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PMI VECTORLINK SIERRA LEONE ANNUAL ENTOMOLOGICAL MONITORING REPORT

MARCH 1, 2019 – FEBRUARY 29, 2020

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The views expressed in this document do not necessarily reflect the views of the United States Agency for International Development or the United States Government.

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ACRONYMS

Ace-1	Acetylcholinesterase 1
CDC	U.S. Centers for Disease Control and Prevention
CSRS	Centre Suisse de Recherches Scientifiques
EIR	Entomological Inoculation Rate
ELISA	Enzyme-linked Immunosorbent Assay
gDNA	Genomic DNA
HBR	Human Biting Rate
HLC	Human Landing Catch
IRD	Indoor Resting Density
IRS	Indoor Residual Spraying
ITN	Insecticide-treated Net
kdr	Knockdown Resistance
LLIN	Long-lasting Insecticidal Net
NMCP	National Malaria Control Program
PBO	Piperonyl Butoxide
PCR	Polymerase Chain Reaction
PMI	President's Malaria Initiative
PSC	Pyrethrum Spray Catch
USAID	United States Agency for International Development
VBDIL	Vector Borne Disease Insectary and Laboratory
VCTWG	Vector Control Technical Working Group
WHO	World Health Organization

EXECUTIVE SUMMARY

Malaria remains one of the biggest public health challenges in Sierra Leone. Due to the country's warm and humid climate, malaria is transmitted throughout the year with little seasonal fluctuation. Distribution of insecticide-treated nets (ITNs) is the main vector control intervention used to reduce the malaria burden. In addition NMCP piloted indoor residual spraying (IRS) with lambda-cyhalothrin in selected chiefdoms of four districts (Bo, Bombali, Kono, and Western Rural Area) in 2010-2012 with the World Health Organization financial support.

For the past two years (March 2018-February 2020), the President's Malaria Initiative VectorLink Project in Sierra Leone has supported the National Malaria Control Program in conducting longitudinal entomological monitoring in four districts representing the country's four regions (Northern, Southern, Eastern, and Western). The susceptibility status of the main malaria vector in Sierra Leone, *Anopheles gambiae* s.l. to insecticides used in public health was also tested.

Vector Bionomics: *An. gambiae* s.l. was the predominant (97.5%) species collected, followed by *An. funestus* s.l. (2.1%) and *An. coustani* (0.4%). *Anopheles funestus* s.l. was collected in low numbers in Bo, Kono, and Bombali districts and none were caught from Western Rural Area. A total of 3,383 *An. gambiae* s.l. collected in 2019–2020 were analyzed for specie identification using the polymerase chain reaction (PCR) method; 79.11% (2677/3383) were *An. gambiae* and 20.89% (707/3383) were *An. coluzzii*. A total of 104 *An. funestus* s.l. collected using human landing catch (HLC) were also processed by PCR, for species identification; 92.3% (96/104) were identified as *An. funestus* s.s. and 7.69% (8/104) as *An. leesoni*. *Anopheles leesoni* were collected biting in Kono and Bo districts.

Human Biting Rate (HBR) and Location: The overall indoor average HBR for the 12 months was 16.7 bites/person/night in the rural sites versus 18.7 bites/person/night in the peri-urban sites. The overall highest HBR was recorded in June in both rural and peri-urban sites. The highest indoor HBR was recorded in Gerihoun (111.8 bites/person/night). In the peri-urban sites, *An. gambiae* s.l. was biting more outdoors than indoors. However, biting was higher indoors than outdoors in rural areas. Overall as a complex, *An. gambiae* s.l. was biting more indoors than outdoors but the difference was not statistically significant. In almost all the districts, biting increased after 11:00 p.m., and was highest between 11:00 p.m. and 05:00 a.m. *An. gambiae* showed a preference biting outdoors 50.4% (953/1,890) than indoors 49.6% (937/1,890) but the difference was not statistically significant (p=0.71). *An. coluzzii* preferred also bite outdoors 52.4% (313/658) than indoors 47.6% (345/658) with no statistically significant difference. *An. gambiae* s.l. did more biting indoors than outdoors in the rural sites but the difference was not statistically significant except in Sand Sand Water in Western Rural Area. Overall, if given equal opportunities of non-protected humans, the vector seems to bite more or less equally indoors and outdoors.

Infection Rate and Entomological Inoculation Rate (EIR): *Plasmodium* infection was detected in 4.7% of *An. gambiae* s.l. and 1.5% of *An. funestus* s.l. The overall *An. gambiae* s.l. infection rate during the study period was similar indoors (4.8%) and outdoors (4.6%). The highest indoor infection rate was observed in Sand Sand Water (10.6%), followed by Tombo (6.3%) in Western Rural Area. The highest indoor EIR was observed in rural areas in June with 56.4 infective bites/person/month. The highest indoor EIR in peri-urban areas was observed in May, with 52.9 infective bites/person/month.

Blood Meal Source: The overall human blood index was 69.8% indicating that about a third of the *An. gambiae* s.l. mosquitoes feeding on other animals.

Insecticide Resistance: *An. gambiae* s.l. is fully susceptible to pirimiphos-methyl, chlorfenapyr, and clothianidin, whereas analysis showed a high level of resistance to the pyrethroids: deltamethrin, permethrin, and alpha-cypermethrin. After pre-exposure to piperonyl butoxide, an increase in mortality of at least 40% was observed for the three pyrethroids tested at all sites except for Gbanti Kamaranka (Bombali), where the increase for deltamethrin was 29%.

Conclusion: Given the preference of the vectors to bite late at night, high coverage and sustained use of ITNs is expected to reduce human-vector contact and provide protection from malaria infection. Moreover, the synergist assay data indicate that the deltamethrin- and permethrin +PBO nets that will be distributed in May 2020 may have a greater impact than conventional nets in reducing mosquito populations and therefore impact malaria transmission. Indoor Residual Spraying just before the peak mosquito and malaria transmission season with a long-lasting insecticide also may help to reduce the malaria burden.

I. INTRODUCTION

Malaria is endemic in Sierra Leone with stable and perennial transmission. Malaria prevalence was 40% in 2016 among children aged 6–59 months. It is currently the main cause of morbidity and mortality among children under 5 years. Malaria prevalence is two times higher in rural areas (49%) than urban areas (25%) (NMCP 2016). Malaria vector control in Sierra-Leone is based on the use of insecticide-treated nets.

In 2011 and 2012, indoor residual spraying (IRS) with lambda-cyhalothrin was piloted in selected chiefdoms in Bo, Bombali, Kono, and Western Rural Area districts, with 97% of the structures sprayed and 309,209 people protected. Lack of financing forced the discontinuation of this IRS after 2012, and no IRS program has been implemented in Sierra Leone since then. The National Malaria Control Program (NMCP) is planning to conduct IRS in selected districts in 2021.

In September 2017, the United States Agency for International Development (USAID)/President's Malaria Initiative (PMI) contracted Abt Associates to implement the five-year PMI VectorLink Project. VectorLink started providing vector control and entomological technical support to NMCP since 2018.

In Year 2, VectorLink Sierra Leone continued to provide technical support to NMCP and implemented entomological monitoring activities that included collecting data on vector bionomics, insecticide resistance, and the establishment of an insectary in Freetown, and the maintenance of the Makeni (Bombali District) Vector Borne Disease Insectary and Laboratory (VBDIL), that was established in 2018. Monthly entomological monitoring activities were conducted in four districts selected in consultation with the NMCP: Bo, Sierra Leone's second-largest district, represents the south of the country; Bombali represents the north; Kono, where there is large-scale mining activity, represents the east; and Western Rural Area represents the coastal area. The vector bionomic monitoring was done in two chiefdoms per district with one community per chiefdom for a total of eight sentinel sites. Insecticide susceptibility tests were performed in one rural chiefdom per district where more *Anopheles gambiae* s.l. breeding sites were available.

In 2019, VectorLink Sierra Leone continued to support NMCP maintenance of a functional VBDIL insectary in Makeni and establishing a susceptible Kisumu strain colony of *Anopheles gambiae* for use in Sierra Leone. In addition, the project established an insectary at its office in Freetown. The project also supported the monitoring of vector bionomics, providing information on vector density, distribution, and behaviors. It assessed the susceptibility of *An. gambiae* s.l. to the insecticides used in public health, and the data were shared through the Vector Control Technical Working Group (VCTWG) for decision making regarding malaria vector control. Based on the findings from the insecticide resistance monitoring and the vector bionomics, VectorLink Sierra Leone provided technical assistance to the NMCP and supported the VCTWG to provide evidence-based recommendations for piperonyl butoxide (PBO) net distribution and the insecticide to be used for 2021 IRS campaign.

2. METHODOLOGY

2.1 LONGITUDINAL MONITORING

Adult mosquitoes were collected monthly from March 2019 to February 2020 in eight sentinel sites from four districts: Bo in the southern region, Bombali in the northern region, Kono in the eastern region, and Western Rural Area in the western region (Figure 1). In each district, mosquitoes were collected in one peri-urban and one rural sentinel site (Table 1). The peri-urban sites are close to a metropolitan municipality. As such, they are transition zones between the urban and the rural areas, with a mix of traditional rural dwellings and relatively modern houses, the presence of agricultural lands, and a highly concentrated population. The rural sites are located in communities away from the urban centers with traditional houses made of mud and cement, and agriculture is the main occupation.

FIGURE 1: *ENTOMOLOGICAL MONITORING SITES*

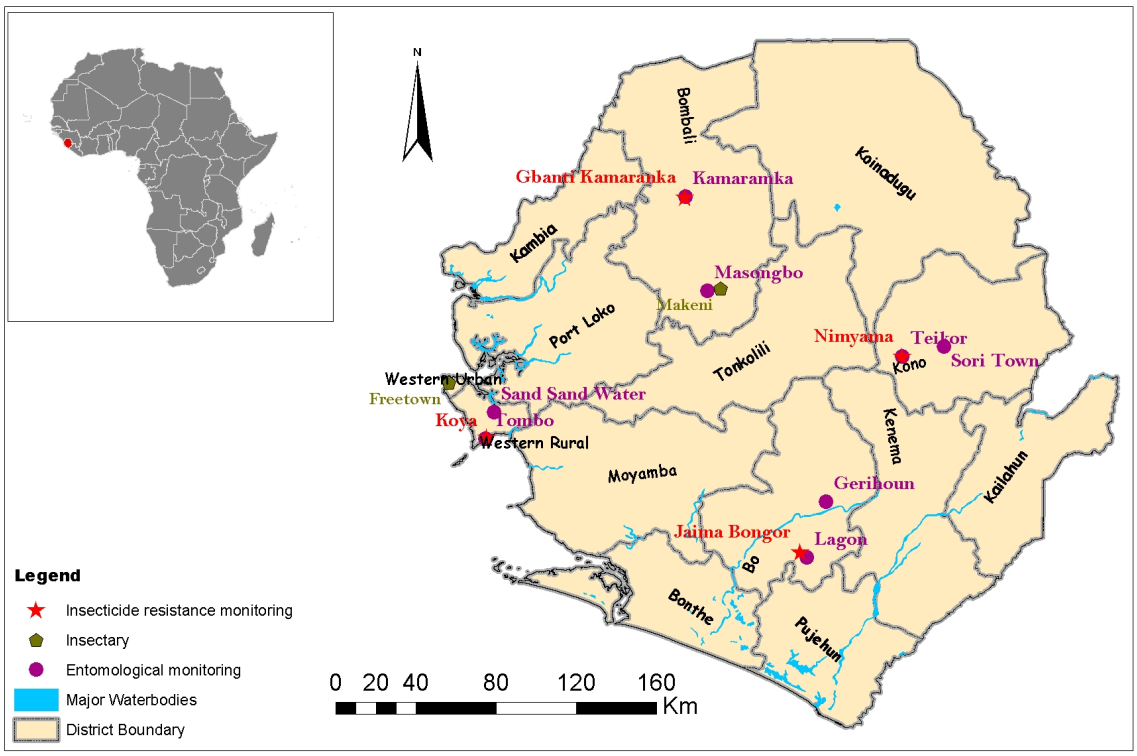


TABLE 1: SENTINEL SITES FOR ENTOMOLOGICAL MONITORING

District	Chiefdom	Sentinel Site	Location	Activity	
				Vector bionomic monitoring	Vector susceptibility
Bo	Jaima Bongor	Lagon	Rural	✓	✓
	Boma	Gerihoun	Peri-urban	✓	
Bombali	Gbanti Kamaranka	Kamaranka	Rural	✓	✓
	Makari Gbanti	Masongbo	Peri-urban	✓	
Kono	Nimyama	Teikor	Rural	✓	✓
	Nimikoro	Sori Town	Peri-urban	✓	
Western Rural Area	Koya Rural	Sand Sand Water	Rural	✓	✓
	York Rural	Tombo	Peri-urban	✓	

Three sampling methods were used to assess the vectors bionomics (Table 2): Pyrethrum spray collections (PSCs), human landing catches (HLCs), and U.S. Centers for Disease Control and Prevention (CDC) light traps. Table 2 describes the methods.

TABLE 2: LONGITUDINAL MONITORING ADULT MOSQUITO COLLECTION METHODS

Collection Method	Time	Frequency	Sample*
PSC	5:00 a.m. to 9:00 a.m.	3 days per site per month	15 houses per site (5 houses each day for 3 consecutive days)**
HLC	6:00 p.m. to 7:00 a.m.	2 nights per month	2 houses for 2 consecutive nights (both inside and outside) per site
CDC light trap	6:00 p.m. to 7:00 a.m.	4 nights every month	4 houses for 4 consecutive nights (inside) per site***

*Same house each month

**PSC were conducted in Masongbo in Bombali District in 14 houses in March 2019, 12 in May, 10 in June, and 12 in July, instead of the targeted 15 houses. Some household owners would not allow the team to enter their houses.

***CDC light traps were set in Gerihoun in Bo District in March 2019 for three days instead of four days, and two light traps were set instead of four on one day of the three days. The bulb and fan in some CDC light traps were faulty in March; the bulbs were then replaced by new ones. CDC light traps were set for three days instead of four days in Western Rural Area in August due to heavy rains and flooding, which prevented the team from reaching the houses.

The project entomology team conducted PSCs based on the VL protocol (SOP03/01) from five houses per day for three consecutive days each month in each sentinel site. The PSCs were performed in the morning hours (5:00 a.m. to 9:00 a.m.) to determine indoor resting mosquito species and densities. Collections took place in rooms where people had slept the preceding night. A white cloth was spread on the floor from wall to wall in the selected house while other open spaces were blocked. The eaves, windows, and other mosquito entry and escape routes around the house were sprayed with a commercial aerosol containing PBO, and then the inside of the house was sprayed. Ten minutes after spraying, all mosquitoes knocked down were collected from the white cloth. The number and different gonotrophic stages (unfed, blood fed, half-gravid, gravid) of female *Anopheles* mosquitoes collected were recorded, and the samples were placed in moist petri dishes and transported to the hotel or laboratory (VBDIL) for morphological identification. Vector species collected were preserved in labeled 1.5 ml screw-top tubes with silica gel. The same sentinel sites and houses were visited for the monthly collections throughout the study period. The mosquitoes collected were used to assess vector species composition, abundance, and indoor resting density. The data collected from the rural sites were compared with the data from peri-urban sites.

Monthly HLCs were also performed according to VL SOP SOP02/01. A team of two trained collectors performed hourly collections simultaneously in two houses from 6:00 p.m. to 12:00 a.m. and is replaced by

another team of two for the collection from 12:00 a.m. to 7:00 a.m. over two nights. The houses were selected in May 2018 taking into account features such as proximity to the breeding site and materials from which houses were made. One of the two houses selected was located close to the breeding site and the other was located away from the breeding site. One of the houses was a mud house and the other was a cement house. The same sentinel sites and houses were visited for the monthly collections throughout the study period.

On each night, collectors (one inside and another one outside the house) sat on chairs with their legs exposed up to the knee (a total of 16 person-nights/district). Mosquitoes attempting to feed on the collectors were caught using mouth aspirators and flashlights. Each hour, the mosquitoes collected were transferred to labeled paper cups. A different paper cup was used for indoor and outdoor collections and for each hourly catch. The collectors worked overnight and exchanged positions indoors and outdoors every hour to reduce possible collection bias due to differences in their attractiveness to mosquitoes.

In each house, one person collected indoors, inside a room, while the other collected mosquitoes outdoors, in the open courtyard of the compound.

The data were used to determine the human biting rate (HBR) (mean number of mosquito bites per person per unit of time), as well as feeding locations and peak biting times. Subsamples of the *An. gambiae* s.l. and *An. funestus* s.l. collected were further analyzed by polymerase chain reaction (PCR). A subsample of unfed *An. gambiae* s.l. collected were dissected to determine parity rate.

CDC light traps (SOP01/01) were set up indoors in four randomly selected houses where people slept under an untreated mosquito net for four consecutive nights each month. The same sentinel sites and houses were visited for the monthly collections throughout the study period. The consent of the head of each household was acquired beforehand. The traps were placed approximately 150 cm above the sleeper's legs and about 50 cm from the human sleeping under an insecticide-treated net (ITN). The traps operated from 6:00 p.m. to 7:00 a.m. for four nights in each house (4 houses x 2 sites x 4 nights = 32 house-nights/per district). Mosquitoes were collected from the traps once, in the morning. Mosquitoes trapped were transported to the VBDIL or hotel for morphological identification.

A taxonomic key (Coetzee 2020) was used to morphologically identify all *Anopheles* mosquitoes collected by each method. All mosquitoes collected were preserved in 1.5 ml Eppendorf tubes with desiccant for further analysis at the *Centre Suisse de Recherches Scientifiques* (CSRS) laboratory in Cote d'Ivoire.

2.2 Insecticide Susceptibility Tests

World Health Organization (WHO) tube tests (SOP06/01) and CDC bottle assays (SOP04/01) were performed to assess the susceptibility of local vector populations to the insecticides used for IRS and for ITNs. All the sentinel sites have a long history of ITN coverage, gained through mass distribution campaigns and maintained through routine distribution at antenatal care and Expanded Program on Immunization visits, and during Maternal and Child Health Week. Alpha-cypermethrin-treated nets were distributed during the 2017 mass campaign in Bombali and Bo districts and deltamethrin-treated nets in Western Rural Area and Kono districts. IRS is planned in 2021 with clothianidin in selected districts.

Methodologies are described below.

2.2.1 WHO Tube Tests

The project team performed insecticide susceptibility tests using the WHO tube method in one rural chiefdom per district: Gbanti Kamaranka (Bombali District), Nimyama (Kono District), Jaima Bongor (Bo District), and Koya (Western Rural Area District). Larvae and pupae of *Anopheles* mosquitoes for susceptibility tests were collected from breeding sites in and around the sentinel sites and reared to adults at the Makeni insectary or in Freetown. Mosquitoes were morphologically identified at the adult stage and only *An. gambiae* s.l. were used for the susceptibility tests. Synergist assays with PBO using WHO tube tests were conducted with deltamethrin 0.05%, permethrin 0.75%, and alpha-cypermethrin 0.05% in the four sites.

The team also conducted susceptibility tests of *An. gambiae* s.l. to pirimiphos-methyl 0.25% using the WHO tube method.

After the 24-hour holding period, the numbers of dead and alive mosquitoes in both the exposure and the control tubes were recorded. For all tests, mortalities were corrected using Abbott's formula if the control mortalities were >5% but ≤20%, but tests were discarded and repeated if control mortalities were >20% (Abbott 1925).

Additionally, the team determined the susceptibility status of wild *An. gambiae* s.l. from selected sites to 13.2mg (per 15x12cm paper) of clothianidin, using papers that were impregnated in country. Knockdown was recorded after a 60-minute exposure and mortalities recorded at one, two, three, four, five, six, and seven days post-exposure.

The team evaluated susceptibility levels of *An. gambiae* s.l. on the basis of the WHO criteria of test mortality (WHO 2013): 98–100% mortality after the holding period indicates susceptibility. Mortality of <98% suggests the existence of resistance and the need for further investigation. If the observed mortality (corrected if necessary) is >90% but <98%, the presence of resistance in the vector population must be confirmed with a repeat test. Mortality of <90% confirms the presence of resistant individuals in the vector population.

2.2.2 CDC Bottle Assays

The project team conducted the CDC bottle assay with chlorfenapyr, using a method described by Brogdon and Chan (2010) with some modifications (60 minutes of exposure time). *An. gambiae* s.l. reared from larvae were exposed to 250ml Wheaton bottles treated with a diagnostic concentration of 100µg/bottle. Tests with *An. gambiae* Kisumu were run in parallel as positive controls. Mosquitoes were introduced in batches of 20–25 in one replicate of each concentration. After the exposure period, mosquitoes were released into clean cages and then gently aspirated into labeled paper cups covered with untreated netting and provided with 10% sugar solution. Knockdown was recorded 60 minutes after the start of the test, while mosquitoes were still in the bottle. Mortality was recorded one, two, and three days after the end of exposure. A negative control was tested at the same time and mortality recorded at one, two, and three days so that corrected mortality could be calculated.

2.3 Resistance Intensity Assays

The project team used a simplified version of the CDC bottle bioassay resistance Intensity Rapid Diagnostic Test (I-RDT) (Brogdon and Chan 2010) to assess the intensity of resistance (5x and 10x) observed using discriminating concentrations of deltamethrin, permethrin, and alpha-cypermethrin in Koya and Gbanti Kamaranka. In Nimyama, where the number of mosquitoes reared was not enough to test all insecticides, only the intensity of alpha-cypermethrin was tested. In Jaima Bongor, intensity assays were not conducted for alpha-cypermethrin due to the low number of larvae collected. Four replicates of 500µl of acetone were added to each insecticide vial and washed off into a 50ml graduated falcon tube. The falcon tube was topped up to the 50ml mark. The prepared insecticide solutions were stored in a refrigerator at 4°C until use. The control bottle was prepared by adding 1ml of acetone into a 250ml Wheaton bottle and coated as described by Brogdon and Chan (2010). Four test bottles were then coated with 1ml of different concentrations of the prepared insecticide solutions to get one bottle each of the required insecticide concentration. Between 20 and 25 mosquitoes were introduced into each of the four replicates. A control bottle (coated with acetone only) was run alongside the tests. The knockdown rate was recorded at 15-minute intervals in each bottle.

2.4 Synergist Assays

Synergist assays with PBO using the WHO procedure were conducted with deltamethrin 0.05%, permethrin 0.75%, and alpha-cypermethrin 0.05% in the four chiefdoms.

2.5 Analysis and Molecular Evaluations

CSRS performed molecular analyses to:

- Determine sporozoite rates and calculate entomological inoculation rates (EIRs)
- Identify members of the *An. gambiae* s.l. and the *An. funestus* s.l. complex to species (Scott et al. 1993)

- Determine the host blood meal

2.5.1 Mosquitoes Species Identification

DNA extraction

Prior to species identification and genotyping experiments, genomic DNA (gDNA) was extracted from samples of mosquitoes according to the Livak method (Livak 1984) using a LIVAK grind buffer (1.6 ml 5M NaCl, 5.48 g sucrose, 1.57g Tris, 10.16 ml 0.5M EDTA, 2.5 ml 20% SDS). Each individual mosquito was ground in 100µl of preheated LIVAK grind buffer and incubated at 65°C for 30 minutes. 14 µl 8M K-acetate was added and gently mixed. After 30 minutes of incubation on ice, the thicker mixture was centrifuged at 13,000 rpm for 20 minutes (4°C). 200 µl 100% EtOH was added to the supernatant (transferred in new Eppendorf tube 1.5 ml) and the mixture was subjected to a new centrifugation at 13,000 rpm for 15 minutes (4°C). After discarding the supernatant, the pellet was rinsed with 100 µl ice cold 70% EtOH. Dried pellets were then re-suspended in 100 µl TAE buffer. The DNA was used for species identification and target site genotyping.

Polymerase chain reaction

Members of *An. gambiae* s.l. and *An. funestus* s.l. groups were identified to species by PCR, following the protocols developed in Scott et al. (1993) and Santolamazza et al. (2008) for *An. gambiae* s.l. and *An. funestus* s.l. (Koekemoer et al. 2002). A total volume of 24.75 µl was used per reaction for the master mix containing 18.83 µl DNase free water, 2.5 µl Buffer 10X, 0.75 µl MgCl2 (25mM), 1 µl dNTP (10mM), 1 µl Sine 6.1a (10 µM) 5'-CGCTTCAAGAATTCGAG ATAC-3', 1 µl Sine 6.1b (10 µM) 5'-TCGCCTTAGA CCTTGCGTTA-3' and 0.17 µl Kappa Taq. Each PCR product had 23 µl of mix and 2 µl of genomic DNA. The PCR product was amplified at 94°C for 3 minutes followed by 35 cycles of 94°C, 62°C, and 72°C for 30 minutes respectively, and a last cycle of 5 minutes at 72°C. Products were then run on 1.5% agarose gels.

2.5.2 Determination of Infection Rate

Enzyme-linked immunosorbent assay (ELISA) (Wirtz et al, 2010) was used to determine the sporozoite infection rate. ELISA plates were coated with 50 µl of 2A10 anti circumsporozoite antibodies per well. The plates were covered for 24 hours (at least one night) and incubated on the bench. The next day, the plates were emptied and filled with 200 µl of blocking solution for 1 hour. Then the contents of the wells were removed and 50 µl of crushed head-thorax mosquito added. After 2 hours of incubation, the plates were washed twice with PBS-Tween. Then wells were filled with 50 µl of peroxidase-labeled anti-circumsporozoite antibody (2A10-PER) and incubated for 1 hour. After 1 hour, the plates were washed four times with PBS-Tween. The wells of the plates were filled with 100 µl of substrate. After 30 minutes in the dark, the reaction was stopped with 50 µl of sulfuric acid. The plates were read in a spectrophotometer at 620 nm.

2.5.3 Determination of Blood Meal Origin

The host preference was assessed by analyzing the blood meal among the potential hosts in the study areas, using ELISA blood meal (Beier et al., 1988). Each mosquito sample (abdomen) was analyzed for the possible host blood: human, bovine, porcine, and chicken. A plate was sensitized with 50 µl of anti-human diluted to 1/2,000 in PBS (pH 7.2), the second plate was sensitized with anti-bovine 1/1,000 in PBS (pH 7.2), the third plate with anti-porcine (1/300 in PBS, pH 7.2), and the fourth with anti-chicken (1/2,000 in PBS, pH 7.2). All these plates were stored for 24 hours (at least one night) at +4°C. Plates were washed three times after 24 hours with 0.05% PBS-Tween. Plate wells were then filled with 200 µl of 0.1% PBS-Tween and stored for 30 minutes at 37°C. After 1 hour, the wells were filled with 50 µl of human serum, 0.1% PBS-Tween, or samples to be tested. The same sample was tested in all plates. Then wells were incubated for 2 hours at room temperature (20–25 °C) and washed three times with 0.05% PBS-Tween. Then wells were filled with 50 µl of anti-human HRPO diluted to 1/30,000 in PBS-Tween, 50 µl HRPO anti-bovine 1/5,000, and finally 50 µl HRPO anti-chicken to 1/20,000. The plates were incubated for 1 hour at 37°C. After the incubation, they were washed three times with 0.05% PBS-Tween and then once with distilled water. They were immediately filled with 50 µl of substrate per well. Four to six minutes later, the reaction was stopped with 25 µl of $H_2SO_4$8N per well. The plates were read in a spectrophotometer at

492 nm. The optical density of the positive samples ranges between 900 and 1,200 nm. Each plate contained positive controls and negative controls.

2.5.5 Analysis of Data

The following parameters were estimated:

- HBR per night = the total number of vectors collected/number of collectors/number of nights of capture (reported as bites per person per night)
- Sporozoite rate = the proportion of *Anopheles* found positive for *Plasmodium* infection
- EIR = number of infectious bites per person per unit time
- Daily EIR = daily HBRs x sporozoite rates
- Monthly EIR = daily EIR x number of days in the month
- Annual EIR = Σ Monthly EIRs

3. RESULTS

3.1 LONGITUDINAL MONITORING

A total of 22,911 *Anopheles* mosquitoes were collected during the monthly collections conducted from March 2019 to February 2020 using PSCs, HLCs, and CDC light traps in both rural and peri-urban sites in the four districts. A total of 22,340 *An. gambiae* s.l. were collected: 4,068 (18.2%) from PSCs, 14,418 (64.5%) from HLCs, and 3,854 (17.3%) from CDC light traps. HLCs yielded the highest number of mosquitoes, followed by PSC and CDC light trap methods. *An. gambiae* s.l. was the predominant (97.5%) *Anopheles* species collected, followed by *An. funestus* s.l. (2.1%) and *An. coustani* (0.4%) (Table 3).

A total of 475 *An. funestus* s.l. mosquitoes were collected, and, again, the greatest number came from HLCs: 279 (58.7%) from HLCs; 156 (32.8%) from PSCs, and 40 (8.5%) from CDC light traps. The *An. funestus* s.l. were mainly collected from four sites: Teikor and Sori Town in Kono District and Gerihoun and Lagon in Bo District, where the Sewa River offers permanent breeding sites suitable for *An. funestus* s.l. This indicates that the distribution of *An. funestus* s.l. is restricted to specific ecological conditions in Sierra Leone.

Table 3 provides the breakdown of each *Anopheles* species collected in the peri-urban and rural sites. Annexes A, B, and C provide more detailed information by site and collection method.

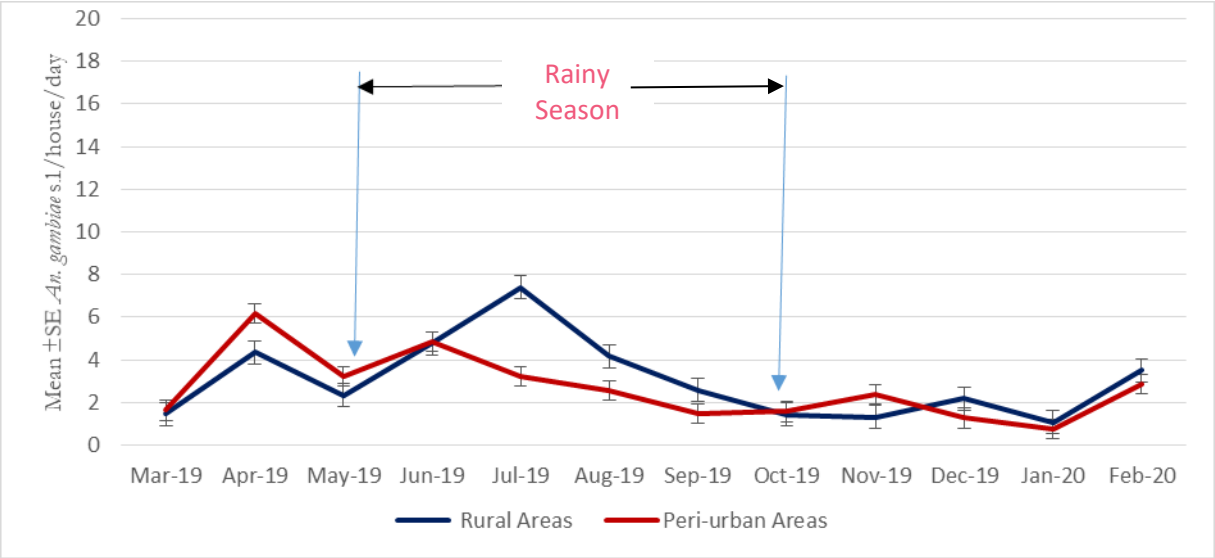
TABLE 3 : TOTAL *ANOPHELES* SPECIES COLLECTED (PSC, HLC, AND CDC-LT), BY SITE

District	Sites	*An. gambiae* s.l.		*An. funestus s.l.*		*An. coustani*	
		n	%	n	%	n	%
Bombali	Kamaranka (Rural)	1982	8.9	0	0.0	2	2.1
	Masongbo (Peri-urban)	3746	16.8	2	0.4	43	44.8
Bo	Lagon (Rural)	3426	15.3	34	7.2	12	12.5
	Gerihoun (Peri-urban)	4816	21.6	337	70.9	18	18.8
Kono	Teikor (Rural)	3030	13.6	56	11.8	13	13.5
	Sori Town (Peri-urban)	2962	13.3	46	9.7	4	4.2
Western Rural Area	Sand Sand Water (Rural)	1652	7.4	0	0	4	4.2
	Tombo (Peri-urban)	726	3.2	0	0	0	0.0
Total		22340	100	475	100	96	100.0

3.1.1 INDOOR RESTING DENSITY (PSC COLLECTION)

The Indoor Resting Density (IRD) of *An. gambiae* s.l. per house per day between March 2019 and February 2020 across all sites is shown in Figure 2, split by rural and peri-urban areas. The average IRD of *An. gambiae* s.l. per house per day was similar in rural (3.0/house/day) and peri-urban sites (2.6/house/day). The highest overall IRD was observed in July in the rural sites (7.4/house/day) and in April in the peri-urban sites (6.2/house/day). The mean IRD of *An. gambiae* s.l. in the rural sites (3.8/house/day) was 1.8 times higher during the wet season than the IRD in the dry season (2.0/house/day). The trend is similar in the peri-urban sites where the IRD during the wet season (2.8/house/day) was higher than the IRD during the dry season (1.8/house/day).

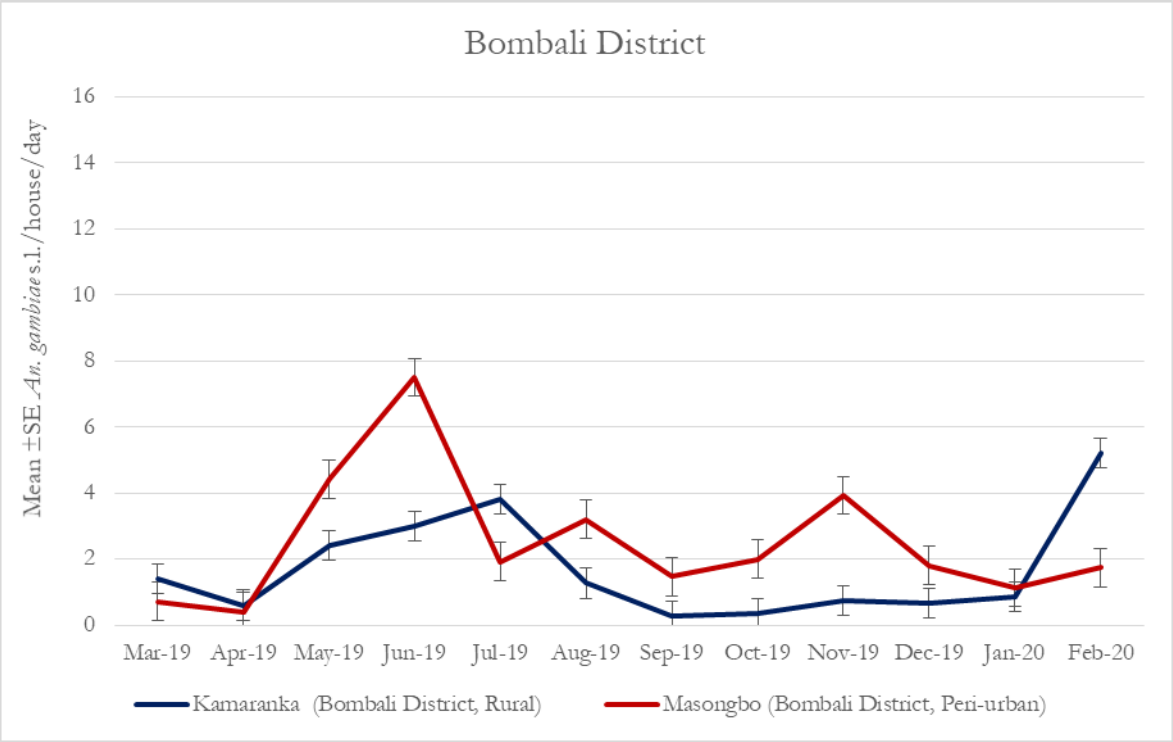
FIGURE 2: MEAN IRD OF *AN. GAMBIAE* S.L. BY MONTH FROM ALL SENTINEL SITES, GROUPED BY RURAL VS PERI-URBAN SITES MARCH 2019–FEBRUARY 2020

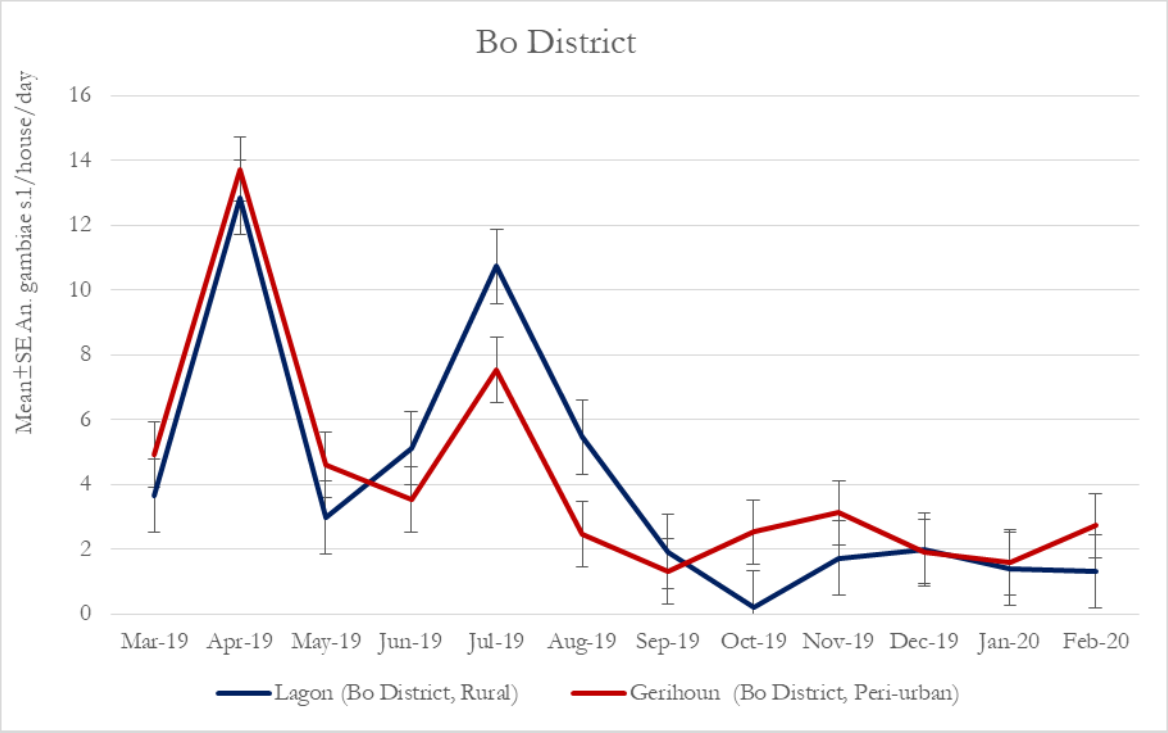


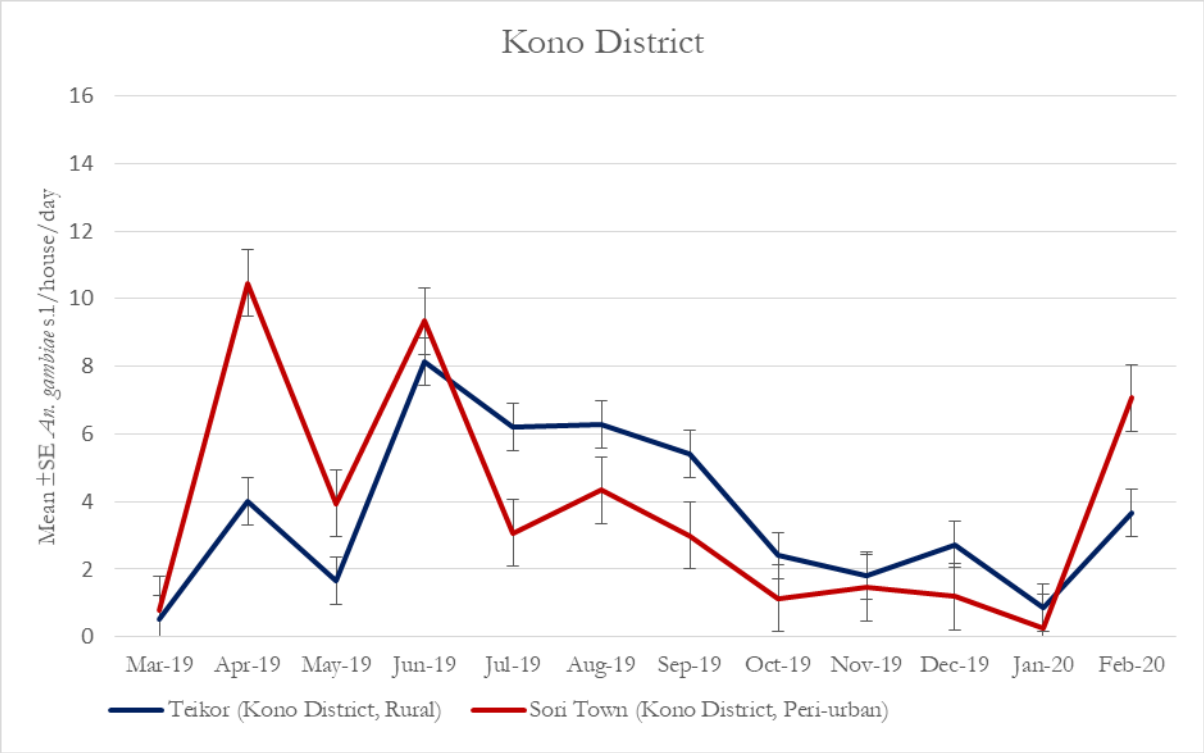
In Bombali, the mean IRD was higher in the peri-urban site (2.4/house/day) than the rural site (1.7/house/day). In Bo District, the mean IRD was similar in the two sites: rural site (4.1/house/day) and peri-urban (4.2/house/day). This also was the case in Kono District: rural site (3.6/house/day) and peri-urban site (3.8/house/day). The average IRD of *An. gambiae* s.l. in the rural site of Western Rural District (2.7/house/day) was higher than the average density in the peri-urban site (0.2/house/day).

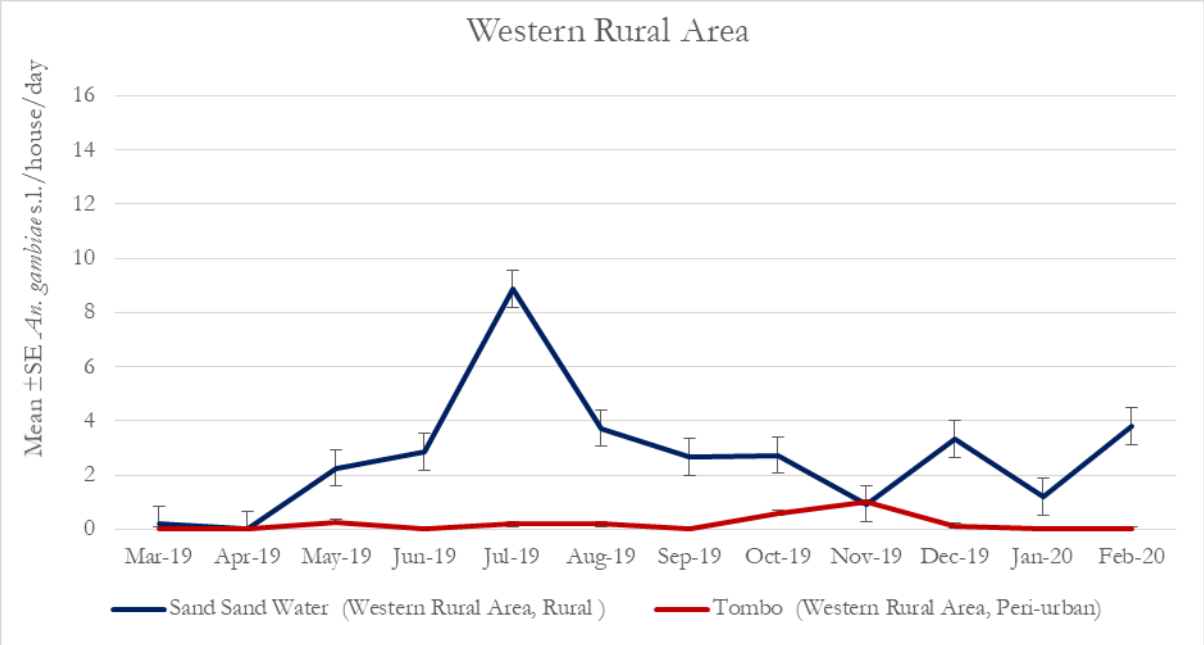
In Bombali District, the highest mean IRD of *An. gambiae* s.l. was observed in rural sites in February (5.2/house/day) and in peri-urban sites in June (7.5/house/day) (Figure 3). In Bo District, the highest IRD was in April, in both rural (12.9/house/day) and peri-urban sites (13.7/house/day). In Kono District, June was the peak (8.1/house/day) for the rural site, and April (10.5/house/day) for the peri-urban site. In Western Rural Area District the highest IRD was observed in July in the rural site (8.9/house/day) and was low (<1.0/house/day in November) in the peri-urban site throughout the collection period.

FIGURE 3: MEAN IRDS OF *AN. GAMBIAE* S.L. IN EIGHT SENTINEL SITES COMPARING RURAL VS PERI-URBAN SITES, MARCH 2019–FEBRUARY 2020





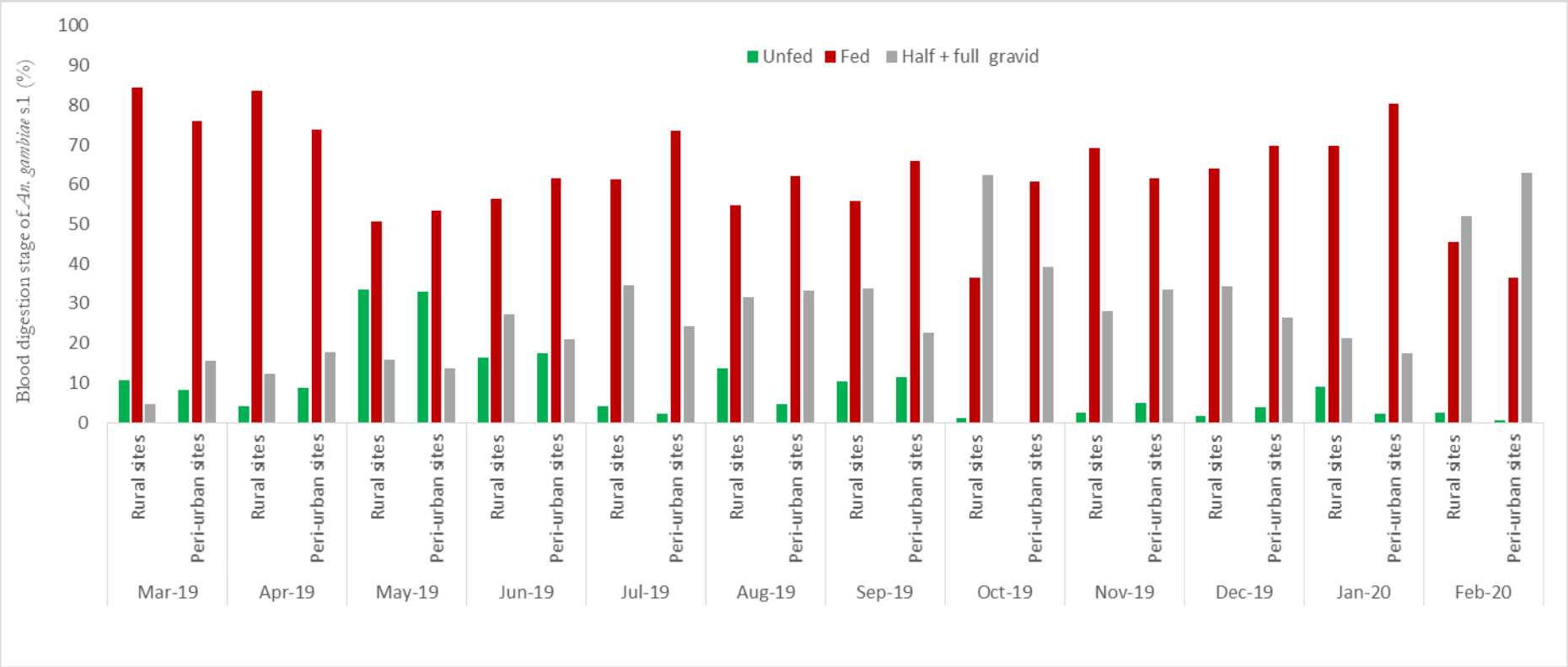




3.1.2 ABDOMINAL CONDITION OF *AN. GAMBIAE* S.L. COLLECTED BY PSC

Figure 4 shows the abdominal condition of *An. gambiae* s.l. collected across rural and peri-urban sites by PSC (indoors). The proportion of half- and full-gravid *An. gambiae* s.l. in the rural sites 30.3% (664/2194) was higher than the proportion in the peri-urban sites 26.6% (498/1874). The proportion of fed *An. gambiae* s.l. collected was high at both rural 60.5% (1328/2194) and peri-urban sites 63.8% (1195/1874).

FIGURE 4: ABDOMINAL CONDITIONS OF *AN. GAMBIAE* S.L. COLLECTED BY PSC FROM ALL SENTINEL SITES COMPARING RURAL VS PERI-URBAN SITES,
MARCH 2019–FEBRUARY 2020



3.1.3 CDC Light Trap Collection

The highest average density of An. *gambiae* s.l. per trap per night was recorded in June in both rural (6.53/trap/night) and peri-urban (6.39/trap/night) areas. In the rural sites, the average density of *An. gambiae* s.l. was low in March (0.70/trap/night) and April (1.92/trap/night) before the wet season and increased to 6.53/trap/night in June (Figure 5). Less than one *An. gambiae* s.l. was collected per trap per night in October during the rainy season and from November to January during the dry season. The overall density of *An. gambiae* s.l. in the peri-urban sites (3.06/trap/night) was 1.5 times higher than the density in rural sites (2.03/trap/night). Broken down by site and district (Figure 6), the highest densities of *An. gambiae* s.l. were recorded around June for most sites except Sori Town (peri-urban site) in Kono District, which recorded the highest density of An. *gambiae* s.l. (14.52/trap/night) in April (Figure 6).

FIGURE 5: MEAN DENSITY OF *AN. GAMBIAE* S.L. FROM CDC LIGHT TRAP COLLECTIONS IN ALL SENTINEL SITES COMPARING RURAL SITES VS PERI-URBAN SITES, MARCH 2019–FEBRUARY 2020

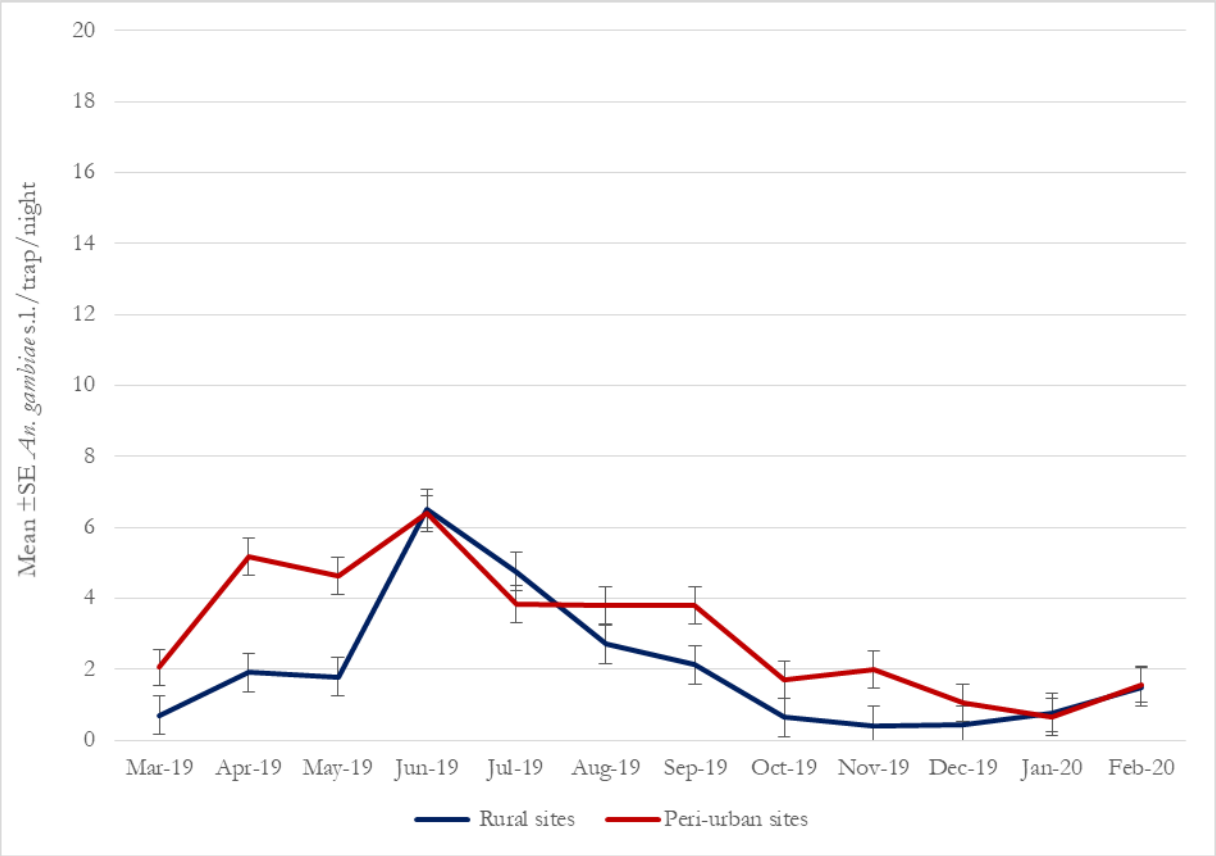
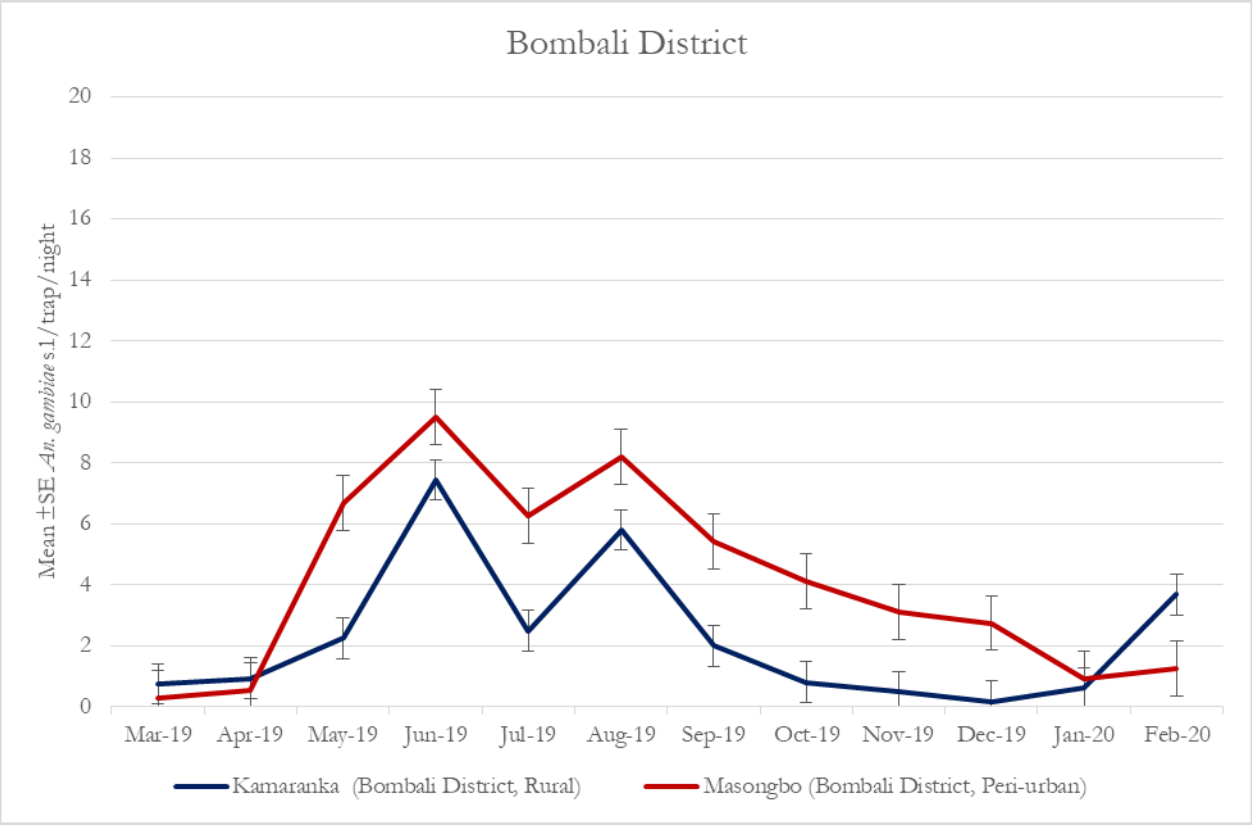
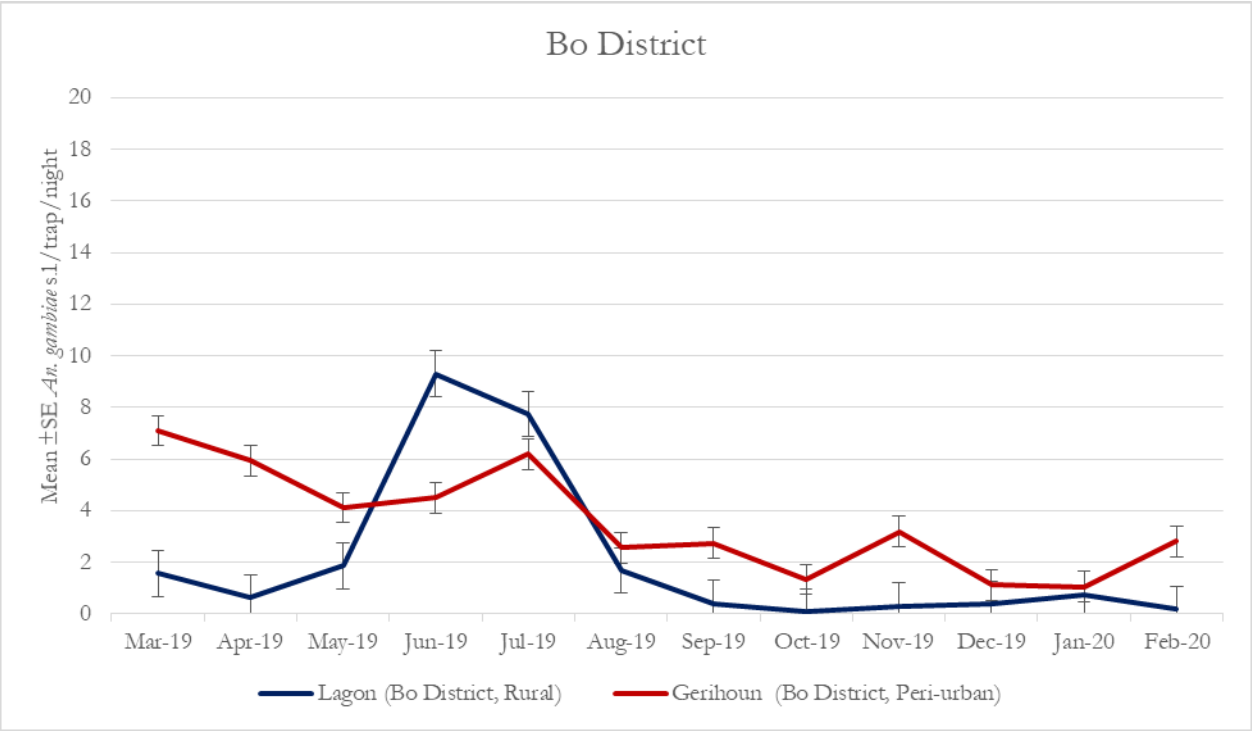
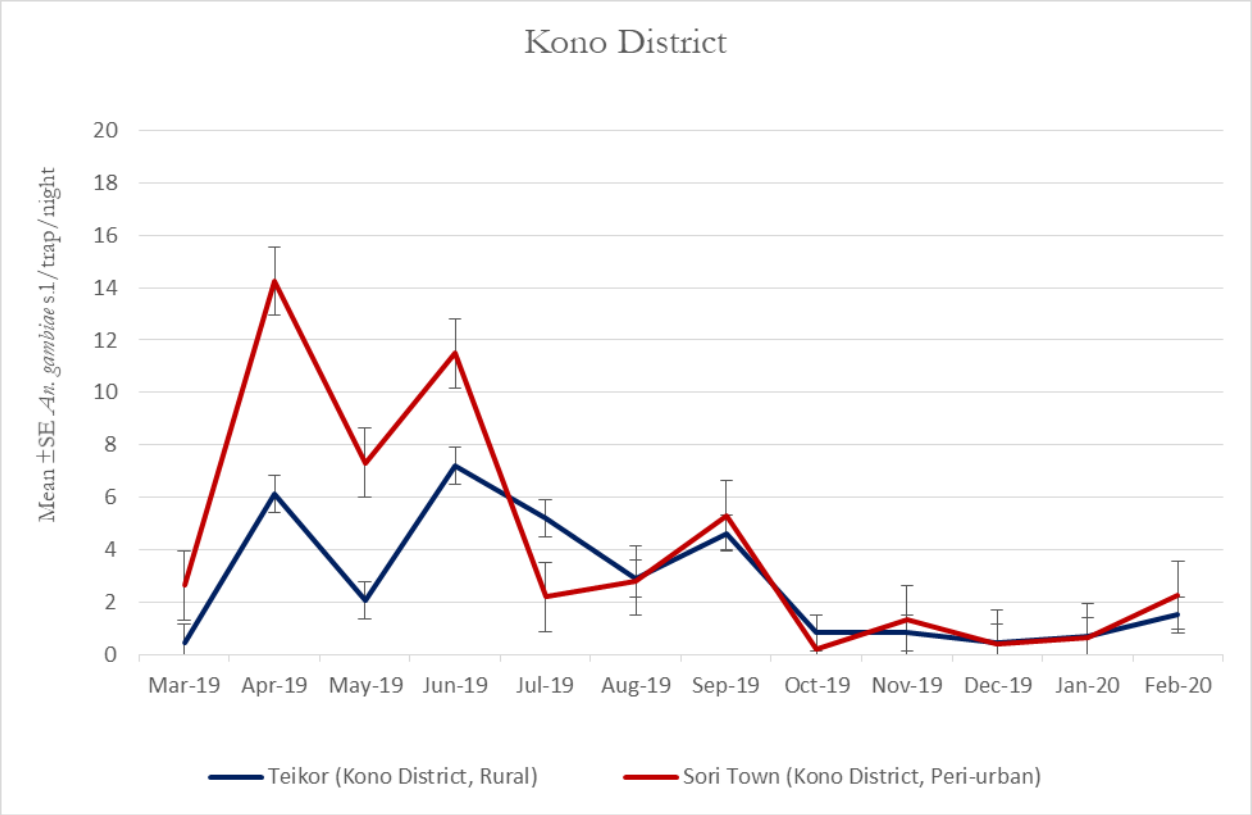
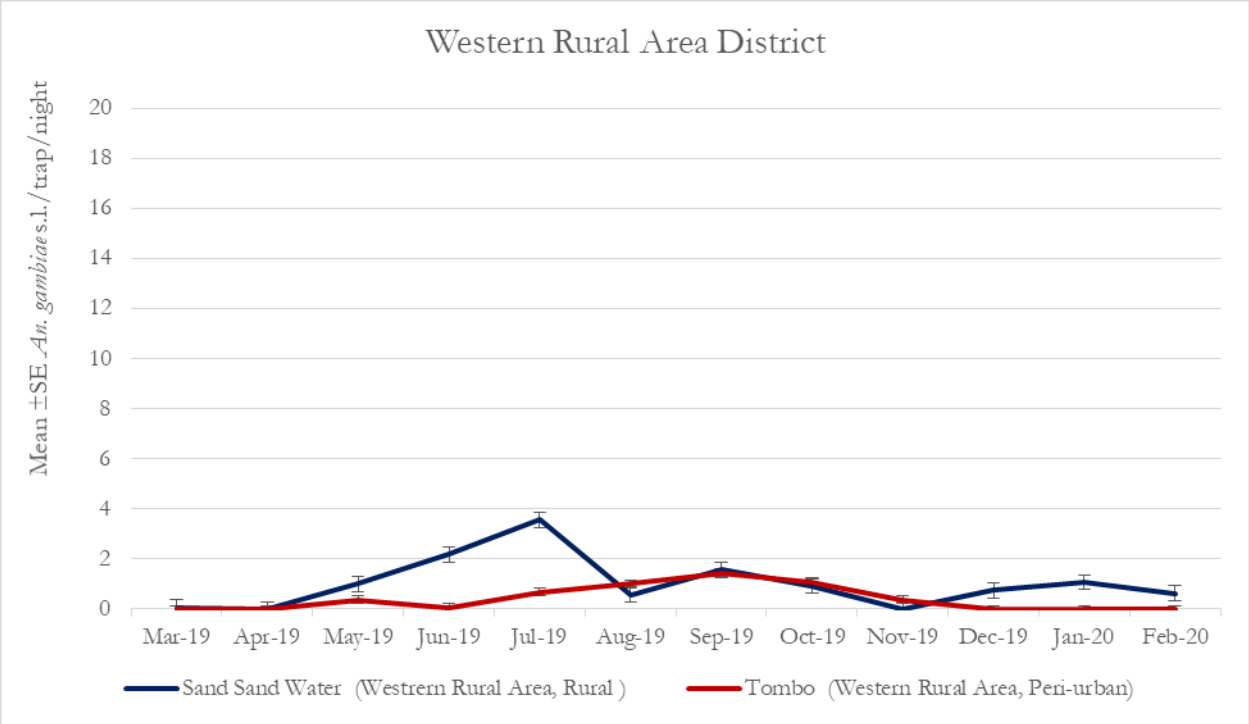


FIGURE 6: DENSITY OF *AN. GAMBIAE* S.L. FROM CDC LIGHT TRAP COLLECTIONS IN EIGHT SENTINEL SITES COMPARING RURAL VS PERI-URBAN SITES, MARCH 2019–FEBRUARY 2020





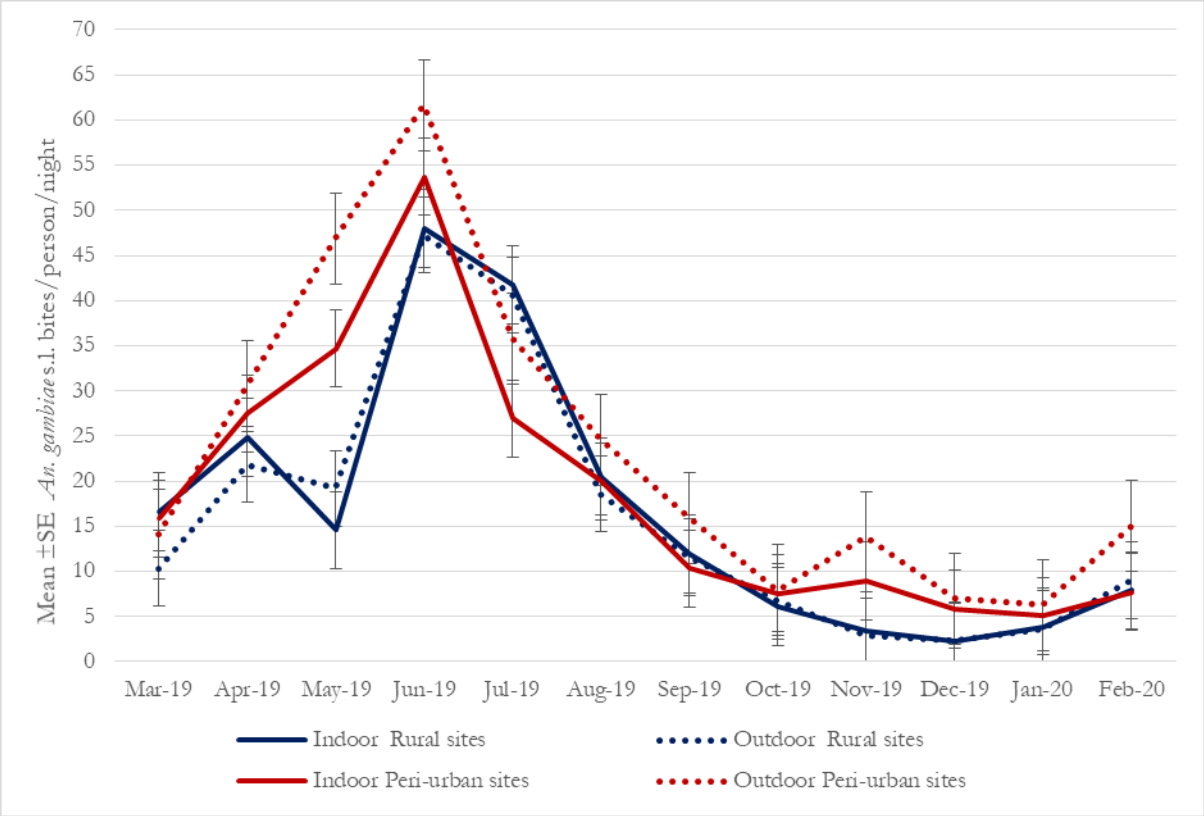

Kono District
Mean ±SE An. gambiae s.l./trap/night
20
18
16
14
12
10
8
6
4
2
0
Mar-19
Apr-19
May-19
Jun-19
Jul-19
Aug-19
Sep-19
Oct-19
Nov-19
Dec-19
Jan-20
Feb-20
Teikor (Kono District, Rural)
Sori Town (Kono District, Peri-urban)


Western Rural Area District
Mean ±SE An. gambiae s.l./trap/night
20
18
16
14
12
10
8
6
4
2
0
Mar-19
Apr-19
May-19
Jun-19
Jul-19
Aug-19
Sep-19
Oct-19
Nov-19
Dec-19
Jan-20
Feb-20
Sand Sand Water (Westrern Rural Area, Rural)
Tombo (Western Rural Area, Peri-urban)

3.1.4 HUMAN LANDING COLLECTIONS

Human Biting Rate: The overall *An. gambiae* s.l. HBR in the rural sites was 16.8 bites/person/night indoors and 16.2 bites/person/night outdoors. The overall for peri-urban sites was 18.7 and 23.3 indoors and outdoors, respectively (Figure 7). The highest indoor HBR/person/night in rural sites was recorded in June, 47.9 and 47.3, indoor vs outdoor, respectively. The June HBR/person/night for peri-urban sites was 53.7 vs 61.6, indoor vs outdoor, respectively. The lowest HBR was recorded in December in rural (2.3 bites/person/night) and in January in peri-urban sites (5.1 bites/person/night).

FIGURE 7: *AN. GAMBIAE* S.L. MEAN HBR IN ALL SENTINEL SITES, COMPARING RURAL VS PERI-URBAN SITES MARCH 2019–FEBRUARY 2020

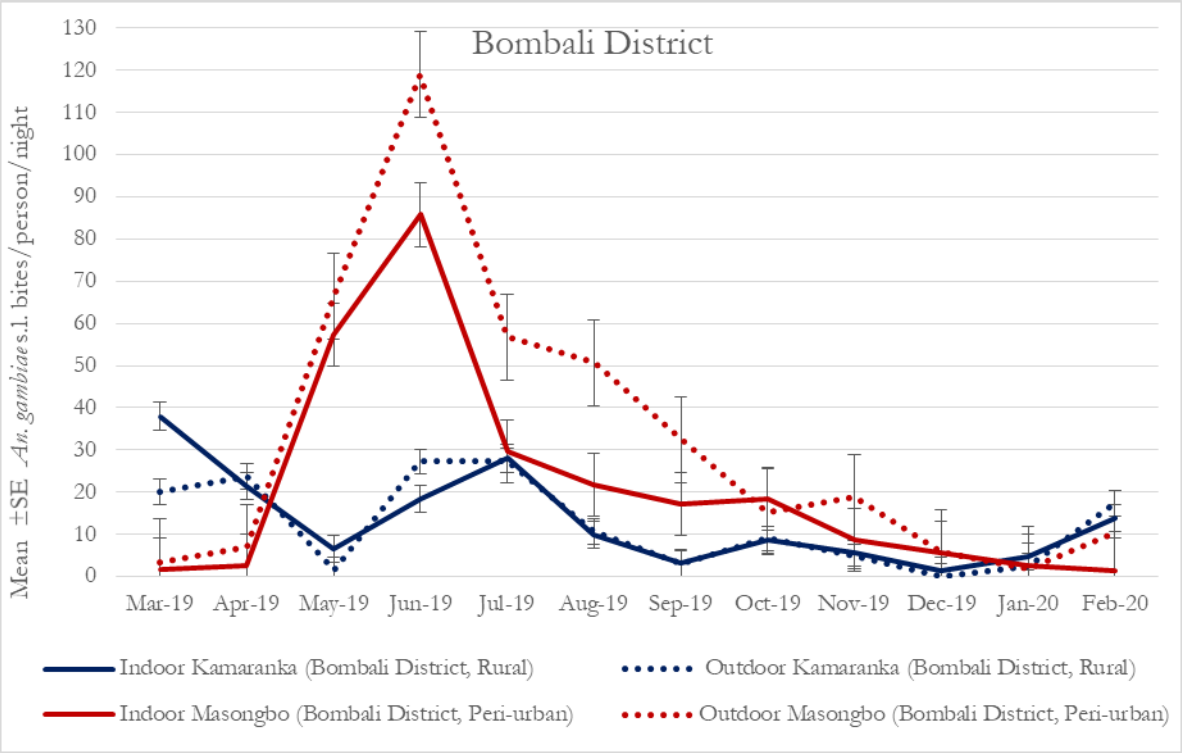


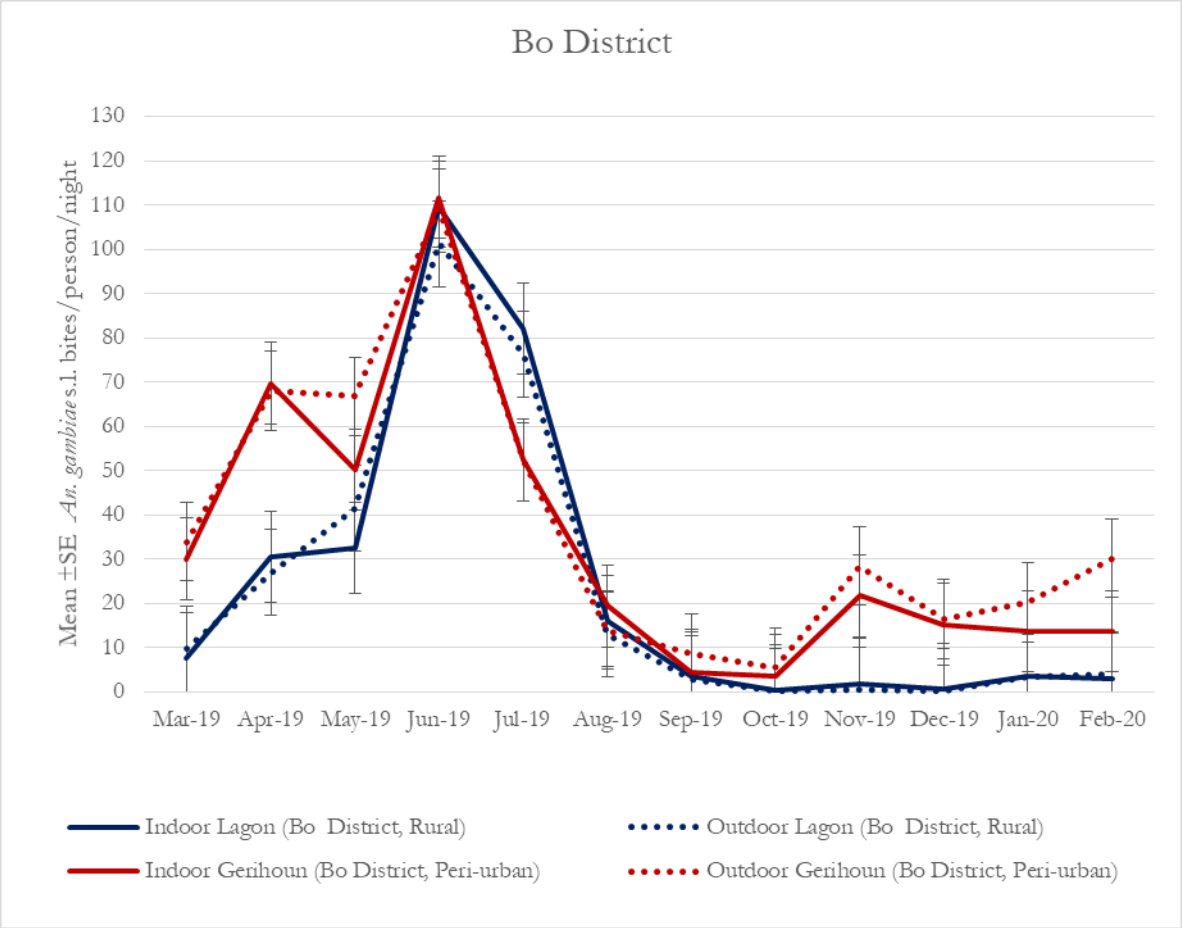
Biting Location: The highest indoor *An. gambiae* s.l. HBR was recorded in Gerihoun in Bo District (111.8 bites/person/night) and outdoors in Masongbo (119.0 bites/person/night) in Bombali, both in June (Figure 8).

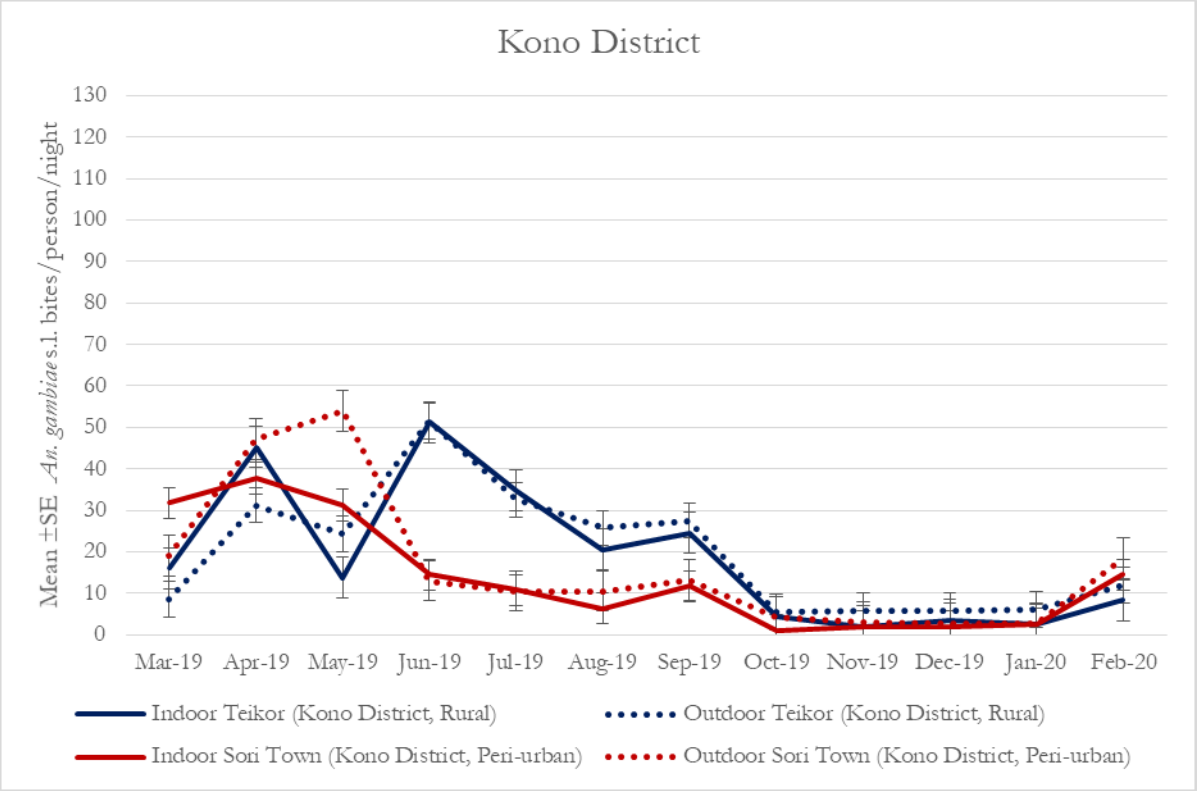
The indoor and outdoor biting rates of *An. gambiae* s.l. in Bo District were two to three times higher than in Kono and Western Rural Area districts. In the rural sites, biting was slightly higher indoors than outdoors except in Teikor. However, the difference between indoor and outdoor biting rates was not statistically significant except in Sand Sand Water. In the peri-urban site, the *An. gambiae* s.l. HBR was significantly higher outdoors than indoors, except in Tombo, where the biting rate was not different between indoors and outdoors.

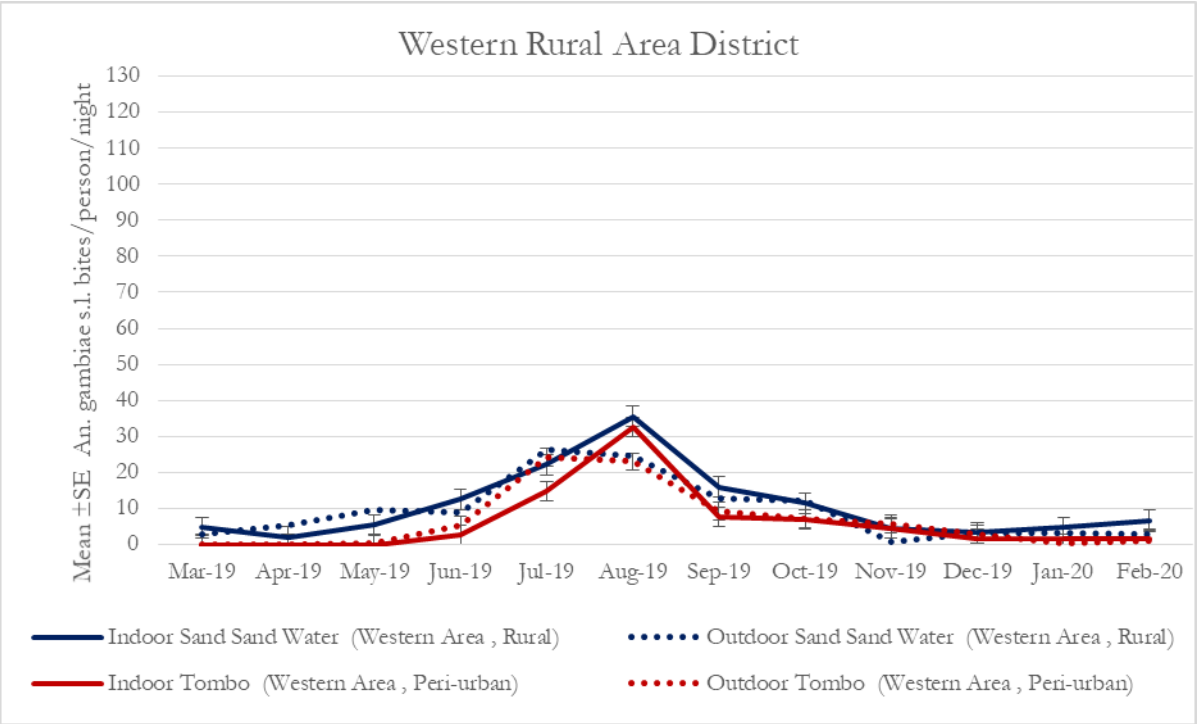
In Bombali District, the highest mean indoor HBR of *An. gambiae* s.l. in Kamaranka (rural) was observed in March (38.0 bites/person/night) and in Masongbo (peri-urban) was in June (85.8 bites/person/night). The highest mean indoor HBR in Bo District was recorded in June for both rural (109.5 bites/person/night) and peri-urban (111.8 bites/person/night) sites. In Kono District, the peak HBR of *An. gambiae* s.l. biting indoors was observed in Teikor (rural) in June (51.3 bites/person/night) and in Sori Town in April (37.8 bites/person/night). In Western Rural Area District, Sand Sand Water (rural) and Tombo (peri-urban), the highest mean indoor HBR of *An. gambiae* s.l. was observed in August (35.5 bites/person/night) and July (14.5 bites/person/night), respectively.

FIGURE 8: MEAN *AN. GAMBIAE* S.L. HBRS IN THE EIGHT SENTINEL SITES COMPARING RURAL VS PERI-URBAN SITES, MARCH 2019–FEBRUARY 2020









An. gambiae s.l. were biting more indoors than outdoors at all rural sites, except in Teikor; but the difference observed was not statistically significant except in Sand Sand Water in Western Rural Area District. In contrast, biting outdoors was higher than indoors in the peri-urban sites (Table 4).

TABLE 4: INDOOR AND OUTDOOR HLC COLLECTION OF AN. GAMBIAE S.L., ALL SENTINEL SITES COMPARING RURAL VS PERI-URBAN SITES, MARCH 2019–FEBRUARY 2020

Sentinel Site	# *An. gambiae* s.l. Biting Indoor	# *An. gambiae* s.l. Biting Outdoor	Endophagic Index	Exophagic Index	χ^2	p-value
Rural Sites						
Kamaranka (Bombali District)	641	593	0.52	0.48	2.2	0.14
Lagon (Bo District)	1166	1121	0.51	0.49	0.93	0.34
Teikor (Kono District)	906	945	0.49	0.51	0.74	0.39
Sand Sand Water (Western Area)	514	451	0.53	0.47	4.11	0.04*
Total Rural Sites	3227	3110	0.51	0.49	2.4	0.12
Peri-urban Sites						
Masongbo (Bombali District)	1011	1553	0.39	0.61	113.8	<0.001*
Gerihoun (Bo District)	1626	1815	0.47	0.53	10.75	0.001*
Sori Town (Kono District)	664	795	0.46	0.54	11.59	0.006*
Tombo Western Area)	296	318	0.48	0.52	1.03	0.3
Total Peri-Urban Sites	3597	4481	0.45	0.55	97.65	<0.001*

* Difference between # of *An. gambiae* s.l. collected indoor and outdoor is statistically significant at 0.05 level.

A total of 279 *An. funestus* s.l. were collected using HLCs; 170 were collected biting inside the houses versus 109 outside (Figure 10. *An. funestus* s.l. did more biting indoors than outdoors ($p<0.001$). Most of the *An. funestus* s.l. (81%) were recorded from HLCs in Bo District (Annex B).

An. funestus s.l. was collected in Bombali District (Masongbo only), Bo District (Lagon and Gerihoun), and Kono District (Teikor and Sori Town) (Figure 10). The highest mean indoor HBR of *An. funestus* s.l. from a rural site was recorded in June in Teikor (1.5 bites/person/night), and from a peri-urban site in August in Gerihoun (7.5 bites/person/night). However, the numbers were too low to make a meaningful comparison between sites and locations (Figure 11). No *An. funestus* s.l. was collected from Western Rural Area District.

FIGURE 9: *AN. FUNESTUS* S.L. MEAN HBR IN ALL SENTINEL SITES COMPARING RURAL VS PERI-URBAN SITES, MARCH 2019–FEBRUARY 2020

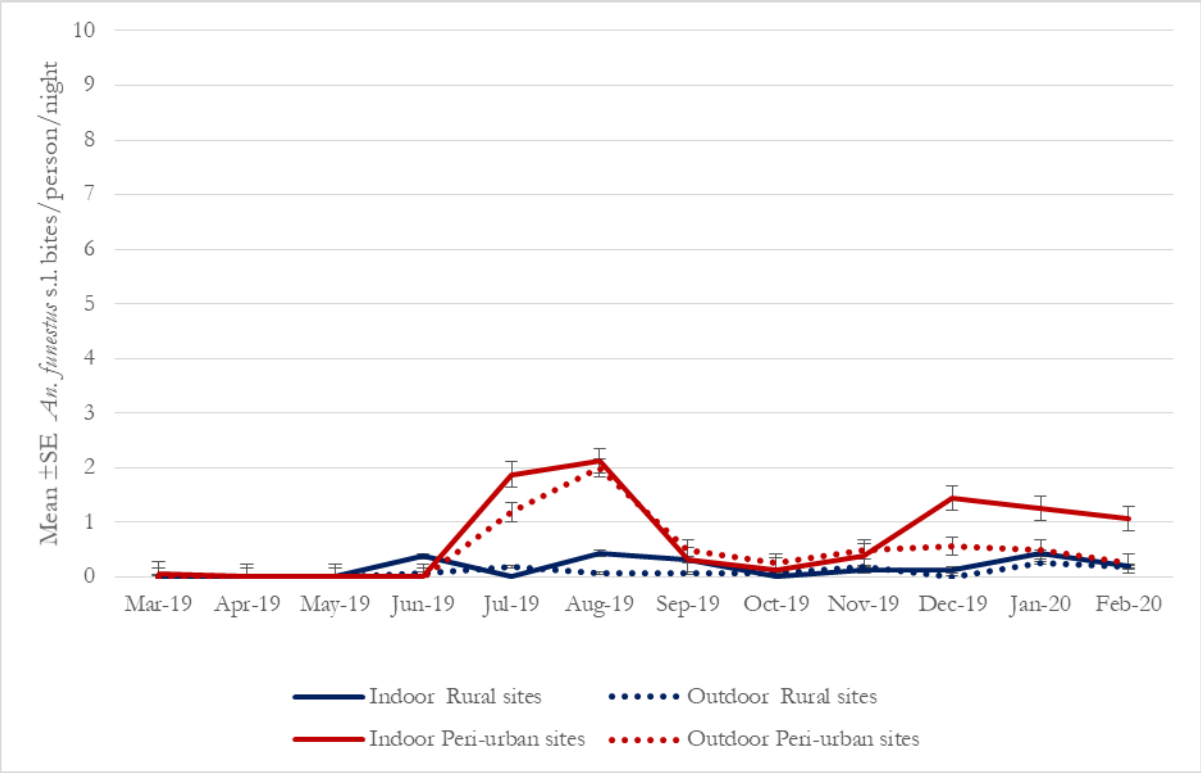
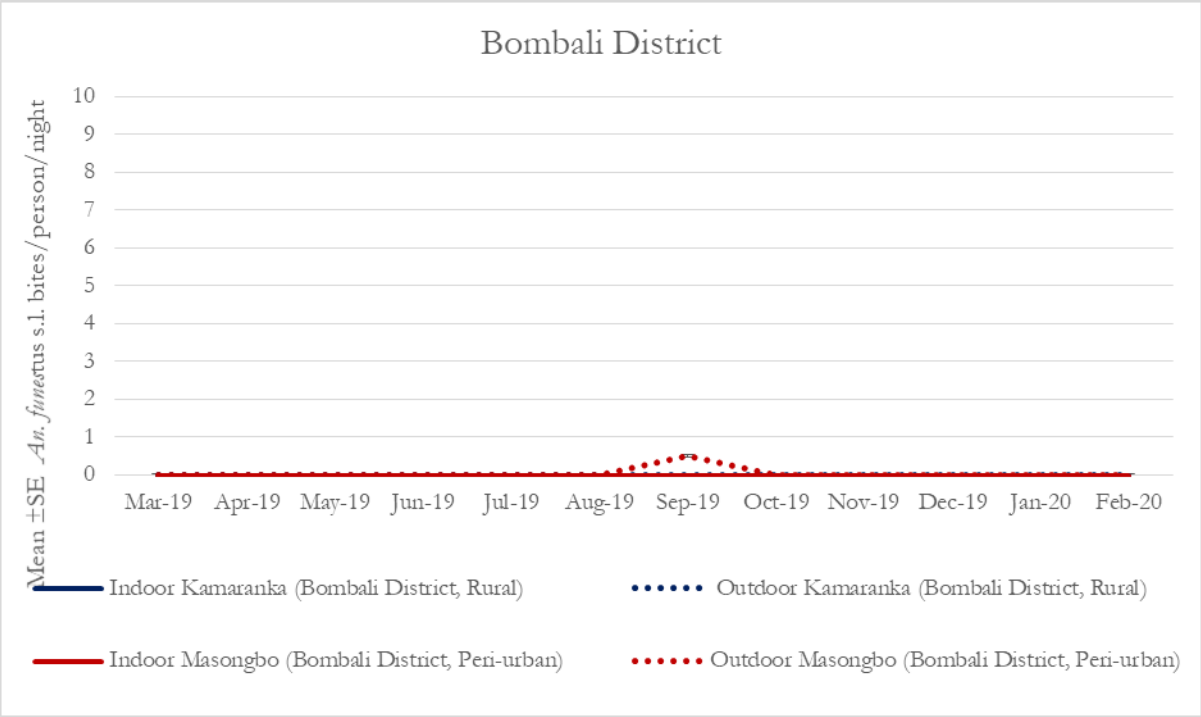
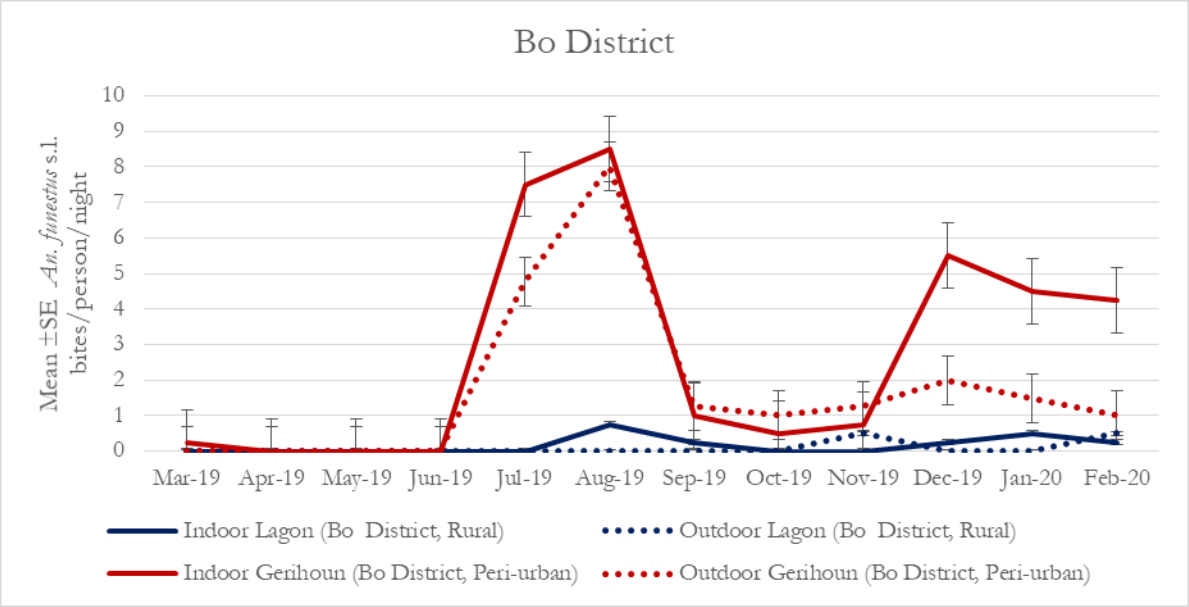
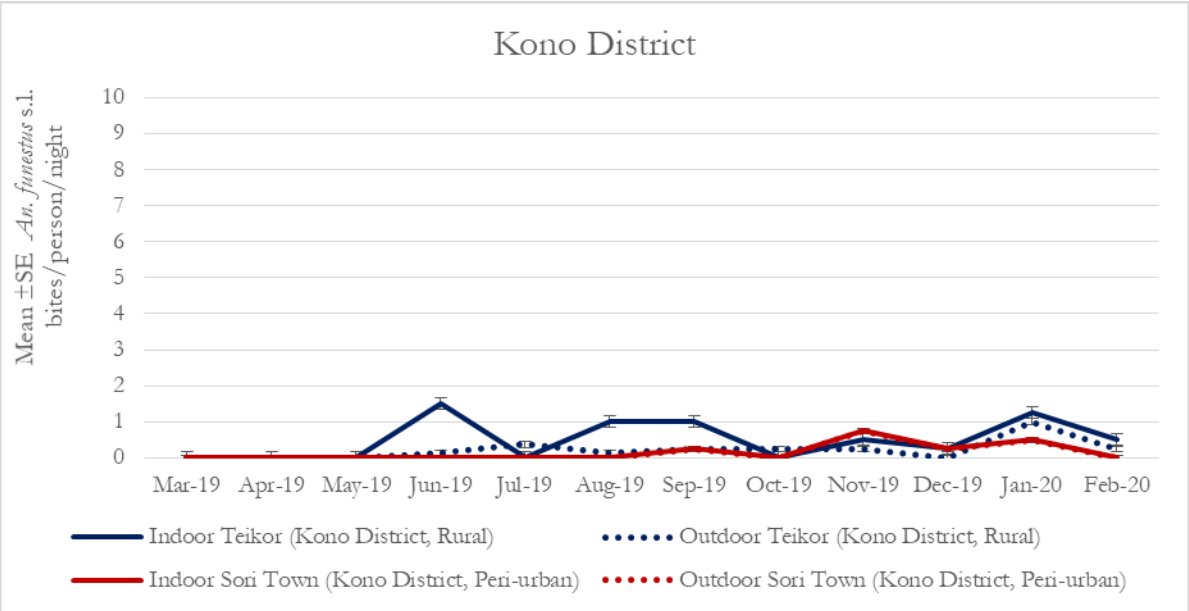


FIGURE 10: *AN. FUNESTUS* S.L. HBR IN BOMBALI, BO, AND KONO DISTRICTS COMPARING RURAL AND PERI-URBAN SITES, MARCH 2019–FEBRUARY 2020

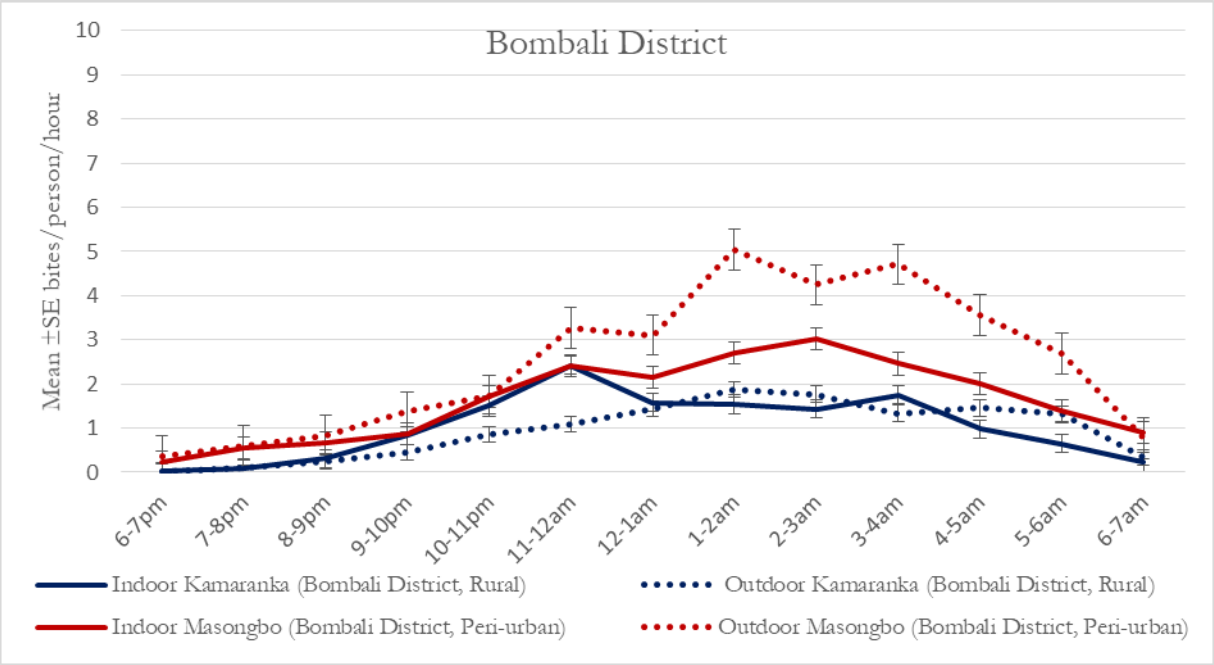


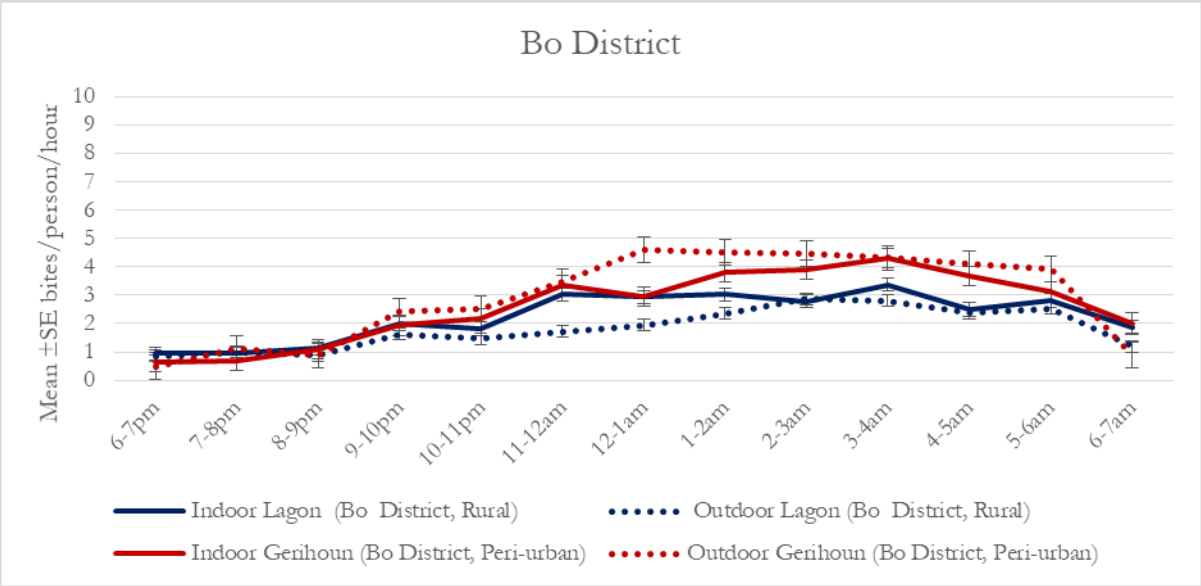


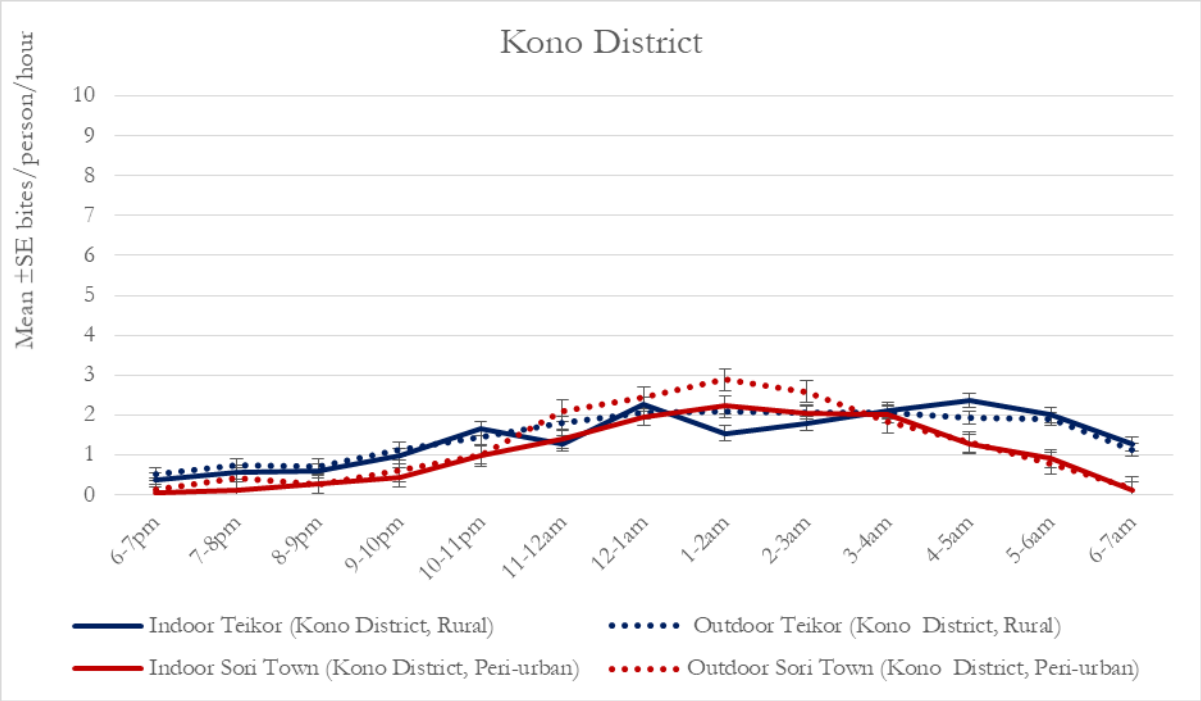


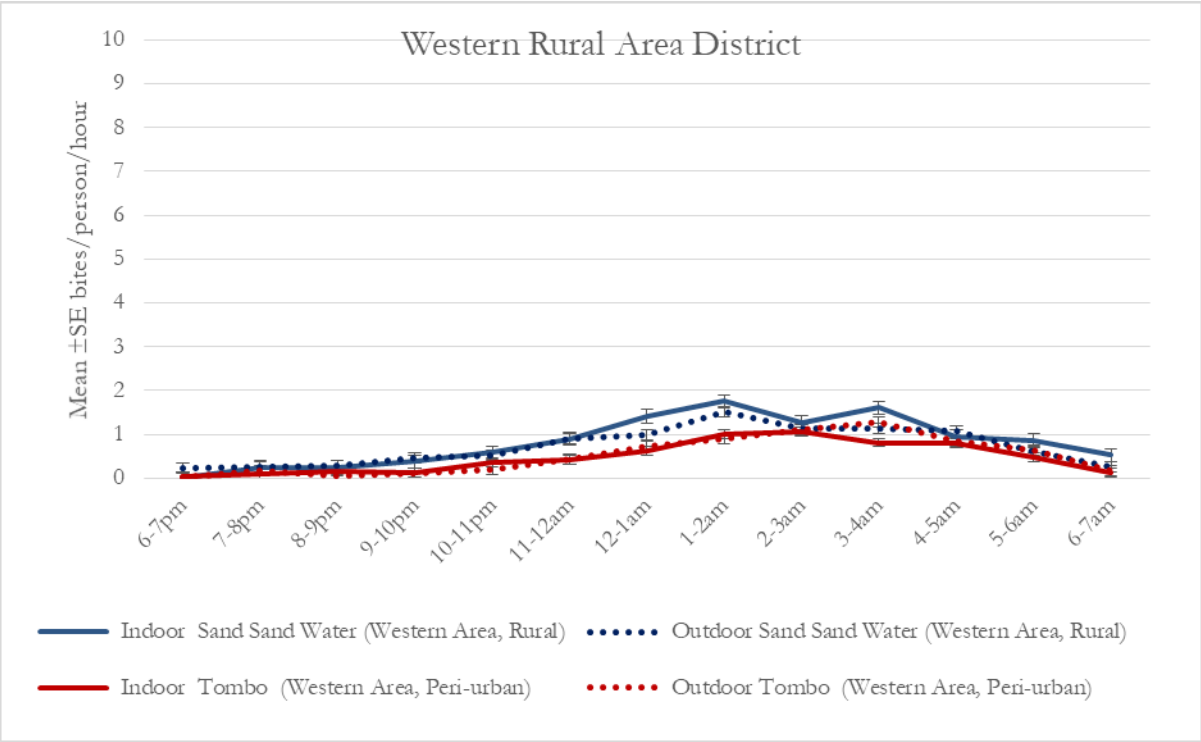
Biting Time: Biting activity started to peak after 10 p.m. in all the districts and sites, as well as indoors and outdoors, with the majority of biting occurring during the second half of the night (Figure 12). This behavior should have a positive impact on the effectiveness of ITNs, as vector activity seems to be higher after people are in bed indoors.

FIGURE 11: *AN. GAMBIAE* S.L. BITING TIME IN THE EIGHT SENTINEL SITES COMPARING RURAL VS PERI-URBAN SITES, MARCH 2019–FEBRUARY 2020









3.2 Laboratory Analysis for Species Identification, Infection Detection, and Blood Meal Origin Determination

The project team sent samples of malaria vectors collected from PSC, HLC, and CDC light trap collections and insecticide resistance monitoring to CSRS for species identification. Samples collected from HLCs were also tested for detection of *Plasmodium* infection. In addition, subsamples collected from PSC and CDC light traps were sent for blood meal origin determination.

3.2.1 SPECIES IDENTIFICATION

A total of 3,384 *An. gambiae* s.l. from 2019 monitoring were successfully amplified using PCR; 79.11% (2,677/3,384) were identified as *An. gambiae* and 20.89% (707/3,384) were identified as *An. coluzzii*. Another 200 *An. gambiae* s.l. collected by HLC and that failed to amplify and therefore could not be identified as *An. gambiae* or *An. coluzzii* were retested for the presence of *An. melas* in the collection. The test identified 14% (28/200) were *An. melas* and the remaining 86% (172/200) were either other members of the *An. gambiae* complex or amplification failures. *An. melas* was reported biting in Bo (19/28), Bombali (8/28), and Western Rural Area (1/28). The study had expected that most *An. melas* would be collected in the coastal areas (Western Rural Area), where brackish water favorable for *An. melas* breeding is available. The report of *An. melas* away from the coastal areas could be due to some salinity of these waters, which is unlikely, or to misclassification.

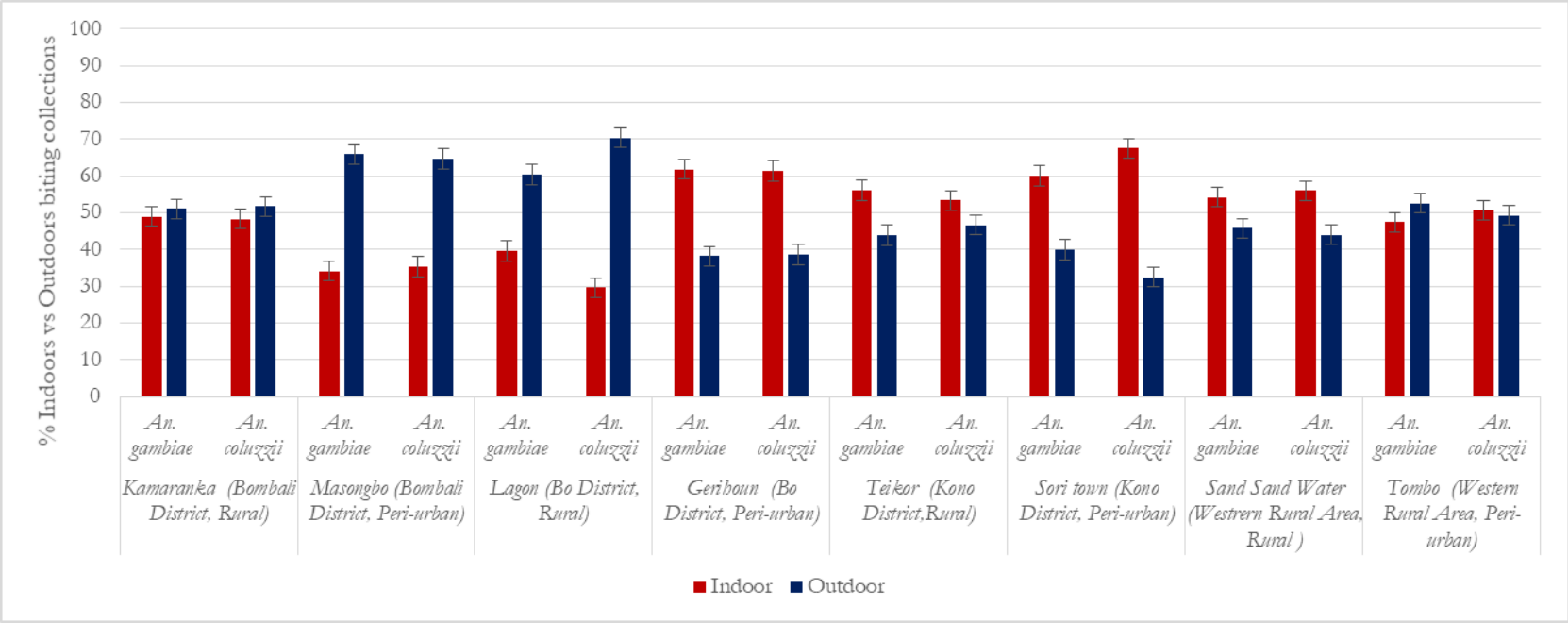
When separated by preference of biting by location, *An. gambiae* s.s. has preference biting inside while An. *coluzzii* showed a preference to bite more outdoor than indoor.

A total of 104 An. *funestus* s.l. from 2019 PSC, HLC, and CDC light trap collections were also processed for species identification using PCR; 92.3% (96/104) were identified as *An. funestus* s.s. and 7.69% (8/104) as *An. leesoni*. *An. leesoni* was collected biting both indoors and outdoors in Kono District and outdoors in Bo District.

3.2.2 *PLASMODIUM* INFECTION

A total of 9,496 *An. gambiae* s.l. and 209 *An. funestus* s.l. were tested for *Plasmodium* infection, and 463/9,705(4.8 %) were found to be positive (Annexes D and E). *Plasmodium* infection was detected in 4.8% of *An. gambiae* s.l. and 3.8% of *An. funestus* s.l.

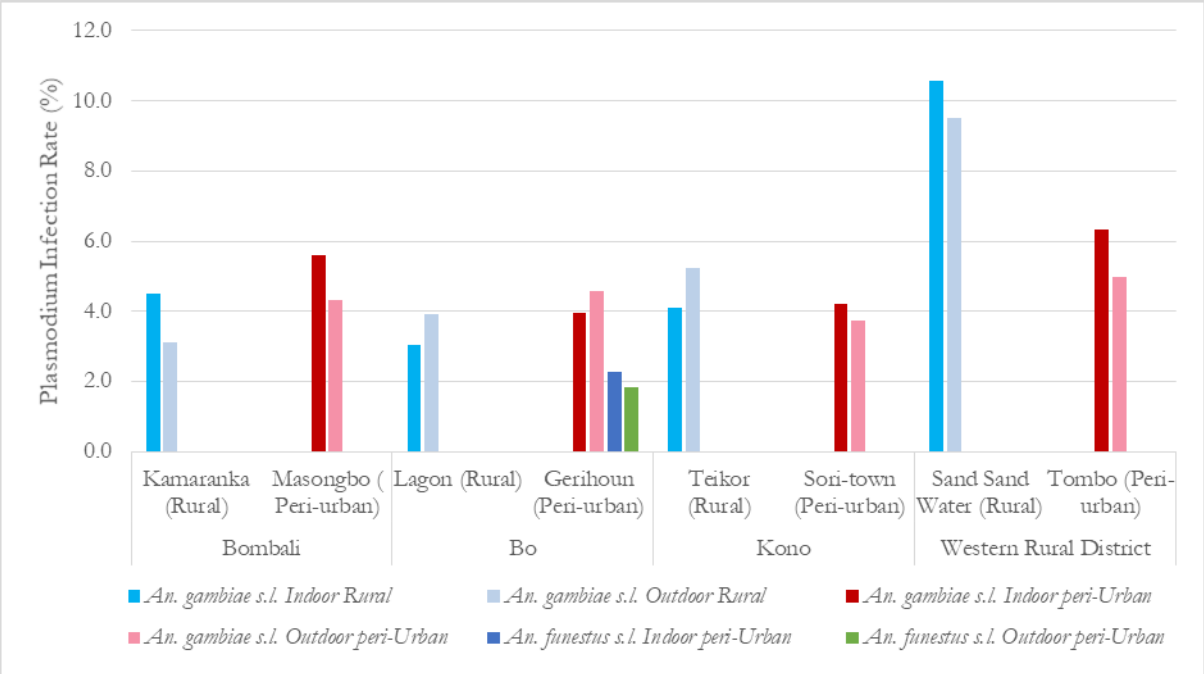
FIGURE 12: *AN. GAMBIAE*, *AN. COLUZZII* AND AN. COLUZZII/GAMBIAE BY BITING LOCATION (PROPORTION)



The overall *An. gambiae* s.l. infection rate during the study period was 4.7% (446/9,524) and was similar indoors 4.8% (223/4,665) and outdoors 4.6% (223/4,859)). The highest indoor infection rate was observed in Sand Sand Water (10.6%), followed by Tombo (6.3%), in Western Rural Area. The lowest indoor *An. gambiae* s.l. infection rate was recorded in Lagon (3%) in Bo District (Figure 13 & Annex D). The highest outdoor infection rate was recorded in Sand Sand Water (8.4%) in Western Rural Area followed by Teikor (5.2%) in Kono District. The lowest outdoor infection rate (3.1%) was recorded in Kamaranka in Bombali District…

The *An. funestus* s.l. overall infection rate is similar indoors (1.7%) and outdoors (1.3%) (Annex E).

FIGURE 13: RATE OF *PLASMODIUM* INFECTION BY SPECIES COMPLEX BY BITING LOCATION COMPARING RURAL VS PERI-URBAN SITES



3.2.3 ENTOMOLOGICAL INOCULATION RATE

The highest EIR was observed in rural areas, indoors, in June, with 56.4 infective bites/person/month. The highest indoors EIR in peri-urban areas was observed in May, with 52.9 infective bites/person/month (Figure 15).

The indoor EIR of *An. gambiae* s.l. in April before the rainy season was 28.1 infective bites/person/month, and it increased to 56.4 infective bites/person/month in June (Annex F). A similar trend was observed in the peri-urban sites, where the indoor EIR in April was 13.6 infective bites/person/month, increased in May to 52.9 infective bites/person/month, and remained high in July at 49.5 infective bites/person/month (Figure 15, Annex G).

The indoor EIR during the wet season (May–October) was high in both rural (average infective bites of 38.3/person/month) and peri-urban sites (36.4 average infective bites/person/month) and was about 3.7 times higher than the EIR recorded during the dry season (November–February) in rural sites (10.4 average infective bites/person/month) and 2.4 times higher than the EIR recorded in peri-urban sites (15.4 average infective bites/person/month) (Annex G & H).

The overall indoor EIR in the rural sites was 305.0 infective bites/person/year and 308.3 infective bites/person/year in the peri-urban sites.

In Bombali District, the overall indoor EIR in peri-urban Masongbo (410.9 infective bites/person/year) was about two times higher than the EIR recorded in rural Kamaranka (216.0 infective bites/person/year). The average EIR in Masongbo during the wet season (59.8 infective bites/person/month) was about six times higher than the EIR recorded during the dry season (10.6 infective bites/person/month) (Figure 16, Annex I). The EIR in Kamaranka during the wet season (average infective bites of 27.4 bites/person/month) was two times higher than the EIR during the dry season (12.9 infective bites/person/ month) (Annex I).

The overall indoor EIR in Kono District was 283.1 and 233.5 infective bites/person/year in Teikor, the rural site, and in Sori Town, the peri-urban site, respectively (Annexes J and K).

The overall indoor EIR in Bo District was 258.2 and 452.0 infective bites/person/year in Lagon, the rural site, and in Gerihoun, the peri-urban site, respectively (Annexes L and M). The highest indoor *An. gambiae* s.l. EIR in Lagon, the rural site (87.7 infective bites/person/month), was recorded in July; the highest EIR in Gerihoun (115.1 infective bites/person/month), the peri-urban site, was in June. The indoor EIR in wet season in Lagon (34.9 infective bites/person/month) was about 19 times higher than the EIR during the dry season (1.8 infective bites/person/month).

The overall indoor EIR in Sand Sand Water, the rural site in Western Rural Area District, was 410.9 infective bites/person/year and 139.1 infective bites/person/year in peri-urban Tombo (Annexes N and O). The highest EIR was recorded in August in both sites, with 98.9 infective bites/person/month in Sand Sand Water and 72.8 infective bites/person/month in Tombo.

FIGURE 14: MONTHLY EIR OF *AN. GAMBIAE* S.L. BY RURAL/PERI-URBAN; ALL SITES, MARCH 2019–FEBRUARY 2020

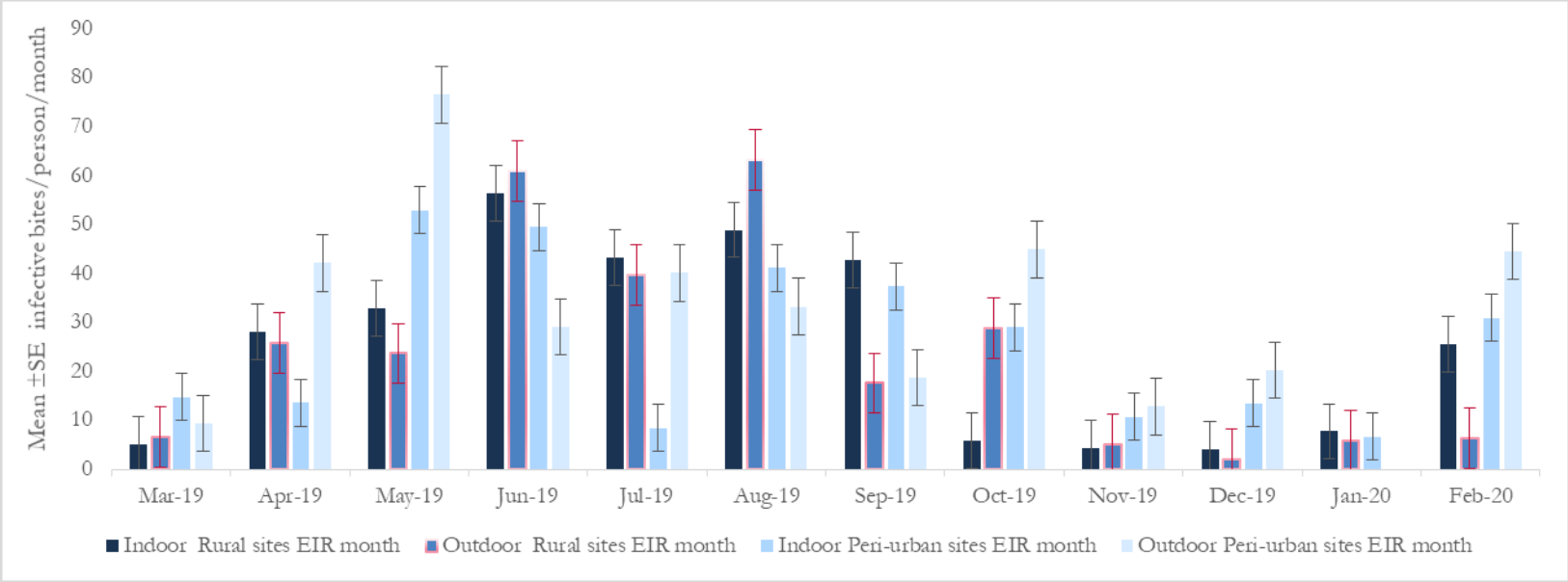
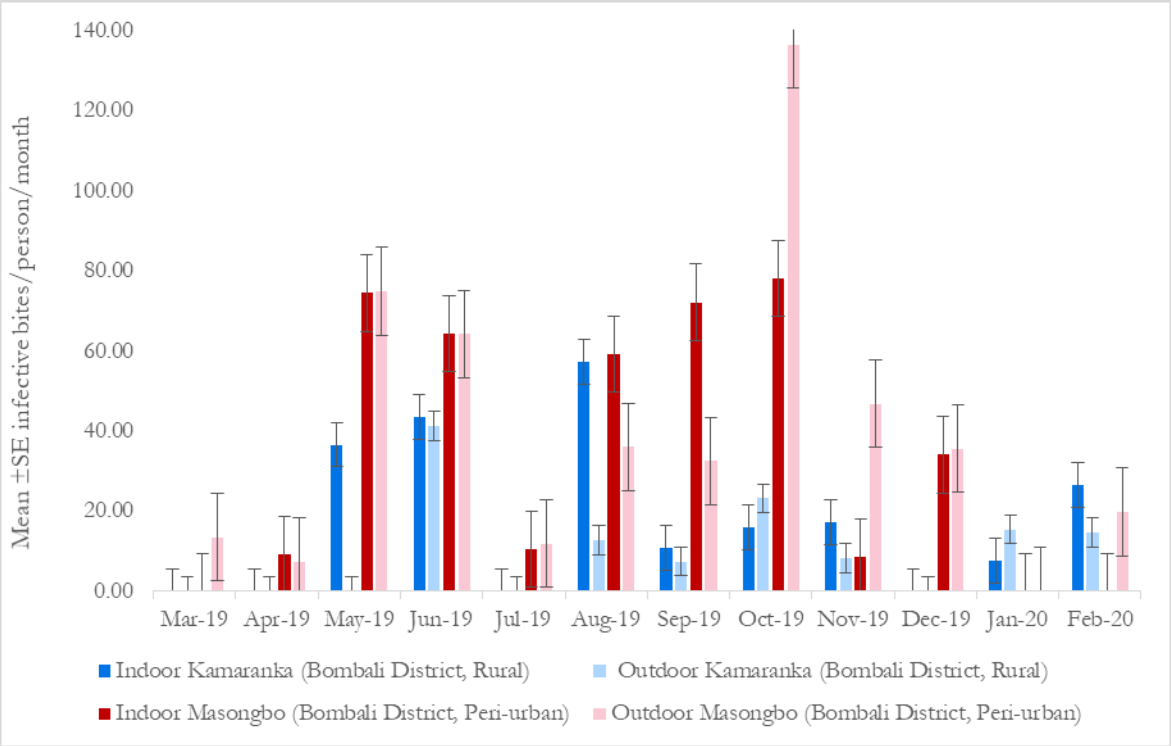
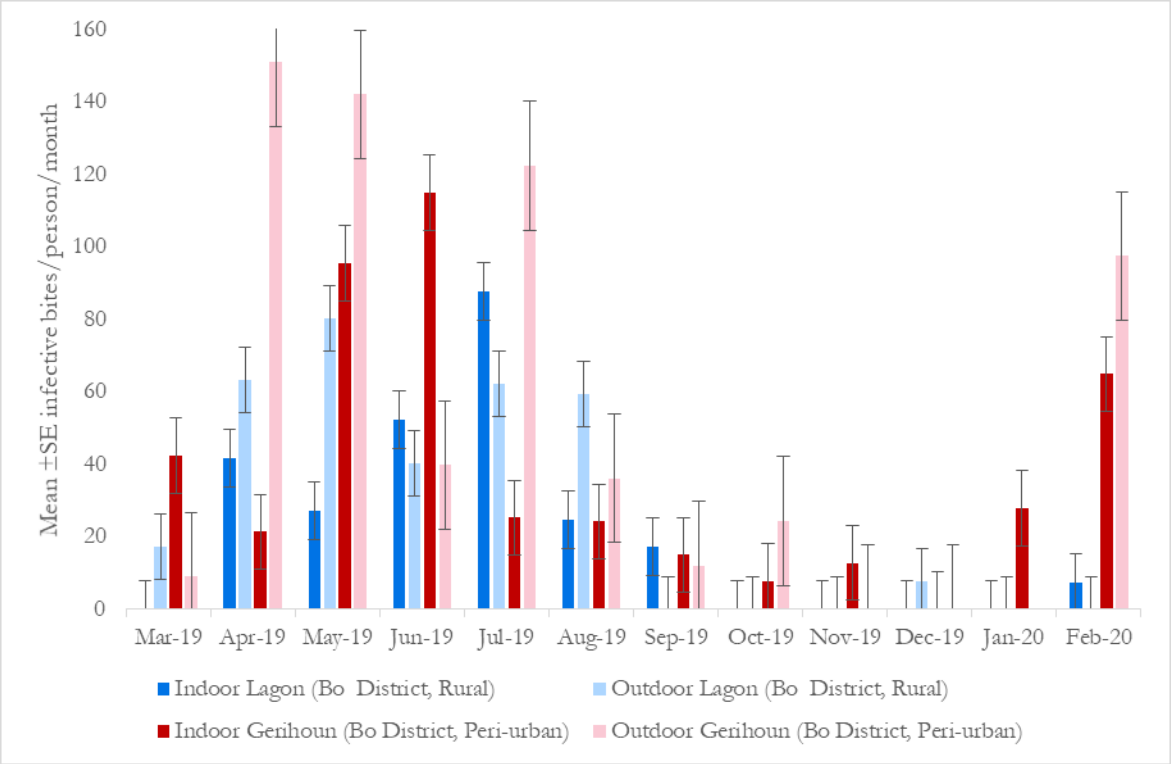
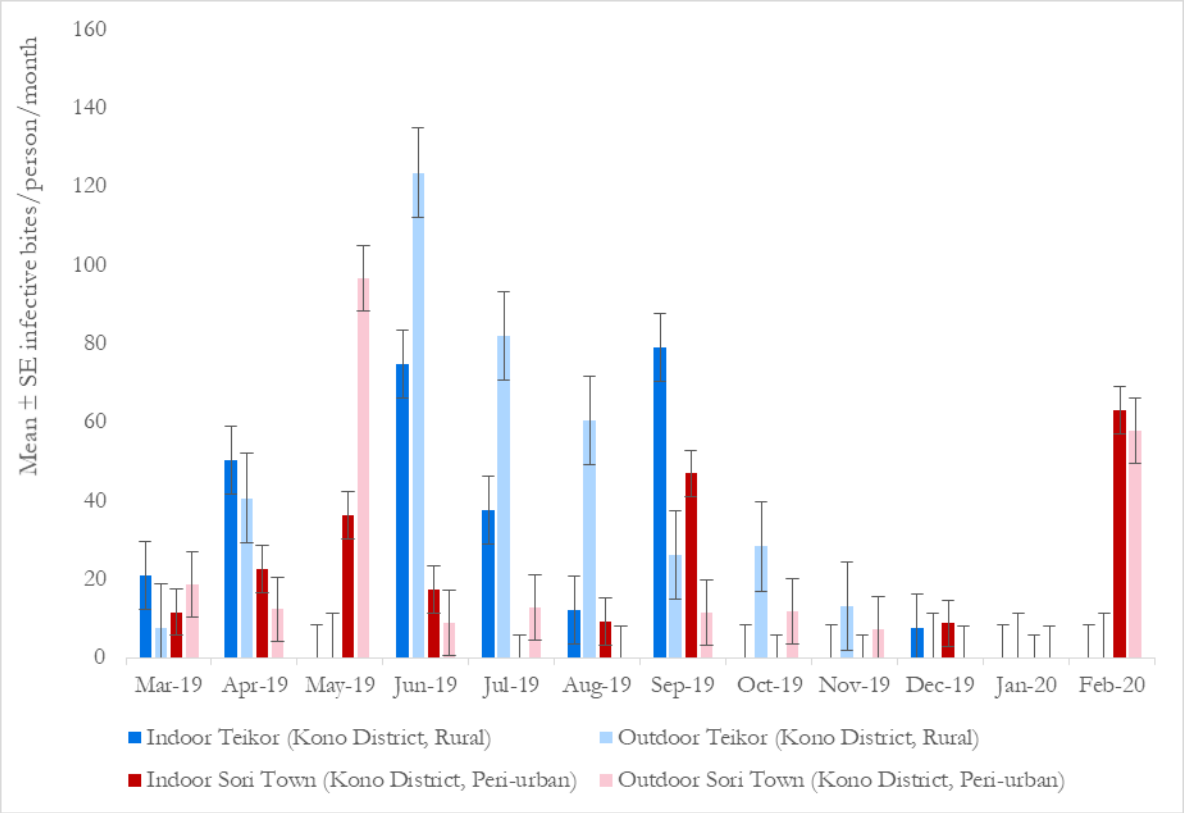
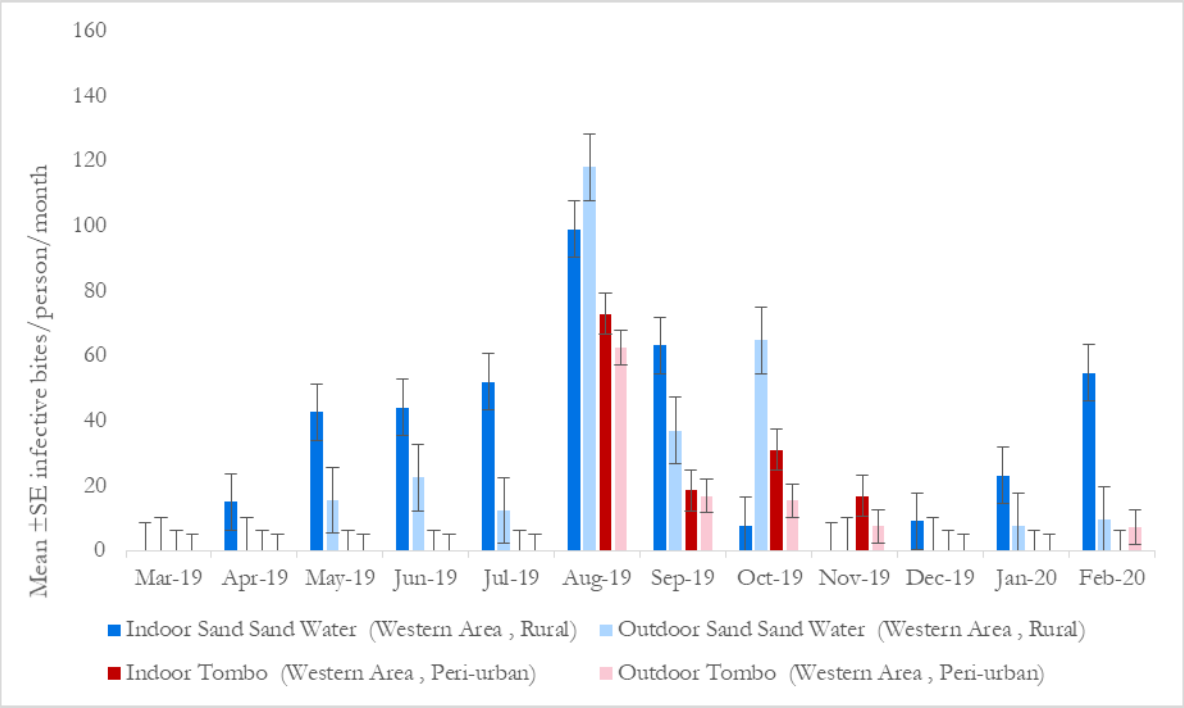


FIGURE 15: MONTHLY EIR OF *AN. GAMBIAE* S.L. BY RURAL/PERI URBAN SITE BY SENTINEL SITE, MARCH 2019–FEBRUARY 2020






Mean ± SE infective bites/person/month
160
140
120
100
80
60
40
20
0
Mar-19
Apr-19
May-19
Jun-19
Jul-19
Aug-19
Sep-19
Oct-19
Nov-19
Dec-19
Jan-20
Feb-20
Indoor Teikor (Kono District, Rural)
Outdoor Teikor (Kono District, Rural)
Indoor Sori Town (Kono District, Peri-urban)
Outdoor Sori Town (Kono District, Peri-urban)


Mean ±SE infective bites/person/month
160
140
120
100
80
60
40
20
0
Mar-19
Apr-19
May-19
Jun-19
Jul-19
Aug-19
Sep-19
Oct-19
Nov-19
Dec-19
Jan-20
Feb-20
Indoor Sand Sand Water (Western Area , Rural)
Outdoor Sand Sand Water (Western Area , Rural)
Indoor Tombo (Western Area , Peri-urban)
Outdoor Tombo (Western Area , Peri-urban)

3.2.4 Blood Meal Origin

In 2019, of the 202 blood-fed malaria vectors collected from CDC light traps and tested to identify the origin of the blood meal; 69.8% (141/202) showed evidence of human blood. Similar findings were observed for the PSC samples with 89.9% (178/198) samples having human blood. (Tables 5 and 6). An additional test conducted for chicken and bovine blood detection was negative.

TABLE 5: HUMAN BLOOD INDEX CDC LIGHT TRAP COLLECTION 2019

District	Sites	Total Tested	Human Blood Positive	Human Blood Index (%)
Bombali	Kamaranka (Rural)	28	16	57.143
	Masongbo (Peri-urban)	34	22	64.71
	Subtotal 2	62	38	61.29
Bo	Lagon (Rural)	14	13	92.86
	Gerihoun (Peri-urban)	31	22	70.97
	Subtotal 1	45	35	77.78
Kono	Teikor (Rural)	37	25	67.57
	Sori Town (Peri-urban)	37	31	83.78
	Subtotal 3	74	56	75.68
Western Rural Area	Sand Sand Water (Rural)	19	10	52.63
	Tombo (Peri-urban)	2	2	100.00
	Subtotal 4	21	12	57.14
Total		202	141	69.80

TABLE 6: HUMAN BLOOD INDEX PSC COLLECTION 2019

District	Sites	Total Tested	Human Blood Positive	Human Blood Index (%)
Bombali	Kamaranka (Rural)	12	11	91.67
	Masongbo (Peri-urban)	25	20	80
	Subtotal 2	37	31	**83.78**
Bo	Lagon (Rural)	26	24	92.31
	Gerihoun (Peri-urban)	38	35	92.11
	Subtotal 1	64	59	**92.19**
Kono	Teikor (Rural)	30	28	93.33
	Sori Town (Peri-urban)	35	31	88.57
	Subtotal 3	65	59	**90.77**
Western Rural Area	Sand Sand Water (Rural)	25	23	92
	Tombo (Peri-urban)	7	6	85.71
	Subtotal 4	32	29	**90.63**
Total		198	178	**89.9**

3.3 INSECTICIDE RESISTANCE MONITORING

3.3.1 SYNERGIST ASSAYS

An. gambiae s.l. exposed to the pyrethroid insecticides deltamethrin, permethrin, and alpha-cypermethrin in Koya (Western Rural Area), Jaima Bongor (Bo), Nimyama (Kono), and Gbanti Kamaranka (Bombali) during the PBO assays were found to be resistant to the insecticides. *An. gambiae* s.l. was resistant to all the pyrethroids tested in all the districts. Mortality ranged from 7.2% to 36.5% for deltamethrin; 10.4% to 33% for permethrin, and 9.4% to 25.3% for alpha-cypermethrin (Figures 16, 17, and 18 and Annex P). After pre-exposure to PBO, the mortality rate increased to between 60% and 72% for deltamethrin, between 69.8% and 76.7% for permethrin, and between 63.5% and 87.1% for alpha-cypermethrin. The increase of *An. gambiae* s.l. mortality after pre-exposure to PBO was at least 40% at all sites for all three pyrethroids except for deltamethrin in Bombali, where the increase was 29%. Nevertheless, mortality remained below the 90% cut-off point for confirmed resistance. Therefore, the results indicate that a monooxygenase-based resistance mechanism is partially involved, but is not fully responsible for the pyrethroid resistance observed.

FIGURE 16: MORTALITY OF *AN. GAMBIAE* S.L. FOR DELTAMETHRIN 0.05% AFTER PBO AND NO PBO EXPOSURE, 2019

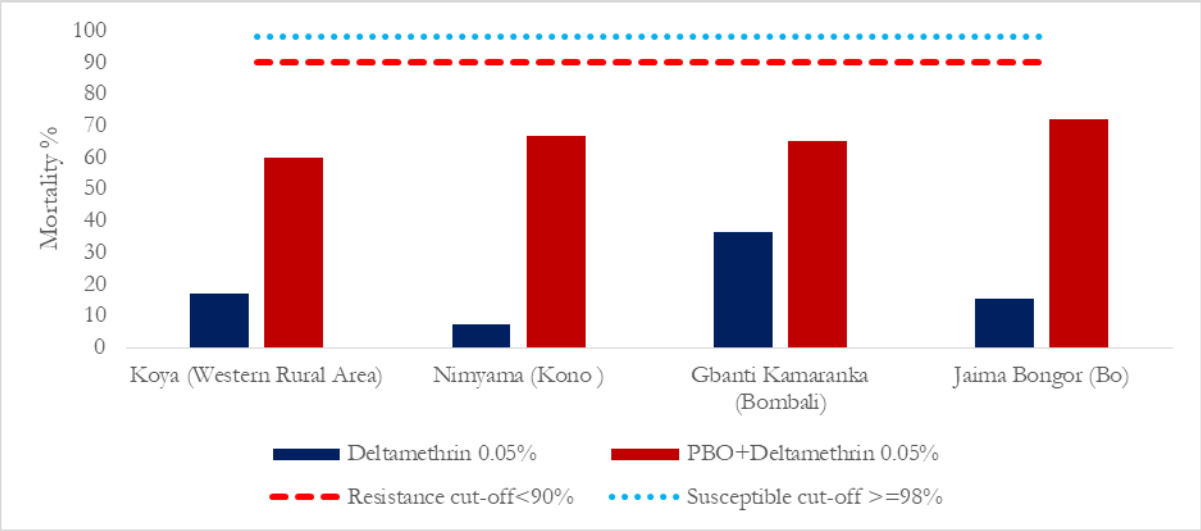


FIGURE 17: MORTALITY OF *AN. GAMBIAE* S.L. FOR PERMETHRIN 0.75% AFTER PBO AND NO PBO EXPOSURE, 2019

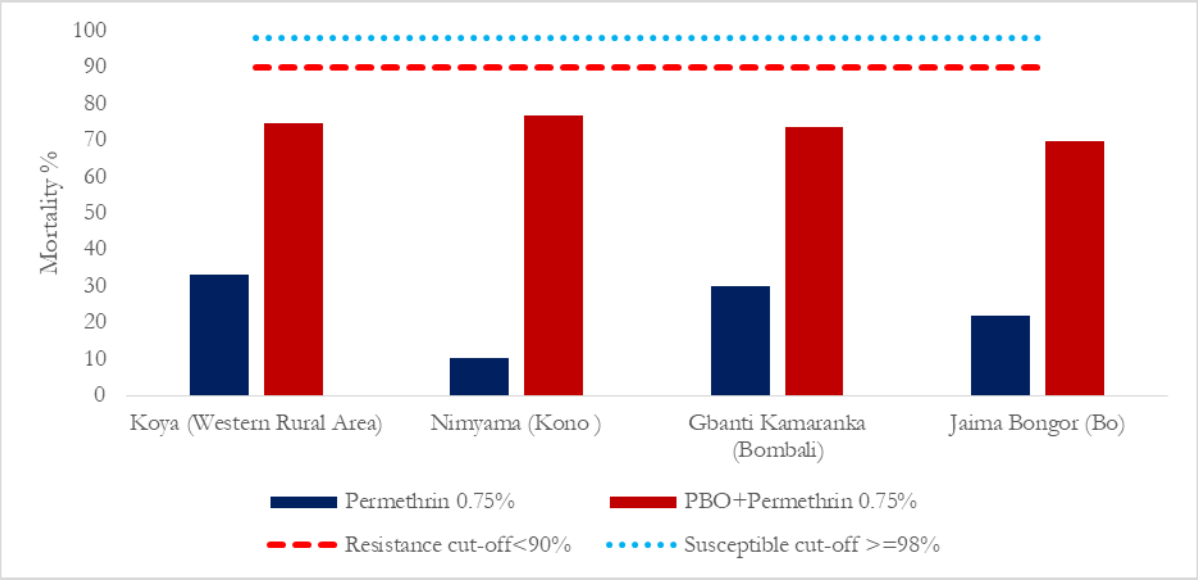
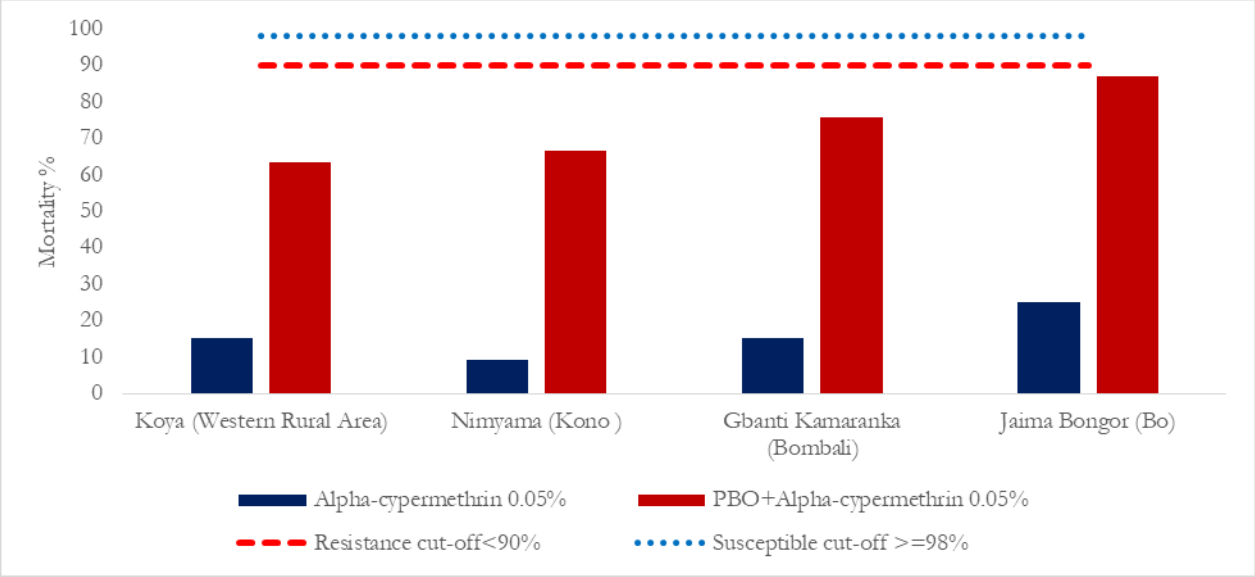


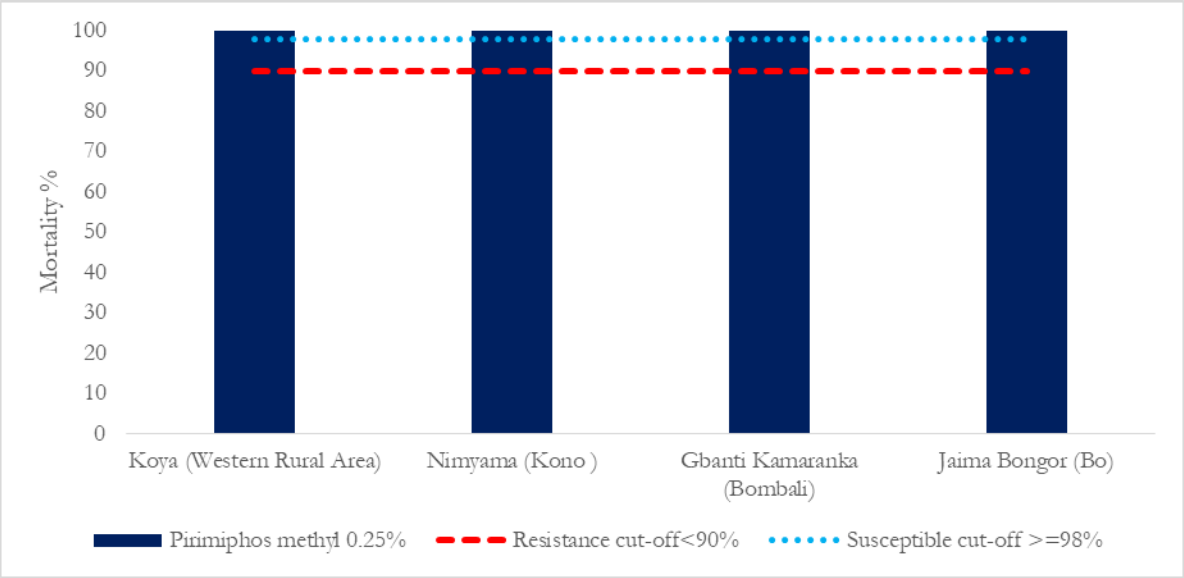
FIGURE 18: MORTALITY OF *AN. GAMBIAE* S.L. FOR ALPHA-CYPERMETHRIN 0.05% AFTER PBO AND NO PBO EXPOSURE 2019



3.3.2 *AN. GAMBIAE* S.L. SUSCEPTIBILITY TO ORGANOPHOSPHATES

An. gambiae s.l. exposed to pirimiphos-methyl in Koya, Jaima Bongor, Nimyama, and Gbanti Kamaranka were fully susceptible to the organophosphate insecticide pirimiphos-methyl (Figure 19).

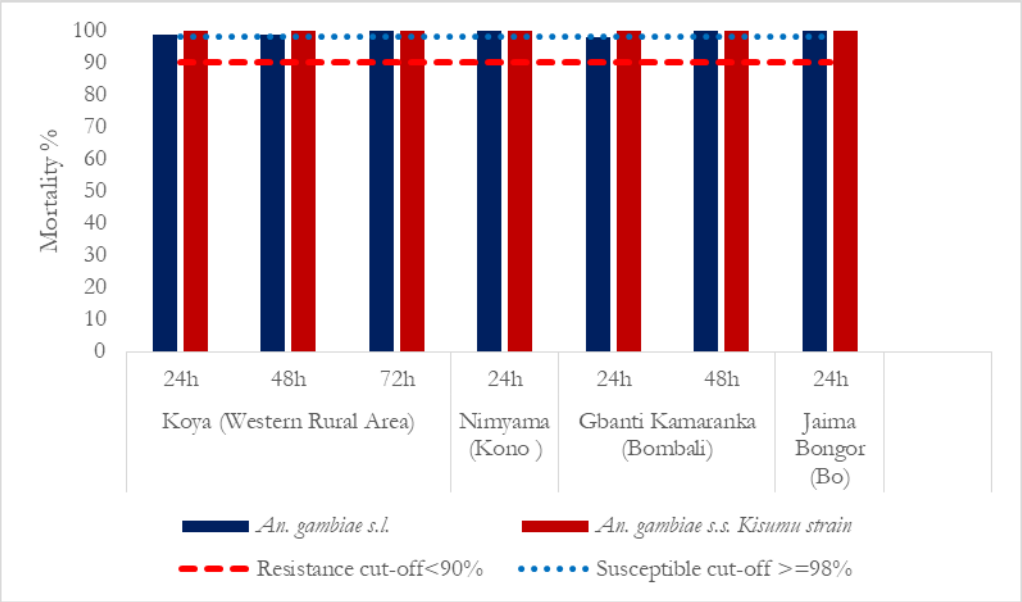
FIGURE 19: SUSCEPTIBILITY STATUS OF *AN. GAMBIAE* S.L. TO PIRIMIPHOS-METHYL (0.25%)



3.3.3 *AN. GAMBIAE* S.L. SUSCEPTIBILITY TO CHLORFENAPYR

Figure 20 shows the results from tests on wild *An. gambiae* s.l. and the laboratory standard *An. gambiae* Kisumu strain. *An. gambiae* s.l. was fully susceptible to chlorfenapyr in all four sites. Mortality of 100% was observed, respectively, within the holding periods of 24 hours (Nimyama and Jaima Bongor), 48 hours (Gbanti Kamaranka), and 72 hours (Koya) after exposure to chlorfenapyr.

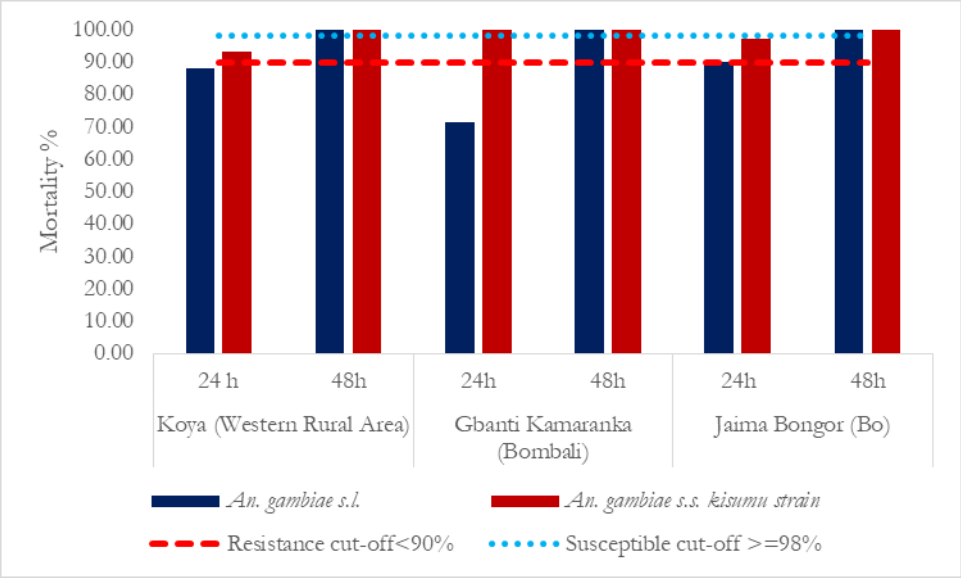
FIGURE 20: MORTALITY OF *AN. GAMBIAE* S.L. FOR CHLORFENAPYR (100 µG/BOTTLE), 2019



3.3.4 *AN. GAMBIAE* S.L. SUSCEPTIBILITY TO CLOTHIANIDIN

Figure 21 shows the results from tests on wild *An. gambiae* s.l. and the laboratory standard *An. gambiae* Kisumu strain. *An. gambiae* s.l. was fully susceptible to clothianidin in Koya, Jaima Bongor, and Gbanti Kamaranka: 100% mortality was achieved within the 48-hour holding period after exposure to clothianidin in the three sites.

FIGURE 21: MORTALITY *OF AN. GAMBIAE* S.L. FOR CLOTHIANIDIN (13.2 MG/PAPER), 2019



3.3.5 DETERMINATION OF THE INTENSITY OF RESISTANCE

High resistance intensity to deltamethrin and permethrin was observed when *An. gambiae* s.l. was exposed to 5 and 10 times the diagnostic concentration in Koya, Gbanti Kamaranka, and Jaima Bongor with the mortality of *An. gambiae* s.l. below 98% (Figures 22 and 23). The intensity of the resistance was also high in Koya, Nimyama, and Gbanti Kamaranka when *An. gambiae* s.l. was exposed to 5 and 10 times the diagnostic dose concentration of alpha-cypermethrin (Figure 24).

FIGURE 22: MORTALITY OF *AN. GAMBIAE* S.L. EXPOSED TO DELTAMETHRIN 5X (62.5 µG/BOTTLE) AND 10X (125 µG/BOTTLE)

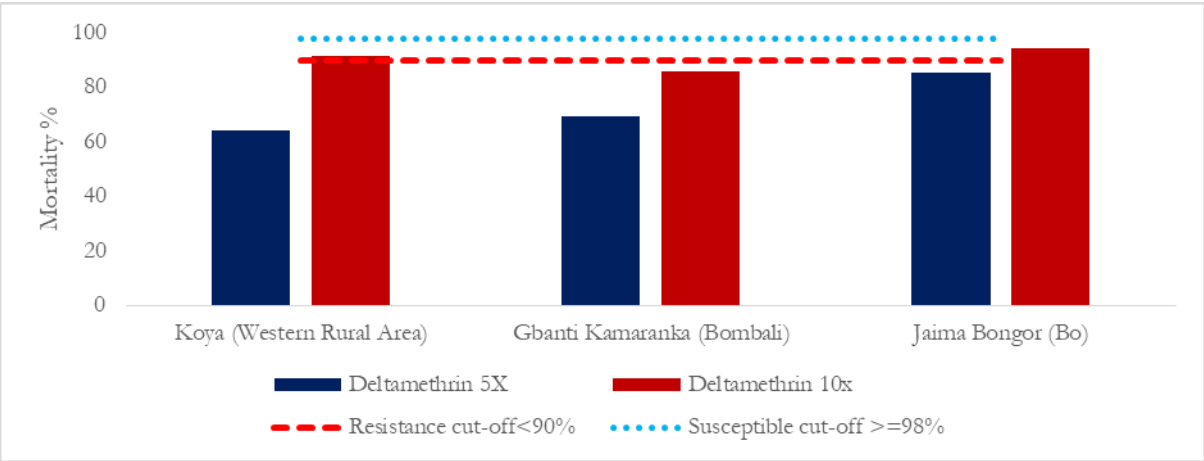


FIGURE 23: MORTALITY OF *AN. GAMBIAE* S.L. EXPOSED TO PERMETHRIN 5X (107.5 µG/BOTTLE) AND 10X (215 µG/BOTTLE)

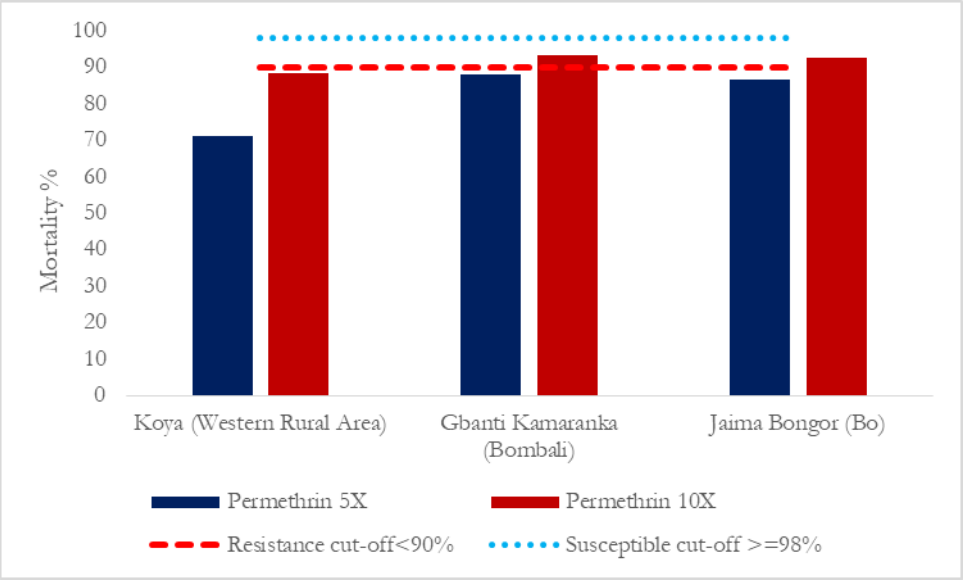
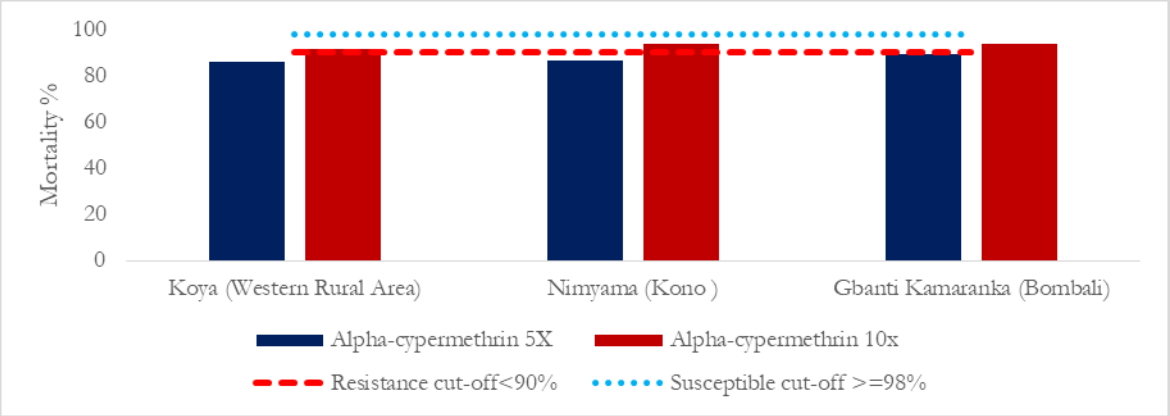


FIGURE 24: MORTALITY OF *AN. GAMBIAE* S.L. EXPOSED TO ALPHA-CYPERMETHRIN 5X (62.5 µG/BOTTLE) AND 10X (125 µG/BOTTLE)



4. CAPACITY BUILDING

- In July 2019, VectorLink Sierra Leone supported by a CDC Atlanta entomologist conducted a refresher training for government supervisors assisting with the entomological monitoring. A total of 17 environmental health officers from each of the sentinel districts (Bo, Bombali, Kono, and Western Rural Area) and two NMCP/Ministry of Health and Sanitation staff were trained in Makeni on aspects of entomological monitoring, including the morphological identification of *Anopheles* mosquitoes, susceptibility tests, and cone bioassays. The in-class sessions of the training were conducted in the midwifery school conference hall in Makeni, and the microscopes mobilized from PMI/VectorLink, CDC and the Ministry of Health and Sanitation Division of Neglected Tropical Diseases allowed the participants to have more time for practice on mosquito identification and dissection. The practical sessions, for the cone bioassay and susceptibility tests, were conducted in the VBDIL laboratory. A practical session was also conducted in Masongbo, and participants were able to practice trap setting CDC light traps and conducting HLCs. A pre-assessment was conducted before the trainings, and the scores ranged from 0 to 5 out of 11 with an average of 2.3. At the end of the training, the scores ranged from 5 to 10; with an average of 7.0. Ttwo participants scored 5.5 and 12 scored above 5.5.
- The NMCP with support from the Global Fund conducted a durability test in 2018 on the long-lasting insecticide-treated nets (LLINs) distributed during the 2017 mass campaign. Samples of LLINs were kept at the insectary for the cone bioassay. In March 2019, VectorLink Sierra Leone supported the LLIN bio-efficacy tests the NMCP conducted on the LLINs distributed in 2017 by providing *An. gambiae* Kisumu strain for the cone bioassay.
- VectorLink Sierra Leone supported the NMCP in reviewing the Integrated Vector Management policy and strategic plan and in holding the quarterly VCTWG and Integrated Vector Management National Steering Committee meetings.
- VectorLink provided support to the VCTWG to review the monitoring, insecticide resistance, and malaria epidemiological data so it could make recommendations on the IRS insecticide to be used in 2021 and the best time to spray.

5. CONCLUSIONS AND RECOMMENDATIONS

- The monitoring results indicate that *An. gambiae* s.l. is the predominant malaria vector in all sentinel sites. *An. funestus* s.l. was collected in low numbers in some sites, with the most found in Gerihoun (Bo District), where the Sewa River offers *An. funestus* s.l. breeding sites.
- The highest indoor *An. gambiae* s.l. HBR was recorded in the peri-urban Gerihoun in Bo District (111.8 bites/person/night). The biting rate of *An. gambiae* s.l. in Bo was two to three times higher than in Kono and Western Rural Area districts. In the rural sites, biting was higher indoors than outdoors. In peri-urban sites, the *An. gambiae* s.l. HBR was higher outdoors than indoors except in Western Rural Area, where the biting was similar indoors and outdoors.
- The proportion of fed *An. gambiae* s.l. collected indoors was high at both rural and peri-urban sites with a high human blood index indicating that the vector feeds mainly on humans rather than on animals. This probably indicates either low community coverage or utilization of ITNs. IEC/BCC (information, education and communication/behavior change communication) on ITN use during and after the ITN mass campaign is necessary to reinforce net use, and reduce human-vector contact.
- The highest indoor EIR was observed in rural areas, indoors, in June with 56.4 infective bites/person/month. The highest indoors EIR in peri-urban areas was observed in May, with 52.9 infective bites/person/month.
- Given that abundance and biting rate indicators show that mosquito population is highest in June with the rain season starting in May and ending in October, future IRS with long lasting insecticide with at least six month remanence should be implemented in April, before the peak of malaria transmission.
- Most human vector contact occurred during the second part of the night (after midnight). Given this vector behavior, high coverage and utilization of ITNs should have a significant impact on malaria prevention.
- Resistance to pyrethroids was observed at all sites at a high frequency. Synergist assays indicate that monooxygenase-based resistance mechanism is partially involved in the resistance to pyrethroids. *Anopheles gambiae* s.l. is fully susceptible to chlorfenapyr and clothianidin. PBO-deltamethrin and PBO-permethrin ITNs that will be distributed in May 2020 may have an effect on malaria transmission in Sierra Leone as it will likely increase the mortality of the malaria vector with *kdr* resistance but without monooxygenase based resistance mechanism.
- *An. gambiae* s.l. is fully susceptible to pirimiphos-methyl and clothianidin, but given the PBO net distribution countrywide, IRS with clothianidin is another viable intervention for malaria reduction and insecticide resistance management in Sierra Leone.

5.1 RECOMMENDATIONS

For VectorLink Sierra Leone:

- The team did not conduct enough ovary dissections during the study period due to limited skills of the field technicians staff. VectorLink entomologists will conduct a refresher training on ovary dissection and slide reading to increase the number ovary dissections which will result in more robust parity data.
- Keep wild and Kisumu mosquitoes in separate rooms to avoid contamination
- Conduct an assessment of the purity of Kisumu from Freetown and Makeni insectaries with the support of CDC Atlanta

- Conduct refresher training for the laboratory technicians on laboratory protocol and emphasize good practices to ensure colony purity.
- Carry out a refresher training to field technicians and supervisors and emphazise quality data collection and data entry and strenthen quality assurance systems.

For the NMCP and VectorLink Sierra Leone:

- Ensure community sensitization for effective use of ITNs to reduce vector-human contact as the Human Blood Index during the study period was high.
- Conduct future rounds of IRS as close as possible to the rainy season (April-May), before the vector population, and malaria transmission, peaks.
- Ensure high-quality application of IRS for the IRS campaign in 2021 so that the insecticide will remain efficacious during peak transmission season; do this by conducting training under master trainers, and reinforcing the direct observation of the spraying by the team leaders and supervisors.
- Assess the residual life of clothianidin in Sierra Leone context after the IRS in 2021.

ANNEX A: NUMBER OF *ANOPHELES* COLLECTED BY PSC, MARCH 2019–FEBRUARY 2020

District	Sites	*PSC*	
		An. gambiae s.l.	*An. funestus s.l.*
Bombali	Masongbo (Peri-urban)	396	0
	Kamaranka (Rural)	308	0
Kono	Teikor (Rural)	655	17
	Sori Town (Peri-urban)	691	29
Bo	Lagon (Rural)	742	18
	Gerihoun (Peri-urban)	751	92
Western Rural Area	Sand Sand Water (Rural)	489	0
	Tombo (Peri-urban)	36	0
Total		4068	156

District	Sites	*PSC*	
		An. gambiae s.l.	*An. funestus s.l.*
Bombali	Kamaranka (Rural)	308	0
	Masongbo (Peri-urban)	396	0
Kono	Teikor (Rural)	655	17
	Sori Town (Peri-urban)	691	29
Bo	Lagon (Rural)	742	18
	Gerihoun (Peri-urban)	751	92
Western Rural Area	Sand Sand Water (Rural)	489	0
	Tombo (Peri-urban)	36	0
Total		4068	156

Annex B: Number of *Anopheles* Collected by CDC Light Traps, March 2019–February 2020

District	Sites	CDC Light Trap		
		An. gambiae s.l.	*An. funestus* s.l.	*An. coustani*
Bombali	Masongbo (Peri-urban)	786	0	7
	Kamaranka (Rural)	440	0	1
Kono	Teikor (Rural)	524	2	0
	Sori Town (Peri-urban)	812	3	0
Bo	Lagon (Rural)	397	4	1
	Gerihoun (Peri-urban)	624	31	1
Western Rural Area	Sand Sand Water (Rural)	195	0	0
	Tombo (Peri-urban)	76	0	0
Total		3854	40	10

District	Sites	CDC Light Trap		
		An. gambiae s.l.	*An. funestus* s.l.	*An. coustani*
Bombali	Kamaranka (Rural)	440	0	1
	Masongbo (Peri-urban)	786	0	7
Kono	Teikor (Rural)	524	2	0
	Sori Town (Peri-urban)	812	3	0
Bo	Lagon (Rural)	397	4	1
	Gerihoun (Peri-urban)	624	31	1
Western Rural Area	Sand Sand Water (Rural)	195	0	0
	Tombo (Peri-urban)	76	0	0
Total		3854	40	10

Annex C: Number of Anopheles Collected by HLC, March 2019-February 2020

District	Sites	*An. gambiae* s.l.		*An. funestus* s.l.		*An. coustani*	
		In	**Out**	**In**	**Out**	**In**	**Out**
Bombali	Masongbo (Peri-urban)	1011	1553	0	2	3	33
	Kamaranka (Rural)	641	593	0	0	0	1
Kono	Teikor (Rural)	906	945	24	13	2	11
	Sori Town (Peri-urban)	664	795	7	7	0	4
Bo	Lagon (Rural)	1166	1121	8	4	3	8
	Gerihoun (Peri-urban)	1626	1815	131	83	5	12
Western Rural Area	Sand Sand Water (Rural)	517	451	0	0	0	4
	Tombo (Peri-urban)	296	318	0	0	0	0
Total		6827	7591	170	109	13	73

District	Sites	*An. gambiae* s.l.		*An. funestus* s.l.		*An. coustani*	
		In	**Out**	**In**	**Out**	**In**	**Out**
Bombali	Kamaranka (Rural)	641	593	0	0	0	1
	Masongbo (Peri - urban)	1011	1553	0	2	3	33
Kono	Teikor (Rural)	906	945	24	13	2	11
	Sori Town (Peri-urban)	664	795	7	7	0	4
Bo	Lagon (Rural)	1166	1121	8	4	3	8
	Gerihoun (Peri-urban)	1626	1815	131	83	5	12
Western Rural Area	Sand Sand Water (Rural)	517	451	0	0	0	4
	Tombo (Peri-urban)	296	318	0	0	0	0
Total		6827	7591	170	109	13	73

ANNEX D: RATE OF *AN. GAMBIAE* S.L. *PLASMODIUM* INFECTION BY BITING LOCATION

District	Site	*An. gambiae* s.l.								
		Indoors			Outdoors			Total		
		EX	Pos	% Pos	Ex	Pos	% Pos	Ex	# Pos	% Pos
Western Rural District	Sand Sand Water	379	40	10.6	346	29	8.4	725	69	9.5
	Tombo	205	13	6.3	221	11	5.0	426	24	5.6
Kono	Teikor	611	25	4.1	630	33	5.2	1241	58	4.7
	Sori Town	474	20	4.2	510	19	3.7	984	39	4.0
Bombali	Kamaranka	468	21	4.5	416	13	3.1	884	34	3.8
	Masongbo	678	38	5.6	998	43	4.3	1676	81	4.8
Bo	Lagon	759	23	3.0	690	27	3.9	1449	50	3.5
	Gerihoun	1091	43	3.9	1048	48	4.6	2139	91	4.3
Total		**4665**	**223**	**4.8**	**4859**	**223**	**4.6**	**9524**	**446**	**4.7**

District	Site	*An. gambiae* s.l.								
		Indoors			Outdoors			Total		
		Tested	Pos	% Pos	Tested	Pos	% Pos	Tested	# Pos	% Pos
Bombali	Kamaranka	468	21	4.5	416	13	3.1	884	34	3.8
	Masongbo	678	38	5.6	998	43	4.3	1676	81	4.8
Bo	Lagon	759	23	3	690	27	3.9	1449	50	3.5
	Gerihoun	1091	43	3.9	1048	48	4.6	2139	91	4.3
Kono	Teikor	611	25	4.1	630	33	5.2	1241	58	4.7
	Sori Town	474	20	4.2	510	19	3.7	984	39	4
Western Rural District	Sand Sand Water	379	40	10.6	346	29	8.4	725	69	9.5
	Tombo	205	13	6.3	221	11	5	426	24	5.6
Total		**4665**	**223**	**4.8**	**4859**	**223**	**4.6**	**9524**	**446**	**4.7**

ANNEX E: RATE OF *AN. FUNESTUS* S.L. *PLASMODIUM* INFECTION BY BITING LOCATION

District	Site	An. funestus s.l.								
		Indoors			Outdoors			Total		
		EX	Pos	% Pos	Ex	Pos	% Pos	Ex	# Pos	% Pos
Western Rural District	Sand Sand Water									
	Tombo									
Kono	Teikor	19	0	0	13	0	0	32	0	0
	Sori Town	7	0	0.0	5	0	0	12	0	0
Bombali	Kamaranka				2	0				
	Masongbo				2	0				
Bo	Lagon	6	0		2	0	0	1	0	0
	Gerihoun	88	2	2.3	55	1	1.8	143	3	2.1
Total		120	2	1.7	79	1	1.3	199	3	1.5

District	Site	*An. funestus s.l.*								
		Indoors			Outdoors			Total		
		Tested	Pos	% Pos	Tested	Pos	% Pos	Tested	# Pos	% Pos
Bombali	Kamaranka				2	0				
	Masongbo				2	0				
Bo	Lagon	6	0		2	0	0	1	0	0
	Gerihoun	88	2	2.3	55	1	1.8	143	3	2.1
Kono	Teikor	19	0	0	13	0	0	32	0	0
	Sori Town	7	0	0	5	0	0	12	0	0
Western Rural District	Sand Sand Water									
	Tombo									
Total		120	2	1.7	79	1	1.3	199	3	1.5

Annex F: Sporozoite Rate and EIR of *An. gambiae* s.l. in Rural Sites, March 2019–February 2020

Position	Indicators	March	April	May	June	July	August	September	October	November	December	January	February
							All rural sites						
	Total collected	266	397	233	767	668	328	190	100	56	36	62	127
Indoor	Total *An. gambiae* s.l. tested	200	291	151	459	449	208	125	99	49	34	62	90
	#SPZ+	2	11	11	18	15	16	15	3	2	2	4	10
	Sporozoite rate	0.0	0.0	0.1	0.0	0.0	0.1	0.1	0.0	0.0	0.1	0.1	0.1
	HBR/night	16.63	24.81	14.56	47.94	41.75	20.50	11.88	6.25	3.50	2.25	3.875	7.9375
	EIR month	**5.15**	**28.14**	**32.89**	**56.40**	**43.24**	**48.88**	**42.75**	**5.87**	**4.29**	**4.10**	**7.75**	**25.58**
	Total collected	165	349	307	756	650	297	189	107	47	37	61	145
Outdoor	Total *An. gambiae* s.l. tested	145	229	201	466	413	191	121	101	34	36	61	84
	#SPZ+	3	9	8	20	13	21	6	14	2	1	3	2
	Sporozoite rate	0.02	0.04	0.04	0.04	0.03	0.11	0.05	0.14	0.06	0.03	0.05	0.02
	HBR/night	10.31	21.81	19.19	47.25	40.63	18.56	11.81	6.69	2.94	2.31	3.81	9.06
	EIR month	**6.61**	**25.72**	**23.67**	**60.84**	**39.64**	**63.27**	**17.57**	**28.74**	**5.18**	**1.99**	**5.81**	**6.26**

Annex G: Sporozoite Rate and EIR of *An. gambiae* s.l. in Peri-urban Sites, March 2019–February 2020

Position	Indicators	March	April	May	June	July	August	September	October	November	December	January	February
							All peri- urban sites						
	Total collected	254	440	555	859	431	320	165	121	148	97	81	126
Indoor	Total *An. gambiae* s.l. tested	201	304	386	488	296	211	116	105	104	70	71	96
	#SPZ+	6	5	19	15	3	14	14	13	4	5	3	13
	Sporozoite rate	0.03	0.02	0.05	0.03	0.01	0.07	0.12	0.12	0.04	0.07	0.04	0.14
	HBR/night	15.88	27.50	34.69	53.69	26.94	20.00	10.31	7.56	9.25	6.06	5.0625	7.875
	EIR month	**14.69**	**13.57**	**52.93**	**49.51**	**8.46**	**41.14**	**37.34**	**29.03**	**10.67**	**13.42**	**6.63**	**30.93**
	Total collected	226	491	750	986	574	393	255	128	224	112	101	241
Outdoor	Total *An. gambiae* s.l. tested	188	306	418	573	388	252	179	105	197	43	19	108
	#SPZ+	4	14	22	9	14	11	7	19	6	4	0	11
	Sporozoite rate	0.021	0.046	0.053	0.016	0.036	0.044	0.039	0.181	0.030	0.093	0.000	0.102
	HBR/night	14.13	30.69	46.88	61.63	35.88	24.56	15.94	8.00	14.00	7.00	6.31	15.06
	EIR month	**9.32**	**42.12**	**76.48**	**29.04**	**40.13**	**33.24**	**18.70**	**44.88**	**12.79**	**20.19**	**0.00**	**44.49**

ANNEX H: SPOROZOITE RATE AND EIR OF *AN. GAMBIAE* S.L. IN KAMARANKA RURAL SITE IN BOMBALI DISTRICT, MARCH 2019–FEBRUARY 2020

Position	**Indicators**	March	April	May	June	July	August	September	October	November	December	January	February
							Kamaranka						
	Total collected	152	86	26	74	112	40	13	35	23	6	19	55
Indoor	Total *An. gambiae* s.l. tested	112	60	22	51	78	27	9	34	20	6	19	30
	#SPZ+	0	0	4	4	0	5	1	2	2	0	1	2
	Sporozoite rate	0	0	0.18182	0.078	0	0.1852	0.1111	0.05882	0.1	0	0.0526316	0.0666667
	HBR/night	38.00	21.50	6.50	18.50	28.00	10.00	3.25	8.75	5.75	1.50	4.75	13.75
	EIR month	**0.00**	**0.00**	**36.64**	**43.53**	**0.00**	**57.41**	**10.83**	**15.96**	**17.25**	**0.00**	**7.75**	**26.58**
	Total collected	80	95	6	109	110	43	16	36	19	0	10	69
Outdoor	Total *An. gambiae* s.l. tested	66	55	6	79	71	26	16	36	17	0	10	34
	#SPZ+	0	0	0	4	0	1	1	3	1	0	2	1
	Sporozoite rate	0.00	0.00	0.00	0.05	0.00	0.04	0.06	0.08	0.06	0	0	0.03
	HBR/night	20	23.75	1.5	27.25	27.5	10.75	4	9	4.75	0	2.5	17.25
	EIR month	**0.00**	**0.00**	**0.00**	**41.39**	**0.00**	**12.82**	**7.50**	**23.25**	**8.38**	**0**	**15.50**	**14.71**

ANNEX I: SPOROZOITE RATE AND EIR OF *AN. GAMBIAE* S.L. IN MASONGBO PERI-URBAN SITE IN BOMBALI DISTRICT MARCH 2019–FEBRUARY 2020

Position	**Indicators**	March	April	May	June	July	August	September	October	November	December	January	February
							Masongbo						
	Total collected	7	10	229	343	119	87	69	74	35	22	10	6
Indoor	Total *An. gambiae* s.l. tested	7	8	143	200	87	57	43	66	31	20	10	6
	#SPZ+	0	1	6	5	1	5	6	9	1	4	0	0
	Sporozoite rate	0.00	0.13	0.04	0.03	0.01	0.09	0.14	0.14	0.03	0.20	0.00	0.00
	HBR/night	1.75	2.50	57.25	85.75	29.75	21.75	17.25	18.50	8.75	5.50	2.5	1.5
	EIR month	**0.00**	**9.38**	**74.47**	**64.31**	**10.60**	**59.14**	**72.21**	**78.20**	**8.47**	**34.10**	**0.00**	**0.00**
	Total collected	14	30	266	476	227	203	130	61	75	23	7	41
Outdoor	Total *An. gambiae* s.l. tested	8	30	165	278	147	131	90	45	48	20	6	30
	#SPZ+	1	1	6	5	1	3	3	13	4	4	0	2
	Sporozoite rate	0.13	0.03	0.04	0.02	0.01	0.02	0.03	0.29	0.08	0.20	0.00	0.07
	HBR/night	3.5	7.5	66.5	119	56.75	50.75	32.5	15.25	18.75	5.75	1.75	10.25
	EIR month	**13.56**	**7.50**	**74.96**	**64.21**	**11.97**	**36.03**	**32.50**	**136.57**	**46.88**	**35.65**	**0.00**	**19.82**

ANNEX J: SPOROZOITE RATE AND EIR OF *AN. GAMBIAE* S.L. IN TEIKOR RURAL SITE IN KONO DISTRICT, MARCH 2019–FEBRUARY 2020

Position	**Indicators**	March	April	May	June	July	August	September	October	November	December	January	February
							Teikor						
	Total collected	64	181	55	205	139	82	98	17	8	14	10	33
Indoor	Total *An. gambiae* s.l. tested	47	135	35	123	86	52	65	17	4	14	10	23
	#SPZ+	2	5	0	6	3	1	7	0	0	1	0	0
	Sporozoite rate	0.0426	0.03704	0	0.04878	0.0349	0.0192	0.1077	0	0	0.07143	0	0
	HBR/night	16.00	45.25	13.75	51.25	34.75	20.50	24.50	4.25	2.00	3.50	2.5	8.25
	EIR month	**21.11**	**50.28**	**0.00**	**75.00**	**37.58**	**12.22**	**79.15**	**0.00**	**0.00**	**7.75**	**0.00**	**0.00**
	Total collected	34	125	97	206	130	103	110	22	23	23	24	48
Outdoor	Total *An. gambiae* s.l. tested	35	92	61	125	86	66	63	18	13	22	24	25
	#SPZ+	1	4	0	10	7	5	2	3	1	0	0	0
	Sporozoite rate	0.03	0.04	0.00	0.08	0.08	0.08	0.03	0.17	0.08	0.00	0.00	0.00
	HBR/night	8.5	31.25	24.25	51.5	32.5	25.75	27.5	5.5	5.75	5.75	6	12
	EIR month	**7.53**	**40.76**	**0.00**	**123.60**	**82.01**	**60.47**	**26.19**	**28.42**	**13.27**	**0.00**	**0.00**	**0.00**

ANNEX K: SPOROZOITE RATE AND EIR OF *AN. GAMBIAE* S.L. IN SORI TOWN PERI-URBAN SITE IN KONO DISTRICT, MARCH 2019–FEBRUARY 2020

Position	**Indicators**	March	April	May	June	July	August	September	October	November	December	January	February
							Sori Town						
	Total collected	127	151	125	58	43	25	47	4	8	8	10	58
Indoor	Total *An. gambiae* s.l. tested	84	100	80	50	44	21	30	3	6	7	9	40
	#SPZ+	1	2	3	2	0	1	4	0	0	1	0	6
	Sporozoite rate	0.01	0.02	0.04	0.04	0.00	0.05	0.13	0.00	0.00	0.14	0.00	0.15
	HBR/night	31.75	37.75	31.25	14.50	10.75	6.25	11.75	1.00	2.00	2.00	2.5	14.5
	EIR month	**11.72**	**23.41**	**36.33**	**17.98**	**0.00**	**9.23**	**48.57**	**0.00**	**0.00**	**8.86**	**0.00**	**67.43**
	Total collected	76	189	216	52	42	42	53	17	12	11	11	74
Outdoor	Total *An. gambiae* s.l. tested	63	114	121	43	25	28	34	11	12	11	11	37
	#SPZ+	2	1	7	1	1	0	1	1	1	0	0	4
	Sporozoite rate	0.0317	0.00877	0.06	0.02	0.04	0	0.03	0.09	0.08	0.00	0.00	0.11
	HBR/night	19	47.25	54	13	10.5	10.5	13.25	4.25	3	2.75	2.75	18.5
	EIR month	**18.70**	**12.43**	**96.84**	**9.07**	**13.02**	**0.00**	**11.69**	**11.98**	**7.5**	**0**	**0**	**58**

ANNEX L: SPOROZOITE RATE AND EIR OF *AN. GAMBIAE* S.L. IN LAGON RURAL SITE IN BO DISTRICT, MARCH 2019–FEBRUARY 2020

Position	**Indicators**	March	April	May	June	July	August	September	October	November	December	January	February
							Lagon						
	Total collected	31	122	130	438	328	64	15	2	7	3	14	12
Indoor	Total *An. gambiae* s.l. tested	23	88	74	251	232	40	13	2	7	3	14	12
	#SPZ+	0	4	2	4	8	2	2	0	0	0	0	1
	Sporozoite rate	0.00	0.05	0.03	0.02	0.03	0.05	0.15	0.00	0.00	0.00	0.00	0.08
	HBR/night	7.75	30.50	32.50	109.50	82.00	16.00	3.75	0.50	1.75	0.75	3.5	3
	EIR month	**0.00**	**41.59**	**27.23**	**52.35**	**87.66**	**24.80**	**17.31**	**0.00**	**0.00**	**0.00**	**0.00**	**7.25**
	Total collected	39	108	166	405	305	52	12	1	2	1	14	16
Outdoor	Total *An. gambiae* s.l. tested	35	64	96	226	190	34	11	1	2	1	14	16
	#SPZ+	2	5	6	3	5	5	0	0	0	1	0	0
	Sporozoite rate	0.06	0.08	0.06	0.01	0.03	0.15	0.00	0.00	0.00	1.00	0.00	0.00
	HBR/night	9.75	27	41.5	101.25	76.25	13	3	0.25	0.5	0.25	3.5	4
	EIR month	**17.27**	**63.28**	**80.41**	**40.32**	**62.20**	**59.26**	**0.00**	**0.00**	**0.00**	**7.75**	**0.00**	**0.00**

Annex M: Sporozoite Rate and EIR of *An. gambiae* s.l. in Gerihoun Peri-urban Site in Bo District, March 2019–February 2020

Position	Indicators	March	April	May	June	July	August	September	October	November	December	January	February
							Gerihoun						
	Total collected	120	279	201	447	210	78	18	15	87	61	55	55
Indoor	Total *An. gambiae* s.l. tested	110	196	163	233	129	50	18	15	51	37	46	43
	#SPZ+	5	2	10	8	2	2	2	1	1	0	3	7
	Sporozoite rate	0.05	0.01	0.06	0.03	0.02	0.04	0.11	0.07	0.02	0.00	0.07	0.16
	HBR/night	30.00	69.75	50.25	111.75	52.50	19.50	4.50	3.75	21.75	15.25	13.75	13.75
	EIR month	**42.27**	**21.35**	**95.57**	**115.11**	**25.23**	**24.18**	**15.00**	**7.75**	**12.79**	**0.00**	**27.80**	**64.91**
	Total collected	136	272	267	437	208	56	35	22	114	66	81	121
Outdoor	Total *An. gambiae* s.l. tested	117	162	131	247	158	36	22	21	114	3		36
	#SPZ+	1	12	9	3	12	3	1	3	0	0		4
	Sporozoite rate	0.0085	0.07407	0.0687	0.01215	0.0759	0.0833	0.0455	0.14286	0	0		0.1111111
	HBR/night	34	68	66.75	109.25	52	14	8.75	5.5	28.5	16.5	20.25	30.25
	EIR month	**9.0**	**151.1**	**142.2**	**39.8**	**122.4**	**36.2**	**11.9**	**24.4**	**0**	**0**		**97.47**

Annex N: Sporozoite Rate and EIR of *An. gambiae* s.l. in Sand Sand Water Rural Site in Western Rural Area District March 2019–February 2020

Position	Indicators	March	April	May	June	July	August	September	October	November	December	January	February
							Sand Sand Water						
	Total collected	19	8	22	50	89	142	64	46	18	13	19	27
Indoor	Total *An. gambiae* s.l. tested	18	8	20	34	53	89	38	46	18	11	19	25
	#SPZ+	0	2	5	4	4	8	5	1	0	1	3	7
	Sporozoite rate	0	0.25	0.25	0.118	0.0755	0.0898876	0.1316	0.02174	0	0.09091	0.1578947	0.28
	HBR/night	4.75	2.00	5.50	12.50	22.25	35.50	16.00	11.50	4.50	3.25	4.75	6.75
	EIR month	**0.00**	**15.00**	**42.63**	**44.12**	**52.06**	**98.92**	**63.16**	**7.75**	**0.00**	**9.16**	**23.25**	**54.81**
	Total collected	12	21	38	36	105	99	51	48	3	13	13	12
Outdoor	Total *An. gambiae* s.l. tested	9	18	38	36	66	65	31	46	2	13	13	9
	#SPZ+	0	0	2	3	1	10	3	8	0	0	1	1
	Sporozoite rate	0.00	0.00	0.05	0.08	0.02	0.15	0.10	0.17	0.00	0.00	0.08	0.11
	HBR/night	3	5.25	9.5	9	26.25	24.75	12.75	12	0.75	3.25	3.25	3
	EIR month	**0.00**	**0.00**	**15.50**	**22.50**	**12.33**	**118.04**	**37.02**	**64.70**	**0.00**	**0.00**	**7.75**	**9.67**

ANNEX O: SPOROZOITE RATE AND EIR OF *AN. GAMBIAE* S.L. IN TOMBO PERI-URBAN SITE IN WESTERN RURAL AREA DISTRICT MARCH 2019–FEBRUARY 2020

Position	Indicators	March	April	May	June	July	August	September	October	November	December	January	February
							Tombo						
	Total collected	0	0	0	11	59	130	31	28	18	6	6	7
Indoor	Total *An. gambiae* s.l. tested	0	0	0	5	36	83	25	21	16	6	6	7
	#SPZ+				0	0	6	2	3	2	0	0	0
	Sporozoite rate	0.00	0.00	0.00	0.00	0.00	0.07	0.08	0.14	0.13	0.00	0.00	0.00
	HBR/night	0.00	0.00	0.00	2.75	14.75	32.50	7.75	7.00	4.50	1.50	1.5	1.75
	EIR month	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**72.83**	**18.60**	**31.00**	**16.88**	**0.00**	**0.00**	**0.00**
	Total collected	0	0	1	21	97	92	37	28	23	12	2	5
Outdoor	Total *An. gambiae* s.l. tested	0	0	1	5	58	57	33	28	23	9	2	5
	#SPZ+			0	0	0	5	2	2	1	0	0	1
	Sporozoite rate	0	0	0	0	0	0.0877193	0.0606	0.07143	0.0435	0	0	0.2
	HBR/night	0	0	0.25	5.25	24.25	23	9.25	7	5.75	3	0.5	1.25
	EIR month	**0**	**0**	**0**	**0**	**0**	**62.54386**	**16.818**	**15.5**	**7.5**	**0**	**0**	**7.25**

ANNEX P: WHO SUSCEPTIBILITY TEST AND CDC BOTTLE ASSAYS RESULTS

Insecticides tested	Gbanti Kamaranka (Bombali)			Jaima Bongor (Bo)			Nimyama (Kono)			Koya (Western Rural Area)		
	# exposed	Mortality %	# replicates	# exposed	Mortality %	# replicates	# exposed	Mortality %	# replicates	# exposed	Mortality %	# replicates
Pirimiphos methyl (0.25%)	100	100%	4	90	100%	4	99	100%	4	87	100%	4
Alpha-cypermethrin 0.05%	99	*15.15%*	4	95	*25.26%*	4	107	*9.35%*	4	78	*15.38%*	4
Alpha-cypermethrin 62.5 µg	113	*89.38%*	4				91	*86.81%*	4	108	*86.11%*	4
Alpha-cypermethrin 125 µg	111	*93.69%*	4				95	*93.68%*	4	95	*91.58%*	4
PBO+Alpha-cypermethrin 0.05%	103	*75.73%*	4	85	*87.06%*	4	93	*66.67%*	4	74	*63.51%*	4
Deltamethrin 0.05%	96	*36.46%*	4	103	*15.53%*	4	97	*7.22%*	4	94	*17.02%*	4
Deltamethrin 62.5 µg	101	*69.31%*	4	96	*85.42%*	4				89	*64.04%*	4
Deltamethrin 125 µg	107	*85.98%*	4	86	*94.19%*	4				93	*91.40%*	4
PBO+Deltamethrin 0.05%	98	*65.31%*	4	100	*72.00%*	4	96	*66.67%*		90	*60.00%*	4
Permethrin 0.75%	100	*30.00%*	4	86	*22.09%*	4	96	*10.42%*		78	*33.00%*	4
Permethrin 107.5 µg	108	*87.96%*	4	83	*86.75%*	4				100	*71.00%*	4
Permethrin 215 µg	104	*93.27%*	4	95	*92.63%*	4				96	*88.54%*	4
PBO+Permethrin 0.75%	102	*73.53%*	4	96	*69.79%*	4	86	*76.74%*		98	*74.49%*	4
Chlorfenapyr 100 µg	97	*100.00%*	**4**	**63**	*100.00%*	**4**	**69**	*100.00%*	**4**	**95**	*100.00%*	**4**
Clothianidin 13.2mg	102	*100.00%*	**4**	**80**	*100.00%*	**4**				**101**	*100.00%*	**4**
		Susceptible	*Possible R*	*Resistant*	*Not Done*							

REFERENCES

Abbott, WS. 1925. A method of computing the effectiveness of an insecticide. *Journal of Economic Entomology* 18: 265–267.

Bass C, Nikou D, Vontas J, Donnelly MJ, Williamson MS, Field LM. 2010. The Vector Population Monitoring Tool (VPMT): High-Throughput DNA-Based Diagnostics for the Monitoring of Mosquito Vector Populations. Malaria Research and Treatment 2010:190434.

Bass C, Nikou D, Blagborough AM, Vontas J, Sinden RE, Williamson MS, Field LM. 2008. PCR-based detection of *Plasmodium* in Anopheles mosquitoes: a comparison of a new high-throughput assay with existing methods. *Malaria Journal.* 17, p. 177 (9 pages).

Beier JC, Perkins VP, Wirtz RA, Koros J, Diggs D, Gargan TP, Koech DK. 1988. Blood meal Identification by Direct Enzyme-Linked Immunosorbent Assay (ELISA), Tested on *Anopheles* (Diptera: Culicidae) in Kenya. *Journal of Medical Entomology.* 1988; 25(1):9-16.

Brogdon W, Chan A. 2010. Guidelines for Evaluating Insecticide Resistance in Vectors using the CDC Bottle Bioassay/Methods in *Anopheles* Research. Second edition. CDC technical report 343.

Cohuet A, Simard F, Toto JC, Kengne P, Coetzee M, Fontenille D. 2003. Species identification within the *Anopheles funestus* group of malaria vectors in Cameroon and evidence for a new species. *The American Journal of Tropical Medicine and Hygiene. 2003; 69(2):200-205.*

Coetzee, M. 2020. Key to the females of Afrotropical *Anopheles* mosquitoes (Diptera: Culicidae). *Malaria Journal* 19, 70. https://doi.org/10.1186/s12936-020-3144-9

Fanello C, Santolamazza F, della Torre A. 2002. Simultaneous identification of species and molecular forms of the *Anopheles gambiae* complex by PCR-RFLP. *Medical and Veterinary Entomology* 16(4):461-4

Gillies MT, Coetzee M. 1987. A supplement to the Anophelinae of Africa south of the Sahara (Afrotropical Region). *Publication of the South African Institute for Medical Research* 55: 33–81.

Jones CM, Liyanapathirana M, Agossa FR, Weetman D, Ranson H, Donnelly MJ, Wilding CS. 2012. Footprints of Positive Selection Associated with A Mutation (N1575Y) in the Voltage-Gated Sodium Channel of *Anopheles gambia*e. P*roceedings of the National Academy of Sciences of the United States of America* 2012 109 (17) 6614-6619.

Koekemoer LL, Kamau L, Hunt RH, Coetzee M. 2002. A cocktail polymerase chain reaction assay to identify members of the *Anopheles funestus* (Diptera: Culicidae) group. *American Journal of Tropical Medicine and Hygiene* 66: 804–811.

Lynd, Amy, Oruni Ambrose, van't Hof Arjen E, Morgan John C, Bwazumo Naego Leon, Pipini Dimitra, O'Kines Kevin A, Bobanga Thierry L, Donnelly Martin J, Weetman David. 2018. Insecticide resistance in *Anopheles gambiae* from the northern Democratic Republic of Congo, with extreme knockdown resistance (*kdr*) mutation frequencies revealed by a new diagnostic assay. *Malaria Journal* 2018; 17(1):412.

Livak, KJ. 1984 Organization and mapping of a sequence on the *Drosophila melanogaster* X and Y chromosomes that is transcribed during spermatogenesis. *Genetics* 107: 611-634.

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.

Riveron, JM, Watsenga F, Irving H, Irish SR, Wondji CS. 2018. High *Plasmodium* infection rate and reduced bed net efficacy in multiple insecticide-resistant malaria vectors in Kinshasa, Democratic Republic of Congo. *Journal of Infectious Dis*ease. 217(2):320-328.

Santolamazza F, Mancini E, Simard F, Qi Y, Tu Z, della Torre A. 2008. Insertion polymorphisms of SINE200 retrotransposons within speciation islands of *Anopheles gambiae* molecular forms. *Malaria Journal. 78: 169-175*

Scott JA, Brogdon WG, Collins FH. 1993. Identification of single specimens of the *Anopheles gambiae* complex by the polymerase chain reaction. *American Journal of Tropical Medicine and Hygiene* 49: 520–529.

Wirtz RD, Burkot TR, Graves PM, Andre RG. July 1987. Field evaluation of enzyme-linked immunosorbent assays for *Plasmodium falciparum* and *Plasmodium vivax* sporozoites in mosquitoes in mosquitoes (Diptera: Culicidae) from Papua New Guinea. *Journal of Medical Entomology*. 1987; 24(4):433-437.

World Health Organization. 2013. Test procedures for insecticide resistance monitoring in malaria vector mosquitoes. Geneva: WHO. http://www.who.int/malaria/ publications/atoz/9789241505154/en/.