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PMI VECTORLINK SIERRA LEONE ANNUAL ENTOMOLOGICAL MONITORING REPORT

MAY 1, 2018 – FEBRUARY 28, 2019

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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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TABLE OF CONTENTS

Acronyms ii

Executive Summary v

1. Introduction 1

2. Methodology 2

2.1 Longitudinal Monitoring 2

2.2 Insecticide Susceptibility Tests 3

2.2.1 WHO Tube Tests 3

2.2.2 CDC Bottle Assays 4

2.3 Resistance Intensity Assays 4

2.4 Synergist Assays 5

2.5 Analysis and Molecular Evaluations 5

2.5.1 PCR for Sporozoite Rate Detection 6

2.5.2 Analysis of Data 6

3. Results 7

3.1 Longitudinal Monitoring 7

3.1.1 Indoor Resting Density (PSC Collection) 8

3.1.2 Abdominal Condition of *An. gambiae* s.l. Collected by PSC 11

3.1.3 CDC Light Trap Collection 1

3.1.4 Human Landing Collections 3

3.2 Laboratory analysis for Species Identification and Infection Detection 9

3.3 Insecticide Resistance Monitoring 16

3.3.1 *An. gambiae* s.l. susceptibility to pyrethroids 16

3.3.2 *An. gambiae* s.l. susceptibility to chlorfenapyr 17

3.3.3 *An. gambiae* s.l. susceptibility to Clothianidin 17

3.3.4 Determination of the intensity of resistance and synergist assays 18

3.4 PCR Analysis for Mechanism of Resistance 20

4. Capacity Building 22

5. Conclusions 23

Annex A: Number of Anopheles collected by PSC, May 2018-February 2019 25

Annex B: Number of Anopheles Collected by CDC Light Traps, September 2018–February 2019 26

Annex C: Number of Anopheles Collected by HLC, May 2018-February 2019 27

References 43

Acronyms

b/p/n	Bites/Person/Night
CDC	U.S. Centers for Disease Control and Prevention
CRID	Centre for Research in Infectious Diseases
EIR	Entomological Inoculation Rate
HBR	Human Biting Rate
HLC	Human Landing Catch
IRD	Indoor Resting Density
IRS	Indoor Residual Spraying
Kdr	Knockdown Resistance
Pf	Plasmodium falciparum
LLIN	Long-lasting Insecticidal Net
NMCP	National Malaria Control Program
OVM	*ovale/vivax/malariae*
PBO	Piperonyl Butoxide
PCR	Polymerase Chain Reaction
PMI	President's Malaria Initiative
PSC	Pyrethrum Spray Catch
USAID	United States Agency for International Development
WHO	World Health Organization

LIST OF TABLES

Table 1. Sentinel Sites for Entomological Monitoring 2
Table 2. Longitudinal Monitoring Adult Mosquito Collection Methods 2
Table 3. Indoor and Outdoor HLC collection of An. gambiae s.l. , all sentinel sites , May 2018 – February 2019 6
Table 4. Mechanism of Resistance 21

LIST OF FIGURES

Figure 1, *Anopheles* Species Collected by the Three Methods, by Site 7
Figure 2. IRD of *An. gambiae* s.l. from All Sentinel Sites, May 2018-February 2019 9
Figure 3. IRD of *An. gambiae* s.l. in Eight Sentinel Sites, May 2018-February 2019 10
Figure 4: Abdominal conditions of *An. gambiae* s.l. collected by PSC from All Sentinel Sites (May-February 2019) 1
Figure 5 Density of *An. gambiae* s.l. from CDC Light Trap Collections in All Sentinel Sites, September 2018-February 2019 1
Figure 6. Density of *An. gambiae* s.l. from CDC Light Trap Collections in Eight Sentinel Sites, September 2018-February 2019 2
Figure 7 *An. gambiae* s.l. HBR in All Sentinel Sites, May 2018-February 2019..... 3
Figure 8. *An. gambiae* s.l. HBRs in the Eight Sentinel Sites, May 2018-February 2019 4
Figure 9. *An. funestus* s.l. HBR in All Sentinel Sites, May 2018-February 2019..... 5
Figure 10. *An. funestus* s.l. HBR in Bombali, bo AND kono districts, May 2018-February 2019..... 5
Figure 11. *An. gambiae* s.l. Biting Time in the Eight Sentinel Sites, May 2018-February 2019 7
Figure 12 12: Parity rate in the eight sentinel sites (May-October 2018) 8
Figure 13: Rate of *Plasmodium* Infection by Species Complex by Biting Location 10
Figure 14. *An. gambi*ae and *An. coluzzi*i by Biting Location and Infection Rate..... 11
Figure 15. *An. gambiae* and *An. coluzzii* by BIting Location (Proportion) 12
Figure 16: Monthly EIR of *An. gambiae* s.l. by rural/Peri-Urban; all sites (May 2018 – February 2019).... 13
Figure 17: Monthly EIR of *An. gambiae* s.l. by sentinel site (May 2018 – February 2019) 14
Figure 18. Susceptibility Status of *An. gambiae* s.l. to Deltamethrin (0.05%), Permethrin (0.75%), and Alpha-cypermethrin (0.05%) 16
Figure 19. Susceptibility Status of *An. gambiae* s.l. to Pirimiphos-methyl (0.25%) and Bendiocarb (0.1%) 16
Figure 20: Mortality of *An. gambiae* s.l. for Chlorfenapyr (100 µg/bottle), October-November 2018 17
Figure 21. Mortality *of An. gambiae* s.l. for Clothianidin (13.2 mg), November 2018..... 17
Figure 22. Mortality of *An. gambiae* s.l. Exposed to 5x (62.5 µg/bottle) and 10X (125 µg/bottle) Alpha-cypermethrin 18
Figure 23. Mortality of *An. gambiae* s.l. for Alpha-cypermethrin 0.05% after PBO and No PBO Exposure 18
Figure 24. Mortality of *An. gambiae* s.l. for Alpha-cypermethrin 5x (62.5 µg/bottle) after PBO and No PBO Exposure..... 19
Figure 25. Mortality of *An. gambiae* s.l. for Deltamethrin 0.05% after PBO and No PBO Exposure, February 2019 19
Figure 26. Mortality of *An. gambiae* s.l. exposed to Deltamethrin 5x (62.5 µg/bottle) and 10X (125 µg/bottle) and to Deltamethrin 5x (62.5 µg/bottle) after PBO and No PBO exposure 19

EXECUTIVE SUMMARY

Malaria is a public health challenge in Sierra Leone. Long-lasting insecticidal net (LLIN) distribution is the main vector control intervention implemented in Sierra Leone for malaria burden reduction. Indoor residual spraying (IRS) has been piloted in selected districts. PMI Vectorlink Sierra Leone provided support to National Malaria Control Program (NMCP) to conduct a longitudinal monitoring from May 2018 to February 2019 in four districts representing the four regions (Northern, Southern, Eastern and Western) in Sierra Leone to assess the malaria vectors bionomics. The susceptibility status of the main malaria vector in Sierra Leone, *An. gambiae* s.l., to insecticides used in Public Health was been also tested.

Vector Bionomics: *An. gambiae* s.l. is the predominant vector in all sentinel sites. A total of 1,028 *An. gambiae* s.l. were processed using the polymerase chain reaction method; 74.7% (768) were *An. gambiae* s.s. and 25.3% (260) were *An. coluzzii*. *An. funestus* s.l. was collected in low numbers in three out of the total eight sites in four districts. It was mainly collected from Gerihoun in Bo District and Teikor in Kono District. *An. funestus* s.l. numbers were generally too low to make any meaningful analysis on abundance and behavioral trends. The peak mosquito season was June, except in Western Rural District even though the vectors were collected throughout the reporting period, May 2018 to February 2019.

Human Biting Rate and Location: The overall indoor average human biting rate for the 10 months was 19.7 bitess/person/night. The overall indoor average human biting rate in June was 73.4 bites/person/night which seems high. Overall, both *An. gambiae* s.s. and *An. coluzzii* slightly prefer to bites outdoors than indoors. In almost all the districts, biting started to increase late in the evening, after 11:00 pm, and was higher around late night.

Parity rate: The parity rate of *An. gambiae* s.l. was 69.5 % [61.6%-77.5%] in the rural sites versus 91.2% [85.7.1%-96.7%] in the peri-urban sites. There is a statistically significant difference between the *An. gambiae* s.l. parity rate in the rural sites and the peri-urban sites ($p<0.05$). The parity rate is higher in the peri-urban sites than the rural sites.

Infection Rate and Entomological Inoculation Rate (EIR): *Plasmodium* infection was detected in 4.1% of *An. gambiae* s.l. and 2.7% of *An. funestus* s.l.. For *An. gambiae* s.l. overall, infection rates were slightly higher outdoors (4.3%) than indoors (3.9%). The *Plasmodium* infection rate for *An. coluzzii,* 11.9% (27/226), was slightly higher than for *An. gambiae* s.s., 9.6% (58/603). In rural areas, the entomological inoculation rate (EIR) indoors peaked at 62.4 infective bitess per month in August in peri-urban areas, it peaked at 36.5 infective bitess per month in July.

Insecticide Resistance: *An. gambiae* s.l. is fully susceptible to pirimiphos-methyl, chlorfenapyr, and clothianidin, whereas it showed a high intensity resistance to the pyrethroids deltamethrin, permethrin, and alpha-cypermethrin. Synergist assays with piperonyl butoxide (PBO) were done to understand mechanisms of resistance and inform LLINs procurement decisions. Monooxygenase-based resistance mechanism is partially involved in the resistance to pyrethroids.

Resistance Mechanism: In general, the frequency of *kdr-w* allele mutation was found in more than 98% of the samples screened. Mutations for *kdr-e* and *ace-1* allele were observed in 2.5% and 13% of the samples tests, respectively. The homozygote resistant is highly predominant.

Conclusion: deltamethrin or alpha-cypermethrin PBO LLINs or nets impregnated with other non-pyrethroid insecticides may be considered for malaria vector control in Sierra-Leone. IRS with pirimiphos-methyl or clothianidin is an option for malaria vector control.

I. INTRODUCTION

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.

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 first cause of morbidity and mortality among children under 5 years. Malaria prevalence is two times higher in rural areas (49%) than urban areas (25%).[1]

In 2011 and 2012, indoor residual spraying (IRS) with lambda-cyhalothrin was piloted in selected chiefdoms in Bo, Bombali, Kono, and Western Area Rural Districts, with 97% of the structures sprayed and 309,209 people protected. This was discontinued after 2012 for logistical reasons. 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.

Under Task Order 1 (Year 1), VectorLink Sierra-Leone implemented entomological monitoring activities that included collecting data on vector bionomics, insecticide resistance, and establishment and maintenance of an insectary. Monthly entomological monitoring activities were conducted in four districts selected in consultation with the NMCP: Bo, the second-largest district in the country, which represents the south of the country; Bombali, which represents the north; Kono District, where there is large-scale mining activity represents the east; and Western Area Rural District, which represents the coastal areas. The vector bionomic monitoring was done in two chiefdoms per district for a total of eight sentinel sites. Insecticide susceptibility tests were performed in one rural chiefdom per district where more *An. gambiae* s.l. breeding sites were available

In 2018, VectorLink Sierra-Leone supported the NMCP by establishing a functional insectary in Makeni (Bombali District) and making a susceptible Kisumu strain of *Anopheles gambiae* available in Sierra-Leone for the first time. The project also supported the monitoring of the 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 served as the basis for the review of the Insecticide Resistance Monitoring and Management Plan (IRMMP), which is currently under review. Based on the findings from the insecticide resistance monitoring, VectorLink Sierra-Leone provided technical assistance to the NMCP. The information is being used by the NMCP to select the most appropriate LLINs for the 2020 mass campaign.

[1] Sierra Leone Malaria Indicator Survey (MIS 2016)

2. METHODOLOGY

2.1 LONGITUDINAL MONITORING

Adult mosquitoes were collected monthly 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 in the western region, from May 2018 to February 2019. In each district mosquitoes were collected in one peri urban and one rural sentinel site (Table 1). The peri-urban site is an area closer to a metropolitan municipalilty. It is a transition zone between the urban and the rural area with a mix of traditional rural dwellings and relatively modern houses with a 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 with agriculture as the main occupation.

Table 1. Sentinel Sites for Entomological Monitoring

District	Chiefdom	Sentinel Site	Location	Activity	
				Vector bionomic monitoring	Vector susceptibility
Bo	Jaima Bongor	Lagon	Rural	✓	✓
	Boama	Gerihoun	Peri-urban	✓	
Bombali	Gbanti Kamaranka	Kamaranka	Rural	✓	✓
	Makari Gbanti	Masongbo	Peri-urban	✓	
Kono	Nimyama	Teikor	Rural	✓	✓
	Nimikoro	Sori Town	Peri-urban	✓	
Western Rural	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) and human landing catches (HLCs) started in May 2018 in Bombali and Kono, and in Bo and Western Rural in June. U.S. Centers for Disease Control and Prevention Light Trap (CDC-LT) collections started in September 2018, due to the delay in the arrival of the traps in-country. 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.	4 nights every month from May to October and 2 nights from November to February	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 from September to February	4 houses for 4 consecutive nights (inside) per site.

For PSC collections, the project entomology team collected mosquitoes in five houses per day for three consecutive days each month in each of the sentinel sites. The PSCs were performed in the morning hours (5:00-9:00 am) 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 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, followed by the space inside the house.. 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 noted, and the samples were kept in moist petri dishes and transported to the laboratory for morphological identification. Vector species collected were preserved in properly labeled 1.5 ml screw-top tubes with silica gel. The same sentinel sites and houses were visited for the monthly collection 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.

For the monthly HLCs, a team of two trained collectors performed hourly collections simultaneously in two houses from 6:00 p.m. to 7:00 a.m. over four nights from May to October 2018, and for two nights from November 2018 to February 2019. . The houses were selected in May 2018 taking into account characteristics such as proximity to the breeding site and materials from which houses were made. One of the two houses 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) were seated 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 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 from indoors and outdoors every hour to reduce possible differences in their attractiveness to mosquitoes.

The data was used to determine the human biting rate (mean number of mosquito bites per person per night/month), as well as feeding locations and times. Subsamples of the *An. gambiae* s.l. and *An. funestus* s.l. collected were further analyzed by PCR. A subsample of unfed *An. gambiae* s.l. collected were dissected to determine parity rate. In each house, one person collected indoors, inside a room, while the other collected mosquitoes outdoors, in the open courtyard of the compound.

CDC Light Traps were set up indoors in four randomly selected houses where people slept under an untreated mosquito net for four consecutive nights each month, from September 2018 to February 2019. 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 centimeters from a human sleeping under a bed net. The traps operated from 6:00 p.m. to 7:00 a.m. for four nights in each house (16 house-nights /site in total). Mosquitoes were collected from the traps once – in the morning.. Mosquitoes trapped were transported to the laboratory for morphological identification

A taxonomic key (Gillies and Coetzee 1987) 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 for Research in Infectious Diseases (CRID) laboratory.

2.2 Insecticide Susceptibility Tests

World Health Organization (WHO) tube tests and CDC bottle assays were performed to assess the susceptibility of local vector populations to insecticides used for IRS and LLINs. All the sentinel sites have a history of LLIN coverage, which has been maintained over the years through mass distribution campaigns, as well as Antenatal Care (ANC) and Expanded Program of Immunization (EPI) clinics including Maternal and Child Health Week (MCHW). .

Methodologies are described below.

2.2.1 WHO Tube Tests

The project team performed insecticide susceptibility tests using the WHO tube method in selected sentinel sites. 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 adult stage and only *An. gambiae* s.l. were used for the susceptibility tests.

WHO tube tests were conducted to assess *An. gambiae* s.l. susceptibility to deltamethrin 0.05%, permethrin 0.75%, alpha-cypermethrin 0.05%, and pirimiphos-methyl 0.25% in four sites: Gbanti Kamaranka (Bombali District), Nimyama (Kono District), Jaima Bongor (Bo District) and in Koya (Western Area District). Bendiocarb 0.1% was also tested in all sites except in Gbanti Kamaranka (Bombali District).

Additionally, the team determined the susceptibility status of wild *An. gambiae* s.l. from selected sites to 13.2mg (per one impregnated paper, 15x12cm) of clothianidin, using papers that were impregnated in country. After the 24-hour holding period, the numbers of dead mosquitoes in both the exposure and the control tubes were recorded. Mortalities were corrected using Abbott's formula if the control mortalities were greater or equal to 5% and less than 20%, but tests were discarded and repeated if control mortalities were greater or equal 20% (Abbott, 1925). For clothianidin, knockdown was recorded after 30 and 60 minutes and mortalities recorded at 1, 2, 3, 4, 5, 6, and 7 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 24 hours indicates susceptibility. Mortality of less than 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 repeat test; if mortality is <90%, then the presence of resistant individuals in the vector population is confirmed.

The project team carried out additional synergist assays with piperonyl butoxide (PBO) using the WHO procedure with deltamethrin 0.05% (1x) in Gbanti Kamaranka, Jaima Bongor, and Nimyama in February 2019. Whenever the control mortality was ≥5% and <20%, the observed mortality was corrected using Abbott's formula.

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 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 and alpha-cypermethrin at all sites except Nimyama (Kono District), where the number of mosquitoes reared was not enough to conduct deltamethrin 10x assay. In addition, the team conducted a CDC bottle assay with PBO pre-exposure for alpha-cypermethrin 62.5 ug/bottle (5x) and deltamethrin 62.5 ug/bottle (5x) in Gbanti Kamaranka, Jaima Bongor, and Nimyama Districts. 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 alpha-cypermethrin 0.05% (1x) in Gbanti Kamaranka, Jaima Bongor, and Nimyama and with deltamethrin 0.05% (1x) in Koya chiefdom. In addition, a CDC bottle assay was conducted with PBO pre-exposure for alpha-cypermethrin 62.5ug/bottle (5x) and deltamethrin 62.5ug/bottle (5x) in Gbanti Kamaranka, Jaima Bongor, and Nimyama.

Two bottles (synergist-exposure and control) were prepared to run each synergist assay. One bottle was coated with 1ml of the synergist (PBO) stock solution and served as the synergist-exposure bottle; the other bottle was coated with 1ml of acetone and served as a synergist-control bottle (without synergist). A batch of 100–125 mosquitoes was introduced into the synergist-exposure bottle, and another batch of 100–125 mosquitoes from the same population was introduced into the synergist-control bottle. Both set-ups were held for one hour. After the hour, the mosquitoes were transferred to two holding cages, one for the synergist-exposed mosquitoes and another for the synergist-control mosquitoes.

CDC bottle bioassays were then run using one set of insecticide-coated bottles (one control and four test bottles) for the synergist-exposed mosquitoes and another set (one control and four test bottles) for the synergist-control mosquitoes. The number of dead or alive mosquitoes was monitored at 15-minute intervals as per the CDC bottle bioassay protocol. Data for the two populations of test mosquitoes were then compared.

2.5 Analysis and Molecular Evaluations

CRID performed molecular analyses to:

- Determine sporozoite rates and calculate EIRs
- Identify members of the *An. gambiae* s.l. and the *An. funestus* s.l. complex to species (Scott, 1993)
- Determine the frequency of knockdown resistance (*kdr)* genes and other molecular markers of insecticide resistance

Genomic DNA extraction:

Genomic DNA (gDNA) were extracted from each mosquito sample via the LIVAK method (Livak 1984) and stored in -20ºC following extraction. NanoDrop™ spectrophotometer (ThermoScientific, Wilmington, USA) was used to determine the concentration and purity of the extracted DNA (Riveron et al. 2018).

Molecular identification of mosquito species by PCR

Identification of *An. gambiae* s.l**:** Identification of members of *An. gambiae* complex was done using the simple SINE PCR following the protocol of Santomalazza et al (Santolamazza et al. 2008) allowing the detection of *An. gambiae, An. coluzzii* and *An arabiensis*. In coastal sites where other species such as *An. melas* were present, the PCR-RFLP protocol described by Fanello et al. was used (Fanello et al. 2002)

Identification of *An. funestus*: *An. funestus* group species was determined using a cocktail PCR according to Koekomoer et al (Koekemoer et al. 2002) with addition of the *An. rivulorum-like* primers (Cohuet et al. 2003). gDNA from 100 randomly selected mosquitoes were processed per each location period as described by Koekoemoer *et al.* (Koekemoer et al. 2002). PCR products were run via electrophoresis through a 1.5% agarose gel with Midori Green® (Gene flow, UK) and visualised using ultraviolet light.

Molecular characterisation of insecticide resistance mechanisms:

i) Genotyping of L1014F and L1014S Kdr mutations:The frequency of the 1014F *kdr* mutation was assessed using TaqMan real-time PCR thermocycler (Agilent Mx3005) following established protocols (Bass et al. 2007; Bass et al. 2010a). In addition, the 1014S *kdr* mutation was also genotyped. The primers *kdr*_F (5'- CATTTTTCTTGGCCACTGTAGTGAT-3') and *kdr*_R (5'- CGATCTTGGTCCATGTTAATTTGCA-) were used without modification. Initially, 9µl made of 5µl of Sensimix (Bioline), 0.25µl of 40x Probe Mix coupled to allelic-specific primers and 4.25µl of dH_20 were

mixed. 1µl of genomic DNA was added to a total volume of 10µl. Thermocycling was carried out using the following condition: initial denaturation of 10min at 95°C, followed by 40 cycles each of 92°C for 15s, and 60°C for 1min. Two probes labeled with fluorochromes FAM and HEX were used to detect the mutant alleles and the wild type susceptible alleles, respectively. FAM to detect the resistant allele for 1014F *kdr* (5'-ACGACAAAATTTC-3') or (5'-ACGACTGAATTTC-3' for 1014S *kdr*)], and HEX (5'-CTTACGACTAAATTTC-3') to detect the susceptible allele. Genotypes were scored from scatter plots of results produced by the Mx3005 v4.10 software. Three positive samples of known genotypes: (i) homozygote resistant for 1014F or 1014S *kdr*; (ii) heterozygote for 1014F or 1014S *kdr*; and (iii) susceptible L1014 were used as positive controls for each of the two experiments. 1µl of ddH_20 was incorporated into the negative control well. The presence of *kdr* allele N1575I in *An. gambiae* (Jones et al. 2012) was also monitored using a TaqMan assay.

ii) Detection of ace-1R mutation

The presence of the G119S ace-1^R mutation (Bass et al. 2010a) was assessed using a Taqman assay (iAChE) (Bass et al. 2010b). 10µl comprise of 1×Sensimix (Bioline), 80x primer/probe mix and 1µl DNA were prepared for each sample. The probes were labeled with specific fluorophores: FAM to detect the mutant allele (S119), and HEX, to detect the susceptible allele (G119). Assay was performed using Agilent MX3005 real-time PCR machine with cycling conditions of 95°C for 10 min, followed by 40 cycles each of 95°C for 15s and 60°C for 1min. In addition, four controls were used: (i) DNA from fully susceptible *An. coluzzii* female (Ngoussou colony); (ii) DNA from fully susceptible *An. gambiae s.s.* female (Kisumu colony); (iii) DNA from a susceptible female (SS-*ace-1*); and a negative control (1µl of ddH_20).

2.5.1 PCR for Sporozoite Rate Detection

The heads and thoraxes of some of the *An. gambiae* s.l. and *An. funestus* s.l. were sorted and tested for the presence of *Plasmodium* sporozoite using the polymerase chain reaction (PCR) method.

A TaqMan assay (Bass et al. 2008), with MX 3005 (Agilent, Santa Clara, USA) was used to identify the *Plasmodium* in the head/thoraces of sub-samples *An. gambiae* s.l. and *An. funestus* s.l. collected from HLC. 1µl of gDNA was used for amplification with the following condition: initial denaturation at 95°C for 10min, followed by 40 cycles each of 15s at 95°C and 1min at 60°C. Primers described previously [PlasF_GCTTAGTTACGATTAATAGGAGTAGCTTG and PlasR_GAAAATCTAAGAATTTCACCTCTGACA (Bass et al. 2008)] was used together with two probes labelled with fluorophores, FAM (Falcip+_TCTGAATACGAATGTC) to detect P. falciparum, and HEX (OVM+_CTGAATACAAATGCC) to detect combination of P. ovale, P. vivax and P. malariae. Positive samples (known FAM+ and OVM+) were used as controls, in addition to a negative control to which 1µl of ddH20 was added.

2.5.2 Analysis of Data

The following parameters were estimated:

- Human biting rate (HBR) = the total number of vectors collected/number of collectors/ number of nights of capture (reported as bites per person per night (b/p/n))
- Sporozoite rate = the proportion of *Anopheles* found positive for the presence of Plasmodium infection
- EIR = number of infectious bitess/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 Monthy EIRs

3. RESULTS

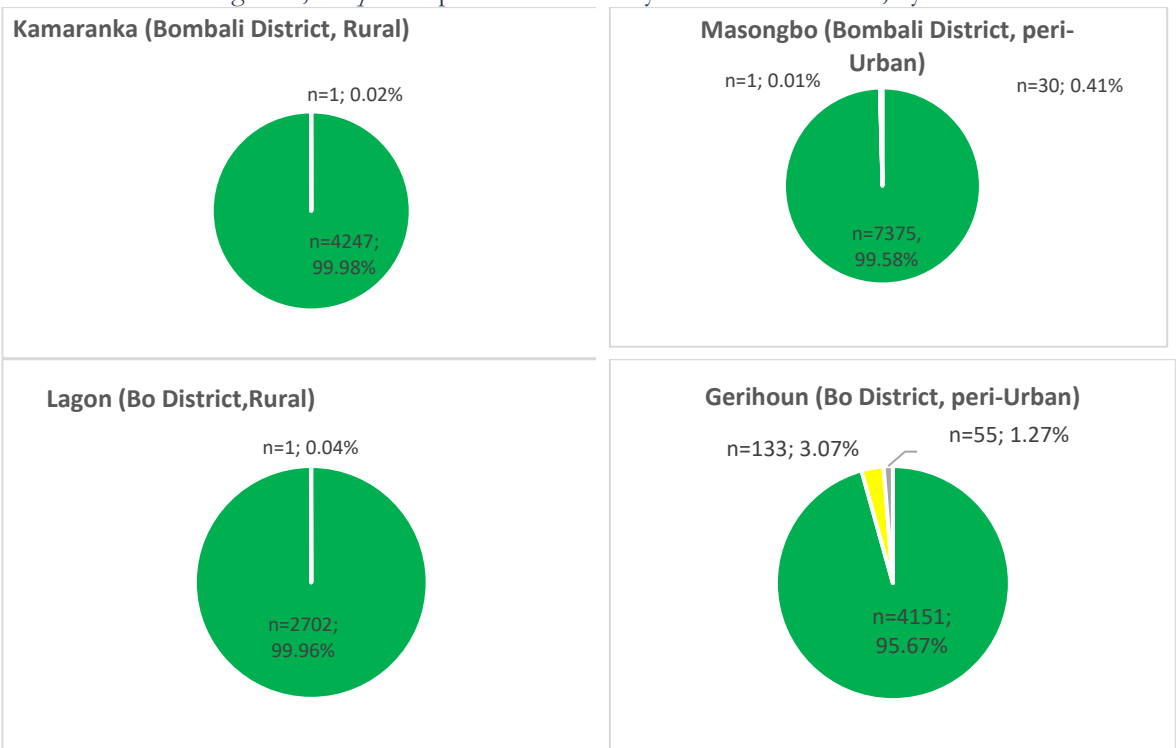
3.1 LONGITUDINAL MONITORING

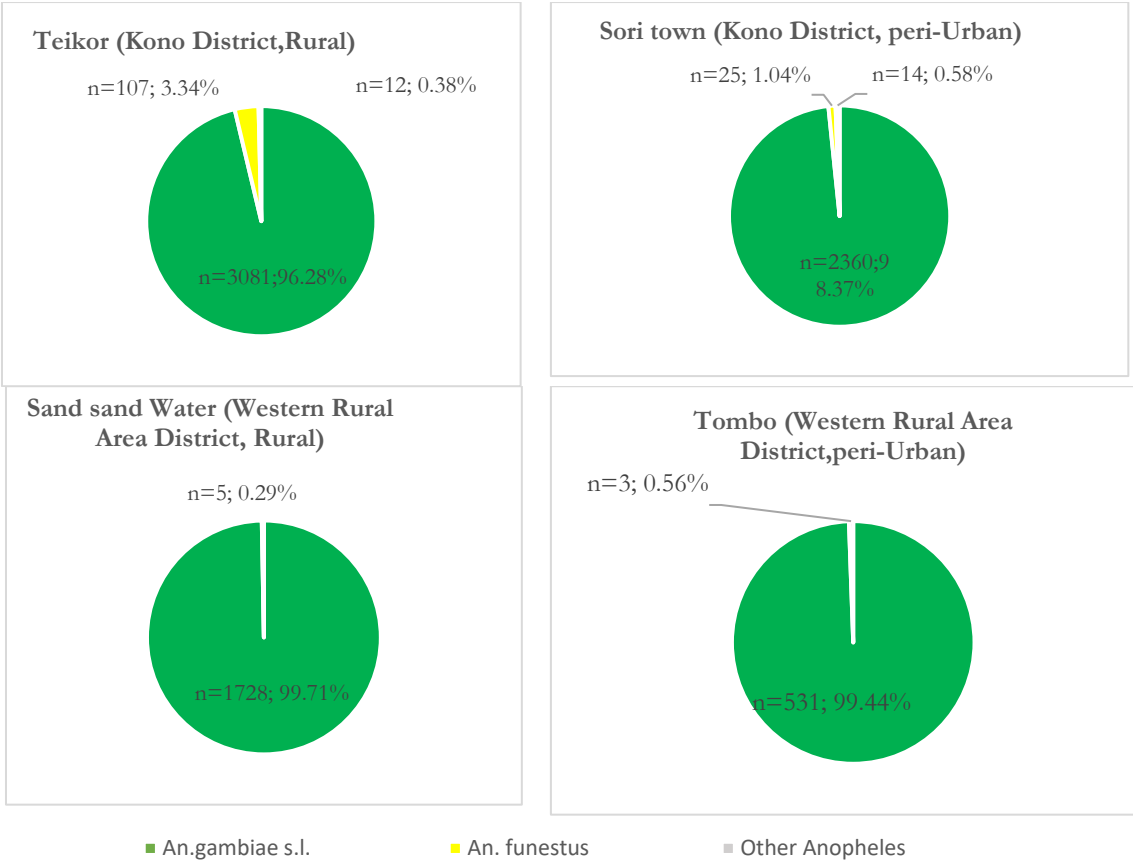
From the monthly collection conducted using HLC, PSC and CDC-LT from both rural and peri-urban sites in 4 districts, overall, a total of 26,175 *An. gambiae* s.l. were collected in the 10 months: 5,392 from PSCs (20.6%), 20,186 (77.1%) from HLCs, and 597(2.3%) from CDC_LT. High numbers were collected from the HLC collections followed by the PSC. The numbers from the CDC LT collections were low mainly because the CDC-LT collections were started late in September. CDC- LT collections were also done only indoors as the team had issues with the lamps in the traps, which were corrected later. *An. gambiae* s.l. was the predominant (98.5%) *Anopheles* species collected, followed by *An. funestus* s.l. (1.0%). *An. coustani, and An. ziemanni* comprised less than 1% (Figure 1). A total of 267 *An. funestus* s.l. were collected: 71 (26.6%) from PSC, 194 (72.7%) using HLCs and 2 (0.7%) from CDC-LTs.

The *An. funestus* s.l. mosquitoes were mainly collected from two sites, Teikor in Kono District and Gerihoun in Bo District where the Sewa River offers *An. funestus* s.l. breeding sites; indicating that the distributions *An. funestus* s.l. is restricted to specific ecological conditions in Sierra Leone.

Figures 1 below provides the breakdown of each *Anopheles* species collected in the peri-urban and rural sites. Annex 1, 2 and 3 provide more detailed information by site and collection method.

Figure 1, *Anopheles* Species Collected by the Three Methods, by Site





3.1.1 Indoor Resting Density (PSC Collection)

The density of *An. gambiae* s.l. per house per day between May 2018 and February 2019 across all sites is shown in Figure 2. The overall density of *An. gambiae* s.l. per house per day was 14.5 in rural sites and 11 in peri-urban areas.

The highest mean indoor resting density (IRD) of *An. gambiae* s.l. was observed in June at all sites (Figure 2). The mean average IRD of *An. gambiae* s.l. in June was higher in the rural sites (33.5/house/day.) than the peri-urban sites (23.2/house/day) (Figure 3). The numbers sharply dropped after June and remained low throughout the collection months. However, IRD was low in the Western Rural District throughout the collection period.

The density of *An. gambiae* s.l. per house per day in the rural sites was higher than the density in the peri-urban sites except in Bombali District where the density of *An. gambiae* s.l. resting inside a house was higher in Masongbo than Kamaranka. The density recorded in June in Masongbo (65.7 *An. gambiae* s.l./house/day) was 2.6 times higher than the density in Kamaranka (25.7 *An. gambiae* s.l./house/day) during the same month. The average density of *An. gambiae* s.l. resting inside house per day in Teikor the rural site in Kono was 16.8 and increased to 51.2 in June and decreased to 6 in July. From November 18 to January 19, the dry season, the density of *An. gambiae* s.l. resting inside houses decreased with less than one *An. gambiae* s.l. per house per day collected. The trend is similar in Sori Town, the peri-urban site. The trend is also similar in Bo District with the highest density record in June 2018.

Figure 2. IRD of *An. gambiae* s.l. from All Sentinel Sites, May 2018-February 2019

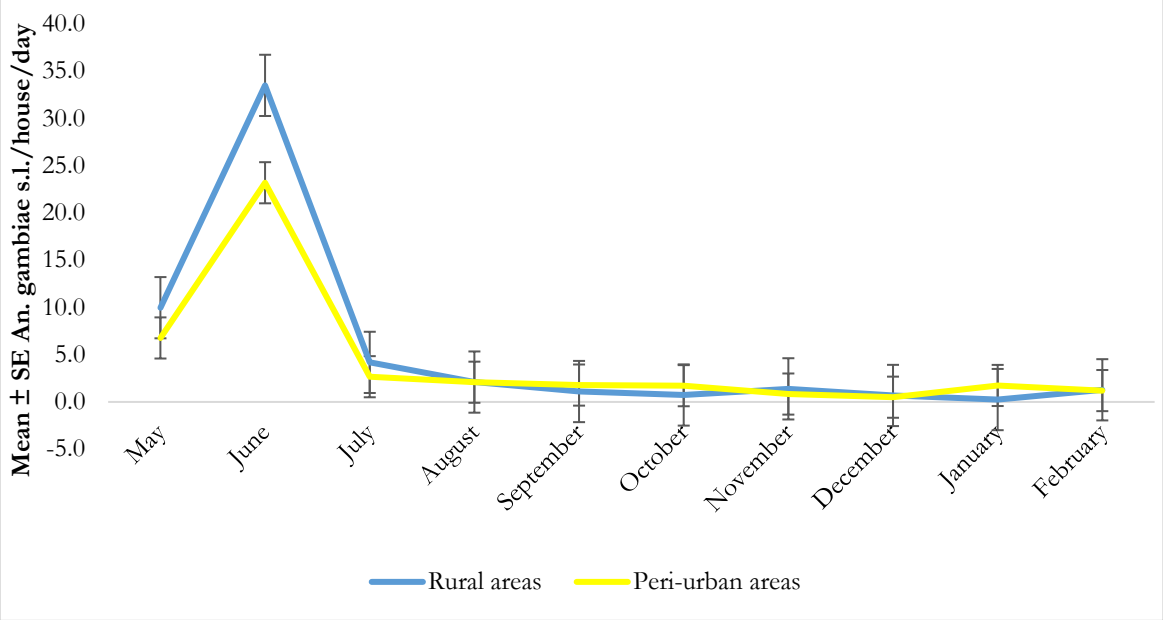
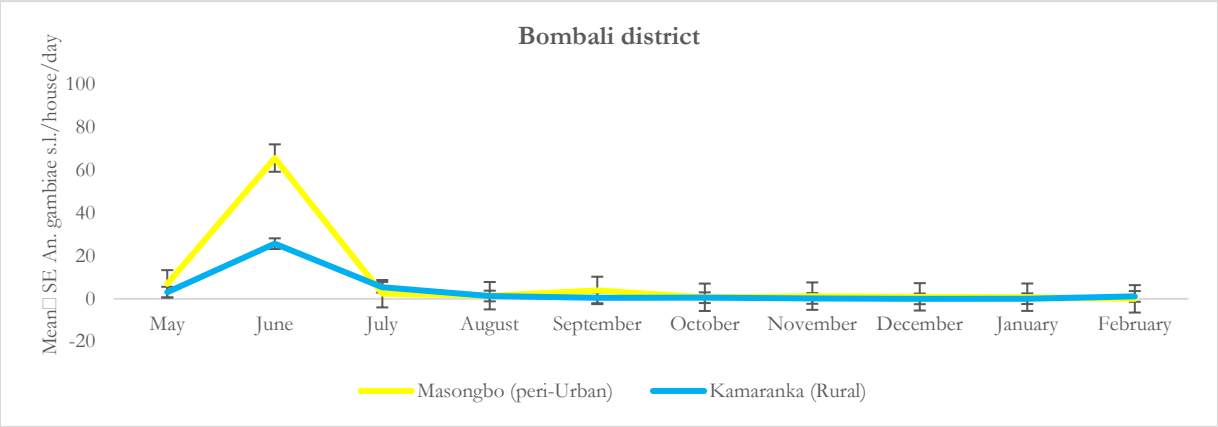
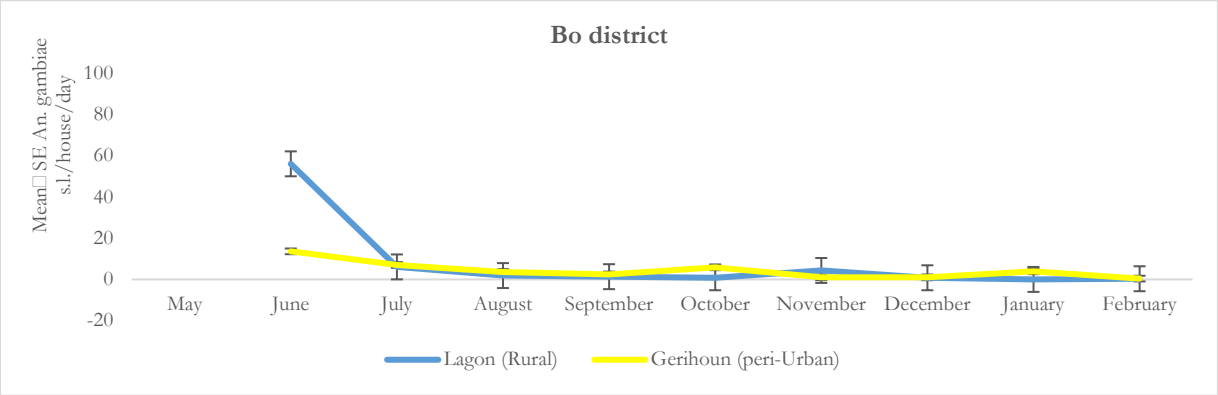
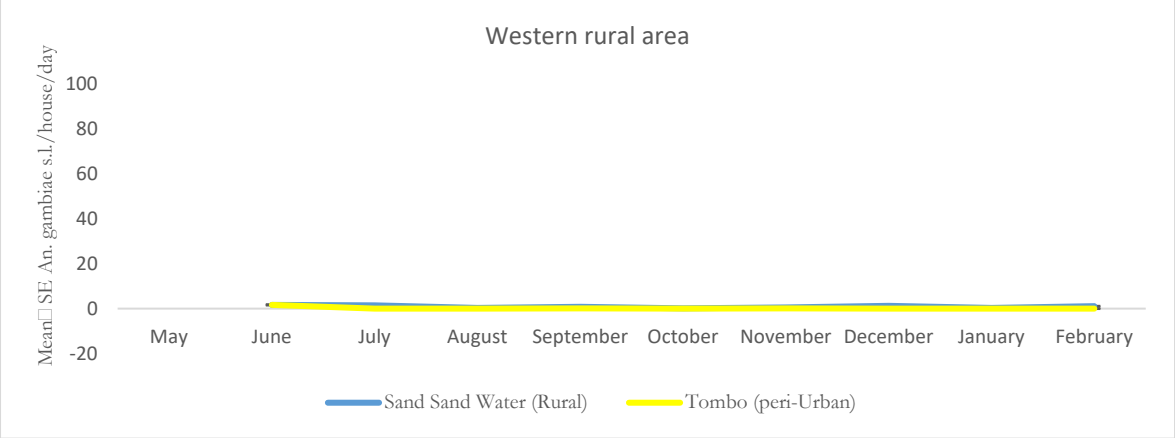
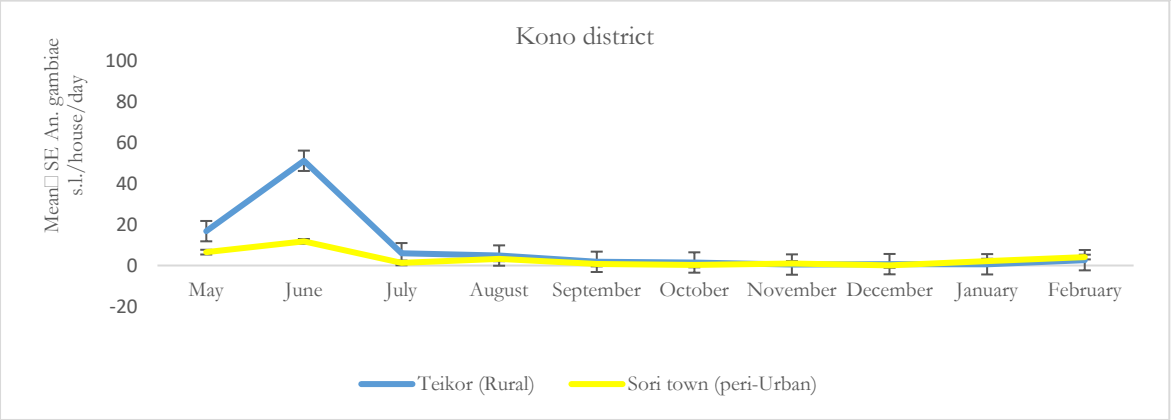


Figure 3. IRD of *An. gambiae* s.l. in Eight Sentinel Sites, May 2018-February 2019





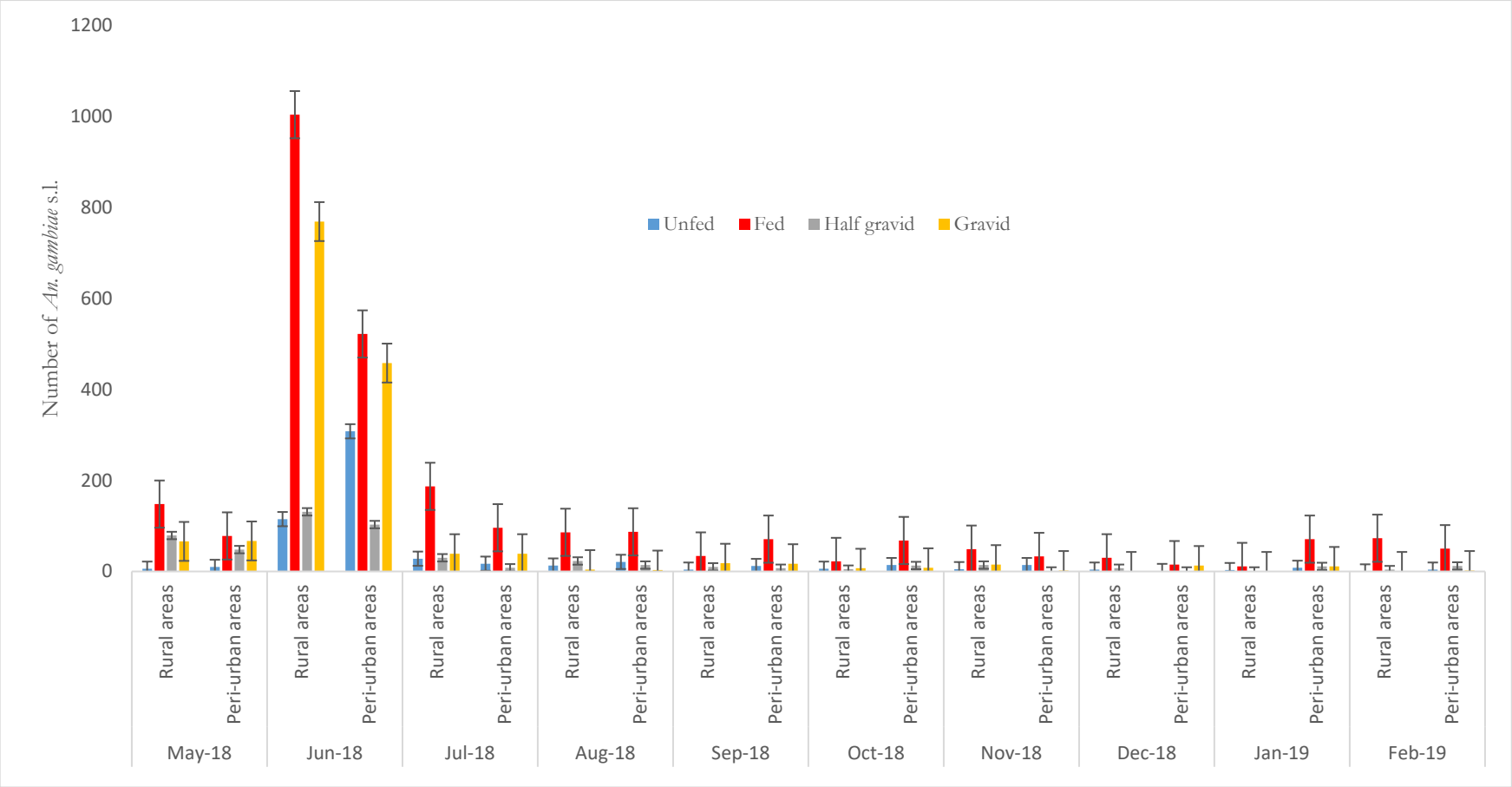




3.1.2 ABDOMINAL CONDITION OF *AN. GAMBIAE* S.L. COLLECTED BY PSC

Figure 4 shows the abdominal condition of *An. gambiae* s.l. collected across rural and peri-urban sites by PSC. The proportion of half and full gravid *An. gambiae* s.l. in the rural sites (40.1%) was higher than the proportion in the peri-urban sites (35.8%). The proportion of fed *An. gambiae* s.l. collected indoors was high at both rural (53.8%) and peri-urban (46.7%). sites.

Figure 4: Abdominal conditions of *An. gambiae* s.l. collected by PSC from All Sentinel Sites (May-February 2019)



3.1.3 CDC Light Trap Collection

The numbers of mosquitoes collected by CDC light traps were low, on average between 1 and 2 *An. gambiae* s.l. per trap per night (Figures 5 and 6). The highest density of *An. gambiae* s.l. (8.3/trap/night) was recorded in February in Sori Town in Kono District.

The CDC Light Trap collections were slightly higher in the peri-urban sites than in the rural ones, though the number are so low that it is impossible to make any meaningful comparison. The CDC Light Traps collections were done indoors only. However, the numbers were low compared to the indoor HLC collections.

Figure 5 Density of *An. gambiae* s.l. from CDC Light Trap Collections in All Sentinel Sites, September 2018-February 2019

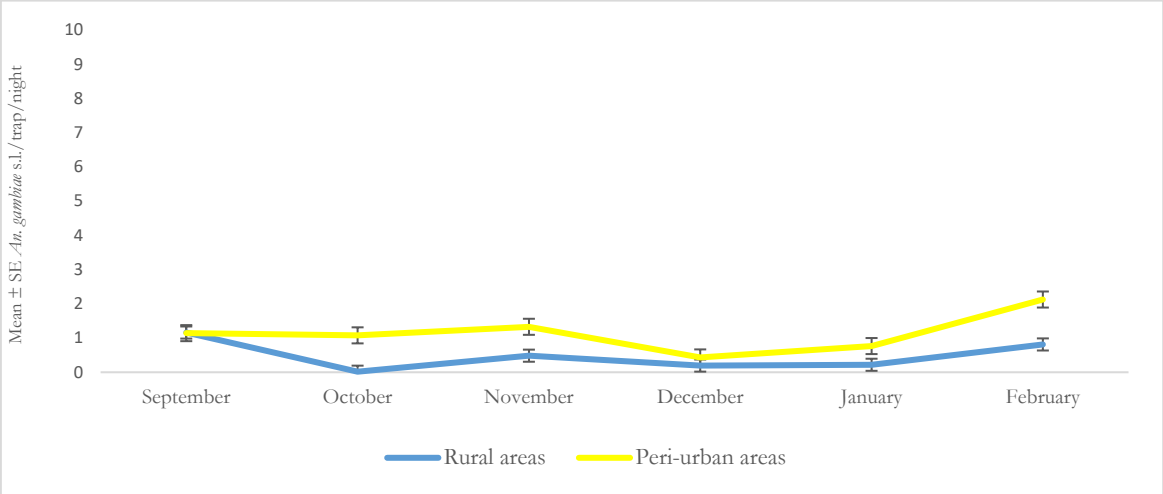
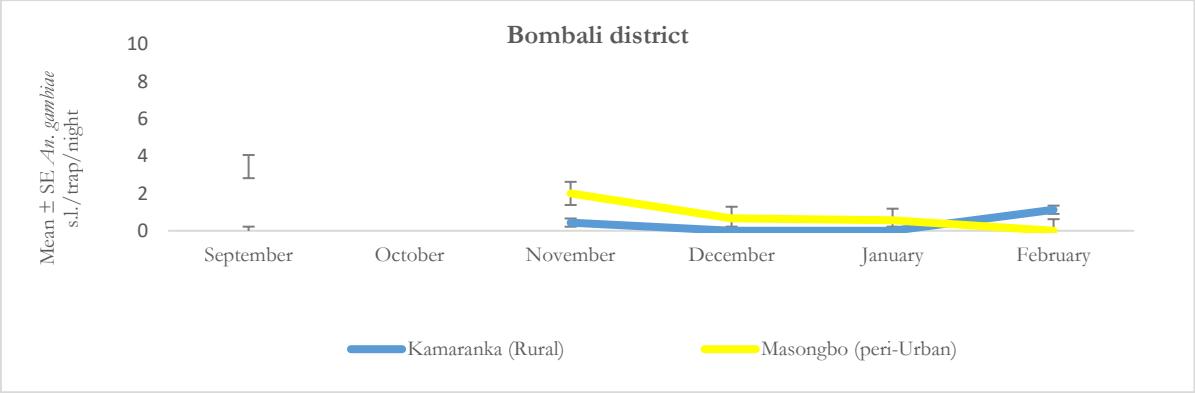
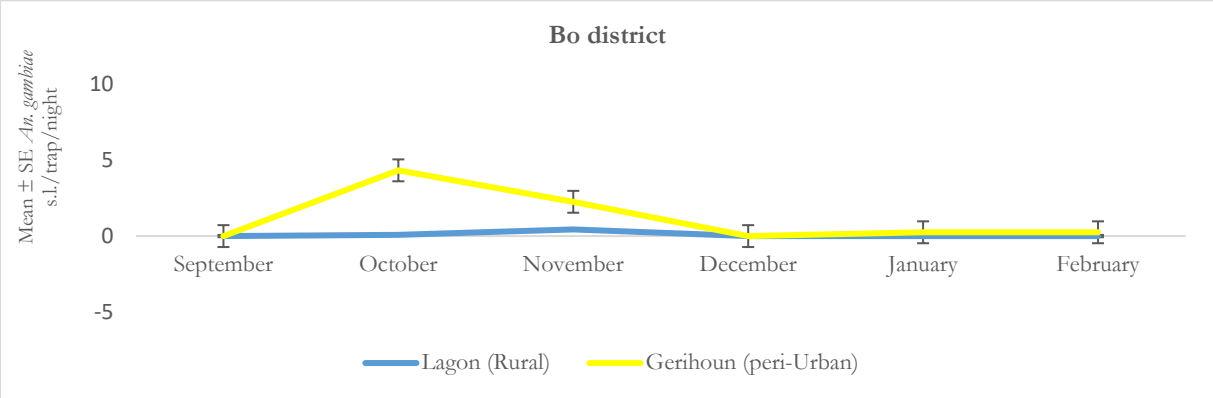
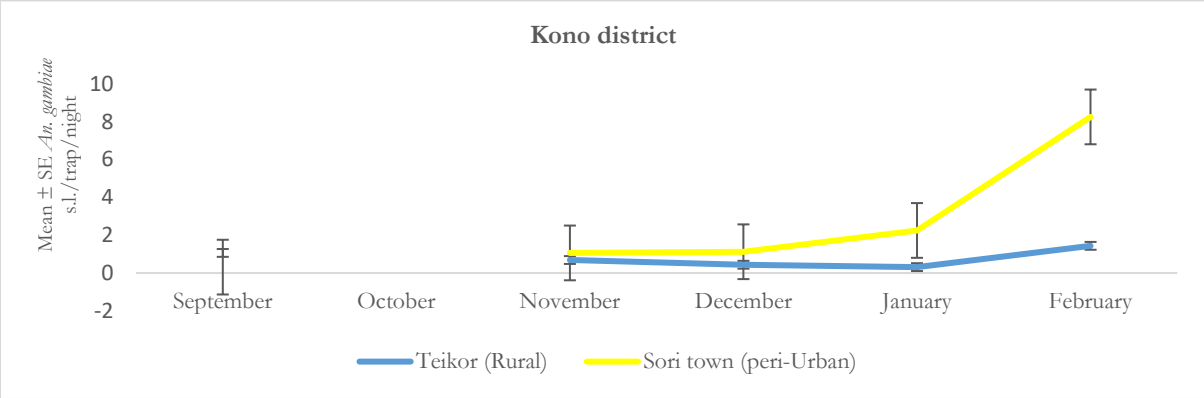
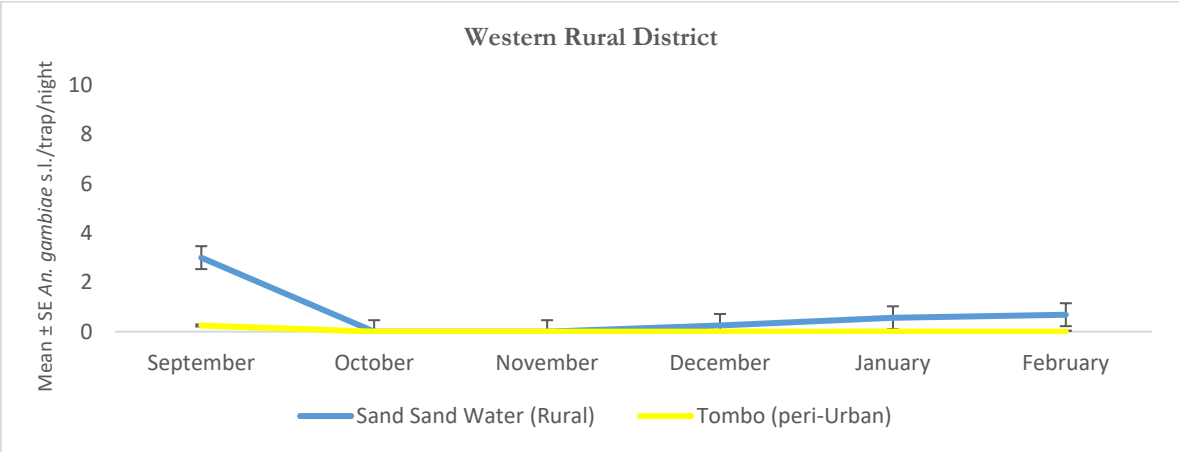


Figure 6. Density of *An. gambiae* s.l. from CDC Light Trap Collections in Eight Sentinel Sites, September 2018-February 2019









3.1.4 HUMAN LANDING COLLECTIONS

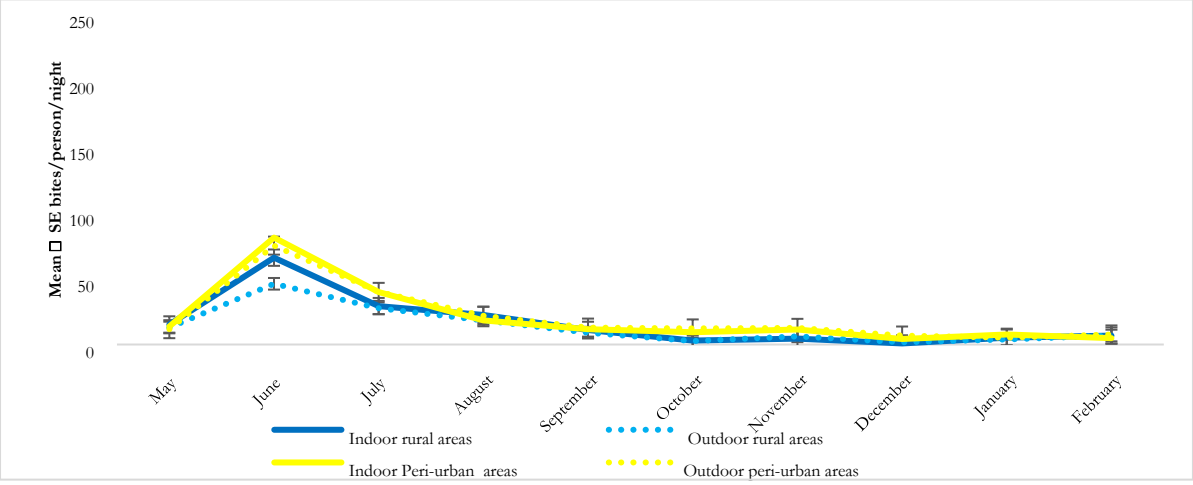
Human Biting Rate: The overall *An. gambiae* s.l. HBR in the rural sites was 19.7 bites/person/night inside house and 15.9 bites/person/night (b/p/n) outside versus 24.1 and 24.5 respectively indoor and outdoor in the peri-urban sites. The average HBR of *An. gambiae* s.l. in May at the beginning of the rainy season was 15.2 per person per night inside the house in rural sites, and 12.9 per person per night outdoors, versus 13.6 per person per night inside and 11.6 per person per night outdoors in the peri-urban sites. The indoor HBR increased in June to 65.8 bites/person/night indoor in rural sites and was found also high (81 bites/person/night) in the peri-urban sites. The lowest HBR rate was recorded indoors in December in both rural (0.8 bites/person/night) and peri-urban sites (4.2 bites/person/night). High numbers of mosquitoes were collected using HLCs in June. The overall highest mean HBR of *An. gambiae* s.l., 73.4 bites/person/night, was recorded indoors in June (Figure 7). In Sand Sand Water and Tombo, the highest mean indoor HBR of *An. gambiae* s.l. was observed in August (40.1 bites/person/night) and July (9.4 bites/person/night), respectively (Figure 8).

Biting Location: The highest indoor *An. gambiae* s.l. HBR was recorded in Masongbo in Bombali District (189 bites/person/night) (Figure 8). The indoor and outdoor biting rates of *An. gambiae* s.l. in Bombali District were at least two to three times higher than all of the other sites. Similar biting rates were observed both indoor and outdoor in Masongbo site.

A total of 194 *An. funestus* s.l. were also collected using HLCs. *An. funestus* s.l. was collected biting inside houses in Teikor, Sori Town, and Gerihoun. The highest mean indoor HBR of *An. funestus* s.l. was recorded in May in Teikor, in June in Sori Town, and in July in Gerihoun. However, the numbers were too low to make a meaningful comparison between sites and locations.

An. funestus s.l. was not collected from Western Rural Area. The highest indoor HBR (4.3 bites/person/night) for *An. funestus* s.l. was recorded from Gerihoun; in Bo District, in July.

Figure 7 *An. gambiae* s.l. HBR in All Sentinel Sites, May 2018-February 2019



In Bombali District, where the highest HBR is recorded, biting was higher indoors than outdoors. Almost in all the sites, urban and peri-urban, biting seems to increase late after 11:00 pm and most of the biting occurred during the second half of the night (Figure 11). This trend is expected to have a positive impact on the effectiveness of LLINs as vector activity seems to be higher after people are indoors.

HBR of *An. Funestus* s.l.: A total of 194 *An. funestus s.l.* were collected from HLCs. *An. funestus s.l.* was collected biting inside house in Teikor,Sori Town and Gerihoun. The highest mean indoor HBR of *An. funestus s.l.* was recorded in May in Teikor, in June in Sori Town and in July in Gerihoun. However, the numbers were low to make a meaningful comparison between sites and locations.

An. funestus s.l. was not collected from Western Rural Area District. The highest indoor HBR (4.3 bites/person/night) for *An. funestus* s.l. was recorded from Gerihoun; in Bo District, in July (*Fig 10*).

A total of 134 *An. funestus* s.l. were collected biting inside the houses versus 60 outside during the collection period.

An. gambiae s.l. was biting more indoor than outdoor at all sites except in Masongbo and in Tombo, the peri-urban sites in Bombali and Western Rural Districts where outdoor biting was higher than indoor biting (Table 3).

Figure 8. *An. gambiae* s.l. HBRs in the Eight Sentinel Sites, May 2018-February 2019

Figure 9. *An. funestus* s.l. HBR in All Sentinel Sites, May 2018-February 2019

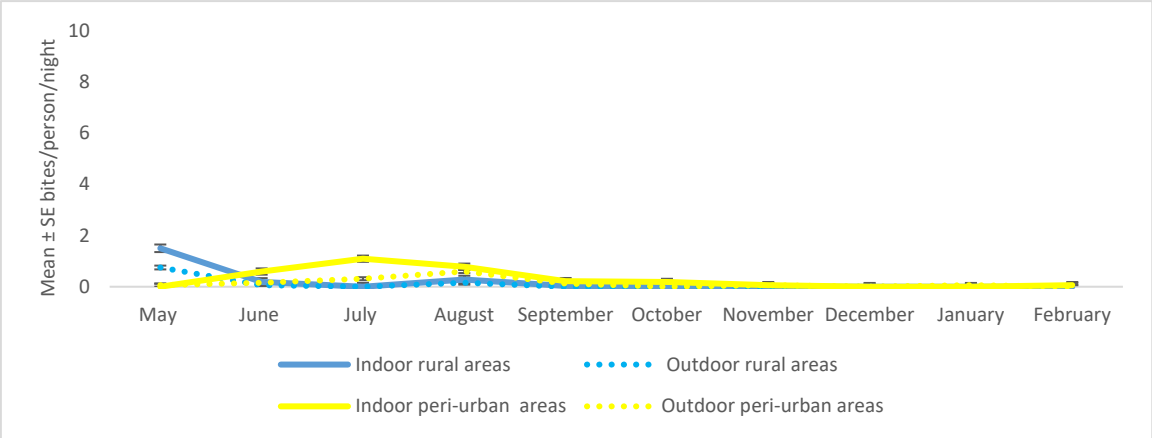


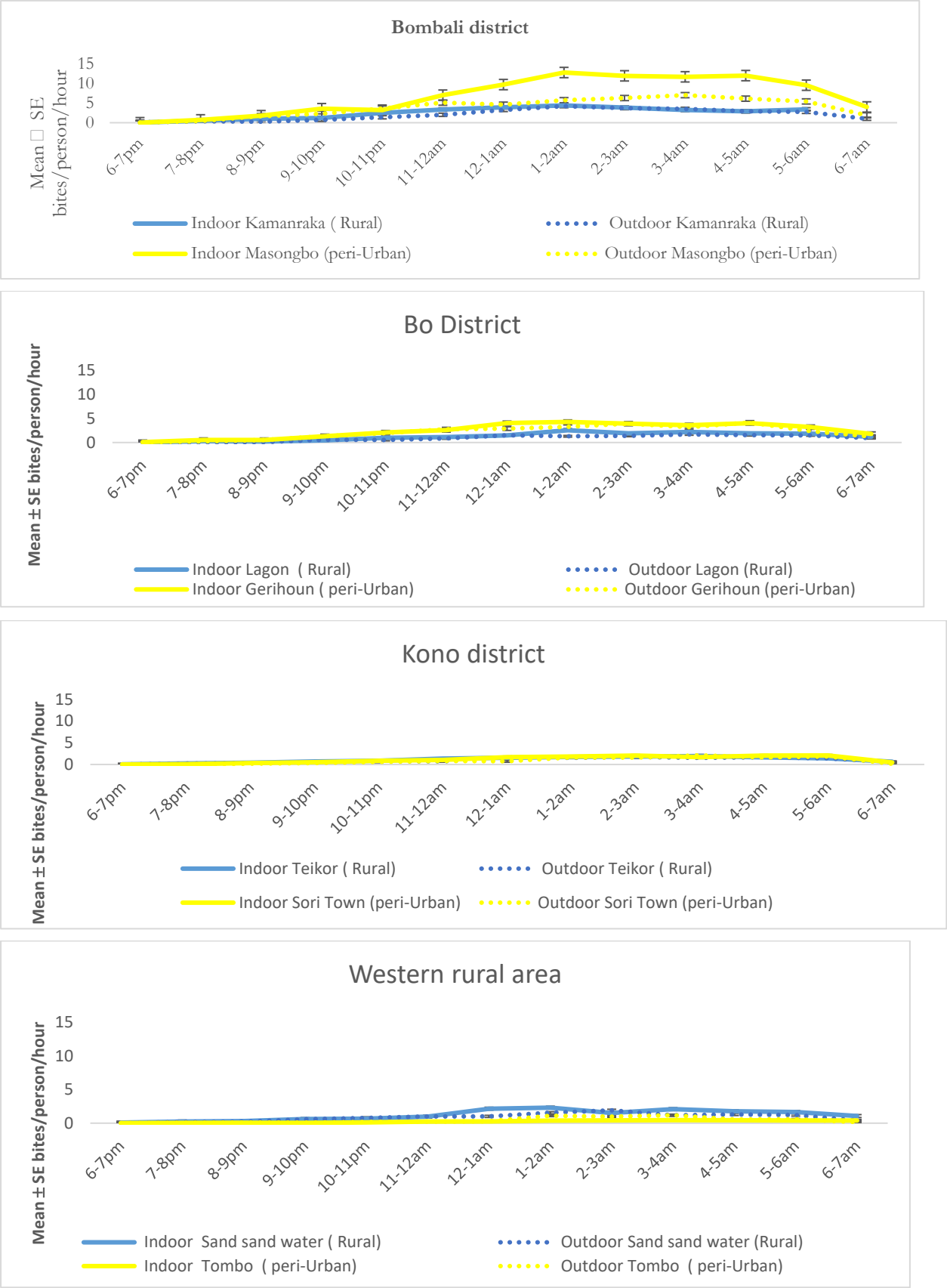
Figure 10. *An. funestus* s.l. HBR in Bombali, and Kono districts, May 2018-February 2019

Table 3.: INDOOR AND OUTDOOR HLC COLLECTION OF AN. GAMBIAE S.L., ALL SENTINEL SITES, MAY 2018 – FEBRUARY 2019

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, Rural)	1980	1672	0.54	0.46	25.9	<0.001*[2]
Lagon (Bo district, Rural)	934	687	0.58	0.42	37.6	<0.001*
Teikor (Kono District, Rural)	917	798	0.53	0.47	8.3	0.004*
Sand sand water (Western Area , Rural)	890	652	0.58	0.42	36.7	<0.001*
Total rural sites	4721	3809	0.55	0.45	97.5	<0.001*
Peri-Urban sites						
Masongbo (Bombali District, Rural)	2808	3202	0.47	0.53	25.8	<0.001*
Gerihoun (Bo district, Rural)	1845	1611	0.53	0.47	15.8	<0.001*
Sori Town (Kono District, Rural)	944	746	0.56	0.44	23.2	<0.001*
Tombo water (Western Area , Rural)	186	314	0.37	0.63	32.8	<0.001*
Total peri-Urban sites	5783	5873	0.50	0.50	0.69	0.41

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

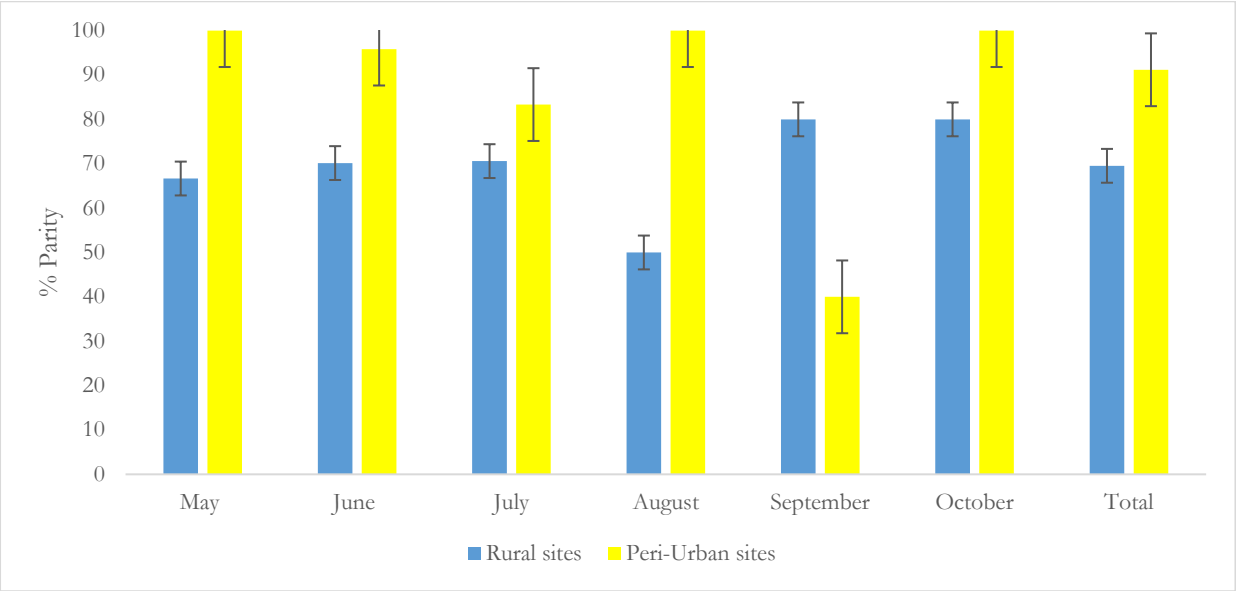
Figure 11. *An. gambiae* s.l. Biting Time in the Eight Sentinel Sites, May 2018-February 2019



Biting Time: Interestingly, biting activity started to peak after 10 p.m., in all the districts and sites as well as indoors and outdoors.

Parity rate: The parity rate of *An. gambiae* s.l. was 69.5 % [61.6%-77.5%] in the rural sites versus 91.2% [85.7.1%-96.7%] in the peri-urban sites (Figure 12). There is a statistically significant difference between the *An. gambiae* s.l. parity rate in the rural sites and the peri-urban sites ($p<0.05$). The parity rate is higher in the peri-urban sites than the rural sites. However few number of malaria vectors were dissected during the study period due to the fact that the new monitoring team dissection skill did not allow to dissect enough mosquitoes

Figure 12 **12: Parity rate in the eight sentinel sites (May-October 2018)**



3.2 Laboratory analysis for Species Identification and Infection Detection

The project team sent sub-samples of malaria vectors collected from HLC collections to CRID for species identification and detection of *Plasmodium* infection. A total of 835 *An. gambiae* s.l. from the HLCs were successfully amplified using PCR; 72.9% (609/835) were identified as *An. gambiae* s.s. and 27.1% (226/835) were identified as *An. coluzzii*. Three specimens were identified as hybrids of *An. gambiae*/*An. coluzzii*. Out of eight sentinel sites *An. coluzzii* dominates the Lagon site of Bo District. When separated by preference of biting by location, both *An. gambiae* and *An. coluzzii* were caught more outdoors than indoors (Annex E and Fig 13).

A total of 105 *An. funestus* s.l. that also were processed for species identification using PCR were found to be all *An. funestus* s.s.

A total of 2,497 *An. gambiae* s.l. and 149 *An. funestus* s.l. were tested for *Plasmodium* infection, and 107 (4.0%) were found to be positive (Figure 13). *Plasmodium* infection was detected in 4.1% of *An. gambiae* s.l. and 2.7% of *An. funestus* s.l. Of these, 97 were infected with *P. falciparum* and 10 with *P. ovale/vivax/malariae (OVM)*. One *An. gambiae* s.l. was found with mixed infection, *P. falciparum/ OVM*. The highest infection rate was observed in Tombo (9.8%) in Western Rural area followed by Lagon (7.9%) in Bo District and Sand sand water (6.6%) in Western Rural Area. The lowest infection rate was recorded in Sori Town (1,5%). For *An. gambiae* s.l. overall, infection rates were slightly higher outdoors (4.3%) than indoors (3.9%). *An. funestus* s.l. had similar infection rates indoors and outdoors.

Infection rates were also separately assessed for *An. gambiae* and *An. coluzzii*. The *Plasmodium* infection rate of 11.9% (27/226) found in *An. coluzzii*, was higher than the 9.6% (58/607) in *An. gambiae* (Figure 14). These infection rates are not a true reflection of the *Plasmodium* sporozoite rates in the natural population because all samples known to be infected with *Plasmodium* were processed by PCR for species identification. As indicated above 2,497 *An. gambiae* s.l samples were tested for sporozoite infection. However, only about 30% those (835) were identified into *An. gambiae* and *An. coluzzii;* which means there was an initial *Plasmodium* infection rate for both species. However, a decision was made to do PCR identifications of all sporozoite positive specimens that were not initially identified *An. gambiae* and *An. coluzzii,* and these were added to the initial infected numbers and rate of infection calculated. As an example, the true value of infection rate for *An. coluzzii* is: (a) = (27- b) / (226 - b); b which were positive for sporozoite and identified as *An. coluzzii* during the second screening and 27- b are those positives from the initial test. The true infection rate values could not presented because separating the positives from the first test and the additional positive samples was not possible. However, the overall infection rate of 4.1% for *An. gambiae* s.l. is the true estimate of infection rate in the vector population. It was slightly higher in *An. coluzzii* than in *An. gambiae.* Infection rates were higher indoors than outdoors for both species.

The highest Entomological Inoculation Rate (EIR) was observed in rural areas in August with 62.4 infective bites/person/month. The highest EIR in peri-urban areas was observed in July with 36.5 infective bites /person/month.

The indoor EIR of *An. gambiae* s.l. in May, at the beginning of the rainy season, was 13.0 infective bites/ person/month in the rural sites and increased to 37.6 infective bites/person/month in June. A similar trend was observed in the peri-urban sites where the indoor EIR was 14.6 infective bites/person/month and increased in June to 35.1 infective bites/person/month (Figure 15).

The indoor EIR during the wet season (May to October) was high in both rural (average infective bites of 28.3/person/month) and peri-urban sites (22.8 average infective bites per person/month) and was about four times higher than the EIR recorded during the dry season (November-February) in rural sites (7.8 average infective bites per person/month) and peri-urban sites (6.5 average infective bites per person/month). (Annex F)

The overall indoor EIR in the rural sites was 201.2 infective bites per person per year and 163 infective bites per person per year in the peri-urban sites.

The overall indoor EIR in Kamaranka, the rural site in Bombali in the northern region of Sierra Leone was 90.1 infective bites/ person/year. The EIR in Kamaranka during the wet season (average infective bites of

11.2 /person/ month) was two times higher than the EIR during the dry season (5.7 infective bites/ person/ month). The overall indoor EIR observed in Masongbo, the peri-urban site was 92 infective bites/person/ year and in average 5 infective bites/person/month during the wet season and 15.5 infective bites/person/month during the dry season. The indoor EIR during the dry season in Masongbo (15.5 infective bites/ person/month) was three times higher than the indoor EIR during the rainy season (5 infective bites/person/month) (Figure 16).

The overall indoor EIR in Kono in the eastern region of Sierra Leone was 116.5 and 60.4 infective bites/ person/year respectively in Teikor the rural site and in SoriTown the peri-urban site.

The overall indoor EIR in Bo in the Southern region of Sierra Leone was 114.9 and 279.9 infective bites per person per year respectively in Lagon the rural and in Gerihoun the peri-urban sites. All the *An. gambiae* s.l. positive to *Plasmodium* in Lagon the rural site were collected during the wet season. The indoor EIR during the dry season in Gerihoun was 7.4 infective bites/person/month

The overall indoor EIR in Sand Sand Water the rural site in Western Rural Area was 147.3 infective bites per person per year. The EIR in the rural site during the wet season (25.8 infective bites/ person/month) was five times higher than the indoor EIR during the dry season (5 infective bites/person/month). The overall indoor EIR observed in Tombo, the peri-urban site was 29.7 infective bites per person per year and 4.4 infective bites/person/month during the wet season and 1.9 infective bites/person/month during the dry season.

Overall biting rate for both *An. gambiae* and *An. coluzzii* was higher indoors than outdoors even though there were some variations by place and species. The proportion of indoor vs. outdoor biting (60.6% vs 39.4%, respectively) for *An. gambiae* was almost exactly the same with that of *An. coluzzii* (59.6% vs 40.4%; respectively) Figure 15. Overall infection rate was also higher in mosquitoes caught indoors than outdoors.

Figure 13: Rate of *Plasmodium* Infection by Species Complex by Biting Location

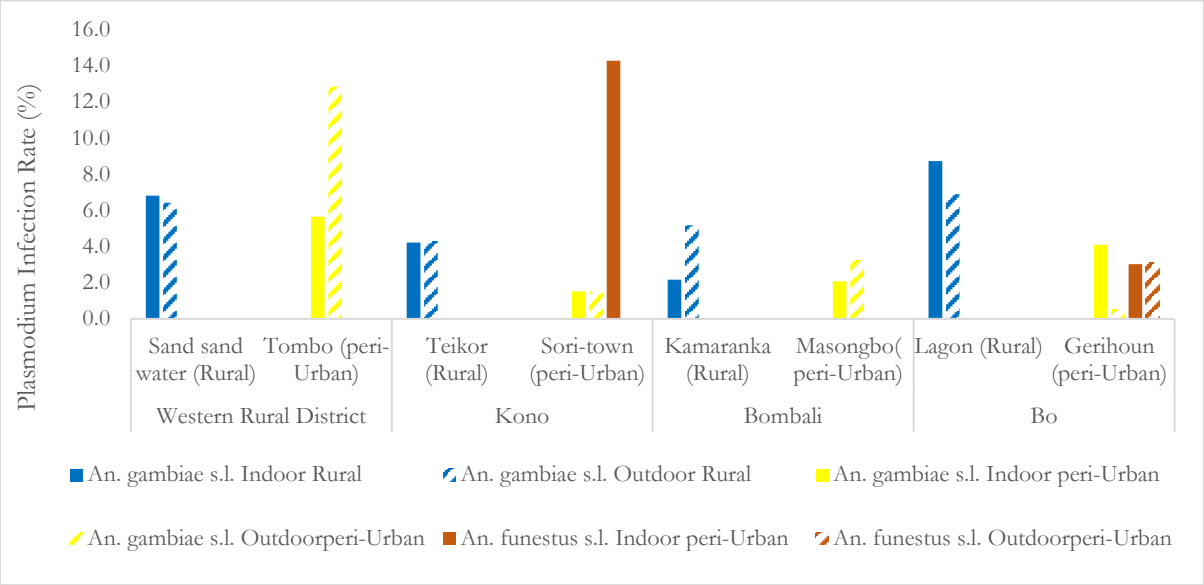


Figure 14. *An. gambiae* and *An. coluzzii* by Biting Location and Infection Rate

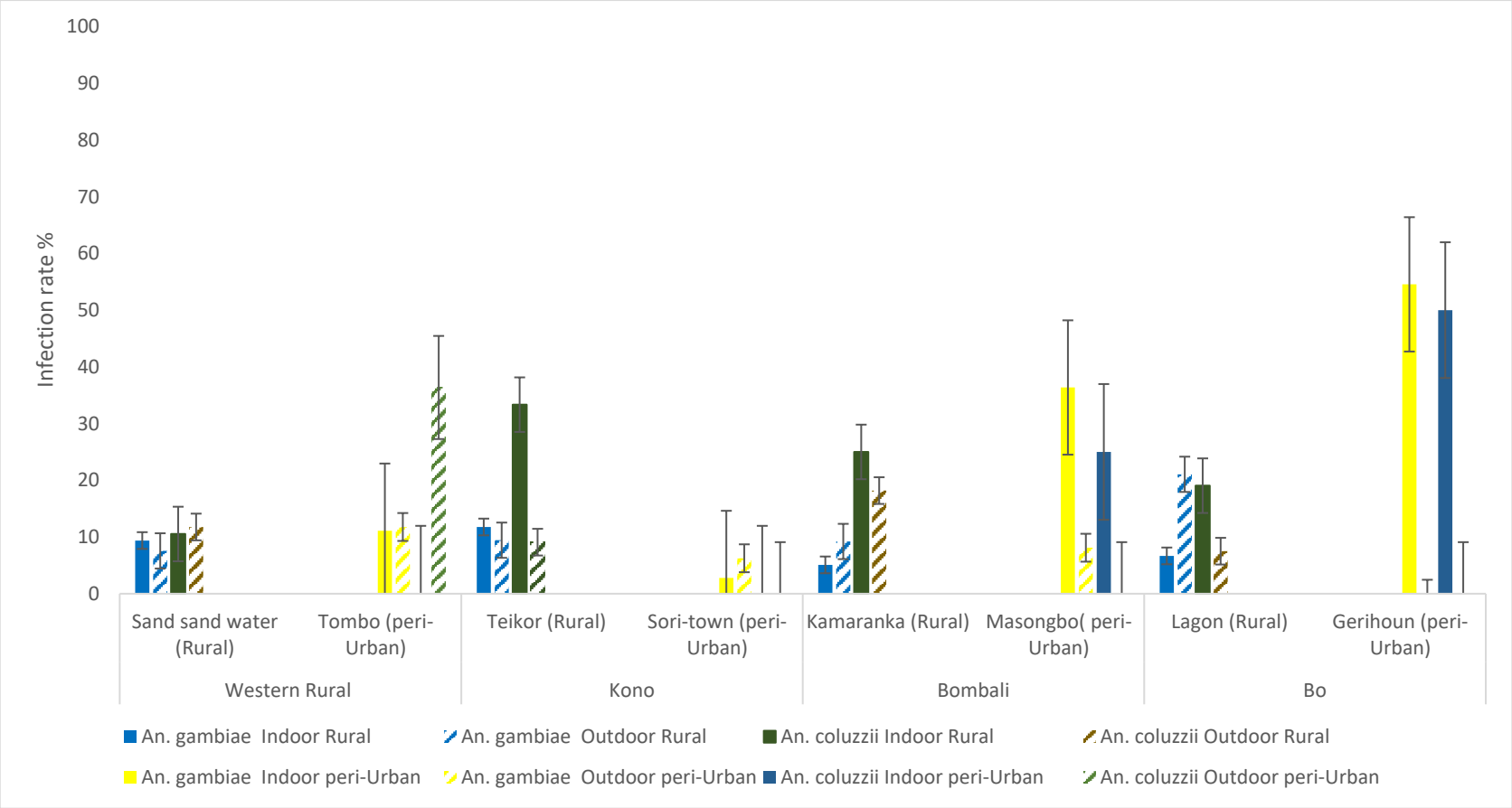


Figure 15. *An. gambiae* and *An. coluzzii* by BIting Location (Proportion)

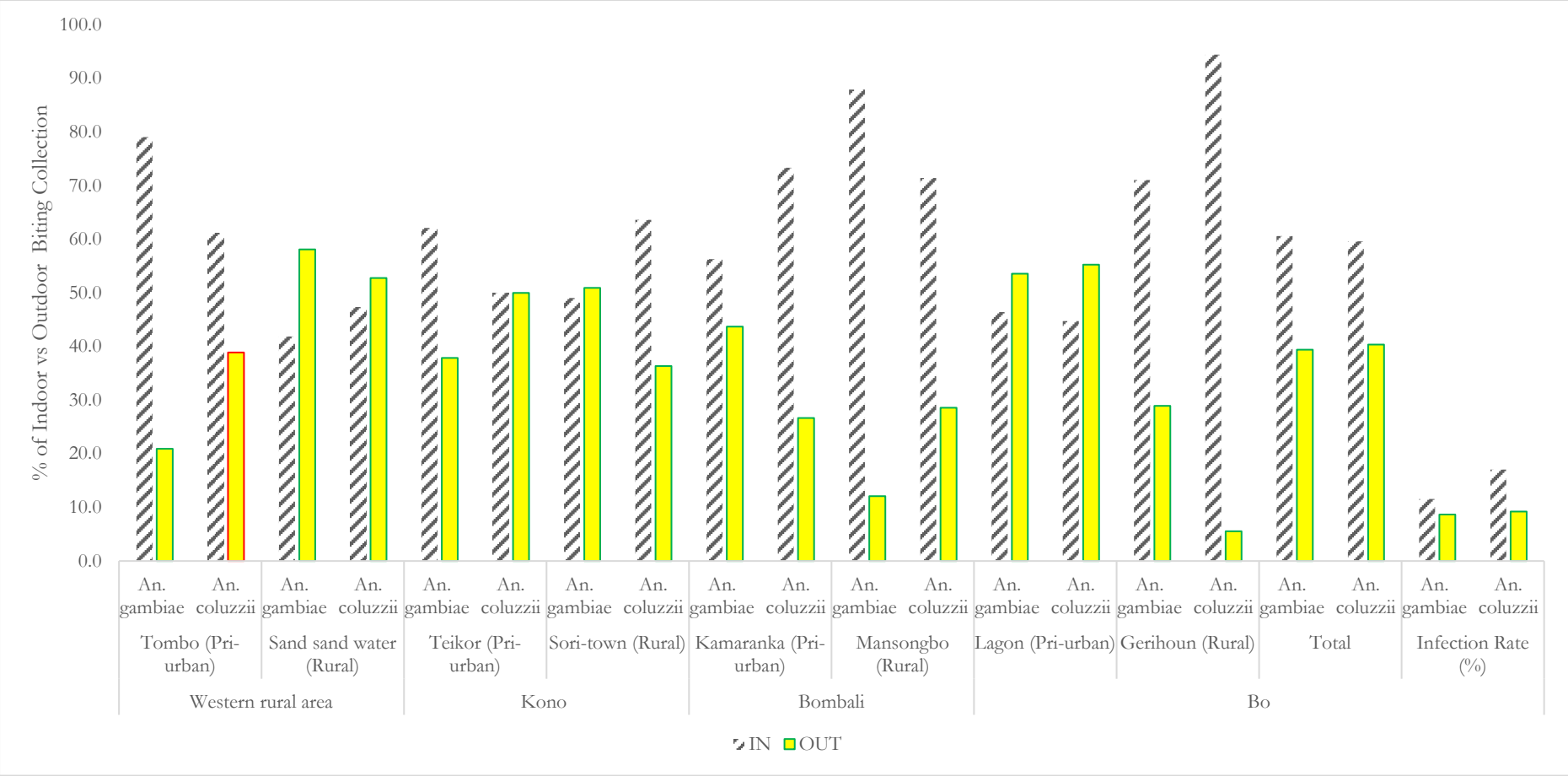


Figure 16: Monthly EIR of *An. gambiae* s.l. by rural/Peri-Urban; all sites (May 2018 – February 2019)

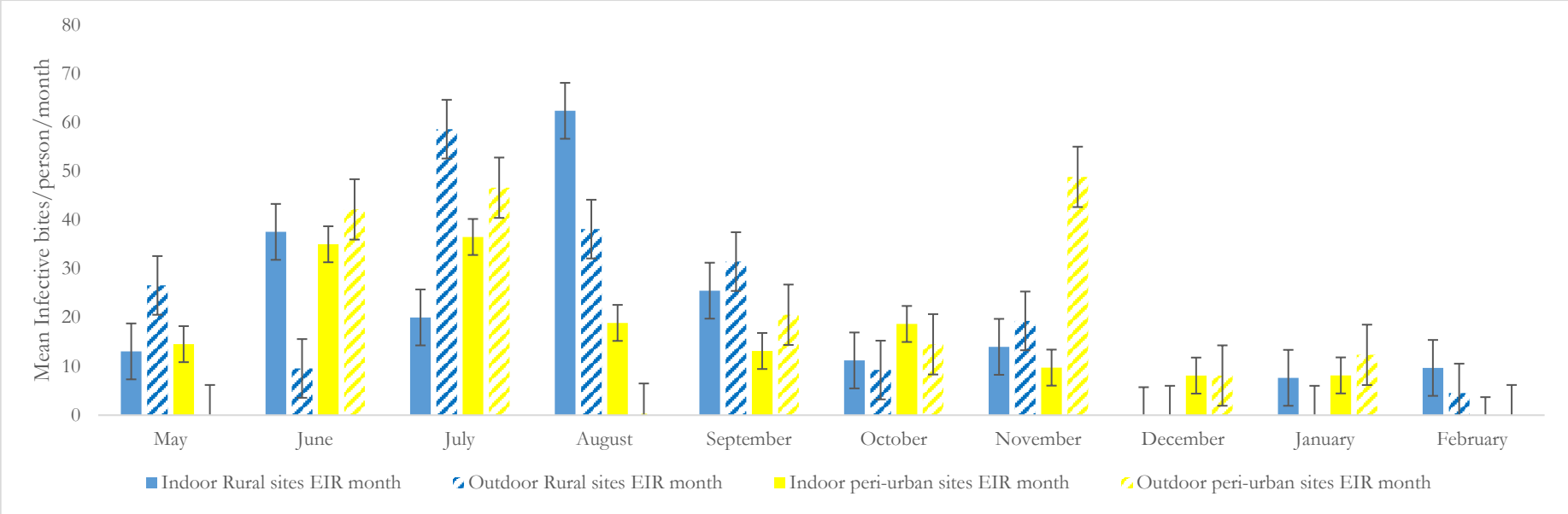
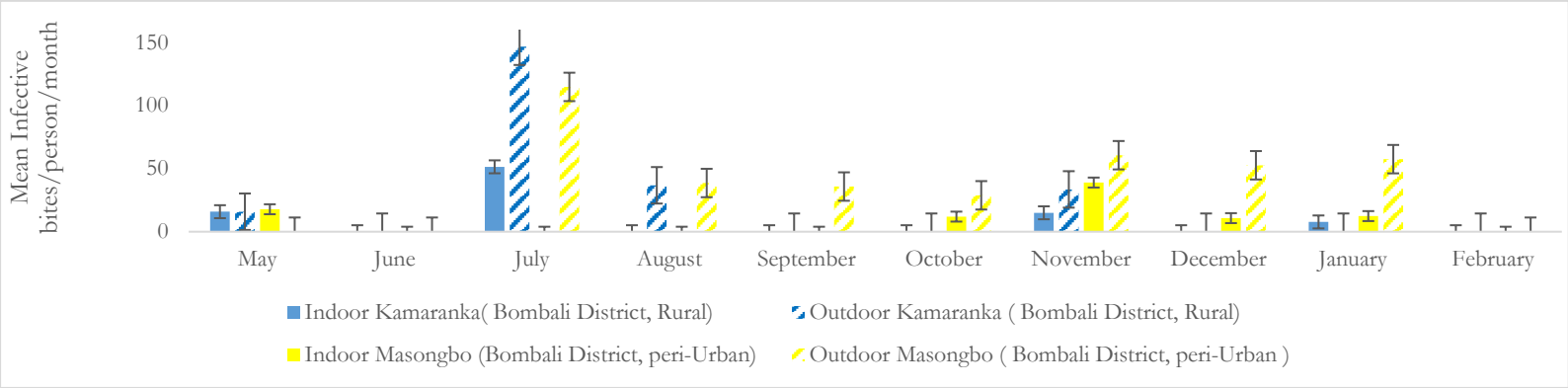
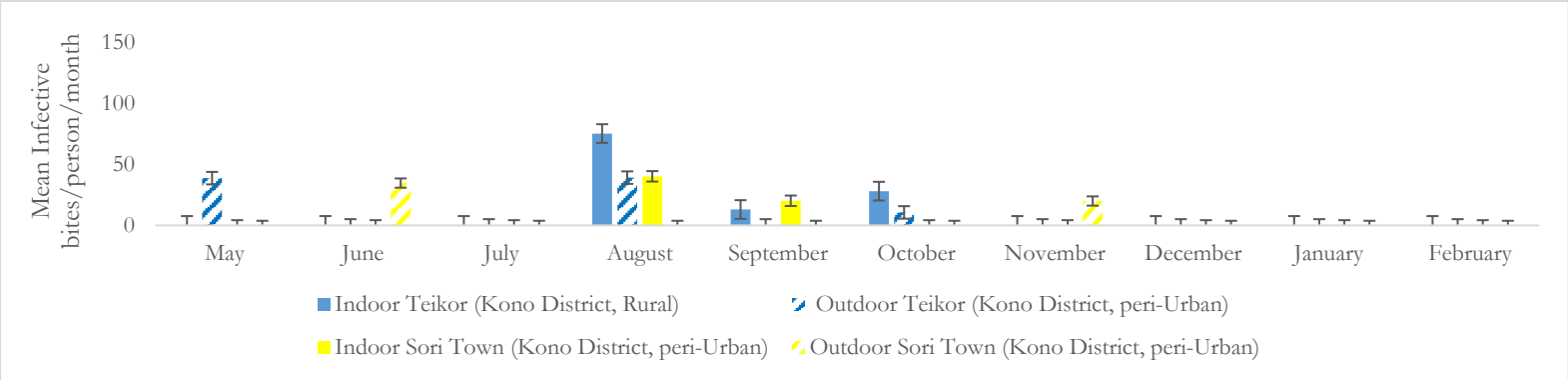
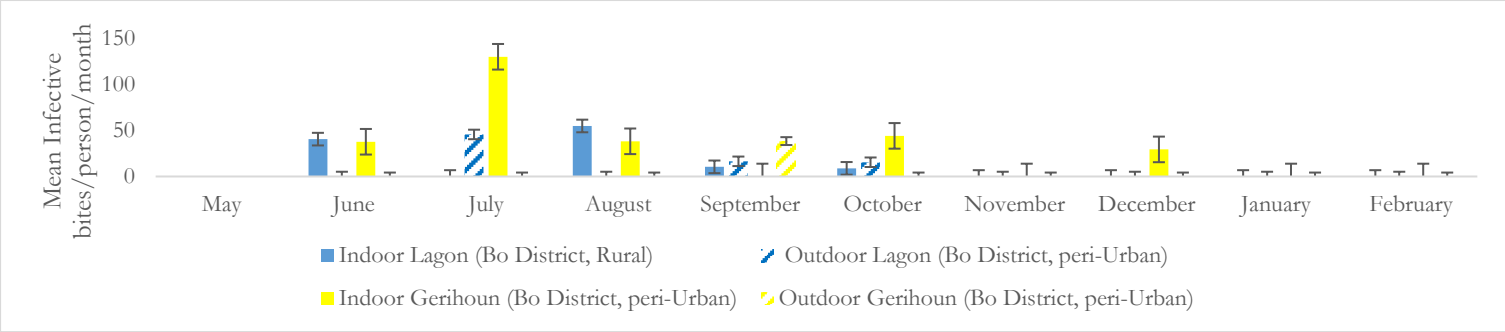
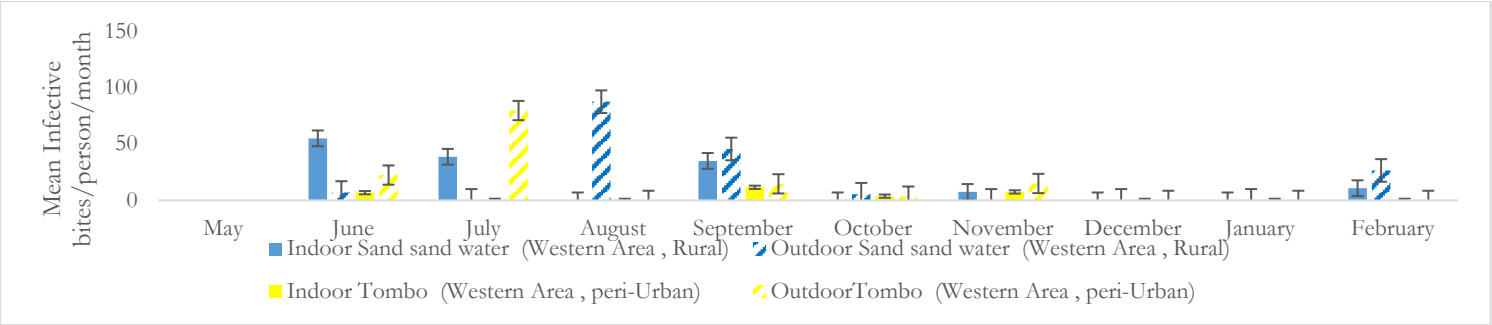


Figure 17: Monthly EIR of *An. gambiae* s.l. by sentinel site (May 2018 – February 2019)






Mean Infective bites/person/month
150
100
50
0
May
June
July
August
September
October
November
December
January
February
Indoor Lagon (Bo District, Rural)
Outdoor Lagon (Bo District, peri-Urban)
Indoor Gerihoun (Bo District, peri-Urban)
Outdoor Gerihoun (Bo District, peri-Urban)

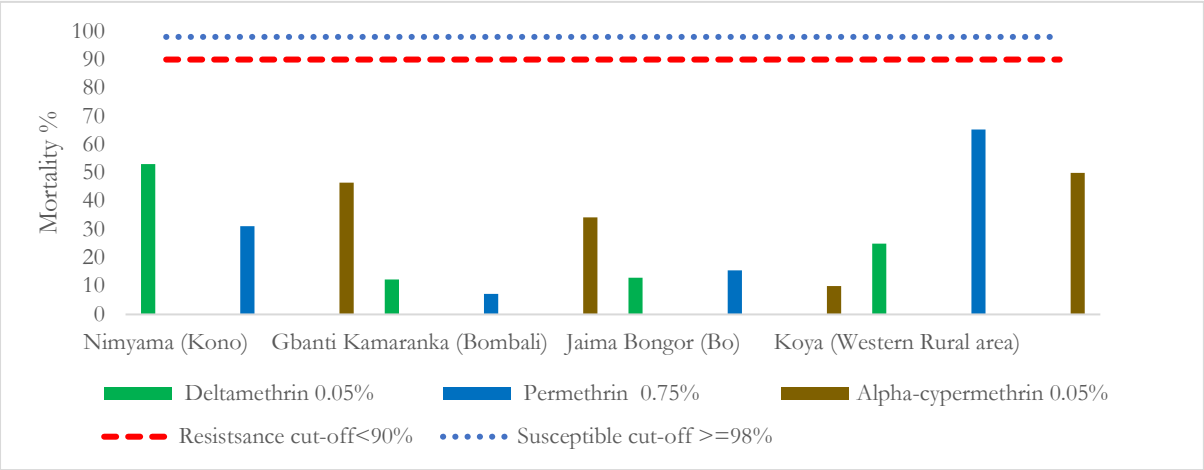

Mean Infective bites/person/month
150
100
50
0
May
June
July
August
September
October
November
December
January
February
Indoor Sand sand water (Western Area , Rural)
Outdoor Sand sand water (Western Area , Rural)
Indoor Tombo (Western Area , peri-Urban)
OutdoorTombo (Western Area , peri-Urban)

3.3 Insecticide Resistance Monitoring

3.3.1 *An. gambiae* s.l. susceptibility to pyrethroids

An. gambiae s.l. exposed to deltamethrin, permethrin, and alpha-cypermethrin in Koya (Western Rural), Jaima Bongor (Bo), Nimyama (Kono), and Gbanti Kamaranka (Bombali) were found to be resistant to the insecticides. *An. gambiae* s.l. mortality ranged from 7% in Gbanti Kamaranka to 65% in Koya after exposure to the diagnostic dosage for permethrin (Figure 17).

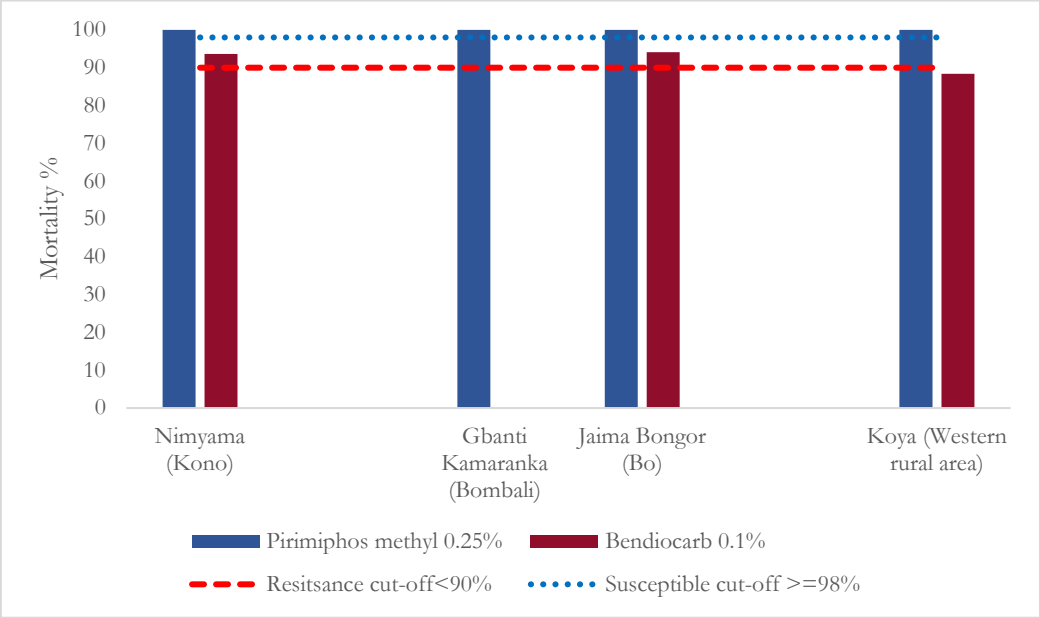
Figure 18. Susceptibility Status of *An. gambiae* s.l. to Deltamethrin (0.05%), Permethrin (0.75%), and Alpha-cypermethrin (0.05%)



3.3.2 *An. gambiae s.l. susceptibility to organophosphates and carbamates*

An. gambiae s.l. exposed to pirimiphos-methyl in Koya, Jaima Bongor, Nimyama, and Gbanti Kamaranka were fully susceptible to the insecticide. *An. gambiae* s.l. was resistant to bendiocarb in Koya (88% mortality), and possibly resistant to bendiocarb in Jaima Bongor and Nimyama, where the test mortality was 94% (Figure 18).

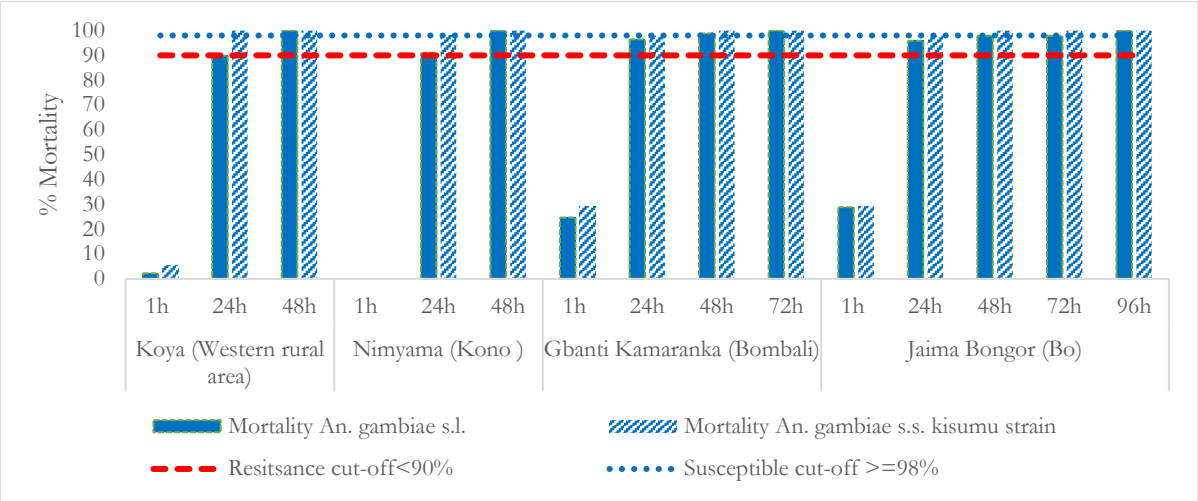
Figure 19. Susceptibility Status of *An. gambiae* s.l. to Pirimiphos-methyl (0.25%) and Bendiocarb (0.1%)



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

Figure 20 shows the results from tests on the wild *An. gambiae* s.l. and the laboratory standard *An. arabiensis* KGB strain. *An. gambiae* s.l. was fully susceptible to chlorfenapyr in Koya, Jaima Bongor, Nimyama, and Gbanti Kamaranka. Mortality of >98% was achieved within the 48-hour holding period after exposure to chlorfenapyr in all the test sites except Jaima Bongor. There, a 100% test mortality was observed at 96 hours holding after exposure to the insecticide.

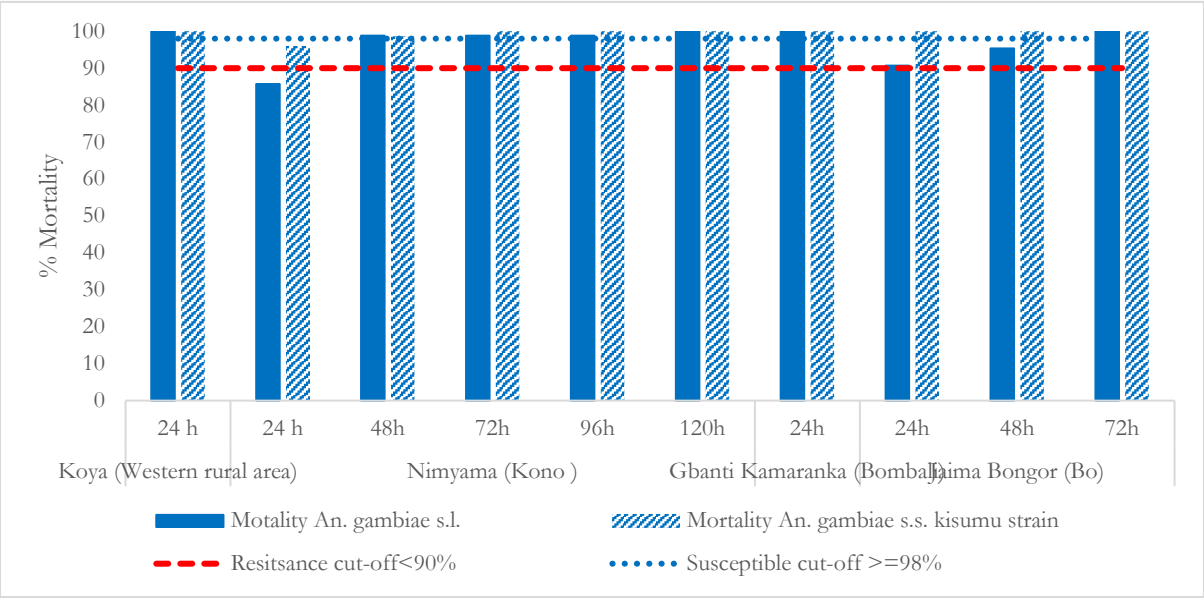
Figure 20: Mortality of *An. gambiae* s.l. for Chlorfenapyr (100 µg/bottle), October-November 2018



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

Figure 21 shows the results from tests on the wild *An. gambiae* s.l. and the laboratory standard *An. arabiensis* KGB strain. *An. gambiae* s.l. was fully susceptible to clothianidin in Koya, Jaima Bongor, Nimyama, and Gbanti Kamaranka. 100% mortality was achieved within the 24-hour holding period after exposure to clothianidin in Koya and Gbanti Kamaranka. However, it took 72 hours to achieve 100% mortality in Jaima Bongor and 120 hours in Nimyama.

Figure 21. Mortality *of An. gambiae* s.l. for Clothianidin (13.2 mg), November 2018



3.3.5 DETERMINATION OF THE INTENSITY OF RESISTANCE AND SYNERGIST ASSAYS

High resistance intensity to alpha-cypermethrin and deltamethrin was observed in Koya, Jaima Bongor, Nimyama, and Gbanti Kamaranka with mortality of *An. gambiae* s.l. below 98% when exposed to 10 times the diagnostic dose concentrations (Figures 22 and 26).

The mortality rate observed with alpha-cypermethrin (0.05%) in Gbanti Kamaranka, Jaima Bongor, and Nimyama and with deltamethrin (0.05%) in Koya after pre-exposure to PBO was higher than those not pre-exposed to PBO. However, the mortality rate with PBO pre-exposure was still less than 98%. Therefore, the results indicate that a monooxygenase-based resistance mechanism is partially involved, but is not fully responsible for the resistance observed (Figures 23 and 25)

The mortality observed with alpha-cypermethrin 62.5 ug/bottle (5x) in Jaima Bongor, Nimyama, and Gbanti Kamaranka, and with deltamethrin 62.5 ug/bottle (5x) in Koya after pre-exposure to PBO was greater or equal to 98% at all sites (Figure 24).

Figure 22. Mortality of *An. gambiae* s.l. Exposed to 5x (62.5 µg/bottle) and 10X (125 µg/bottle) Alpha-cypermethrin

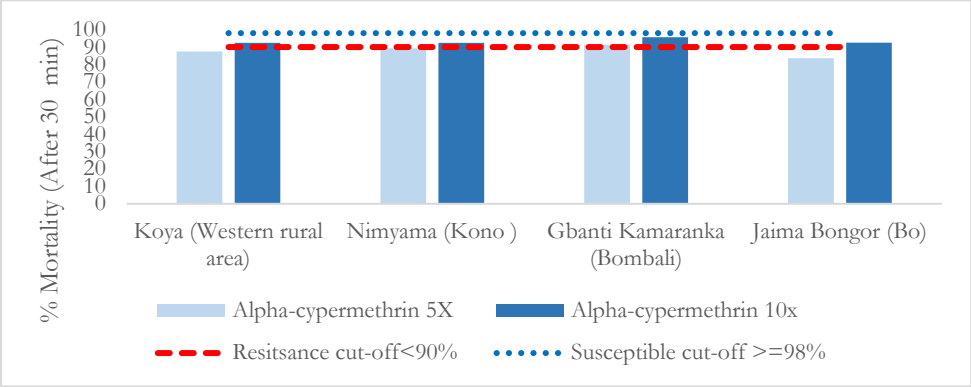


Figure 23. Mortality of *An. gambiae* s.l. for Alpha-cypermethrin 0.05% after PBO and No PBO Exposure

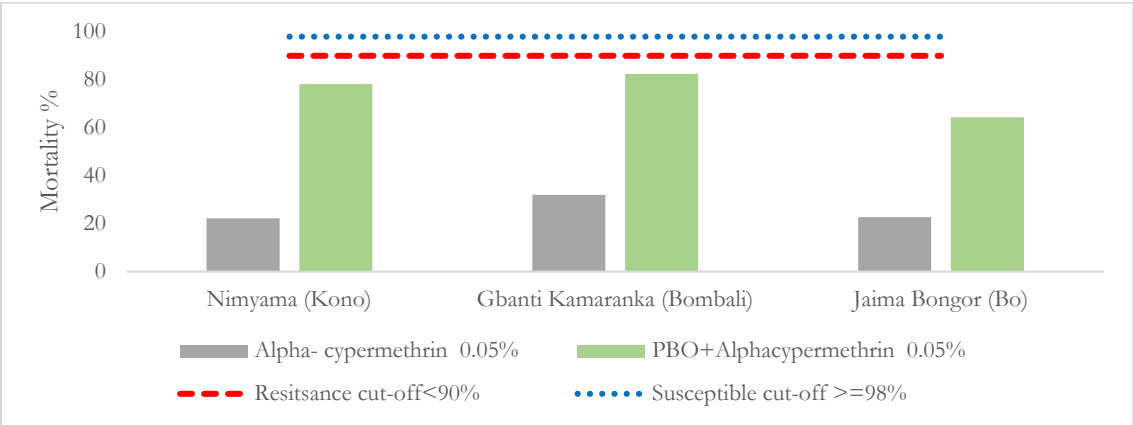


Figure 24. Mortality of *An. gambiae* s.l. for Alpha-cypermethrin 5x (62.5 µg/bottle) after PBO and No PBO Exposure

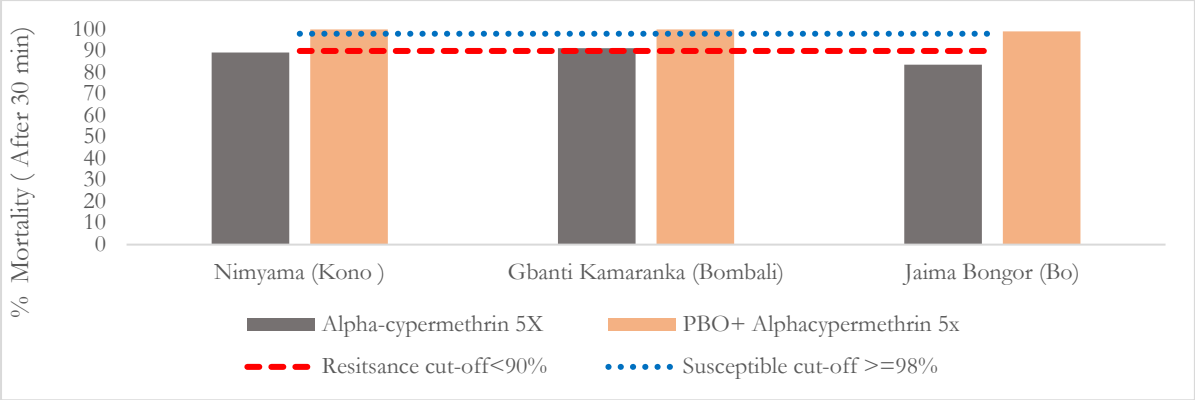


Figure 25. Mortality of *An. gambiae* s.l. for Deltamethrin 0.05% after PBO and No PBO Exposure, February 2019

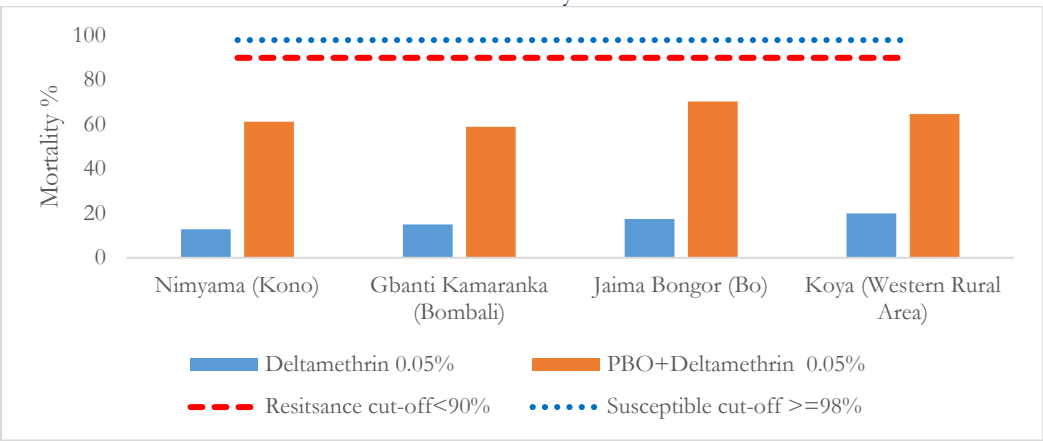
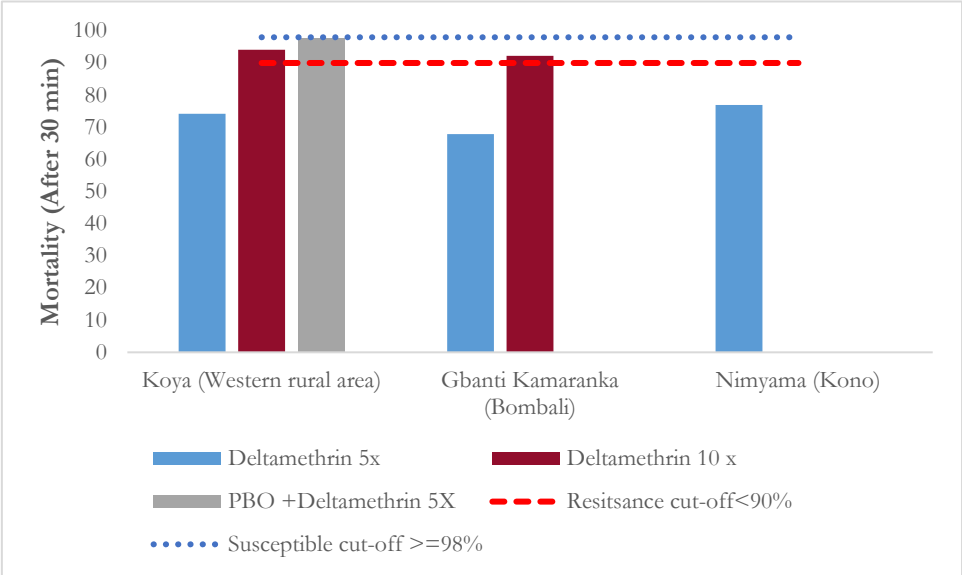


Figure 26. Mortality of *An. gambiae* s.l. exposed to Deltamethrin 5x (62.5 µg/bottle) and 10X (125 µg/bottle) and to Deltamethrin 5x (62.5 µg/bottle) after PBO and No PBO exposure



3.4 PCR Analysis for Mechanism of Resistance

A sample of the mosquitoes from the susceptibility tests was tested for the *kdr-w, kdr-e,* and N1575Y mutation using PCR. In general, the frequency of the *kdr-w* allele mutation was found in more than 98% of the samples screened (Table 4). Mutations for *kdr-e* and *ace*-1 allele were observed in 2.5% and 13% of the samples tested, respectively. The resistance allele frequency for N1575Y was 9%; but all were in the heterozygous form. All the 12 N1575Y mutations were found in samples also homozygotes to the *kdr- w* mutation. This mutation is believed to be involved in the amplification of resistance conferred by *kdr-w* (Jones CM, 2012). This mutation is also detected in both *An. gambiae* and An. *coluzzii.*

Table 4. Mechanism of Resistance

Site	*An. spp*	*kdr-w*			*kdr-e*			*ace-1*			N1575Y		
		RR	**RS**	**SS**	**RR**	**RS**	**SS**	**RR**	**RS**	**SS**	**RR**	**RS**	**SS**
Jaima Bongor (Bo District, Southern)	*An. coluzzii*	16	1	0	0	1	16	0	0	17	0	1	16
	An. gambiae	29	1	0	0	3	27	5	0	25	0	3	27
	An. coluzziiAn.gambiae	1	0	0	0	1	0	0	0	1	0	0	1
	An. gambiae s.l.	2	0	0	0	0	1	0	0	2	0	1	1
Gbanti Kamaranka (Bombali District, Northern)	*An. coluzzii*	6	0	0	0	0	6	0	0	6	0	2	4
	An. gambiae	44	0	0	0	3	41	3	0	41	0	11	33
	An. gambiae s.l.	0	0	0	0	0	0	0	0	0	0	0	0
Koya (Western Rural Area, Western)	*An. coluzzii*	5	2	0	0	0	7	0	0	7	0	1	6
	An. gambiae	40	0	0	0	0	40	13	0	27	0	11	29
	An. gambiae s.l.	3	0	0	0	0	2	0	0	3	0	0	2
Nimyama (Kono District, Eastern)	*An. coluzzii*	3	1	0	0	1	3	0	0	4	0	0	4
	An. gambiae	40	2	0	0	0	43	5	0	38	0	5	39
	An. gambiae s.l.	2	0	0	0	0	2	0	0	2	0	0	2
# of genotypes		**191**	**7**	**0**	**0**	**9**	**188**	**26**	**0**	**173**	**0**	**35**	**164**
# of haplotypes		**382**	**14**	**0**	**0**	**18**	**376**	**52**	**0**	**346**	**0**	**70**	**328**
Total # of alleles		**396**			**394**			**398**			**366**		
Frequency of the resistance (R) allele [(2RR + RS)/(RR+RS+SS)		**98.23%**			**2.28%**			**13.07%**			**9.56%**		

4. CAPACITY BUILDING

- The PMI VectorLink Project established a functional insectary in a space provided at the Makeni Neglected Tropical Disease building in Makeni.
- The new Makeni insectary is now maintaining the *An. gambiae* Kisumu colony transported from VectorLink Ghana.
- Twelve environmental health officers from the sentinel districts (Bo, Bombali, Kono, and Western Rural Area) and two NMCP staff were trained on entomological monitoring, including morphological identification of *Anopheles* at the Makeni facility.
- Sixty-four community health workers from all four districts were trained on PSC, HLC, and larvae collection methods.
- The VectorLink entomology coordinator and the NMCP focal person for integrated vector management attended a six-day regional entomology training in Cote d'Ivoire in August 2018.

5. Conclusions & Recommendations

- The monitoring results indicated that *An. gambiae* s.l. is the predominant malaria vector in all sentinel sites. *An. funestus* s.l. was collected in low numbers in most sites, with the most found in Gerihoun (Bo District), where the Sewa River offers *An. funestus* s.l. breeding sites.
- The overall highest mean HBR of *An. gambiae* s.l., 73.4 bites/person/night, was recorded indoors in June. HBR was high in June for all sites except in Sand sand Water and Tombo, where the highest mean indoor HBRs of *An. gambiae* s.l. was observed in August.
- The proportion of fed *An. gambiae* s.l. collected indoors was high at both rural and peri-urban sites. This probably indicates either low coverage or low utilization rate of bed nets by communities. However, the blood meal sources must be known to confirm if substantial human host-vector contact occurred during the study period. Identification of blood meal sources of malaria vectors is included in the 2018-19 work plan to capture this indicator in Sierra Leone. It is important for the NMCP to increase the availability of LLIN in the households and the sensitization on LLIN use.
- The highest indoor monthly EIR was recorded in August (62.42 infective bites/person/month) in the rural sites and in July in peri-urban sites (36.54 infective bites/person/month).
- Given the abundance and biting rate indicators show that mosquito population at its higher in June, indoor residual spraying (IRS) in the future should be implemented in April-May before the peak of malaria transmission.
- Most of the human vector contact occurred during the second part of the night. Given this late behavior by the vector, probably, high coverage and utilization rate of bednets will have a significant impact on malaria prevention
- A statistically significant difference was found between the parity rate of *An. gambiae s*.l., in the rural and peri-urban sites. But the numbers dissected during this reporting period were low and this result should be interpreted cautiously. The VectorLink Sierra Leone team will put more efforts into dissecting more malaria vectors to adequately estimate the parity rate and have the baseline information to assess the impact of IRS on longevity of malaria vectors.
- Resistance to pyrethroids was observed at all sites at a high frequency. Monooxygenase-based resistance mechanism is partially involved in the resistance to pyrethroids. *An. gambiae* s.l. is fully susceptible to chlorfenapyr and clothianidin. PBO-Deltamethrin and PBO -Alphha-cypermethrin LLIN or nets impregnated with other non-pyrethroid insecticides may be considered for malaria vector control in Sierra-Leone.
- A very high frequency of *kdr- w* mutation was detected in all sampling sites and this probably explains the observed high level of phenotypic resistance to pyrethroids. N1575Y mutation was also detected and its contribution to resistance of the vectors in Sierra Leone remains to be further monitored. The frequency of *kdr-w* allele mutation was found in more than 98% of the samples screened. The homozygote resistant allele was highly predominant.
- IRS with pirimiphos-methyl or clothianidin is another viable intervention for Insecticide Resistance Management in Sierra Leone.

- VectorLink Sierra Leone provided support to NMCP to review the IRMMP and there is a need to take action to preserve the effectiveness of the insecticides used for malaria vector control in Sierra Leone by inviting all vector control stakeholders to follow the IRMMP recommendations.

Recommendations

For VectorLink Sierra Leone

- Work with laboratory to complete the blood meal analysis
- Increase the number of malaria vectors dissected.

For NMCP & VectorLink Sierra Leone

- Consider the implementation of IRS in the future as close as possible to the rainy season (April-May) before peak vector population and malaria transmission season.
- Ensure that all vector control stakeholders follow the IRMMP recommendations for insecticide use in Sierra Leone.
- Consider the procurement of PBO-Deltamethrin or PBO-alpha-cypermethrin LLINs for the 2020 mass campaign.

- Ensure high quality application of IRS for the IRS campaign in 2021 in order for the insecticide to remain efficacious during peak transmission season by conducting training under master trainers, reinforcing the direct observation of the spraying by the team leaders and supervisors.
- Increase sensitization efforts for ITN use.

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ANNEX A: NUMBER OF *ANOPHELES* COLLECTED BY PSC, MAY 2018-FEBRUARY 2019

District	**Sites**	*PSC*			
		An. gambiae s.l.	*An. funestus s.l.*	*An. coustani*	*An ziemanni*
Bombali	Masongbo (peri-urban)	1261	0	11	1
	Kamaranka (Rural)	570	0	0	0
Kono	Teikor (Rural)	1297	49	0	0
	Sori Town (peri-Urban)	468	1	0	0
Bo	Lagon (Rural)	1073	0	0	0
	Gerihoun (peri-Urban)	582	21	0	0
Western Rural Area	Sand Sand Water (Rural)	114	0	0	0
	Tombo (peri-Urban)	27	0	0	0
Total		5392	71	11	1

Annex B: Number of *Anopheles* Collected by CDC Light Traps, September 2018–February 2019

District	Sites	CDC Light Trap			
		An. gambiae s.l.	*An. funestus* s.l.	*An. coustani*	*An ziemanni*
Bombali	Masongbo peri-Urban)	104	0	1	0
	Kamaranka (Rural)	25	0	0	0
Kono	Teikor (Rural)	69	0	1	0
	Sori Town (peri-Urban)	202	1	2	0
Bo	Lagon (Rural)	8	0	0	0
	Gerihoun (peri-Urban)	113	1	0	0
Western Rural Area	Sand Sand Water (Rural)	72	0	0	0
	Tombo (peri-Urban)	4	0	0	0
Total		597	2	4	0

ANNEX C: NUMBER OF *ANOPHELES* COLLECTED BY HLC, MAY 2018-FEBRUARY 2019

District	Sites	*An. gambiae* s.l.		*An. funestus* s.l.		*An. coustani*		*An. ziemanni*	
		In	**Out**	**In**	**Out**	**In**	**Out**	**In**	**Out**
Bombali	Masongbo (peri-Urban)	2,808	3,202	0	1	3	14	0	0
	Kamaranka (Rural	1,980	1,672	0	0	1	0	0	0
Kono	Teikor (Rural)	917	798	40	18	3	8	0	0
	Sori Town (peri-Urban)	944	746	18	5	0	8	2	2
Bo	Lagon (Rural)	934	687	0	1	0	0	0	0
	Gerihoun (peri-Urban)	1,845	1,611	76	35	16	34	2	3
Western Rural Area	Sand Sand Water (Rural)	890	652	0	0	1	4	0	0
	Tombo (peri-Urban)	186	314	0	0	0	2	0	1
	Total	10,504	9,682	134	60	24	70	4	6

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 water (Rural)	132	9	6.8	140	9	6.4	272	18	6.6
	Tombo (peri-Urban)	53	3	5.7	70	9	12.9	123	12	9.8
Kono	Teikor (Rural)	142	6	4.2	139	6	4.3	281	12	4.3
	Sori-town (peri-Urban)	131	2	1.5	131	2	1.5	262	4	1.5
Bombali	Kamaranka (Rural)	230	5	2.2	193	10	5.2	423	15	3.5
	Masongbo (peri-Urban)	240	5	2.1	277	9	3.2	517	14	2.7
	Lagon (Rural)	126	11	8.7	116	8	6.9	242	19	7.9
Bo	Gerihoun (peri-Urban)	195	8	4.1	182	1	0.5	377	9	2.4
Total		1249	49	3.9	1248	54	4.3	2497	103	4.1

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 water (Rural)									
	Tombo (peri-Urban)									
Kono	Teikor (Rural)	27	0	0	14	0	0	41	0	0
	Sori-town (peri-Urban)	7	1	14.3	2	0	0	9	1	11.111
Bombali	Kamaranka (Rural)									
	Masongbo (peri-Urban)									
	Lagon (Rural)				1	0	0	1	0	0
Bo	Gerihoun (peri-Urban)	66	2	3	32	1	3.1	98	3	3.1
Total		**100**	**3**	**3**	**49**	**1**	**2**	**149**	**4**	**2.7**

ANNEX F. RATE OF PLASMODIUM INFECTION IN AN. GAMBIAE AND AN. COLUZZII BY LOCATION

District	Site	IN/OUT	*An. gambiae*			*An. coluzzii*		
			Ex	Pos	% Pos	Ex	Pos	% Pos
Western Rural District	Tombo(peri-Urban)	Indoors	9	1	11.11	7	0	0.00
		Outdoors	34	4	11.76	11	4	36.36
	Sand sand Water (Rural)	Indoors	68	6	8.82	19	2	10.53
		Outdoors	49	4	8.16	17	2	11.76
Kono	Teikor (Rural)	Indoors	25	4	16.00	6	2	33.33
		Outdoors	41	3	7.32	6	1	16.67
	Sori-town (peri-Urban)	Indoors	27	1	3.70	4	0	0.00
		Outdoors	26	2	7.69	7	0	0.00
Bombali	Masongbo (peri-Urban)	Indoors	11	4	36.36	4	1	25.00
		Outdoors	80	6	7.50	10	0	0.00
	Kamaranka (Rural)	Indoors	59	3	5.08	4	1	25.00
		Outdoors	76	7	9.21	11	2	18.18
Bo	Lagon (Rural)	Indoors	15	1	6.67	42	8	19.05
		Outdoors	13	4	30.77	34	3	8.82
	Gerihoun (peri-Urban)	Indoors	11	6	54.55	2	1	50.00
		Outdoors	27	0	0.00	34	0	0.00
Infection rate by species, by location		*An. gambiae* IN	225	26	11.6			
		An. gambiae OUT	346	30	8.7			
				An. coluzzii IN		88	15	17.0
				An. coluzzii OUT		130	12	9.2

ANNEX G : SPOROZOITE RATE AND EIR OF AN. GAMBIAE S.L. IN RURAL SITES (MAY 2018 – FEBRUARY 2019)

Position	Indicators	May	June	July	August	September	October	November	December	January	February
						Rural sites					
Indoor	Total *An. gambiae s.l.* tested	36	210	90	78	65	41	38	12	21	39
	nb SPZ+	1	4	2	7	5	5	4	0	1	2
	Sporozoite rate	0.03	0.02	0.02	0.09	0.08	0.12	0.11	0	0.05	0.05
	HBR/night	15.19	65.78	29.09	22.44	11.06	2.97	4.44	0.81	5.19	6.75
	EIR month	**13.08**	**37.59**	**20.04**	**62.42**	**25.53**	**11.22**	**14.01**	**0**	**7.66**	**9.69**
Outdoor	Total *An. gambiae s.l.* tested	45	144	87	74	60	45	48	23	20	42
	nb SPZ+	3	1	6	5	7	5	5	0	0	1
	Sporozoite rate	0.07	0.01	0.07	0.07	0.12	0.11	0.1	0	0	0.02
	HBR/night	12.88	46	27.44	18.22	9	2.69	6.19	1.69	3.81	6.81
	EIR month	**26.61**	**9.58**	**58.66**	**38.16**	**31.5**	**9.26**	**19.34**	**0**	**0**	**4.54**

ANNEX H : SPOROZOITE RATE AND EIR OF AN. GAMBIAE S.L. IN PERI-URBAN SITES (MAY 2018 – FEBRUARY 2019)

Position	Indicators	May	June	July	August	September	October	November	December	January	February
						Peri-Urban sites					
Indoor	Total *An. gambiae s.l.* tested	29	208	101	60	53	46	35	32	29	26
	nb SPZ+	1	3	3	2	2	3	1	2	1	0
	Sporozoite rate	0.03	0.01	0.03	0.03	0.04	0.07	0.03	0.06	0.03	0
	HBR/night	13.63	81	39.69	18.31	11.63	9.25	11.38	4.19	7.63	4.88
	EIR month	**14.56**	**35.05**	**36.54**	**18.92**	**13.16**	**18.7**	**9.75**	**8.11**	**8.15**	**0**
Outdoor	Total *An. gambiae s.l.* tested	27	213	106	71	56	52	31	26	26	52
	nb SPZ+	0	4	4	1	3	2	4	1	2	0
	Sporozoite rate	0	0.02	0.04	0.01	0.05	0.04	0.13	0.04	0.08	0
	HBR/night	11.63	74.91	39.88	21.81	12.81	12.19	12.63	6.81	5.19	7.63
	EIR month	**0**	**42.2**	**46.65**	**0.31**	**20.59**	**14.53**	**48.87**	**8.12**	**12.37**	**0**

ANNEX I: SPOROZOITE RATE AND EIR OF AN. GAMBIAE S.L. IN KAMARANKA RURAL SITE IN BOMBALI DISTRICT (MAY 2018 – FEBRUARY 2019)

Position	Indicators	May	June	July	August	September	October	November	December	January	February
						Kamaranka					
Indoor	Total *An. gambiae s.l.* tested	20	113	35	10	10	12	11	2	2	15
	nb SPZ+	1	0	1	0	0	0	2	0	1	0
	Sporozoite rate	0.05	0	0.03	0	0	0	0.18	0	0.5	0
	HBR/night	10.25	148.88	58.13	12.75	6.5	3.63	2.75	0.25	0.5	11.25
	EIR	**15.89**	**0**	**51.48**	**0**	**0**	**0**	**15**	**0**	**7.75**	**0**
Outdoor	Total *An. gambiae s.l.* tested	21	65	40	12	10	10	19	6	0	10
	nb SPZ+	1	0	3	1	0	0	5	0		0
	Sporozoite rate	0.05	0	0.08	0.08	0	0	0.26	0		0
	HBR/night	10.75	106.75	63.125	14.25	4	2.625	4.25	0.75	0	10
	EIR	**15.87**	**0**	**146.77**	**36.81**	**0**	**0**	**33.55**	**0**	**0**	**0**

ANNEX J: SPOROZOITE RATE AND EIR OF AN. GAMBIAE S.L. IN MASONGBO PERI-URBAN SITE IN BOMBALI DISTRICT (MAY 2018 – FEBRUARY 2019)

Position	Indicators	May	June	July	August	September	October	November	December	January	February
						Masongbo					
Indoor	Total *An. gambiae s.l.* tested	15	87	44	25	15	11	10	18	10	5
	nb SPZ+	1	0	0	0	0	1	1	1	1	0
	Sporozoite rate	0.07	0	0	0	0	0.09	0.1	0.0556	0.1	0
	HBR/night	8.63	189	87.25	31.75	17.5	4.25	13	6.25	4	2
	EIR	**17.83**	**0**	**0**	**0**	**0**	**11.98**	**39**	**10.76**	**12.4**	**0**
Outdoor	Total *An. gambiae s.l.* tested	15	98	51	34	18	20	11	10	7	13
	nb SPZ+	0	0	2	1	1	1	1	1	2	0
	Sporozoite rate	0	0	0.04	0.03	0.06	0.05	0.09	0.1	0.29	0
	HBR/night	9.25	187.5	94.5	42.38	21.5	18.63	22.25	17	6.5	7.25
	EIR	**0**	**0**	**114.88**	**38.64**	35.83	28.87	**60.68**	**52.7**	**57.57**	**0**

ANNEX K : SPOROZOITE RATE AND EIR OF AN. GAMBIAE S.L. IN TEIKOR RURAL SITE IN KONO DISTRICT (MAY 2018 – FEBRUARY 2019)

Position	Indicators	May	June	July	August	September	October	November	December	January	February
						Teikor					
Indoor	Total *An. gambiae s.l.* tested	16	17	16	25	25	12	9	3	9	10
	nb SPZ+	0	0	0	2	1	3	0	0		0
	Sporozoite rate	0	0	0	0.08	0.04	0.25	0	0		0
	HBR/night	20.13	20.75	20.75	30.38	10.88	3.63	2.25	0.75	2.5	10.75
	EIR month	**0**	**0**	**0**	**75.3**	13.05	28.09	0	0		0
Outdoor	Total *An. gambiae s.l.* tested	24	16	16	18	18	12	8	7	10	10
	nb SPZ+	2	0	0	1	2	1	0	0	0	0
	Sporozoite rate	0.08	0	0	0.06	0	0.08	0	0	0	0
	HBR/night	15	19.88	20	22.75	12.5	4.13	2	1.75	2.5	4.75
	EIR month	**38.75**	**0**		**39.18**	**0**	**10.66**	**0**	**0**	**0**	**0**

ANNEX L. SPOROZOITE RATE AND EIR OF AN. GAMBIAE S.L. IN SORI TOWN PERI-URBAN SITE IN KONO DISTRICT (MAY 2018 – FEBRUARY 2019)

Position	Indicators	May	June	July	August	September	October	November	December	January	February
						Sori-Town					
Indoor	Total *An. gambiae s.l.* tested	14	36	12	13	13	10	10	3	9	11
	nb SPZ+	0	0	0	1	1	0	0	0	0	0
	Sporozoite rate	0	0	0	0.077	0.077	0	0	0		0
	HBR/night	18.63	45.5	13.13	16.88	8.75	3.63	2.75	0.75	16.75	2.75
	EIR month	**0**	**0**	**0**	**40.24**	**20.19**	**0**	**0**	**0**	**0**	**0**
Outdoor	Total *An. gambiae s.l.* tested	12	39	9	12	11	5	6	0	9	28
	nb SPZ+	0	1	0	0	0	0	1		0	0
	Sporozoite rate	0	0.03	0	0	0	0	0.17			0
	HBR/night	14	45.13	5.13	14.5	4.63	1.75	4	0.5	5.5	8
	EIR month	**0**	**34.71**	**0**	**0**	0	0	**20**	**0**	**0**	**0**

ANNEX M: SPOROZOITE RATE AND EIR OF AN. GAMBIAE S.L. IN LAGON RURAL SITE IN BO DISTRICT (MAY 2018 – FEBRUARY 2019)

Position	Indicators	May	June	July	August	September	October	November	December	January	February
						Lagon					
Indoor	Total *An. gambiae s.l.* tested		60	24	11	10	7	10	0	0	4
	nb SPZ+		1	0	5	3	2	0			0
	Sporozoite rate		0.017	0	0.27	0.1	0.29	0			0
	HBR/night		81.25	18.75	6.5	3.5	1	10.75	0	0	0.75
	EIR month		**40.63**	**0**	**54.95**	**10.5**	**8.86**	**0**	**0**	**0**	**0**
Outdoor	Total *An. gambiae s.l.* tested		41	20	11	10	12	10	0	0	12
	nb SPZ+		0	3	0	2	3	0			0
	Sporozoite rate		0	0.1	0	0.2	0.25	0			0
	HBR/night		52.25	14.75	4.75	2.75	2	15.75	0	0	3
	EIR month		**0**	**45.73**	**0**	16.5	15.5	**0**	**0**	**0**	**0**

ANNEX N: SPOROZOITE RATE AND EIR OF AN. GAMBIAE S.L. IN GERIHOUN PERI-URBAN IN BO DISTRICT (MAY 2018 – FEBRUARY 2019)

Position	Indicators	May	June	July	August	September	October	November	December	January	February
						Gerihoun					
Indoor	Total *An. gambiae s.l.* tested		68	35	16	15	20	11	10	10	10
	nb SPZ+		2	3	1	0	1	0	1	0	0
	Sporozoite rate		0.01	0.09	0.06	0	0.05	0	0.1	0	0
	HBR/night		85.63	49	19.75	16.38	28.5	28.75	9.5	9.75	14.75
	EIR month		**37.78**	**130.2**	**38.27**	**0**	**44.175**	**0**	**29.5**	**0**	**0**
Outdoor	Total *An. gambiae s.l.* tested		50	36	15	15	22	10	14	10	10
	nb SPZ+		0	0	0	1	0	0	0	0	0
	Sporozoite rate		0	0	0	0.07	0	0	0	0	0
	HBR/night		60.5	47	17.88	19.25	28.63	23.25	9.25	8.75	15
	EIR month		**0**	**0**	**0**	38.5	0	**0**	**0**	**0**	**0**

ANNEX O: SPOROZOITE RATE AND EIR OF AN. GAMBIAE S.L. IN SAND SAND WATER RURAL SITE IN WESTERN RURAL DISTRICT (JUNE 2018 – FEBRUARY 2019)

Position	Indicators	May	June	July	August	September	October	November	December	January	February
					Sand-Sand Water						
Indoor	Total *An. gambiae s.l.* tested		20	15	32	20	10	8	7	10	11
	nb SPZ+		3	1	0	1	0	2	0	0	2
	Sporozoite rate		0.15	0.07	0	0.05	0	0.13	0	0	0.18
	HBR/night		12.25	18.75	40.13	23.38	3.63	2	1.13	8.88	2.13
	EIR		**55.13**	**38.75**	**0**	**35.06**	**0**	**7.5**	**0**	**0**	**10.82**
Outdoor	Total *An. gambiae s.l.* tested		22	11	33	22	11	11	10	10	10
	nb SPZ+		1	0	3	3	1	0	0	0	1
	Sporozoite rate		0.05	0	0.09	0.09	0.09	0	0	0	0.1
	HBR/night		5.13	11.88	31.13	16.75	2	2.75	4.25	12.75	9.5
	EIR		**6.99**	**0**	**87.72**	**45.68**	**5.45**	**0**	**0**	**0**	**26.6**

ANNEX P: SPOROZOITE RATE AND EIR OF AN. GAMBIAE S.L. IN WESTERN RURAL DISTRICT (JUNE 2018 – FEBRUARY 2019)

Position	Indicators	May	June	July	August	September	October	November	December	January	February
						Tombo					
Indoor	Total *An. gambiae s.l.* tested		17	10	6	10	5	4	1	0	0
	nb SPZ+		1	0	0	1	1	0	0		
	Sporozoite rate		0.06	0	0	0.1	0.2	0.25	0		
	HBR/night		3.88	9.38	4.88	3.88	0.63	1	0.25	0	0
	EIR		**6.84**	**0**	**0**	**11.63**	**3.75**	**7.5**	**0**	**0**	**0**
Outdoor	Total *An. gambiae s.l.* tested		26	10	10	12	5	4	2	0	1
	nb SPZ+		3	2	0	1	1	2	0		0
	Sporozoite rate		0.12	0.2	0	0.08	0.2	0.5	0		0
	HBR/night		6.5	12.875	12.5	5.875	0.625	1	0.5	0	0.25
	EIR		**22.5**	**79.83**	**0**	**14.69**	**3.75**	**15**	**0**	**0**	**0**

ANNEX Q: 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%)	93	100	4	102	100	4	88	100	4	96	100	4
Alpha-cypermethrin 0.05%	73	*34.25%*	4	70	*10.00%*	4	92	*46.56%*	4	64	*50.00%*	4
Alpha-cypermethrin 62.5 µg	115	91.30%	4	122	*83.61%*	4	56	*89.29%*	4	88	*87.50%*	4
Alpha-cypermethrin 125 µg	114	*95.61%*	4	67	*92.54%*	4	106	*92.45%*	4	105	*92.38%*	4
PBO+Alphacypermethrin 0.05%	97	*82.47%*	4	73	*64.38%*	4	78	*78.21%*	4			
PBO+Alpha-cypermethrin62.5 µg	**129**	*100.00%*	**4**	**112**	*99.11%*	**4**	70	*100.00%*	**4**			
Deltamethrin 0.05%	81	*12.35%*	4	85	*12.94%*	4	81	*53.09%*	4	100	*25.00%*	4
Deltamethrin 62.5 µg	81	*67.90%*	4				91	*76.92%*	4	62	*74.19%*	4
Deltamethrin 125 µg	77	*92.21%*	4							102	*94.12%*	4
PBO+Deltamethrin 0.05%	91	*70.33%*	4	112	*58.93%*	4	31	*61.29%*	2	85	*64.71%*	4
PBO+Deltamethrin 62.5 µg										86	*97.67%*	4
Permethrin 0.75%	55	*7.27%*	4	77	*15.58%*	4	77	*31.17%*	4	72	*65.28%*	4
Bendiocarb 0.1%				85	*94.12%*	4	94	*93.62%*	4	129	*88.37%*	4

Chlorfenapyr 100 µg	**85**	*100.00 %*	**4**	**97**	*100.00 %*	**4**	**100**	*100.00 %*	**4**	**89**	*100.00 %*	**4**
Clothianidin 13.2mg	**99**	*100.00 %*	**4**	**86**	*100.00 %*	**4**	**84**	*100.00 %*	**4**	**96**	*100.00 %*	**4**

	Susceptible
	Suspected resistance
	Resistance
	Not tested

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