/e*) and Carbamates/Organophosphates (*Ace-1*), in *An. gambiae* s.l. Sampled in the Rainy Season between May and October 2020 in Sierra Leone.

		Kdr-w					*Kdr*-e					*Ace-1*				
District	Species	WW	WS	SS	f (W)	% WW	EE	ES	SS	f (E)	%EE	AG	AG	GG	f (A)	%AA
Bo	*An. coluzzii*	32	11	0	0.87	74.4	0	0	43	0.0	0	1	2	40	0.0	2.3
	An. gambiae s.s.	49	1	0	0.99	98.0	0	0	51	0.0	0	4	5	41	0.1	8.0
Bombali	*An. coluzzii*	5	0	1	0.83	83.3	0	3	3	0.5	0	0	0	6	0.0	0.0
	An. gambiae s.s.	89	2	0	1.0	97.8	0	0	91	0.0	0	3	2	86	0.0	3.3
Kono	*An. coluzzii*	7	0	0	1.0	100.0	0	0	7	0.0	0	1	0	6	0.1	14.3
	An. gambiae s.s.	74	0	0	1.0	100.0	0	0	74	0.0	0	19	0	55	0.3	25.7
Port Loko	*An. coluzzii*	1	0	0	1.0	100.0	0	0	1	0.0	0	0	0	1	0.0	0.0
	An. gambiae s.s.	68	0	0	1.0	100.0	0	0	68	0.0	0	8	0	60	0.1	11.8
Western rural area	*An. coluzzii*	5	0	0	1.0	100.0	0	0	5	0.0	0	1	0	4	0.2	20.0
	An. gambiae s.s.	93	0	1	0.99	98.9	0	0	94	0.0	0	27	0	66	0.3	29.0
Grand Total		423	14	2	0.98	96.4	0	3	437	0.0	0	64	9	365	0.2	14.6

Frequency (f) of the resistance allele [(2WW + WS)/2(WW+WS+SS)]

W=west mutation (Phenylalanine); E = east mutation (Serine); A =*Ace-1* mutation (Serine); S=susceptible (Leucine); G=susceptible (Glycine)

Table 6: Distribution of Insecticide Resistance Mutation to Pyrethroids (N1575Y), in *An. gambiae* s.l., Sampled in the Rainy Season between May and October 2020 in Sierra Leone.

District	PCR M/S	YY	YN	NN	f (Y)	%YY
Bo	*An. coluzzii*	0	0	24	0	0
	An. gambiae s.s.	1	0	33	0.03	2.94
Bombali	*An. coluzzii*	0	0	6	0	0
	An. gambiae s.s.	1	12	78	0.08	1.10
Total		2	12	141	0.05	1.29

Frequency (f) of the resistance allele [(2YY + YN)/2(YY+YN+NN)]; Y=mutation (Tyrosine)

3.1.5 VERIFICATION OF *AN. GAMBIAE* S.S. KISUMU COLONY

In 2020, a subsample of 100 laboratory colony strain was sent to CRID for molecular analysis. Two mosquitoes were not identified to species level while the remaining 98 were identified as *An. gambiae* s.s. (Table 7). There was no *Kdr-w* mutation in the samples tested. However, the frequency of *Kdr-et* was over 80% for both Ghana and Rwanda sources (Table 7). The source of contamination has not been resolved; however, it is probable that the mosquitoes were brought into Sierra Leone with low frequency of *kdr-east* and the population crash that occurred when the colony in Makeni crashed could have led to genetic drift resulting in fixation of *kdr-e* in the insectary population. The crash in 2021 was due to change in larvae food from tropical fish flakes to cold water flakes, which resulted in slowed larvae development with reduced fecundity in adult females. Sierra Leone is in the process of rearing a new colony from MR4 (CDC-Atlanta) and has now regained full productivity. The MR4 laboratory undertakes routine screening of its insectary mosquitoes for insecticide resistance mutations and the eggs supplied are expected to be free of any resistance mutations. However, the colony will also be regularly screened to detect any contamination as early as possible.

Table 7: Distribution of Insecticide Resistance Mutation to Pyrethroids (*Kdr-w/e*) in Susceptible Laboratory Colony in Makeni, Sierra Leone, 2021

		kdr-w		***kdr-e***				
Strain source	species	SS	Total	EE	ES	SS	f (E)	% EE
Ghana	*An. gambiae* s.s.	45	45	37	8		0.9	82.2
	N. A	5	5	4		1	0.8	80.0
Rwanda	*An. gambiae* s.s.	49	49	31	16	2	0.8	63.3
	N. A	1	1	1			1.0	100.0
Total		100	100	73	24	3	0.9	73.0

Frequency (f) of the resistance allele [(2EE + ES)/2(EE+ES+SS)]
W=west mutation (Phenylalanine); E = east mutation (Serine); S=susceptible (Leucine)

4. Discussion

Molecular analyses revealed that *An. coluzzii* was the predominant species followed by *An. gambiae* s.s. across all sites apart from Western Area and Kono districts. Although *An. coluzzii* was the predominant vector during the sampling period, *An. gambiae* s.s. was more likely to be infected with malaria sporozoites. Between March- May 2020, no sampling took place due to the Covid-19 pandemic. In June, only few sites were sampled as there was travel restrictions to allow proper coordination during the Covid-19 pandemic. There was a delay in laboratory analysis and the project sent over 15,000 samples to CRID to be screened for sporozoite infection... Subsequently, some samples for September were not screened for sporozoite infection. Sierra Leone is in the process of identifying a local laboratory to carry out molecular analysis of mosquito samples and this will boost processing of more samples and also reduce the logistical challenge involved with shipment of samples.

The majority of samples tested for susceptibility to insecticides using WHO tube tests and CDC bottle assays were *An. gambiae* s.s. These were collected from larval breeding grounds and reared to adults before being exposed to insecticides in WHO tubes or CDC bottle assays. This species distribution is different compared to distribution estimated from adults collected during HLC, PSC and CDC-light traps where *An. coluzzii* was the predominant vector (2020 annual entomology report).

The *kdr-w* (L1014F) allele was almost fixed in all the species in all sites, and this agrees with the reported high insecticide resistance to pyrethroids in Sierra Leone. The *ace-1* mutation that confers resistance to carbamates/organophosphates was found in Kono and Western Area districts and is a cause for concern for Sierra Leone. If it spreads, then it may limit the choice of insecticides to use in IRS such as using organophosphates (pirimiphos-methyl) in a rotation strategy for resistance management. The *ace-1* mutation is known to have a strong relationship in mosquitoes that survive exposure to carbamates. Although the 2020 samples that had the *ace-1* mutation died following exposure to pirimiphos-methyl, further/additional testing is needed to ascertain the role of *ace-1* in conferring resistance to organophosphates in Sierra Leone. Additional monitoring of insecticide resistance and their resistance mechanisms is ongoing for 2021 and this will be useful for the NMCP in planning the management of insecticide resistance in Sierra Leone.

To ensure accurate assessment of insecticide resistance and to monitor the durability of ITNs, it is necessary to have a susceptible laboratory colony to compare with wild caught mosquitoes. In 2020, the insectary mosquitoes in Sierra Leone were contaminated by mosquitoes harboring the *kdr-e* (L1014S) allele. This was detected at lower frequency in 2020 but analysis of the colony in mid-2021 indicated that the mutation had become fixed in the insectary colony. From the analysis of wild collected samples, the frequency of *kdr*-e is very low compared to *kdr*-w that is fixed in wild populations. This implies that the contamination may have come from the source laboratories, albeit at lower frequencies, but increased in frequency through a phenomenon called genetic drift. This happens when catastrophic events reduce populations to very small numbers and when the small population re-grows, the surviving mosquitoes form majority of gene pool, making an allele fixed. In Sierra Leone, two crash events occurred in 2020 and in mid-2021 at the Makeni insectary that resulted in the loss of almost the entire colony. The samples analyzed in this report were sampled in July 2021 after the crash.

In conclusion, both *An. gambiae* s.s. and *An. coluzzii* are the principal vectors of malaria in Sierra Leone and the *kdr-w* mutation is fixed in both species. The *kdr-w* mutation is responsible for insecticide resistance in Sierra Leone. NMCP and VectorLink continually monitor insecticide resistance to guide selection of insecticides for use in IRS and for the optimal type of ITN. In May 2021, data on alpha-cypermethrin and chlorfenapyr was used as justification for the country to procure the dual active ingredient Interceptor® G2 Nets (alpha-cypermethrin and chlorfenapyr). The NMCP and the VectorLink project have put in place measures to ensure that sporozoite data is monitored each month in order to document monthly malaria transmission.

Annex A: *Plasmodium* Infection Rate by Species, All sites, March 2020 to February 2021

Molecular IDs	District	Month	Negative	Sporozoite Positive	Total	% Positive
An. coluzzii	Bo	Mar-20	83	9	92	9.8
		Jul-20	433	12	445	2.7
		Sep-20	16	3	19	15.8
	Bombali	Mar-20	19	2	21	9.5
		Jun-20	143	5	148	3.4
	Kono	Mar-20	15	0	15	0.0
		Jul-20	46	4	50	8.0
	Port Loko	Mar-20	81	5	86	5.8
		Jun-20	113	5	118	4.2
		Jul-20	464	10	474	2.1
		Aug-20	159	8	167	4.8
	Western Rural	Mar-20	4	2	6	33.3
		Jun-20	15	1	16	6.3
		Jul-20	52	5	57	8.8
		Sep-20	20	2	22	9.1
Total			**1,663**	**73**	**1,736**	4.2
An. gambliae s.s.	Bo	Mar-20	65	3	68	4.4
		Jul-20	53	1	54	1.9
		Sep-20	23	3	26	11.5
	Bombali	Mar-20	163	14	177	7.9
		Jun-20	237	5	242	2.1
	Kono	Mar-20	155	15	170	8.8
		Jul-20	72	8	80	10.0
	Port Loko	Mar-20	18	1	19	5.3
		Jun-20	38	0	38	0.0
		Jul-20	52	1	53	1.9
		Aug-20	26	2	28	7.1
	Western Rural	Mar-20	40	6	46	13.0
		May-20	1	0	1	0.0
		Jun-20	82	7	89	7.9
		Jul-20	120	7	127	5.5
		Sep-20	65	5	70	7.1
Total			**1,210**	**78**	**1,288**	**6.1**

Molecular IDs	District	Month	Negative	Sporozoite Positive	Total	% Positive
An. melas	Port Loko	Jul-20	20	0	20	0.0
	Western Rural	Jul-20	13	1	14	7.1
Hybrid (*An. coluzzii*/ *An. gambiae*)	Bombali	Jun-20	1	0	1	0.0
	Port Loko	Jul-20	5	0	5	0.0
Total			**39**	**1**	**40**	2.5
An. funestus s.l.	Bo	Mar-20	9	0	9	0.0
		Jul-20	44	1	45	2.2
		Sep-20	31	0	31	0.0
		Oct-20	43	1	44	2.3
		Nov-20	26	1	27	3.7
		Dec-20	59	2	61	3.3
		Jan-21	28	1	29	3.4
	Bombali	Oct-20	9	0	9	0.0
		Dec-20	6	0	6	0.0
	Kono	Mar-20	13	0	13	0.0
		Jul-20	13	0	13	0.0
		Aug-20	9	0	9	0.0
		Nov-20	6	0	6	0.0
		Dec-20	4	0	4	0.0
	Port Loko	Jun-20	8	0	8	0.0
		Jul-20	3	0	3	0.0
		Aug-20	6	0	6	0.0
		Nov-20	2	0	2	0.0
	Western Rural	Oct-20	1	0	1	0.0
		Nov-20	1	0	1	0.0
		Dec-20	1	0	1	0.0
Total			**322**	**6**	**328**	**1.8**
Grand Total			**3,234**	**158**	**3,392**	**4.7**

Annex B: Sporozoite Rate and EIR of *An. gambiae* s.l. and *An. funestus* s.l. in Gerihun Peri-urban Site in Bo District, March 2020 to February 2021

Location	Month	Gerihun												
		Mar 20	**Apr 20**	**May 20**	**Jun 20**	**Jul 20**	**Aug 20**	**Sep 20**	**Oct 20**	**Nov 20**	**Dec 20**	**Jan 21**	**Feb 21**	**Annual EIR (9 months)**
***An. gambiae* s.l. (Indoor)**	Total collected	72				454	214	105	65	252	350	94	162	
	HBR	36				28.38	13.38	6.56	4.06	15.75	21.88	5.88	10.13	
	Total tested	41				93	76	47	17	64	80	18	23	
	Spz +ve	4				2	5	3	1	5	0	0	0	
	Sporozoite rate	0.10				0.02	0.07	0.06	0.06	0.08	0.00	0.00	0.00	
	Nightly Indoor EIR	3.51				0.61	0.88	0.42	0.24	1.23	0.00	0.00	0.00	
	Monthly EIR	**108.88**				**18.92**	**27.28**	**12.70**	**7.41**	**36.91**	**0.00**	**0.00**	**0.00**	
***An. funestus* s.l. (Indoor)**	Total collected	2				132	75	87	39	65	28	19	25	
	HBR	1				8.25	4.69	5.44	2.44	4.06	1.75	1.19	1.56	
	Total tested	2				21	0	31	21	10	28	16	0	
	Spz +ve	0				0	0	0	0	0	2	0	0	
	Sporozoite rate	0.00				0.00	0.00	0.00	0.00	0.00	0.07	0.00	0.00	
	Nightly Outdoor EIR	0.00				0.00	0.00	0.00	0.00	0.00	0.13	0.00	0.00	
	Monthly EIR	**0.00**				**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**3.88**	**0.00**	**0.00**	
Total Monthly EIR		**108.88**				**18.92**	**27.28**	**12.70**	**7.41**	**36.91**	**3.88**	**0.00**	**0.00**	**215.97**
An. gambiae* s.l. *(Outdoor)	Total collected	173				446	155	93	98	232	404	113	165	
	HBR	86.5				27.88	9.69	5.81	6.13	14.50	25.25	7.06	10.31	
	Total tested	102				83	79	50	20	39	79	27	34	
	Spz +ve	6				4	3	2	0	1	0	1	1	
	Sporozoite rate	0.06				0.05	0.04	0.03	0.00	0.03	0.00	0.04	0.03	
	Nightly Indoor EIR	5.09				1.34	0.37	0.18	0.00	0.37	0.00	0.26	0.30	
	Monthly EIR	**157.74**				**41.64**	**11.40**	**5.28**	**0.00**	**11.15**	**0.00**	**8.11**	**8.49**	

Location	Month	Gerihun												
		Mar 20	**Apr 20**	**May 20**	**Jun 20**	**Jul 20**	**Aug 20**	**Sep 20**	**Oct 20**	**Nov 20**	**Dec 20**	**Jan 21**	**Feb 21**	**Annual EIR (9 months)**
***An. funestus* s.l. (Outdoor)**	Total collected	2				105	52	64	27	38	37	23	17	
	HBR	1				6.56	3.25	4.00	1.69	2.38	2.31	1.44	1.06	
	Total tested	2				14	0	0	12	10	25	10	0	
	Spz +ve	0				1	0	0	0	1	0	0	0	
	Sporozoite rate	0.00				0.07	0.00	0.00	0.00	0.10	0.00	0.00	0.00	
	Nightly Outdoor EIR	0.00				0.47	0.00	0.00	0.00	0.24	0.00	0.00	0.00	
	Monthly EIR	**0.00**				**14.53**	**0.00**	**0.00**	**0.00**	**7.13**	**0.00**	**0.00**	**0.00**	
Total Monthly EIR		**157.74**				**56.18**	**11.40**	**5.28**	**0.00**	**18.28**	**0.00**	**8.11**	**8.49**	**265.48**

Annex C: Sporozoite Rate and EIR of *An. gambiae* s.l. in Largor Rural Site in Bo District, March 2020 to February 2021

Location	Month	Largor												Annual EIR (9 months)
		Mar 20	**Apr 20**	**May 20**	**Jun 20**	**Jul 20**	**Aug 20**	**Sep 20**	**Oct 20**	**Nov 20**	**Dec 20**	**Jan 21**	**Feb 21**	
Indoor	Total collected	14				685	366	197	137	60	61	46	12	
	HBR	7				42.81	22.88	12.31	8.56	3.75	3.81	2.88	0.75	
	Total tested	10				193	74	54	34	28	40	33	17	
	Spz +ve	2				6	6	6	5	0	3	0	1	
	Sporozoite rate	0.20				0.03	0.08	0.10	0.15	0.00	0.08	0.00	0.06	
	Nightly EIR	1.4				1.33	1.85	1.25	1.26	0.00	0.29	0.00	0.04	
	Monthly EIR	**43.4**				**41.26**	**57.50**	**37.62**	**39.03**	**0.00**	**8.86**	**0.00**	**1.24**	**228.91**
Outdoor	Total collected	9				478	319	217	146	46	78	56	22	
	HBR	4.5				29.88	19.94	13.56	9.13	2.88	4.88	3.50	1.38	
	Total tested	8				150	73	57	41	28	43	36	22	
	Spz +ve	0				1	2	4	6	0	2	1	3	
	Sporozoite rate	0				0.01	0.03	0.07	0.15	0.00	0.05	0.03	0.14	
	Nightly EIR	0				0.20	0.55	0.95	1.34	0.00	0.23	0.10	0.19	
	Monthly EIR	**0**				**6.17**	**16.93**	**28.55**	**41.40**	**0.00**	**7.03**	**3.01**	**5.25**	**108.35**

Annex D: Sporozoite Rate and EIR of *An. gambiae* s.l. in Kamaranka Rural Site in Bombali District, March 2020 to February 2021

Kamaranka														
Location	**Month**	**Mar 20**	**Apr 20**	**May 20**	**Jun 20**	**Jul 20**	**Aug 20**	**Sep 20**	**Oct 20**	**Nov 20**	**Dec 20**	**Jan 21**	**Feb 21**	**Annual EIR (8 months)**
Indoor	Total collected	163			150	308	65	39	50	29	24	133	173	
	HBR	40.75			37.5	19.25	4.06	2.44	3.13	1.81	1.50	8.31	10.81	
	Total tested	101			47	30	13	16	18	28	9	6	34	
	Spz +ve	10			2	2	2	2	1	0	1	1	0	
	Sporozoite rate	0.10			0.04	0.07	0.15	0.10	0.06	0.00	0.11	0.17	0.00	
	Nightly Indoor EIR	4.03			1.60	1.28	0.63	0.24	0.17	0.00	0.17	1.39	0.00	
	Monthly EIR	**164.41**			**47.87**	**38.50**	**19.38**	**7.08**	**5.38**	**0.00**	**5.17**	**42.95**	**0.00**	**330.73**
Outdoor	Total collected	123			200	287	48	27	45	27	17	124	137	
	HBR	30.75			50	17.93 75	3.00	1.69	2.81	1.69	1.06	7.75	8.56	
	Total tested	74			58	36	14	15	16	25	4	13	23	
	Spz +ve	5			0	1	1	1	1	0	0	1	0	
	Sporozoite rate	0.07			0.00	0.01	0.07	0.07	0.06	0.00	0.00	0.08	0.00	
	Nightly Outdoor EIR	2.08			0.00	0.25	0.21	0.11	0.18	0.00	0.00	0.60	0.00	
	Monthly EIR	**64.41**			**0.00**	**7.72**	**6.64**	**3.38**	**5.45**	**0.00**	**0.00**	**18.48**	**0.00**	**106.08**

Annex E: Sporozoite Rate and EIR of *An. gambiae* s.l. in Masongbo Peri-urban Site in Bombali District, March 2020 to February 2021

							Masongbo							
Location	**Month**	**Mar 20**	**Apr 20**	**May 20**	**Jun 20**	**Jul 20**	**Aug 20**	**Sep 20**	**Oct 20**	**Nov 20**	**Dec 20**	**Jan 21**	**Feb 21**	**Annual EIR (8 months)**
Indoor	Total collected	4			702	303	230	227	162	174	201	272	256	
	HBR	1			175.5	18.94	14.38	14.19	10.13	10.88	12.56	17.00	16.00	
	Total tested	4			209	151	93	69	44	48	58	60	51	
	Spz +ve	1			6	7	7	5	2	0	1	6	2	
	Sporozoite rate	0.25			0.03	0.04	0.08	0.07	0.05	0.00	0.02	0.10	0.04	
	Nightly EIR	0.25			5.04	0.82	1.08	0.93	0.46	0.00	0.22	1.70	0.63	
	Monthly EIR	**7.75**			**151.15**	**24.46**	**33.54**	**28.89**	**14.27**	**0.00**	**6.71**	**52.70**	**17.57**	**337.04**
Outdoor	Total collected	20			282	473	362	293	177	129	158	212	234	
	HBR	5			70.5	29.56	22.63	18.31	11.06	8.06	9.88	13.25	14.63	
	Total tested	19			102	130	158	104	49	23	61	67	34	
	Spz +ve	0			2	4	6	5	4	0	4	2	1	
	Sporozoite rate	0			0.02	0.03	0.04	0.05	0.08	0.00	0.07	0.03	0.03	
	Nightly EIR	0			1.38	0.91	0.86	0.88	0.90	0.00	0.65	0.40	0.43	
	Monthly EIR	**0**			**42.85**	**28.20**	**26.63**	**27.42**	**27.99**	**0.00**	**20.07**	**12.26**	**13.33**	**198.77**

Annex F: Sporozoite Rate and EIR of *An. gambiae* s.l. in Sorie Town Peri-urban Site in Kono District, March 2020 to February 2021

Sorie Town														
Location	**Month**	**Mar 20**	**Apr 20**	**May 20**	**Jun 20**	**Jul 20**	**Aug 20**	**Sep 20**	**Oct 20**	**Nov 20**	**Dec 20**	**Jan 21**	**Feb 21**	**Annual EIR (8 months)**
Indoor	Total collected	103				22	28	76	15	7	5	2	11	
	Routine	25.75				5.5	7	19	3.75	1.75	1.25	0.5	2.75	
	Total tested	46				22	11	12	13	5	4	2	11	
	Spz +ve	5				3	0	1	1	0	1	0	0	
	Sporozoite rate	0.11				0.14	0.00	0.04	0.08	0.00	0.25	0.00	0.00	
	Nightly EIR	2.80				0.75	0.00	0.79	0.29	0.00	0.31	0.00	0.00	
	Monthly EIR	**86.77**				**23.25**	**0.00**	**23.75**	**8.94**	**0.00**	**9.69**	**0.00**	**0.00**	**152.40**
Outdoor	Total collected	77				30	10	58	6	9	7	2	10	
	HBR	19.25				7.5	2.5	14.5	1.5	2.25	1.75	0.5	2.5	
	Total tested	45				22	3	5	6	6	4	2	9	
	Spz +ve	1				2	0	0	0	0	0	0	0	
	Sporozoite rate	0.02				0.09	0.00	0.00	0.00	0.00	0.00	0.00	0.00	
	Nightly EIR	0.43				0.68	0.00	0.00	0.00	0.00	0.00	0.00	0.00	
	Monthly EIR	**13.26**				**21.14**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**34.40**

Annex G: Sporozoite Rate and EIR of *An. gambiae* s.l. in Teikor Rural Site in Kono District, March 2020 to February 2021

Teikor														
Location	**Month**	**Mar 20**	**Apr 20**	**May 20**	**Jun 20**	**Jul 20**	**Aug 20**	**Sep 20**	**Oct 20**	**Nov 20**	**Dec 20**	**Jan 21**	**Feb 21**	**Annual EIR (8 Months)**
Indoor	Total collected	88				41	7	86	42	15	5	18	9	
	HBR	22				10.25	1.75	21.5	10.5	3.75	1.25	4.5	2.25	
	Total tested	49				48	36	39	42	14	5	4	9	
	Spz +ve	4				5	1	1	0	0	0	0	0	
	Sporozoite rate	0.08				0.10	0.03	0.01	0.00	0.00	0.00	0.00	0.00	
	Nightly Indoor EIR	1.80				1.07	0.05	0.28	0.00	0.00	0.00	0.00	0.00	
	Monthly EIR	**55.67**				**33.10**	**7.75**	**8.27**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**104.79**
Outdoor	Total collected	84				51	7	69	8	10	6	15	11	
	HBR	21				12.75	1.75	17.25	2	2.5	1.5	3.75	2.75	
	Total tested	45				38	14	7	0	9	6	6	11	
	Spz +ve	5				2	0	0	0	0	1	0	1	
	Sporozoite rate	0.11				0.05	0.00	0.00	0	0.00	0.17	0.00	0.09	
	Nightly EIR	2.33				0.67	0.00	0.00	0	0.00	0.25	0.00	0.25	
	Monthly EIR	**72.33**				**20.80**	**0.00**	**0.00**	**0.00**	**0.00**	**7.75**	**0.00**	**7.00**	**107.89**

Annex H: Sporozoite Rate and EIR of *An. gambiae* s.l. in Bakolo Rural Site in Port Loko District, March 2020 to February 2021

Bakolo														
Location	**Month**	**Mar 20**	**Apr 20**	**May 20**	**Jun 20**	**Jul 20**	**Aug 20**	**Sep 20**	**Oct 20**	**Nov 20**	**Dec 20**	**Jan 21**	**Feb 21**	**Annual EIR (9 months)**
Indoor	Total collected	3			188	255	80	7	1	2	7	11	8	
	HBR	0.75			47	15.94	5.00	0.44	0.06	0.13	0.44	0.69	0.50	
	Total tested	3			53	78	25	13	1	2	3	2	8	
	Spz +ve	0			3	2	3	2	0	0	0	0	0	
	Sporozoite rate	0			0.06	0.03	0.12	0.12	0.00	0.00	0.00	0.00	0.00	
	Nightly EIR	0			2.66	0.41	0.60	0.05	0.00	0.00	0.00	0.00	0.00	
	Monthly EIR	**0**			**79.81**	**12.67**	**18.60**	**3.35**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**114.43**
Outdoor	Total collected	4			230	254	44	4	3	2	10	18	8	
	HBR	1			57.5	15.88	2.75	0.25	0.19	0.13	0.63	1.13	0.50	
	Total tested	4			50	82	13	7	1	2	1	5	8	
	Spz +ve	0			1	1	0	0	0	0	0	0	0	
	Sporozoite rate	0			0.02	0.01	0	0	0	0	0	0	0	
	Nightly EIR	0			1.15	0.19	0	0	0	0	0	0	0	
	Monthly EIR	**0**			**34.5**	**6.00**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**40.50**

Annex I: Sporozoite Rate and EIR of *An. gambiae* s.l. in New Maforki Peri-urban Site in Bo District, March 2020 to February 2021

New Maforki														
Location	**Month**	**Mar 20**	**Apr 20**	**May 20**	**Jun 20**	**Jul 20**	**Aug 20**	**Sep 20**	**Oct 20**	**Nov 20**	**Dec 20**	**Jan 21**	**Feb 21**	**Annual EIR (9 months)**
Indoor	Total collected	96			85	278	179	44	46	20	8	8	17	
	HBR	24			21.25	17.38	11.19	5.5	2.88	1.25	0.50	0.50	1.06	
	Total tested	58			25	96	53	34	14	12	2	8	17	
	Spz +ve	4			1	3	2	1	0	0	0	0	0	
	Sporozoite rate	0.07			0.04	0.03	0.04	0.03	0.00	0.00	0.00	0.00	0.00	
	Nightly Indoor EIR	1.66			0.85	0.54	0.42	0.16	0.00	0.00	0.00	0.00	0.00	
	Monthly EIR	**51.31**			**26.35**	**16.83**	**13.09**	**5.09**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**112.67**
Outdoor	Total collected	134			63	251	144	75	18	34	14	2	17	
	HBR	33.5			15.75	15.69	9.00	4.69	1.13	2.13	0.88	0.13	1.06	
	Total tested	40			25	75	29	18	7	12	1	1	15	
	Spz +ve	2			0	1	5	3	0	0	0	0	1	
	Sporozoite rate	0.05			0	0.01	0.17	0.14	0	0	0	0	0.07	
	Nightly EIR	1.68			0.00	0.21	1.55	0.65	0.00	0.00	0.00	0.00	0.07	
	Monthly EIR	**51.93**			**0.00**	**6.48**	**48.10**	**19.53**	**0.00**	**0.00**	**0.00**	**0.00**	**1.98**	**128.03**

Annex J: Sporozoite Rate and EIR of *An. gambiae* s.l. in Sand Sand Water Rural Site in Western Area Rural District, March 2020 to February 2021

Sand Sand Water														
Location	**Month**	**Mar 20**	**Apr 20**	**May 20**	**Jun 20**	**Jul 20**	**Aug 20**	**Sep 20**	**Oct 20**	**Nov 20**	**Dec 20**	**Jan 21**	**Feb 21**	**Annual EIR (10 months)**
Indoor	Total collected	11			96	135	122	50	14	22	39	33	25	
	HBR	2.75			24	33.75	30.5	12.5	3.5	5.5	9.75	8.25	6.25	
	Total tested	10			30	71	54	46	21	12	31	31	22	
	Spz +ve	0			4	7	6	4	2	2	1	0	0	
	Sporozoite rate	0			0.13	0.10	0.11	0.09	0.10	0.17	0.03	0.00	0.00	
	Nightly EIR	0			3.20	3.33	3.39	1.09	0.33	0.92	0.31	0	0	
	Monthly EIR	**0**			**96**	**103.15**	**105.06**	**32.61**	**10.33**	**27.50**	**9.75**	**0.0**	**0**	**384.40**
Outdoor	Total collected	18			52	23	52	28	14	13	28	29	24	
	HBR	4.5			13	5.75	13	7	3.5	3.25	7	7.25	6	
	Total tested	19			17	22	36	14	19	6	15	27	22	
	Spz +ve	6			1	0	5	0	2	2	0	2	2	
	Sporozoite rate	0.32			0.06	0.00	0.14	0.00	0.11	0.33	0.00	0.07	0.09	
	Nightly EIR	1.42			0.76	0.00	1.81	0.00	0.37	1.08	0.00	0.54	0.55	
	Monthly EIR	**44.05**			**22.94**	**0.00**	**55.97**	**0.00**	**11.42**	**32.50**	**0.00**	**16.65**	**15.27**	**198.81**

Annex K: Sporozoite Rate and EIR of *An. gambiae* s.l. in Tombo Peri-urban Site in Western Area Rural District, March 2020 to February 2021

Tombo														
Location	**Month**	**Mar 20**	**Apr 20**	**May 20**	**Jun 20**	**Jul 20**	**Aug 20**	**Sep 20**	**Oct 20**	**Nov 20**	**Dec 20**	**Jan 21**	**Feb 21**	**Annual EIR (10 months)**
Indoor	Total collected	38			89	62	51	33	5	16	11	6	12	
	HBR	9.5			22.25	15.5	12.75	8.25	1.25	4	2.75	1.5	3	
	Total tested	6			30	43	45	23	21	13	11	6	12	
	Spz +ve	0			1	4	3	2	3	2	0	0	1	
	Sporozoite rate	0			0.03	0.09	0.07	0.09	0.14	0.15	0.00	0.00	0.08	
	Nightly Indoor EIR	0			0.74	1.44	0.85	0.72	0.18	0.62	0.00	0.00	0.25	
	Monthly EIR	**0**			**22.99**	**44.70**	**26.35**	**22.24**	**5.54**	**19.08**	**0.00**	**0.00**	**7.75**	**148.64**
Outdoor	Total collected	17			78	66	49	21	4	16	0	6	7	
	HBR	4.25			19.5	16.5	12.25	5.25	1	4	0	1.5	1.75	
	Total tested	17			28	67	36	17	17	11	8	6	5	
	Spz +ve	2			2	2	0	1	2	2	1	0	1	
	Sporozoite rate	0.12			0.07	0.03	0.00	0.06	0.12	0.18	0.13	0.00	0.20	
	Nightly EIR	0.5			1.39	0.49	0.00	0.31	0.12	0.73	0.00	0.00	0.35	
	Monthly EIR	**15.5**			**43.18**	**15.27**	**0.00**	**9.57**	**3.65**	**22.55**	**0.00**	**0.00**	**10.85**	**120.56**