PMI VECTORLINK PROJECT

THE DEMOCRATIC REPUBLIC OF CONGO ANNUAL ENTOMOLOGICAL MONITORING REPORT

JANUARY – DECEMBER 2020

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CONTENTS

LIST OF FIGURES

LIST OF TABLES

ACRONYMS

CDC	Centers for Disease Control and Prevention
CI	Confidence Interval
CTAB	Cetyltrimethylammonium bromide
DRC	Democratic Republic of Congo
EIR	Entomological Inoculation Rate
ELISA	Enzyme-linked Immunosorbent Assay
GTLAV	*Groupe de Travail Lutte Anti-Vectorielle*/Vector Control Working Group
HBR	Human Biting Rate
HLC	Human Landing Catch
INRB	*Institut National de Recherche Biomédicale*/National Institute of Biomedical Research
ITN	Insecticide-Treated Net
kdr	Knock-Down Resistance
Nbr	Number
NMCP	National Malaria Control Program
PBO	Piperonyl Butoxide
PCR	Polymerase Chain Reaction
PMI	President's Malaria Initiative
PSC	Pyrethrum Spray Catch
SS	Wild type susceptible
SR	Sporozoite Rate
RS	Heterozygous resistant
RR	Homozygous resistant
WHO	World Health Organization

EXECUTIVE SUMMARY

The President's Malaria Initiative (PMI) VectorLink Project conducted entomological monitoring in the Democratic Republic of Congo (DRC) from January to December 2020. Activities took place in 13 sentinel sites distributed nationwide. The project conducted monthly longitudinal monitoring of malaria vector biting rates, resting densities, and entomological inoculation rates (EIRs) in three sites (Lodja, Kimpese, and Inongo). Indoor resting densities were determined using pyrethrum spray catch (PSC) collections, and human landing catch (HLC) was undertaken indoors and outdoors to determine malaria vector biting rates. To inform the National Malaria Control Program's (NMCP's) choice of insecticides for future insecticide-treated net (ITN) distribution campaigns, insecticide susceptibility tests were conducted in all 13 sites. Resistance intensity bioassays using *Anopheles gambiae* s.l. were conducted with permethrin, deltamethrin, and alpha-cypermethrin at one, five, and 10 times the diagnostic concentration, according to World Health Organization (WHO) protocols. The project used piperonyl butoxide (PBO) synergist bioassays with pyrethroids and bottle bioassays to determine susceptibility status to chlorfenapyr. To prevent delays in entomological laboratory analysis and build local capacity, PMI VectorLink DRC bought equipment for polymerase chain reaction (PCR) and ELISA (Enzyme-linked Immunosorbent Assay) testing for the National Institute of Biomedical Research (*Institut National de Recherche Biomédicale*, INRB). The dedicated entomology molecular laboratory was established in August 2020 and all planned molecular analysis were performed on time. The VectorLink Collect module hosted in DHIS2 was used in DRC for the first time in 2020, and following remote training of INRB and Abt staff, all entomological data collected in 2020 was entered into VectorLink Collect and exported for data analysis to prepare the annual report. An online data visualization platform within VectorLink Collect was developed so that stakeholders, including NMCP, INRB and PMI, will have on-demand access to data summaries and raw data in 2021.

Anopheles gambiae s.l. was the predominant malaria vector throughout the year in Lodja and Inongo, with *An. funestus* the predominant species collected in Kimpese. The abundance in all sites of *Anopheles* species was greater (88%: 13,831/15,718) from HLC than by PSC. This indicates that *An. gambiae* s.l. and *An. funestus* s.l. exit houses early in the morning (before PSC sampling which started at 6 a.m.). Biting rates of *An. gambiae* s.l. were particularly high in Lodja throughout the year and much lower in Inongo. In Lodja, the mean *An. gambiae* s.l. biting rate was 17 bites per person per night indoors and 35 outdoors, with a malaria sporozoite rate of 2.29% (55/2,400). This equates to an annual EIR of 218 infectious bites per person in Lodja. In Kimpese, the mean *An. gambiae* s.l. sporozoite rate was 2.94% (33/1,124), and the annual *An. gambiae* s.l. EIR was 63 infectious bites per person per year. Also in Kimpese, the mean *An. funestus* s.l. biting rate was 21 bites per person per night indoors and 22 outdoors, with a malaria sporozoite rate of 2.5% (6/240), giving an annual *An. funestus* s.l. EIR of 194 infectious bites per person. Combined for both malaria vector species, this gives an annual EIR of 257 infectious bites per person per year in Kimpese. In Inongo, the mean *An. gambiae* s.l. biting rate was low at 0.54 bites per person per night indoors, with a sporozoite rate of 1.9% (2/104). This gives a much lower EIR of 3.8 infectious bites per person per year. The results highlight that there is an extremely high year-round malaria transmission risk in Lodja and Kimpese. They also show that there is heterogeneity across the country, with Inongo in southern DRC having a relatively low transmission risk (in the village where trapping was conducted).

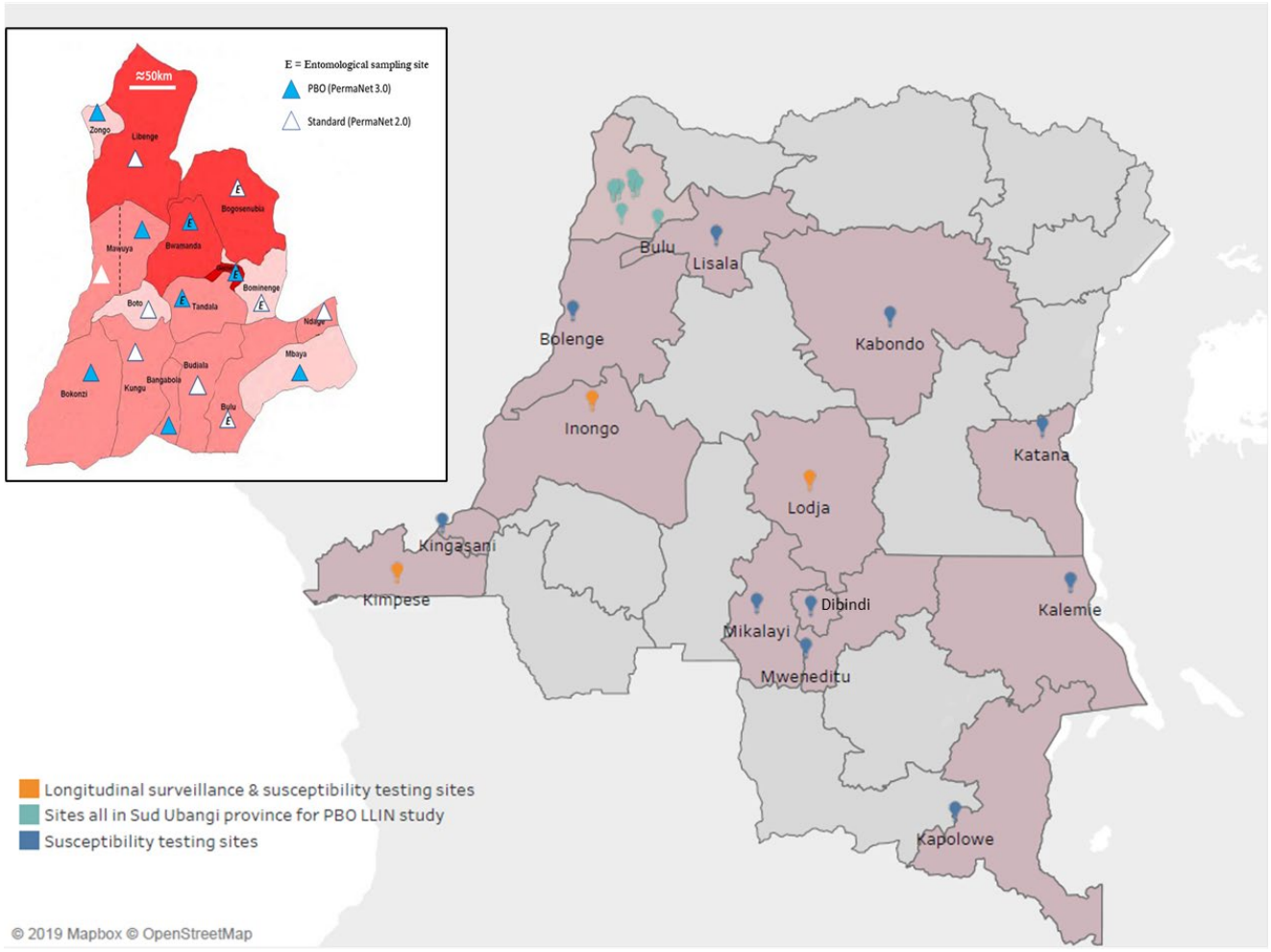
Insecticide susceptibility tests showed that pyrethroid resistance is widespread. In all sites, *An. gambiae* s.l. were resistant to permethrin, deltamethrin and alpha-cypermethrin. Resistance intensity varied by site and by insecticide, but was usually moderate or high. For example, permethrin resistance intensity was low in two sites (Mweneditu, and Dibindi); moderate in three sites (Kabondo, Mbandaka and Lisala); and high (<98% mortality at ×10 dose) in eight sites (Katana, Kingasani, Inongo, Kimpese, Mikalayi, Kapolowe, Kalemie, and Lodja). Despite uncertainty regarding the impact of pyrethroid resistance, WHO states that, "When resistance is confirmed at the 5× and especially at the 10× concentrations, operational failure is likely." Throughout DRC, resistance to the three most common pyrethroids used on ITNs was common at the 5× and 10× concentrations, therefore making it highly likely that pyrethroid ITNs are no longer providing optimal protection against malaria. The high intensity of pyrethroid resistance indicates that the NMCP should consider new types of ITNs for future distribution campaigns. In all sites, bioassays with permethrin following pre-exposure to PBO 4% in WHO tube tests showed an increase in mortality compared with permethrin alone. Despite this, mortality was still <90% in six sites. There was a significant increase in mortality with deltamethrin and alpha-cypermethrin following pre-exposure to PBO 4% in all 13 sites; increases in deltamethrin mortality were particularly large in Katana, Kingasani, Mweneditu, Dibindi, and Mbandaka. Despite an increase in mortality after pre-exposure to PBO, alpha-cypermethrin mortality was still <90% in five sites (Inongo, Mikalayi, Kapolowe, Lodja, and Lisala). The general increase in mortality when a PBO synergist was used indicates that ITNs containing PBO may provide greater control, although susceptibility was not restored. A better option may be Interceptor G2 ITNs, as susceptibility to chlorfenapyr was recorded in all six sites where testing was conducted (Mweneditu, Dibindi, Inongo, Kimpese, Mbandaka, and Lisala).

1. METHODOLOGY

1.1 STUDY AREA

This report details the results of entomological monitoring activities that the PMI's VectorLink Project conducted in 13 sites in 2020 (Figure 1). Results of a PBO net study conducted in Sud Ubangi are presented in a separate report. Susceptibility testing that was initially scheduled for Bolenge was re-located to Mbandaka (both in Equateur Province) due to an Ebola outbreak.

Figure 1: Location of Sentinel Sites in DRC for Entomological Monitoring in 2020.



The activities were conducted according to the PMI VectorLink DRC Year 3 work plan (Table 1).

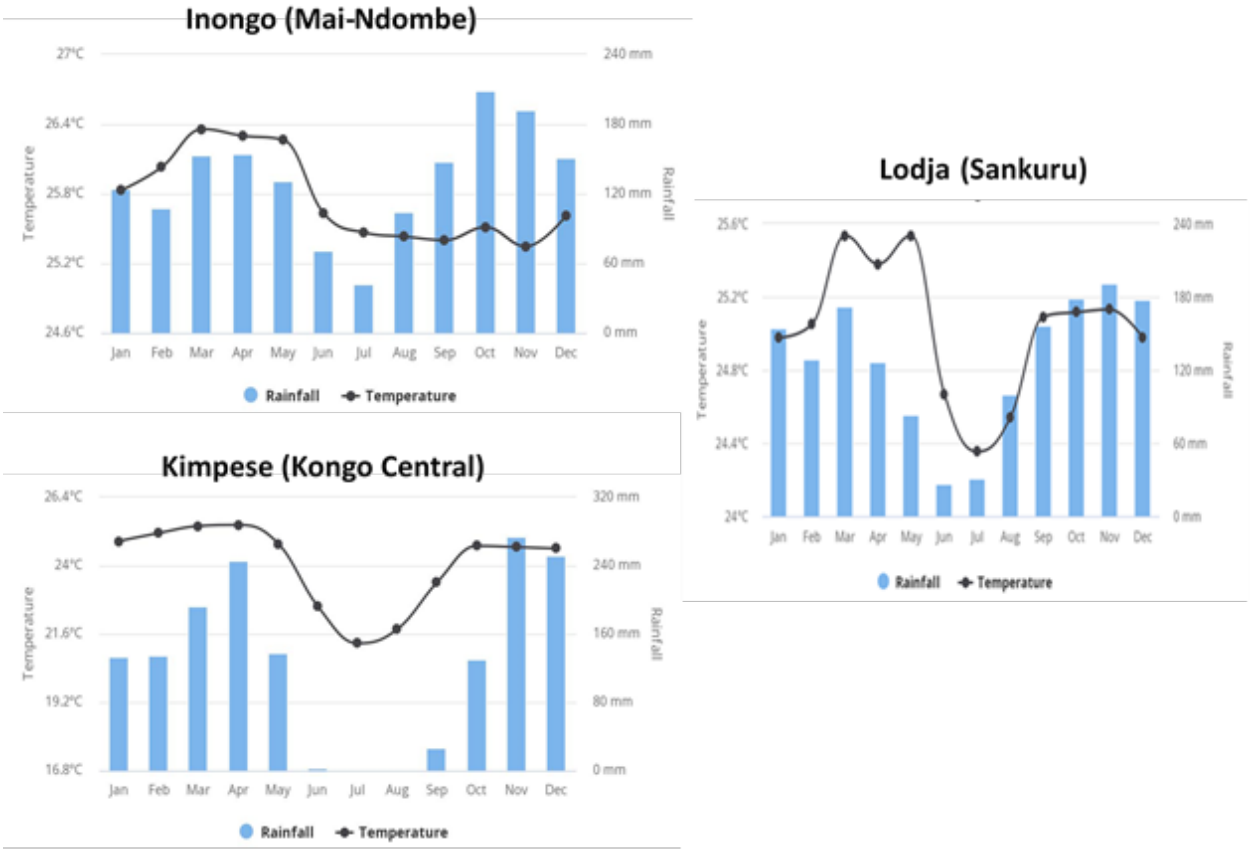
Table 1: Summary PMI VectorLink DRC 2020 Entomological Activities.

Activity	Purpose	Sites	Timeline	Frequency	Status
Vector susceptibility and intensity of resistance	To determine vector susceptibility to three pyrethroid insecticide with/without the synergist PBO. To determine pyrethroid resistance intensity. To determine susceptibility to chlorfenapyr.	Kimpese, Lodja, Inongo, Kabondo, Kalemie, Kapolowe, Mikalayi, Katana, Kingasani, Mbandaka,* Mweneditu,* Lisala,* and Dibindi*	May–December 2020	Thirteen sites, once per site	Completed
Monthly species composition, biting rate, biting times, and indoor resting densities	To gather more detailed longitudinal information on malaria vector dynamics and behavior.	Lodja, Inongo, and Kimpese	January–December 2020	Three sites, every month	Completed
Tanganyika ITN bio-efficacy and durability	To assess the physical and biological durability of the two ITN brands: Veeralin and SafeNet.	Manono and Kalemie	September 2020	Two sites	Baseline survey moved to Year 4 due to delay of ITN distribution
Molecular assays	To identify mosquito species of the *An. gambiae* s.l. species complex, mechanisms of pyrethroid resistance (*kdr*), and sporozoite rates.	Kimpese, Lodja, Inongo, Kabondo, Kalemie, Kapolowe, Mikalayi, Katana, Kingasani, Mbandaka, Mweneditu, Lisala, and Dibindi	January–December 2020	Thirteen sites	Completed
Sud Ubangi ITN monitoring	To collect data on the density of vectors and the susceptibility of *An. gambiae* s.l. to deltamethrin with and without PBO. To conduct ITN bioassay testing of PBO and pyrethroid nets using locally collected *An. gambiae* s.l. from Gemena.	Sud Ubangi in 6 health zones	Six and 12 month surveys scheduled for May and November 2020.	Six sites, once per site	Baseline survey completed in February 2020. six and 12 month surveys carried over to Year 4 due to delay of ITN distribution.

Notes: ITN=insecticide-treated net, *kdr*=knockdown resistance, PBO=piperonyl butoxide, PCR=polymerase chain reaction. *New site for 2020.

Figure 2 shows the mean rainfall and temperature for the three longitudinal monitoring sites (Inongo, Lodja, and Kimpese). All three have a dry season in the middle of the year, which lasts approximately four months in Lodja and Inongo, and six months in Kimpese. Inongo has considerable rainfall for most of the year, with peaks in March/April and October/November. The mean temperature in Inongo is quite stable year-round, with a mean of 25-26°C, providing perfect conditions for malaria vector survival.

Figure 2: Average Monthly Temperature and Rainfall (1991-2016) in Longitudinal Entomological Monitoring Sites.



Source: Climatic Research Unit of University of East Anglia

Mosquitoes were collected monthly using HLC and PSC in Lodja, Inongo, and Kimpese. See Table 2 for a summary of collection methods. Houses (8 for HLC and 10 for PSC) were sampled monthly in the same village each month in the district of Inongo (Maman Yaka: -1.92249, 18.30027), and the same village was used in Lodja each month in the health zone of Asani (-3.53636, 23.58333). In Kimpese, four villages were used in the district of Cataractes, Kimpese health zone with trapping conducted for three months in Yanga Diansonga (-5.57166, 14.42333), for four months in Kilueka (-5.41972, 14.47055), for two months in Malanga (-5.54694, 14.355333), and for three months in Viaza (-5.65111, 14.30972).

Table 2: Summary of Collection Methods.

Collection method	Time	Frequency	Sample
PSC	6:00 am to 9:00 am	Two days per site per month	Ten houses per site per month (five houses per day)
HLC	6:00 pm to 6:00 am	Four days per site per month	Eight houses per site per month (two houses per night)

1.2 Human Landing Catch

HLCs were performed to assess mosquito biting time, feeding behavior, and biting rates, and to monitor species composition and sporozoite rates. Trained residents collected adult mosquitoes over four consecutive nights in two different houses each night (total of eight houses per month), with one person located indoors and another outdoors in each selected house. Collectors rotated indoors and outdoors every hour. The same houses were used every month in Lodja and Inongo but in Kimpese different houses were used as described in section 1.1.. All *Anopheles* mosquitoes collected by HLC were identified to species morphologically in the field and cross-checked by INRB entomologists either in the field or in Kinshasa (depending on the supervision schedule). All *Anopheles* were preserved in 1.5 ml Eppendorf tubes on silica gel for further molecular analysis in Kinshasa by the INRB.

1.3 Pyrethrum Spray Catch

PSCs were conducted from 6:00 a.m. to 9:00 a.m. in the same areas as HLC (in different houses to HLC) to estimate the indoor resting density of mosquito species. Before the PSCs were performed, all occupants were asked to move out of the house. The rooms were sprayed with a commercially available aerosol containing pyrethroid and PBO to knock down mosquitoes resting inside the house. Twenty minutes after spraying, all mosquitoes knocked down were collected from a white sheet lying on the flat surfaces. Female *Anopheles* were classified according to the four abdominal stages (unfed, fed, half-gravid, and gravid). Each mosquito collected was labeled properly, stored in an Eppendorf tube with silica gel, identified to species morphologically in the field, and cross-checked by an INRB entomologist once back at the central lab.

1.4 Insecticide Susceptibility, PBO Synergist, and Resistance Intensity Testing

Insecticide susceptibility and resistance intensity testing were conducted in 13 sentinel sites. As part of de-centralization of entomological surveillance, insecticide susceptibility testing was conducted remotely by sentinel staff in six sites, with no supervision from INRB. The six sites were Katana, Kabondo, Lodja, Kalemie, Kapolowe, and Mikalayi, where local staff are relatively well-trained. Insecticide papers were sent from Kinshasa by plane to the remote sites, and after testing, mosquito samples and data sheets were returned to INRB by plane. As chlorfenapyr testing is more challenging technically and requires controlled test conditions, it was not undertaken in these sites. Susceptibility testing was conducted in another seven sites (Kimpese, Kingasani, Inongo, Bolenge, Mweneditu, Lisala, and Dibindi) with in-person supervision from INRB. This makes a total of 13 sites, four of which were sampled for the first time (Mbandaka, Mweneditu, Lisala, and Dibindi).

In addition to testing at the diagnostic dose, WHO intensity bioassays were also conducted by testing with pyrethroid papers treated with five and ten times the diagnostic dose.

The insecticides tested in 2020 were:

- Deltamethrin ×1, ×5, ×10 (0.05%, 0.25%, 0.5%)
- Permethrin ×1, ×5, ×10 (0.75%, 3.75%, 7.5%)
- Alpha-cypermethrin ×1, ×5, ×10 (0.05%, 0.25%, 0.5%)
- Deltamethrin 0.05% with pre-exposure to PBO 4%
- Permethrin 0.75% with pre-exposure to PBO 4%

- Alpha-cypermethrin 0.05% with pre-exposure to PBO 4%
- Chlorfenapyr 100µg/bottle (chlorfenapyr was tested in Centers for Disease Control and Prevention (CDC) bottle bioassays)

In all sites, susceptibility testing was conducted with adult *An. gambiae* s.l. following the WHO method (with the exception of chlorfenapyr). During the susceptibility tests, female adult mosquitoes were exposed for one hour to insecticide-treated filter papers provided by WHO (Universiti Sains Malaysia). Exposure tests were accompanied by negative control tests in which mosquitoes were exposed to filter papers impregnated with oil or solvent. Testing was undertaken according to WHO protocols, with mortality being the primary outcome measure. Four replicates of 25 *An. gambiae* s.l. were exposed to each concentration.

WHO susceptibility tests were conducted on permethrin, deltamethrin, and alpha-cypermethrin, with pre-exposure to PBO for 60 minutes to determine the change in mortality rates with PBO exposure.

CDC bottle bioassays were completed in six sites (Kimpese, Inongo, Mbandaka, Mweneditu, Lisala, and Dibindi) to determine the susceptibility status of *An. gambiae* s.l. populations to chlorfenapyr using an interim diagnostic dose of 100µg/bottle. Four replicates of 20-25 *An. gambiae* s.l. were exposed for 60 minutes to chlorfenapyr 100ug/bottle. The proportion of mosquitoes knocked down was recorded 60 minutes after the start of the test while the mosquitoes were still in the bottle. After 60 minutes of exposure, the mosquitoes were removed from the bottle, transferred to paper cups, and supplied with a sugar solution. Mortality was recorded every 24 hours for three days following the 60 minute exposure.

1.5 Molecular Analysis

Molecular analyses were conducted in a new molecular laboratory at INRB, which was equipped by the project and dedicated to entomology. The mosquito samples collected from sentinel sites were transported to the INRB for processing and analysis. Technicians conducted laboratory analyses under the supervision of the INRB focal point entomologist, Professor Francis Wat'senga, and PMI VectorLink Entomologist Dr. Rodrigue Fiacre Agossa, following the protocols described in Table 3.

Table 3: Protocols Used for Laboratory Analysis of Malaria Vectors.

Molecular Analysis	Protocol	Output
ELISA	Malaria Research and Reference Reagent Resource Centre document "*Anopheles* in Research" (based on Wirtz et al., 1987)	Sporozoite identification: Identified mosquito samples that were positive for *Plasmodium falciparum* sporozoites
PCR	Santolamazza et al. (2008)	Species identification: Identified *An. gambiae* complex sibling species including *An. coluzzii*, *An. gambiae,* and *An. arabiensis*
	Huynh et al. (2007)	Vgsc-L1014F allele frequency: Monitored pyrethroid target site resistance mechanism frequency

Sporozoite infection rate testing was completed for 3,628 *An. gambiae* s.l. collected from Kimpese, Lodja and Inongo in 2020 using ELISA and 2,608 *An. gambiae* s.l using PCR for species identification and resistance mechanism detection (see detail in Table 4). The results are reported below in Section 2.6 and 2.8. ELISA tests were conducted on a subsample of *Anopheles* collected through HLCs in Lodja, Inongo, and Kimpese (targeted at a sample of 200 per month, or 2,400 total). PCR analysis was performed on a subsample of

Anopheles collected through HLCs and susceptibility tests in 13 sites. The number of *An. gambiae* s.l. analyzed by each method in each sentinel site is shown in Table 4.

Table 4: Number of *An. gambiae* s.l. Samples Analyzed at INRB, Kinshasa, Compared with Number Planned.

Sentinel site	Total *An. gambiae* s.l. collected in 2020 by HLC	HLC sporozoite ELISA monthly monitoring	HLC species ID monthly monitoring	WHO pyrethroid susceptibility test, species ID	WHO pyrethroid susceptibility test, Vgsc 1014F frequency
Kimpese	1,124	1,124/2,400	600/600	100/100	50/50
Lodja	5,003	2,400/2,400	600/600	100/100	50/50
Inongo*	113	104/2,400	108/600	100/100	50/50
Kapolowe	-	-	-	100/100	50/50
Mikalayi	-	-	-	100/100	50/50
Katana	-	-	-	100/100	50/50
Kingasani	-	-	-	100/100	51/50
Kalemie	-	-	-	100/100	44/50
Mbandaka	-	-	-	100/100	50/50
Mweneditu	-	-	-	100/100	50/50
Lisala	-	-	-	100/100	49/50
Dibindi	-	-	-	100/100	50/50
Kabondo	-	-	-	100/100	47/50
Total	6,240	3,628/7,200	1,308/1,800	1,300/1,300	641/650

*Only 113 *An. gambiae* s.l. were collected in Inongo (January–December 2020)

1.6 Data Management and Analysis

The DHIS2-based VectorLink Collect programs for entomological data management were used in DRC for the first time in 2020. The Home Office staff remotely trained and supported INRB and Abt entomologist technicians and database managers on updated data work flows - including field paper collections, technical reviews, data entry, data cleaning, and analytics - to support the generation and use of high quality entomological data. All entomological data collected in DRC in 2020 was managed within VectorLink Collect. The platform includes comprehensive dashboards to synthesize vector bionomics and insecticide resistance summary results. In 2021, stakeholders including NMCP, INRB and PMI, will have ongoing access to these results dashboards to support timely decision-making.

The following formulas were used to calculate entomological indicators:

- The sporozoite rate = (total ELISA positive/total number tested) X 100

- Human biting rate (HBR) = total # of each *Anopheles* species collected by HLCs during a specific period/total number of trap-nights
- Nightly EIR = Nightly HBR X sporozoite rate
- Monthly EIR = Nightly mean EIR X number of nights in the month
- Yearly EIR = Nightly mean EIR X 365

2. RESULTS

2.1 MALARIA VECTOR SPECIES COMPOSITION

Over the study period (January to December 2020), a total of 15,723 *Anopheles* were collected from the three longitudinal monitoring sites through monthly HLC and PSC. Six *Anopheles* species (*An. gambiae* s.l., *An. funestus* s.l., *An. paludis, An. coustani, An, caliginosus and An. tenebrosus*) were collected, with *An. gambiae* s.l. being the most common (n=7,020), followed by *An. funestus* s.l. (n=4,909) and *An. paludis* (n=3,794). In all sites, the abundance of *Anopheles* species was greater from HLC (88%: 13,836/15,723) than from PSC (12%: 1,887/15,723). *Anopheles paludis* was abundant in Inongo, particularly from outdoor HLC, but was not collected in Kimpese. The species composition is presented by site and collection method in Figures 3, 4, and 5. Details about the indoor resting densities and biting rates are presented in Sections 2.2 and 2.3. Molecular species composition is presented in Section 2.8.

Figure 3: Species Composition of *Anopheles* Captured by PSC and HLC (Indoors and Outdoors) in Kimpese from Monthly Collections.

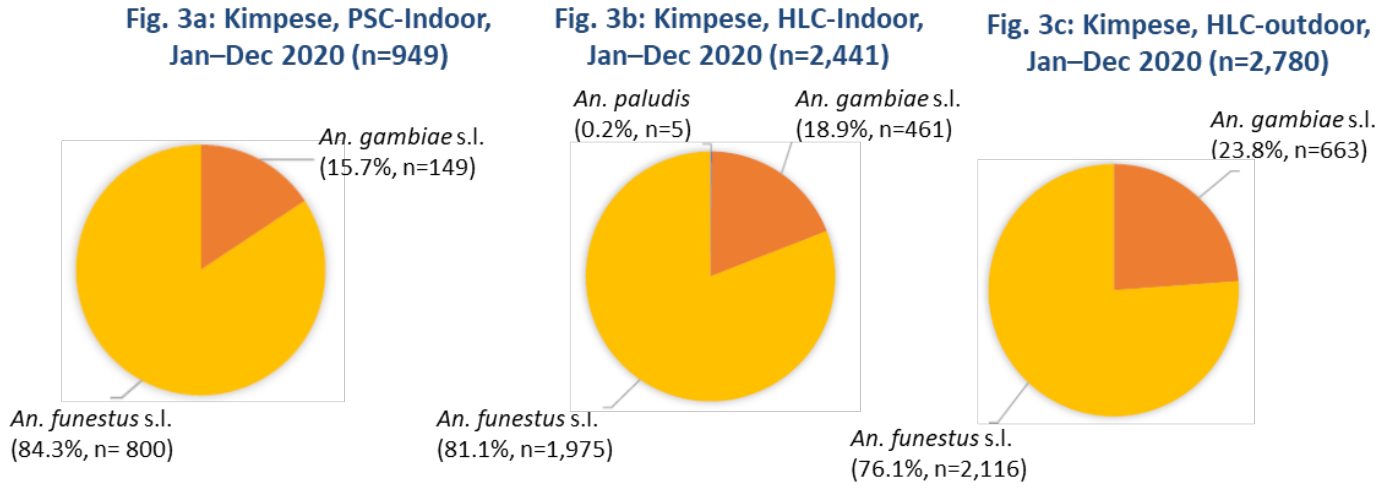


Figure 4: Species Composition of *Anopheles* Captured by PSC and HLC (Indoors and Outdoors) in Lodja from Monthly Collections.

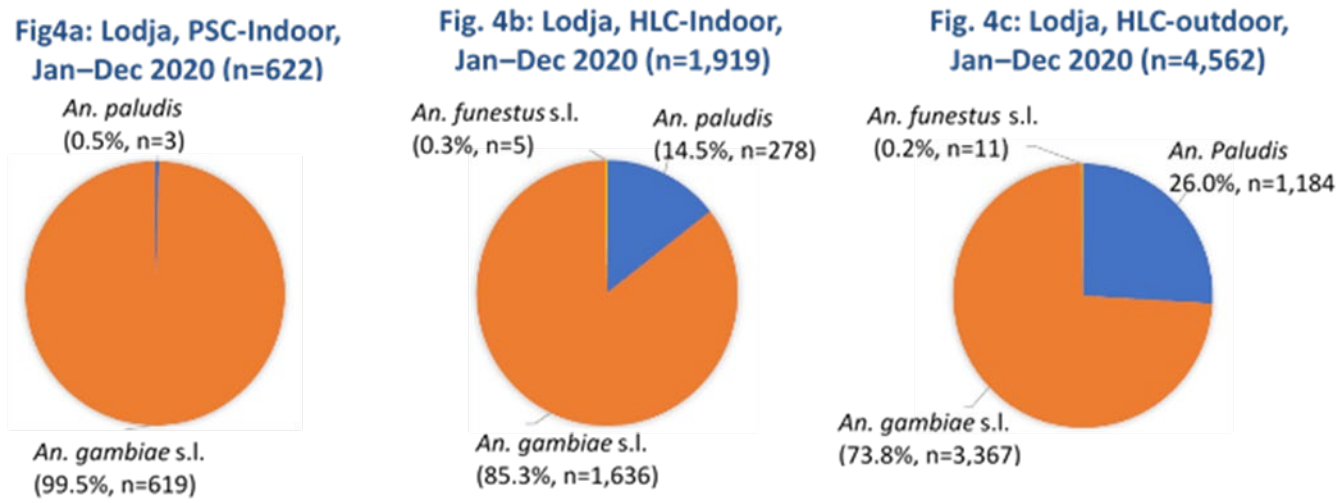
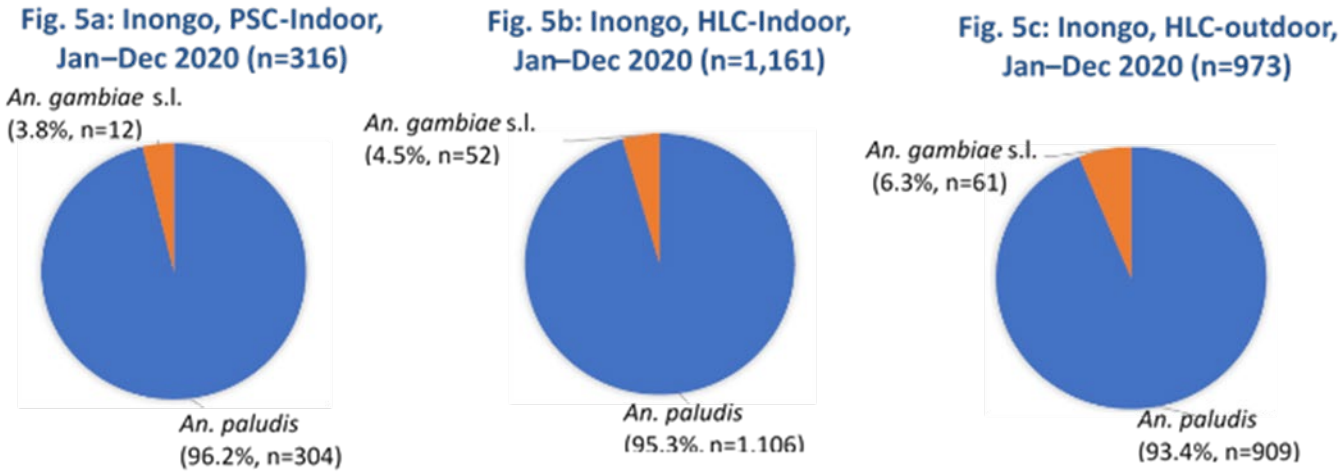


Figure 5: Species composition of *Anopheles* captured by PSC and HLC (Indoors and Outdoors) in Inongo from Monthly Collections.



2.2 Malaria Indoor Vector Resting Density (by PSC)

Figures 6, 7, and 8 show the mean indoor resting density of *An. gambiae* s.l., *An. funestus* s.l. and *An.paludis* per house per day collected by PSC. In Lodja and Inongo, the indoor resting density was fairly stable throughout the year, with the biggest *An. gambiae* s.l. peak in May for Lodja and March for Kimpese. The mean indoor resting density was less than six *An. gambiae* s.l. in Lodja, seven *An. funestus* s.l. in Kimpese, and three *An. paludis* in Inongo per house per day for the entire reporting period. The majority of *Anopheles* collected were blood-fed in Lodja, Kimpese, and Inongo.

Figure 6: Mean Indoor Resting Density per House per Day of *An. gambiae* s.l. Captured by PSC in Lodja (n=619), Kimpese (n=149), and Inongo (n=12) from Monthly Collections.

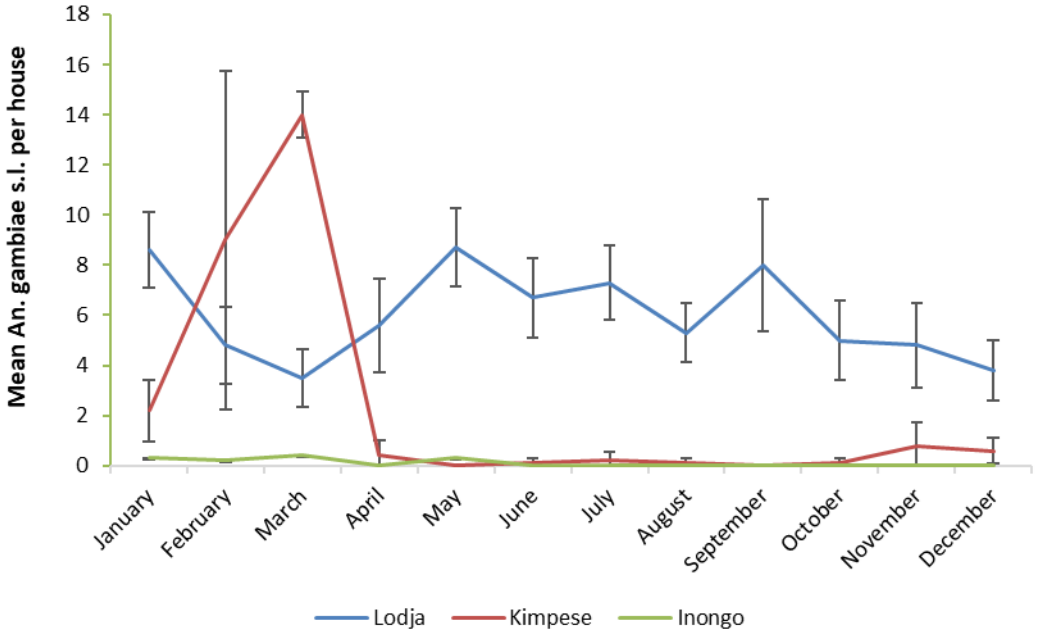


Figure 7: Mean Indoor Resting Density per House per Day of *An. funestus* s.l. Captured by PSC in Kimpese (n=800) from Monthly Collections.

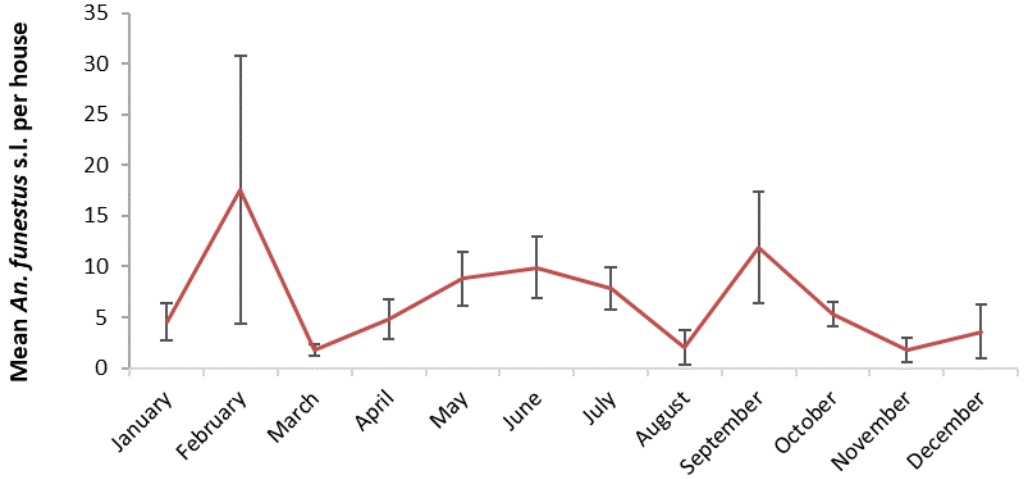
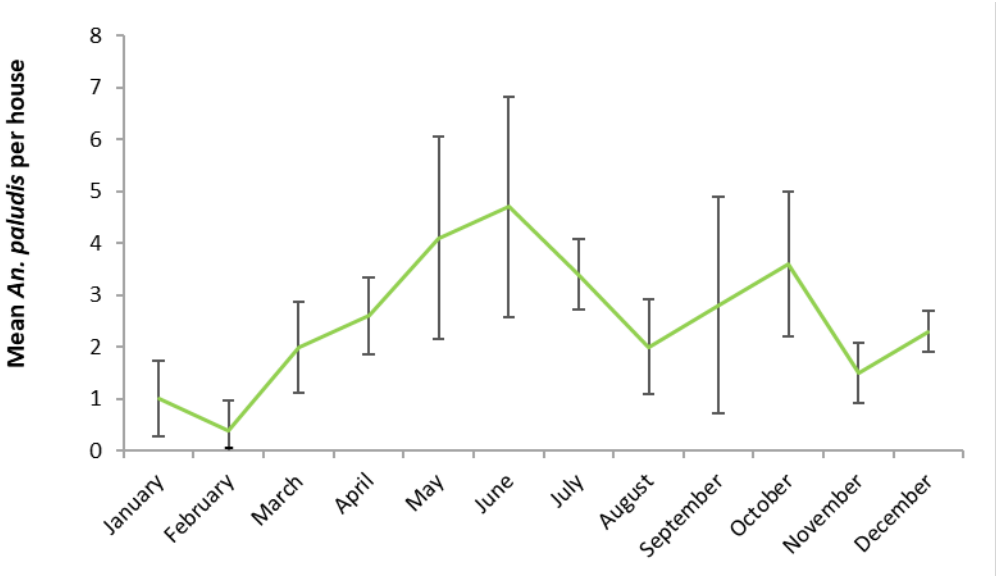


Figure 8: Mean Indoor Resting Density per House per Day of *An. paludis* Captured by PSC Inongo (n=304) from Monthly Collections.



2.3 MALARIA VECTOR HUMAN BITING RATES (BY HLC)

Figures 9 to 14 show the mean monthly biting rates per person per night in Lodja, Kimpese, and Inongo by vector species (further details are in the Annex, Tables 10, 11, and 12). *Anopheles gambiae* s.l. biting rates were particularly high in Lodja, with a mean over the 12-month period of 17 bites per person per night indoors and 35 outdoors (Figure 9). *An. gambiae* s.l. mean biting rates in Inongo were very low year-round, and seasonal in Kimpese with most biting occurring between November and March (Figures 9 and 10). *An. funestus* s.l. was most commonly collected in Kimpese (>20 mean bites per person per night) with peak biting between May and July during the dry season (Figures 11 and 12). The mean *An. gambiae* s.l. biting rates were consistent year-round in Lodja, with no clear seasonality. In Inongo, the biting rates were generally much lower than in Lodja and Kimpese, with a mean *An. gambiae* s.l. biting rate over the 12-month period of 0.5 bites per person per night indoors and 0.6 bites outdoors (Figures 9 and 10). The most common species collected in Inongo was *An. paludis*, which is unlikely to be an important malaria vector species (Figures 13 and 14).

Figure 9: Mean Monthly Indoor *An. gambiae* s.l. Biting Rate in Lodja (n=1,636), Kimpese (n=461), and Inongo (n=52).

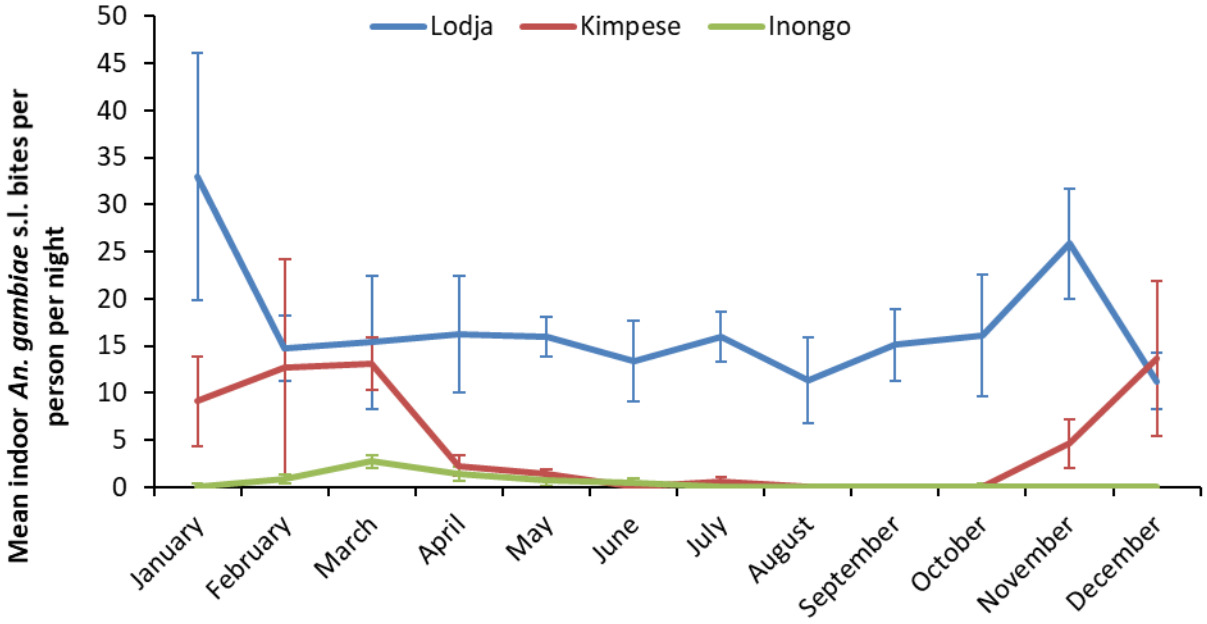


Figure 10: Mean Monthly Outdoor *An. gambiae* s.l. Biting Rate in Lodja (n=3,367), Kimpese (n=663), and Inongo (n=61).

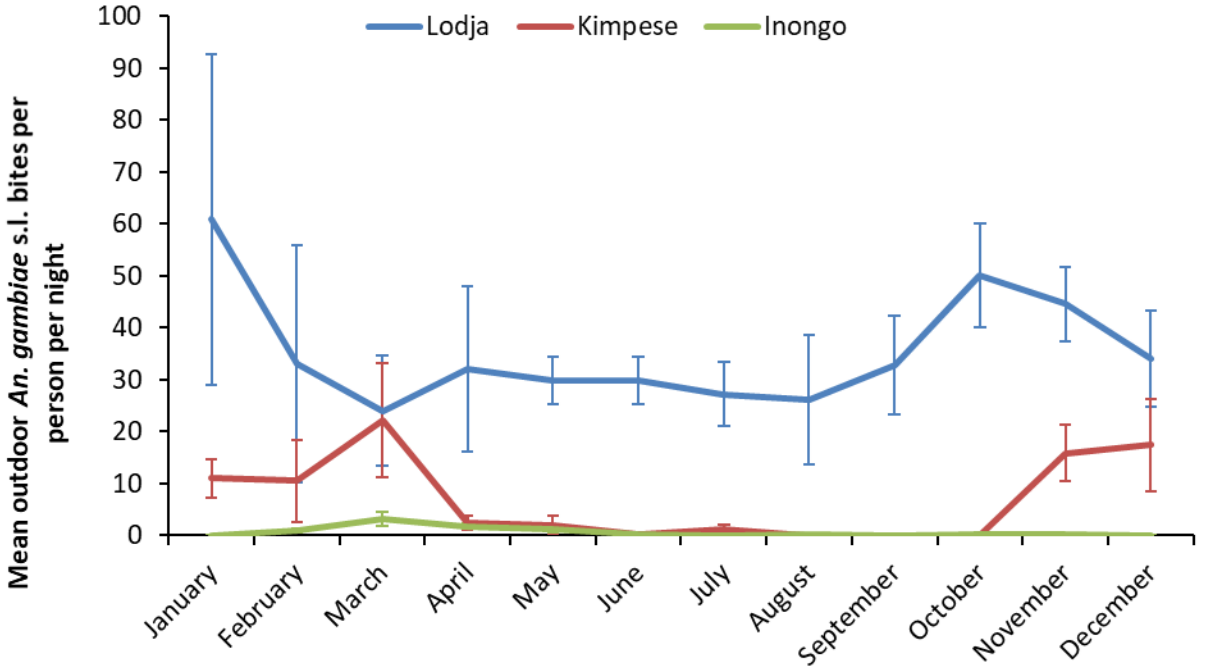


Figure 11: Mean Monthly Indoor *An. funestus* s.l. Biting Rate in Lodja (n=5), Kimpese (n=1,975), and Inongo (n=1).

Figure 12: Mean Monthly Outdoor *An. funestus* s.l. Biting Rate in Lodja (n=11), Kimpese (n=2,116), and Inongo (n=1).

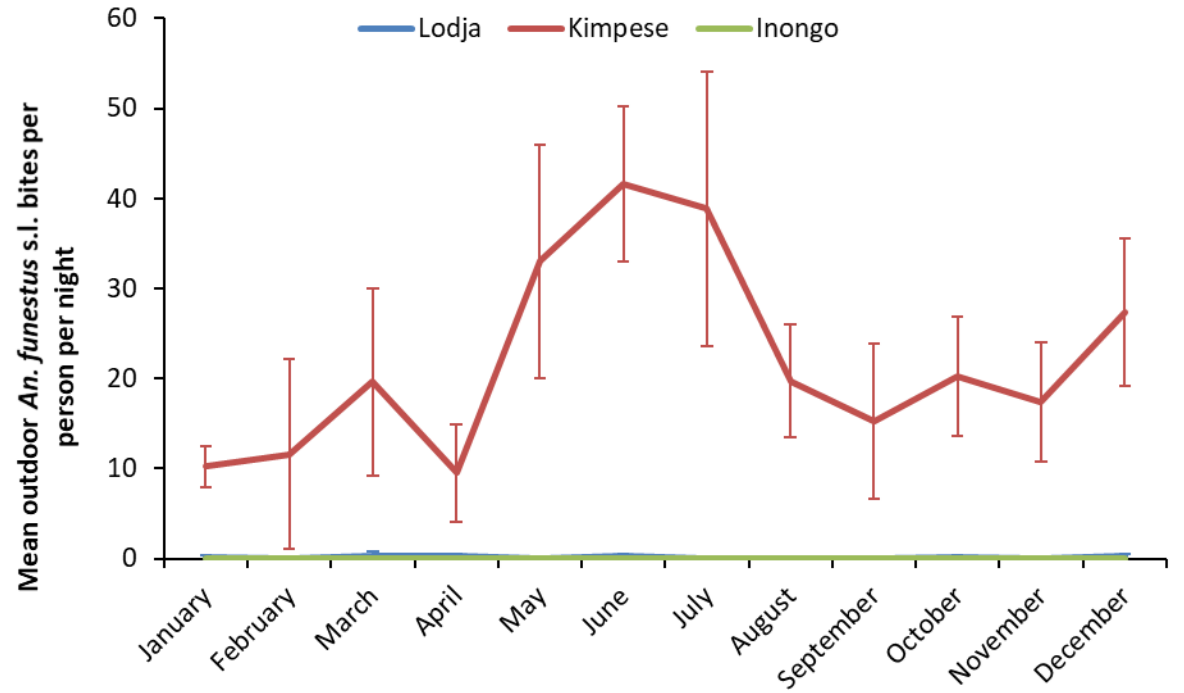


Figure 13: Mean Monthly Indoor *An. paludis* Biting Rate in Lodja (n=278), Kimpese (n=0), and Inongo (n=1,106).

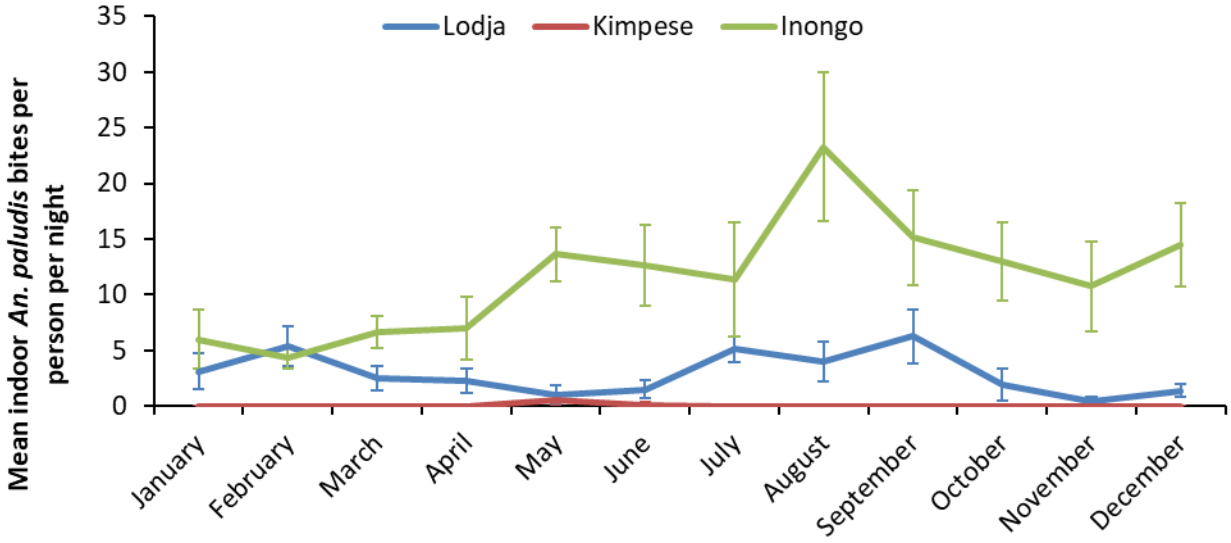
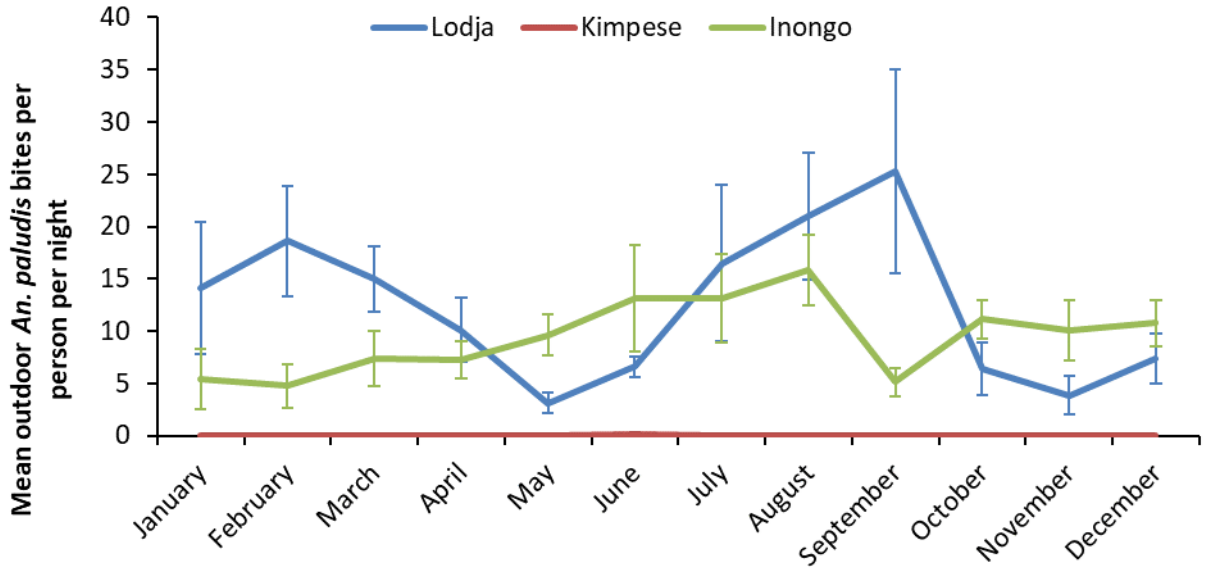


Figure 14: Mean Monthly Outdoor *An. paludis* Biting Rate in Lodja (n=1,184), Kimpese (n=1) and Inongo (n=909).



2.4 Biting Times of Malaria Vectors Collected Indoors and Outdoors by HLC

In general, the peak period of *An. gambiae* s.l. and *An. funestus* s.l. indoor biting was late at night, between 10 p.m. and 5 a.m., which mirrored outdoor biting trends in all sites (Figures 15 to 20). Biting rates in Lodja were substantially greater outdoors than indoors, particularly between 9 p.m. and 2 a.m. The peak period of *An.*

paludis indoor biting was earlier, between 6 p.m. and 11 p.m., which mirrored outdoor biting trends in Inongo and Lodja (Figures 19 and 20).

Figure 15: Mean Hourly Indoor *An. gambiae* s.l. Biting Rates in Lodja (n=1,636), Kimpese (n=461), and Inongo (n=52).

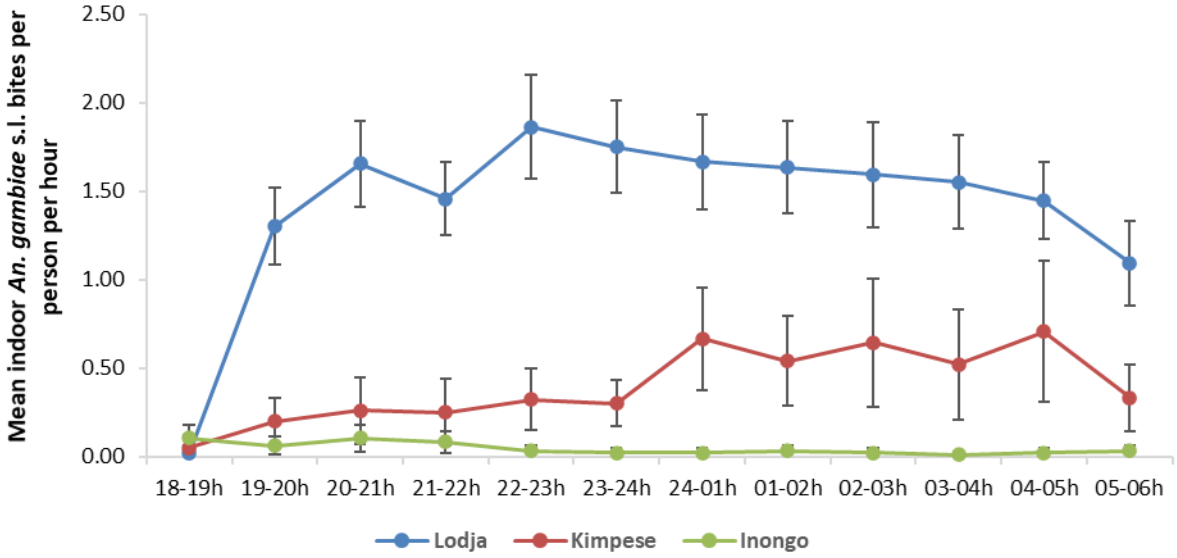


Figure 16: Mean Hourly Outdoor *An. gambiae* s.l. Biting Rates in Lodja (n=3,367), Kimpese (n=663), and Inongo (n=61).

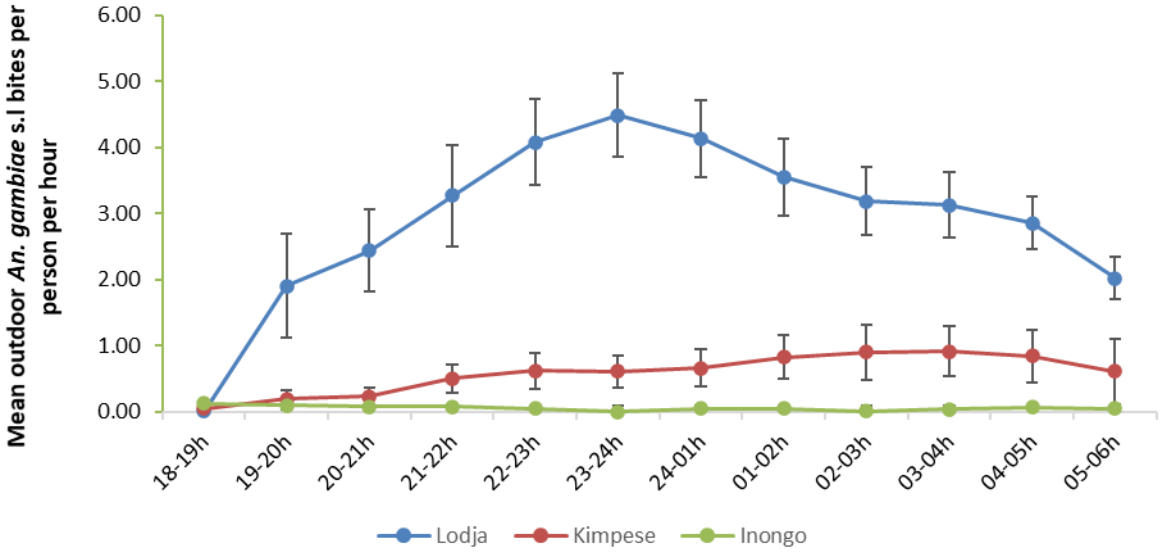


Figure 17: Mean Hourly Indoor *An. funestus* s.l. Biting Rates in Lodja (n=5), Kimpese (n=1,975), and Inongo (n=1).

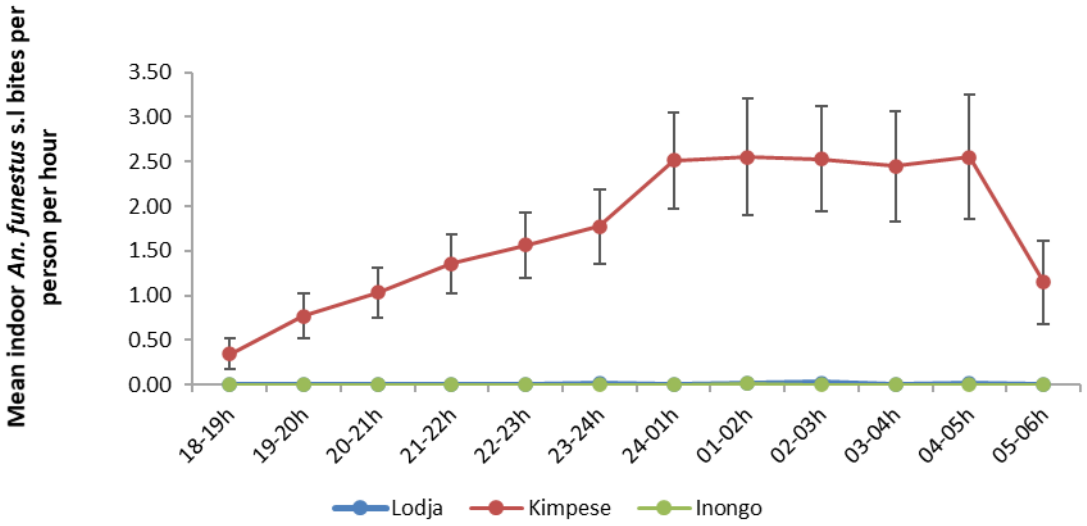


Figure 18: Mean Hourly Outdoor *An. funestus* s.l. Biting Rates in Lodja (n=11), Kimpese (n=2,116), and Inongo (n=1).

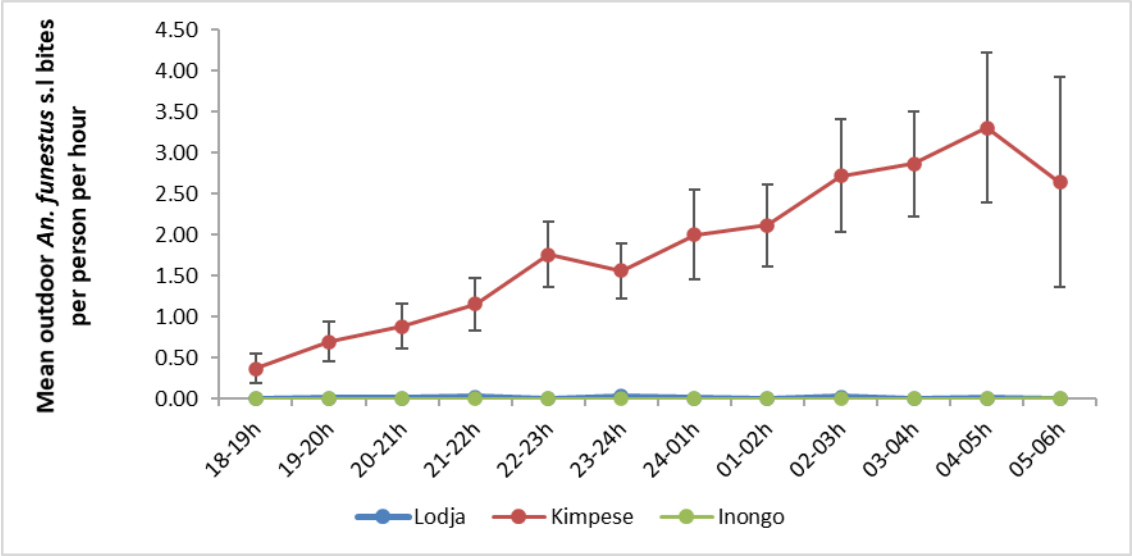


Figure 19: Mean Hourly Indoor *An. paludis* Biting Rates in Lodja (n=278), Kimpese (n=5), and Inongo (n=1,106).

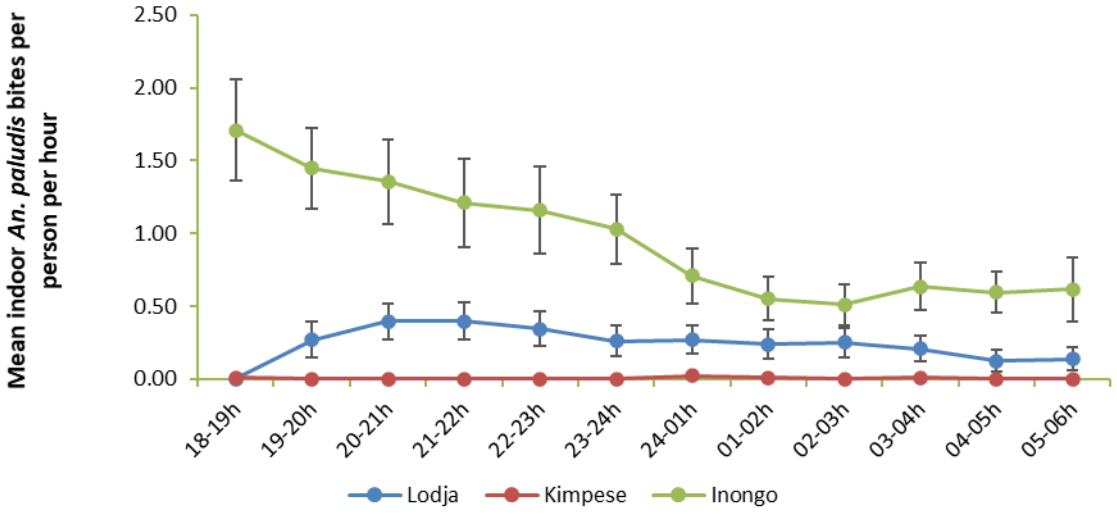
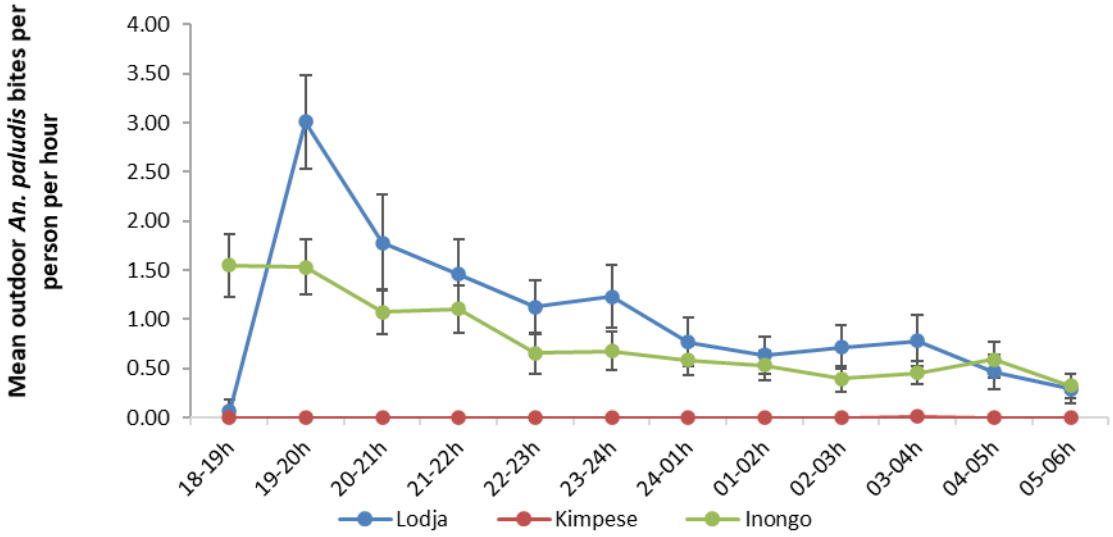


Figure 20: Mean Hourly Outdoor *An. paludis* Biting Rates in Lodja (n=1,184), Kimpese (n=1). and Inongo (n=909).



2.5 Insecticide Susceptibility, PBO Synergist, and Pyrethroid Resistance Intensity

WHO insecticide susceptibility and resistance intensity tests were completed with *An. gambiae* s.l. populations that were collected as larvae in all 13 sites. While following the PMI COVID-19 mitigation plan, provincial technicians in the sites with no INRB supervision (Kabondo, Kalemie, Lodja, Katana, Mikalayi and Kapolowe) were able to conduct insecticide susceptibility testing (including PBO synergist tests) with success. To continue this decentralization process, refresher training should be organized every two or three years in Kinshasa to bring all field teams together for standardized testing. Training would also be needed if additional bioassay

methods were to be implemented, such as chlorfenapyr susceptibility testing using CDC bottle bioassays, which was not conducted in sites with no INRB supervision. Figure 21 shows the percentage mortality in permethrin intensity tests, with resistance to permethrin (<90% mortality, ×1 dose) observed in all sites. Resistance intensity to permethrin was low (>98% mortality at ×5 dose) in two sites (Mweneditu, and Dibindi); moderate (<98% mortality at ×5 dose) in three sites (Kabondo, Mbandaka and Lisala); and high (<98% mortality at ×10 dose) in eight sites (Katana, Kingasani, Inongo, Kimpese, Mikalayi, Kapolowe, Kalemie, and Lodja). As shown in Figure 22, resistance to deltamethrin was recorded in all sites (<90% mortality, ×1 dose). The intensity of deltamethrin resistance was low in two sites (Mweneditu and Mikalayi); moderate in six sites (Kabondo, Dibindi, Inongo, Lodja, Mbandaka, and Lisala); and high in five sites (Katana, Kingasani, Kimpese, Kapolowe, and Kalemie). Resistance to alpha-cypermethrin was also observed in all sites (Figure 23). The intensity of alpha-cypermethrin resistance was high in all sites. Despite uncertainty regarding the impact of pyrethroid resistance, WHO states that, "When resistance is confirmed at the 5× and especially at the 10× concentrations, operational failure is likely."

Bioassays with permethrin (×1 dose) following pre-exposure to PBO 4% in WHO tube tests showed an increase in mortality compared with permethrin alone in 12 out of the 13 sites (Figure 24). There was no significant increase in mortality in Dibindi. Despite an increase in mortality after pre-exposure to PBO, mortality was still <90% in six sites (Kingasani, Dibindi, Inongo, Kapolowe, Kalemie, and Lisala). There was a significant increase in mortality with deltamethrin and alpha-cypermethrin (×1 dose) following pre-exposure to PBO 4% in WHO tube tests in all 13 sites (Figures 25 and 26), except Kapolowe for PBO + alpha-cypermethrin. There were particularly large increases in deltamethrin mortality in Katana, Kingasani, Mweneditu, Dibindi, and Mbandaka (Figure 19). Despite an increase in mortality after pre-exposure to PBO, alpha-cypermethrin mortality was still <90% in five sites (Inongo, Mikalayi, Kapolowe, Lodja and Lisala). See Figure 26.

CDC bottle bioassays using the PMI VectorLink recommended dose of 100µg/bottle as the diagnostic dose for chlorfenapyr (until WHO releases further guidance) produced 100% mortality in all sites tested (Mweneditu, Dibindi, Inongo, Kimpese, Mbandaka, and Lisala) within 48 hours of exposure.

Figure 21: Percentage Mortality of *An. gambiae* s.l. after Exposure to Permethrin at ×1, ×5, and ×10 the Diagnostic Concentration in WHO Tube Tests in 13 Sites.

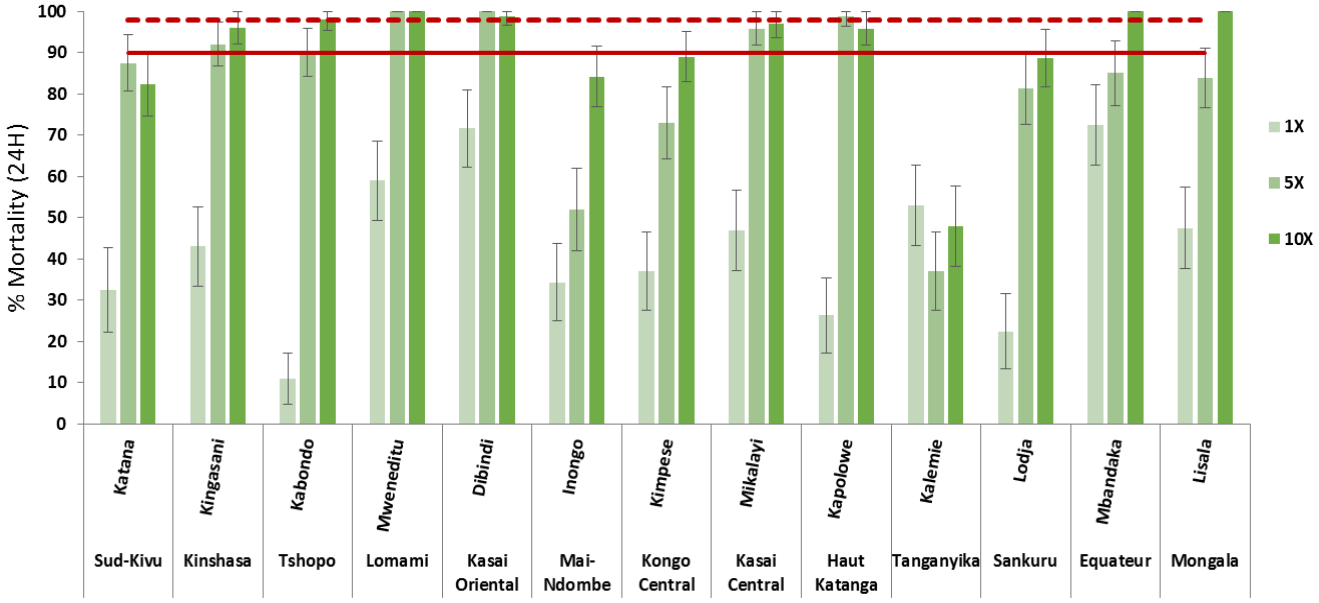


Figure 22: Percentage Mortality of *An. gambiae* s.l. after Exposure to Deltamethrin at ×1, ×5, and ×10 the Diagnostic Concentration in WHO Tube Tests in 13 Sites.

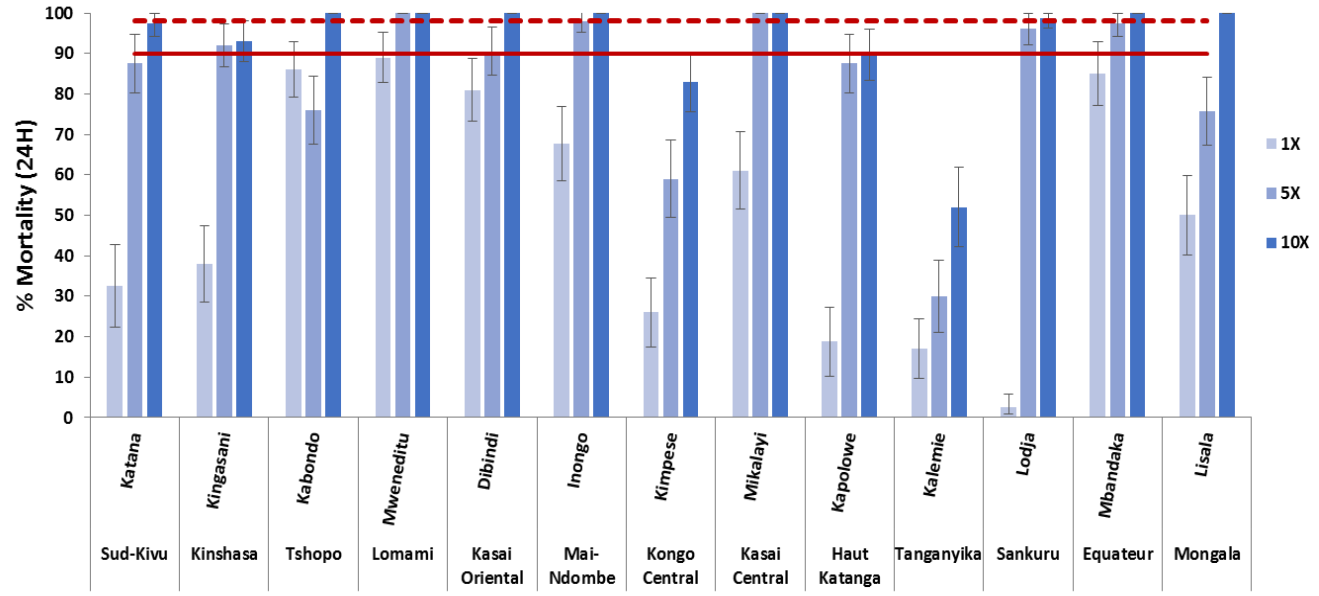


Figure 23: Percentage Mortality of *An. gambiae* s.l. after Exposure to Alpha-Cypermethrin at ×1, ×5, and ×10 the Diagnostic Concentration in WHO Tube Tests in 13 Sites.

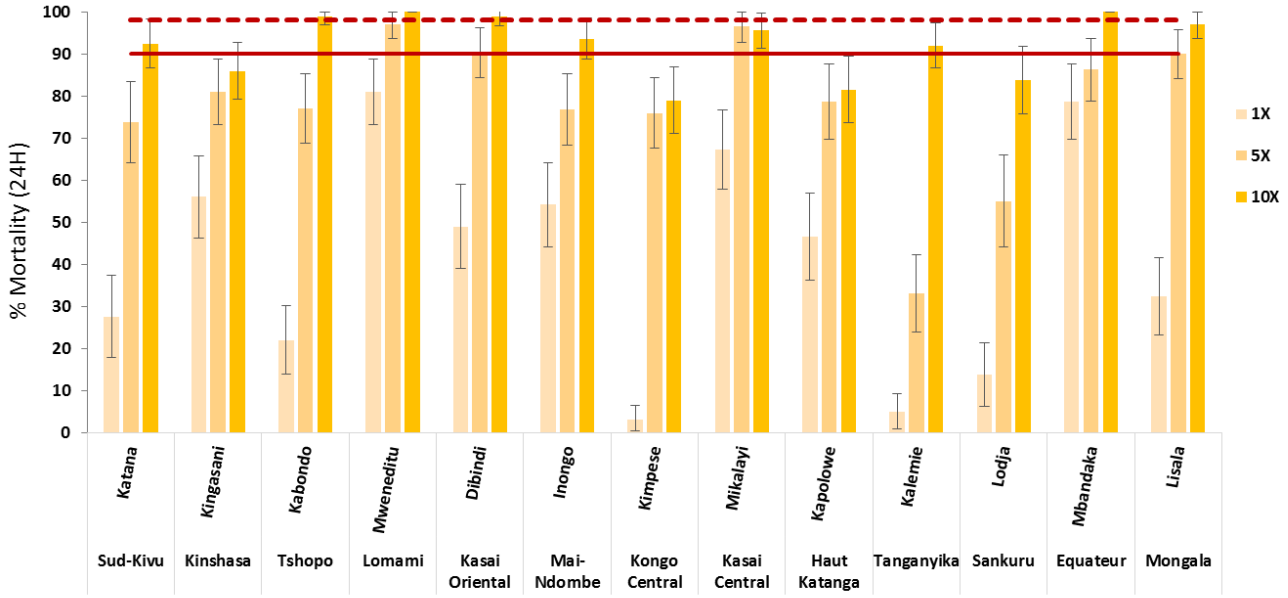
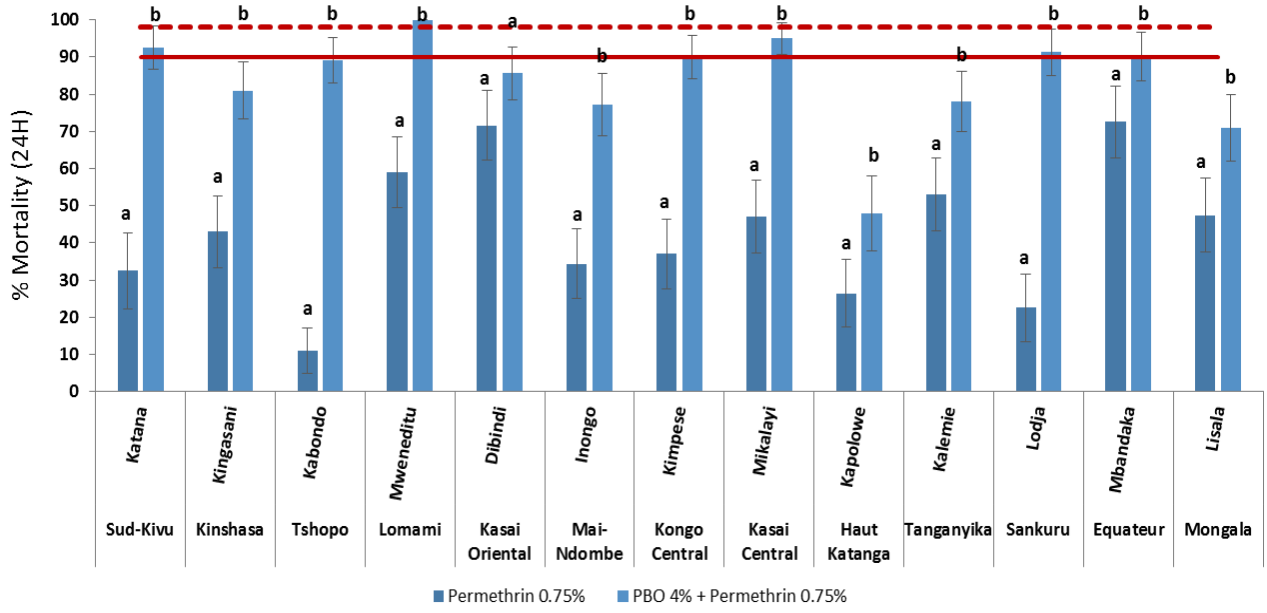
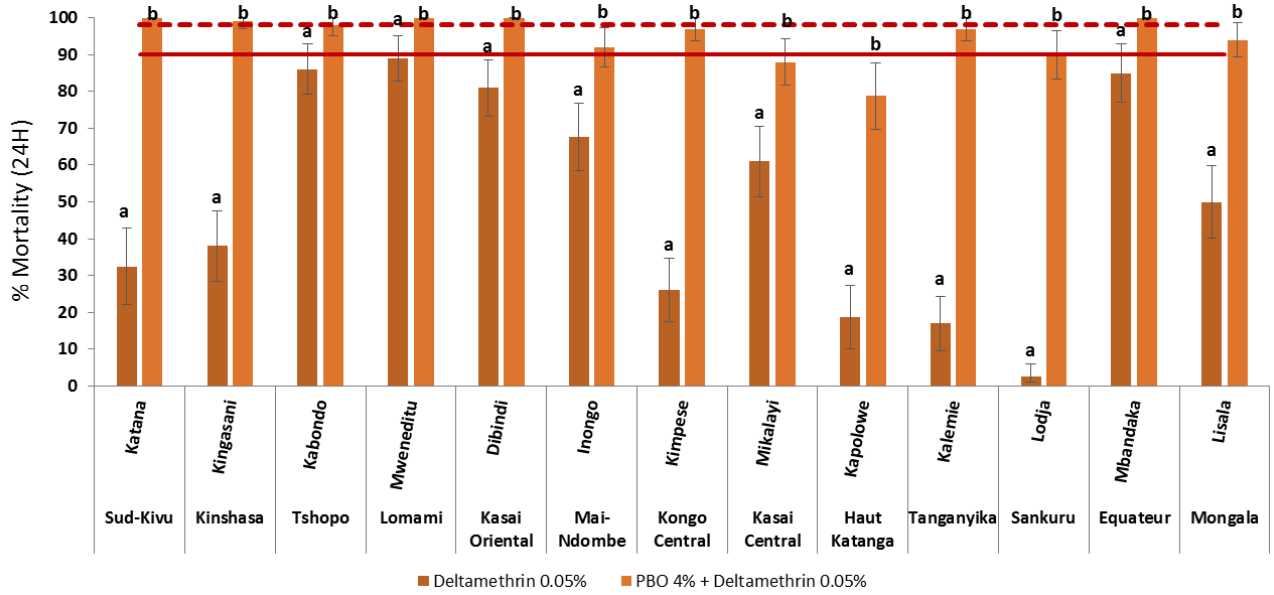


Figure 24: Percentage Mortality of *An. gambiae* s.l. after Pre-Exposure to PBO Followed by Permethrin at the Diagnostic Concentration in WHO Tube Tests in 13 Sites.



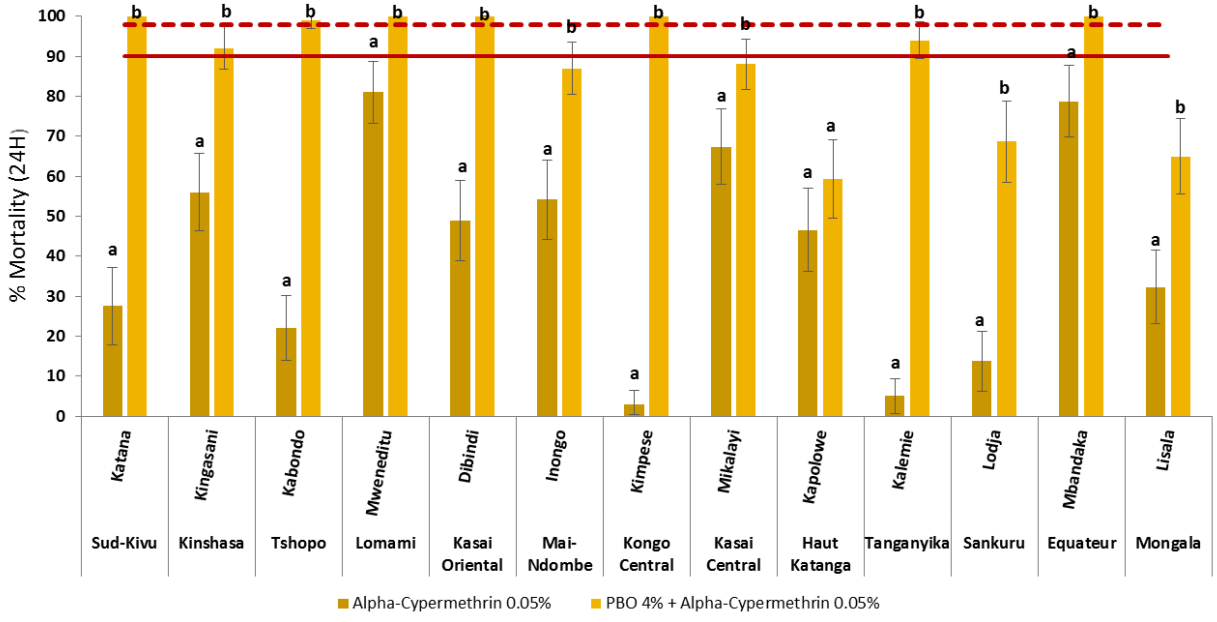
Note: Superscript indicates whether % mortality for permethrin is significantly different to % mortality for permethrin + PBO. a, b = significant difference P<0.05; a, a = no significant difference P>0.05.

Figure 25: Percentage Mortality of *An. gambiae* s.l. after Pre-Exposure to PBO Followed by Deltamethrin at the Diagnostic Concentration in WHO Tube Tests in 13 Sites.



Note: Superscript indicates whether % mortality for deltamethrin is significantly different to % mortality for deltamethrin + PBO. a, b = significant difference P<0.05; a, a = no significant difference P>0.05.

Figure 26: Percentage Mortality of *An. gambiae* s.l. after Pre-Exposure to PBO Followed by Alpha-cypermethrin at the Diagnostic Concentration in WHO Tube Tests in 13 Sites.



Note: Superscript indicates whether % mortality for alpha-cypermethrin is significantly different to % mortality for alpha-cypermethrin + PBO. a, b = significant difference P<0.05; a, a = no significant difference P>0.05.

2.6 *PLASMODIUM FALCIPARUM* SPOROZOITE RATE

The number of *An. gambiae* s.l. and *An. funestus* s.l. analyzed for presence of sporozoites from each site is shown in Table 5. All work plan targets for processing mosquitoes were met, with the exception of Kimpese and Inongo due to an insufficient number of *An. gambiae* s.l. specimens collected through HLC (all 1,124 and 113 *An. gambiae* s.l. collected respectively from Kimpese and Inongo were analyzed in the laboratory).

Table 5: Number of *An. gambiae* s.l. and *An. funestus* s.l. Samples Collected by HLC and Tested for Presence of *P. falciparum* Sporozoites.

Sentinel site	Total *An. gambiae* s.l. collected by HLC	Total *An. gambiae* s.l. tested (tested/planned)	% Mean sporozoite rate (positive/ tested)	Total *An. funestus* s.l. collected by HLC in 2020	Total *An. funestus* s.l. tested	% Sporozoite rate (positive/ tested)
Kimpese	1,124	1,124/2,400 (200 per month)	2.9% (33/1,124)	4,091	240 (20 per month)	2.5% (6/240)
Lodja	5,003	2,400/2,400 (200 per month)	2.3% (55/2,400)	16*	0	n/a
Inongo	113	104/2,400 (all collected)	1.9% (2/104)	2*	0	n/a
Total	7,310	3,628	2.5% (90/3,628)	4,109	208	2.5% (6/240)

Note: *Insufficient *An. funestus* s.l. captured in Lodja and Inongo for testing.

The mean *An. gambiae* s.l. infection rate over 12 months in Lodja was 2.3% (95% confidence interval (CI) ; 1.69-2.89), 2.9% in Kimpese (95% CI; 1.95-3.92), and 1.9% in Inongo (95% CI; 0.72-4.56%). The mean *An. funestus* s.l. sporozoite rate in Kimpese was 2.5% (95% CI; 0.52-4.48), while in Lodja and Inongo no *An. funestus* were tested due to low numbers collected (Table 5). The monthly *An. gambiae* s.l. sporozoite rate for Lodja and Kimpese from HLCs is presented in Figures 27 and 28. There is no figure presented for Inongo due to the low number of *An. gambiae* s.l. collected. Although there appeared to be peaks of infection (e.g., Lodja in March and December) the confidence intervals were quite large as only 200 mosquitoes were tested per month and it was not possible to clearly determine any seasonality in infection rate. As *An. funestus* s.l. was the main species collected in Kimpese, it would be beneficial to increase the number of samples tested for sporozoites to determine the monthly infection rate and more accurately determine the overall EIR for 2020. These additional tests will be conducted using 2020 samples during Year 4 with a view to future publication of the longitudinal data.

Figure 27: Monthly *P. falciparum* sporozoite Rate of *An. gambiae* s.l. Collected by HLC in Lodja.

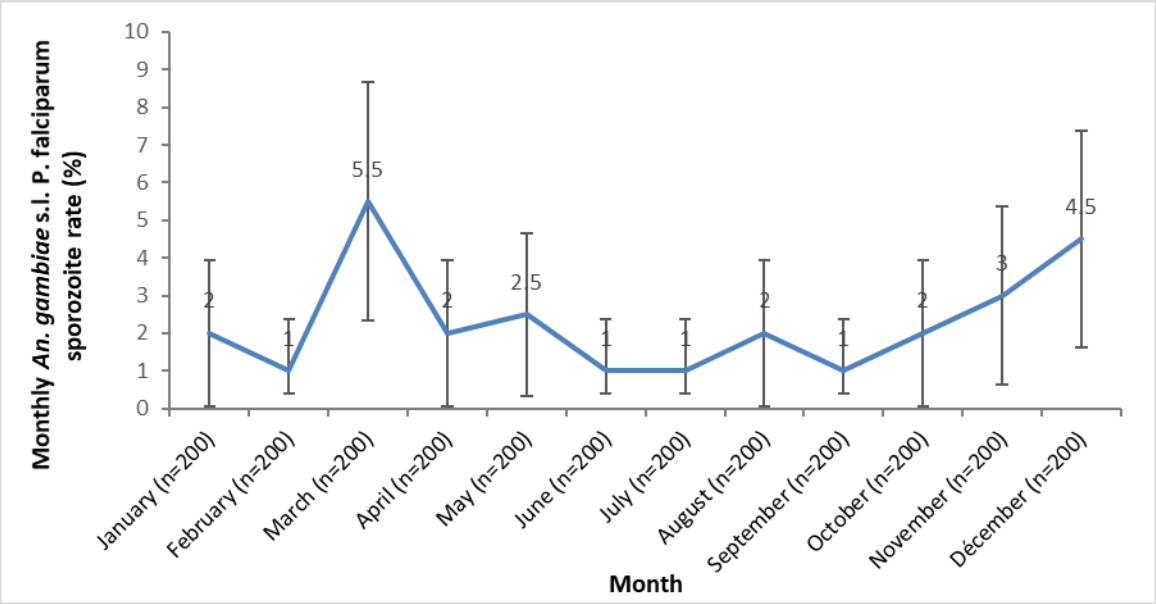
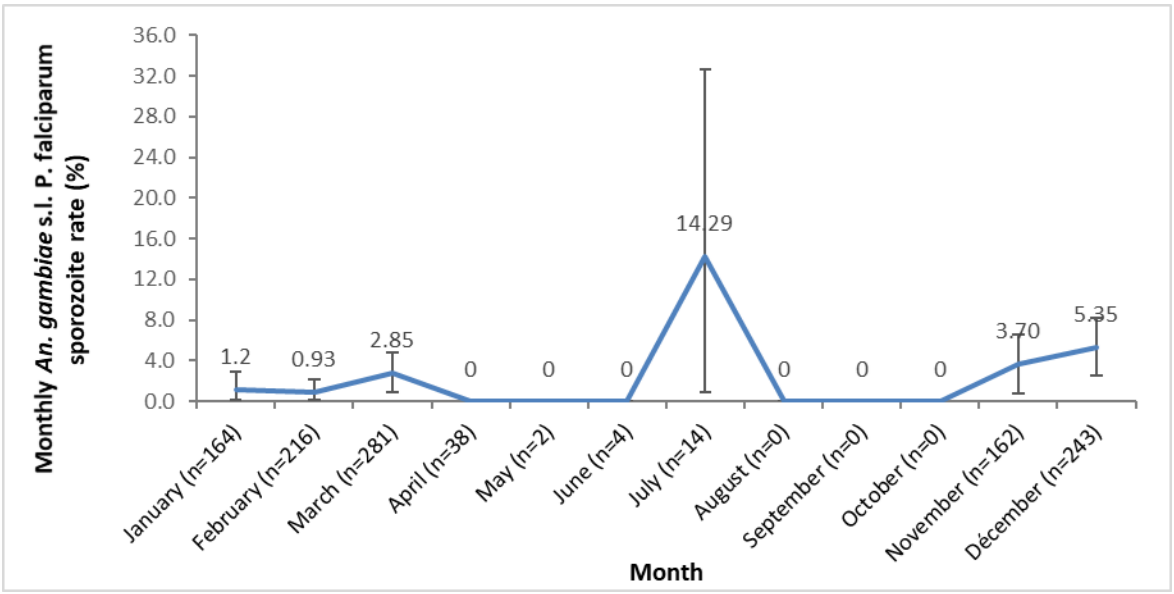


Figure 28: Monthly *P. falciparum* sporozoite Rate of *An. gambiae* s.l. Collected by HLC in Kimpese.



2.7 Entomological Inoculation Rate

Tables 6 and 7 summarize the combined indoor and outdoor monthly EIRs for *An. gambiae* s.l. in Lodja and Kimpese for 2020. Addition of the monthly EIR gave an annual EIR of 218 infectious bites per person per year for Lodja and 63 infectious bites per person per year for Kimpese (by *An. gambiae* s.l. only). Sporozoite positive *An. gambiae* s.l. were detected in Inongo only in April and May 2020, giving an annual EIR of 3.8 infectious bites per person.

As the monthly sporozoite rate (SR) has a wide confidence interval, an alternative way to calculate the annual EIR is to multiply the mean annual sporozoite rate by the mean biting rate. This approach would give an annual EIR of 218 infectious bites per person per year for Lodja ((5,003 bites/192 trap nights × 365 nights) × 2.29% SR) and 63 infectious bites per person (*An. gambiae* s.l. only) for Kimpese ((1,124 bites/192 trap nights × 365 nights) × 2.94% SR). For Inongo, the annual EIR would be 3.8 infectious bites per person per year (((104 bites/192 trap nights) × 365 nights) × 1.9% SR). Sporozoite positive *An. funestus* s.l. were detected in Kimpese only in May, July, September, and October 2020, giving an estimated annual EIR of 194 infectious bites per person (*An. funestus* s.l. only) ((4,091 bites/192 trap nights × 365 nights) × 2.5% SR). This gives a combined annual EIR in Kimpese of 257 infectious bites per person per year (*An. funestus* s.l. and *An. gambiae* s.l.). Overall, these results demonstrate the extremely high malaria transmission risk faced year-round in Lodja and Kimpese, despite the use of pyrethroid ITNs. However, the malaria transmission risk was relatively low in Inongo.

Table 6: Monthly *An. gambiae* s.l. EIR in Lodja.

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	2020 Total
Total *An. gambiae* s.l. (HLC) collected	751	383	315	386	367	315	346	300	384	530	564	362	5,003
HLC trap-nights (indoors + outdoors)	16	16	16	16	16	16	16	16	16	16	16	16	192
HBR per night	47	24	20	24	23	20	22	19	24	33	35	23	26 (mean)
Total *An. gambiae* s.l. tested by ELISA	200	200	200	200	200	200	200	200	200	200	200	200	2,400
Sporozoite rate (%)	2	1	5.5	2	2.5	1	1	2	1	2	3	4.5	2.29 (mean)
EIR per night	0.94	0.24	1.1	0.48	0.58	0.2	0.22	0.38	0.24	0.66	1.05	1.04	0.60 (mean)
EIR per month*	29.14	6.96	34.1	14.4	17.83	6	6.82	11.78	7.2	20.46	31.5	32.09	18.19 (mean)
Kabondo 12 month EIR Jan–Dec 2020 = 218 infectious bites per person (by *An. gambiae* s.l.)													

***Nightly EIR is multiplied by number of nights in that month.

Table 7: Monthly *An. gambiae* s.l. EIR in Kimpese.

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	2020 Total
Total *An. gambiae* s.l. (HLC) collected	161	186	283	38	26	3	14	0	0	0	164	249	1,124
HLC trap-nights (indoors + outdoors)	16	16	16	16	16	16	16	16	16	16	16	16	192
HBR per night	10	12	18	2	2	0	1	0	0	0	10	16	6 (mean)
Total *An. gambiae* s.l. tested by ELISA	164	216	281	38	2	4	14	0	0	0	162	243	1,124
Sporozoite rate (%)	1.22	0.93	2.85	0	0	0	14.29	0	0	0	3.70	5.35	2.94 (mean)
EIR per night	0.12	0.11	0.50	0	0	0	0.13	0	0	0	0.38	0.83	0.18 (mean)
EIR per month*	3.80	3.12	15.61	0	0	0	3.88	0	0	0	11.39	25.81	5.30 (mean)
Lodja 12 month EIR Jan–Dec 2019 = 63 infectious bites per person (by *An. gambiae* s.l.)													

***Nightly EIR is multiplied by number of nights in that month.

2.8 MOLECULAR SPECIES IDENTIFICATION OF THE *AN. GAMBIAE* SPECIES COMPLEX

Out of 2,608 *An. gambiae* s.l. analyzed, 84% were identified as *An. gambiae* s.s. (n=2,182), 7% as *An. coluzzii* (n=185), and 0.12% as *An. arabiensis* (n=3). Nine percent (n=238) did not amplify. No hybrid (*An. gambiae* s.s./*An. coluzzii*) was detected. See Figure 29.

Figure 29: Molecular Species Composition of *An. gambiae* across 13 Sites.

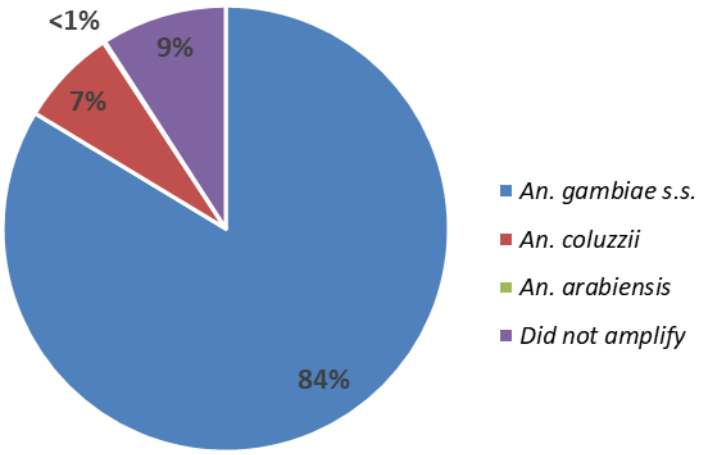
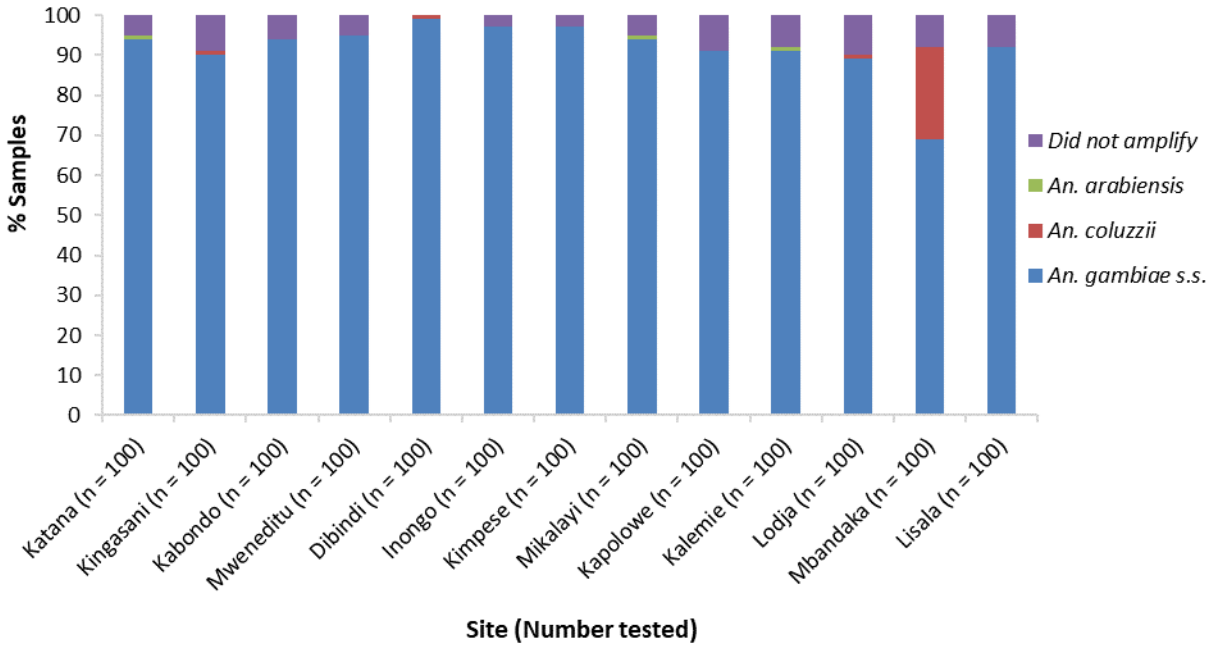


Table 8 shows the proportion of each species per site (collected by HLC) and Figure 30, WHO susceptibility tests. In Kimpese *An. gambiae* was the predominant species collected (99% of amplified samples). In Lodja and Inongo, there were sympatric populations of *An. gambiae* and *An. coluzzii*, with *An. gambiae* the most common species collected in both sites. Samples from monthly HLC collections in Lodja, Kimpese, and Inongo showed no clear seasonal variation in species composition. *An. arabiensis* was detected at very low frequency in Katana, Mikalayi, and Kalemie from *An. gambiae* s.l. collected as larvae for WHO susceptibility tests (1% per site). See Figure 30.

Table 8: Molecular Species Composition of *An. gambiae* s.l. collected by HLC.

Site	*An. gambiae* s.s.	*An. coluzzii*	Hybrid	*An. arabiensis*	Did not amplify	Total
Lodja	410 **(68%)**	126 **(21%)**	0	0	64 **(11%)**	600
Kimpese	518 **(86%)**	3 **(1%)**	0	0	79 **(13%)**	600
Inongo	62 **(57%)**	30 **(28%)**	0	0	16 **(15%)**	108

Figure 30: Molecular Species Composition of *An. gambiae* s.l. Collected as Larvae for WHO Susceptibility Tests.



An. gambiae was the predominant species used in WHO susceptibility tests (collected as larvae) at all 13 sites. *An. coluzzii* were detected in Kingasani (1%), Dibindi (1%), Lodja (1%) and Mbandaka (23%). See Figure 24. The overall amplification rate was generally good at 94% in all 13 susceptibility test sites.

2.9 Frequency of the Voltage Gated Sodium Channel Mutation L1014F

From a total of 641 *An. gambiae/coluzzii* specimens analyzed, 592 (92%) were homozygous for Vgsc-L1014F, and no wild type susceptible (SS) or heterozygous resistant (RS) samples were detected. The remaining 49 (8%) did not amplify (Table 9). A new DNA extraction method with cetyltrimethylammonium bromide (CTAB) was adopted in April 2018 to obtain a better quality of genetic material for PCR. This has been implemented successfully, keeping non-amplification rates relatively low. Unfortunately, these tests were conducted without the use of positive control samples because the frozen samples from BEI Resources were unusable due to DNA degradation (the samples thawed during the customs clearance process). Therefore, results were interpreted according to band size compared to the ladder (Annex Figure 30). It will be important to confirm these trends in 2021 using suitable positive control specimens. To overcome this issue, dried positive control samples will be requested from BEI Resources in 2021. The high proportion of homozygous resistant (RR) samples is in keeping with previous results of *An. gambiae* s.l. collected from 11 sites in 2018 that were tested by CDC, Atlanta, and found to be 85% (883/1,039) RR compared to only 3% SS or RS (28/1,039) with the remainder not amplified.[1]

[1] Wat'senga et al. 2020. Intensity of pyrethroid resistance in *Anopheles gambiae* before and after a mass distribution of insecticide-treated nets in Kinshasa and in 11 provinces of the Democratic Republic of Congo. *Malaria Journal*, 19; 169.

Table 9: Frequency of the Vgsc L1014F Mutation (Formely Kdr-west) In *An. gambiae* s.s. and *An. coluzzii.*

Site	Number tested	*RR*	*RS*	SS	Did not amplify	Frequency 1014F
Katana	50	50	0	0	0	1.00
Kingasani	51	44	0	0	7	1.00
Kabondo	47	41	0	0	6	1.00
Mweneditu	50	47	0	0	3	1.00
Dibindi	50	49	0	0	1	1.00
Inongo	50	48	0	0	2	1.00
Kimpese	50	49	0	0	1	1.00
Mikalayi	50	46	0	0	4	1.00
Kapolowe	50	41	0	0	9	1.00
Kalemie	44	39	0	0	5	1.00
Lodja	50	49	0	0	1	1.00
Mbandaka	50	45	0	0	5	1.00
Lisala	49	44	0	0	5	1.00
Overall	**641**	**592**	**0**	**0**	**49**	**1.00**

Note: RR=homozygous resistant, RS=heterozygote resistant, SS=homozygous sensitive

3. CAPACITY BUILDING

Conferences and meetings.	All in-person training was cancelled because of COVID-19. Remote training was conducted in August for INRB and VectorLink DRC staff on the use of VectorLink Collect. All 2020 entomological data (HLC, PSC, and susceptibility data) were successfully entered and cleaned in VectorLink Collect. VectorLink DRC financially and technically supported the NMCP to plan and hold a national meeting of the Vector Control Working Group (*Groupe de Travail Lutte Anti-Vectorielle,* GTLAV) at Carita Congo Hotel on October 6, 2020. This is a multi-sectoral group composed of participants from the NMCP; the ministries of Health, Agriculture, Environment and Hygiene, and Plant Protection; the directorates of Disease and Pharmacy; and universities, research institutions, civil society, mining companies, and technical partners. Due to COVID-19, the coordinating team of GTLAV has only been able to organize one of the two bi-annual meetings scheduled for 2020.
Scientific support – short-term technical assistance.	All home office technical assistance to support VectorLink DRC activities was conducted remotely in 2020.

Technical support from Rodrigue Agossa (PMI VectorLink).	Dr. Rodrigue Agossa, the in-country entomologist, supported project activities by updating and translating standard operating procedures for susceptibility and intensity tests, WHO cone bioassays, Prokopack aspiration, molecular species identification, and *kdr* mutation detection, into French. Mr. Agossa worked with INRB and the University of Kinshasa to implement activities. He also provided technical assistance to the NMCP to support vector control working group activities.
Training of field staff in Dibindi, Mbandaka, Lisala, and Mweneditu.	VectorLink through INRB identified and trained 12 supervisors (three per site) in the new sites. The training consisted of basic bio-ecology of *Anopheles gambiae* s.l., adult mosquito sampling methodology, and WHO susceptibility tests.
Molecular analysis.	The project transformed the existing space at the INRB entomological unit into a new molecular laboratory dedicated to entomology. The development of this dedicated laboratory for entomology enabled the project to complete molecular data on-schedule without the need for an addendum report.

4. DISCUSSION

The climate of Lodja province in central DRC is particularly favorable for the proliferation of malaria vectors, with year-round high temperatures and only a short dry season. *An. gambiae* s.l. biting rates were high in this province throughout the year. The annual EIR of 218 infectious bites per person in Lodja and 257 infectious bites per person per year in Kimpese highlight the extremely high year-round malaria transmission risk in these provinces. However, the EIR was far lower in Inongo due to low biting rates of *An. gambiae* s.l. It is clear that in high transmission areas such as Lodja and Kimpese, multiple interventions in addition to ITNs are needed to have a significant impact on malaria transmission.

Insecticide susceptibility tests showed that pyrethroid resistance is widespread. In all sites, *An. gambiae* s.l. were resistant to permethrin, deltamethrin, and alpha-cypermethrin. Resistance intensity varied by site and by insecticide, but was commonly moderate or high. Despite uncertainty regarding the impact of pyrethroid resistance, WHO states that, "When resistance is confirmed at the 5× and especially at the 10× concentrations, operational failure is likely." Throughout DRC, resistance to the three most common pyrethroids used on ITNs was common at the 5× and 10× concentrations, making it highly likely that pyrethroid ITNs are no longer providing optimal protection against malaria. The high intensity of pyrethroid resistance indicates that the NMCP should consider alternative ITNs for future net distribution campaigns. Molecular analyses revealed that *An. gambiae* was the predominant species of the *An. gambiae* complex across all sites, with *An. coluzzii* identified only in Lodja, Kimpese, Inongo, Mbandaka, Dibindi, and Kingasani. The Vgsc-L1014F mutation was detected at fixation in all sites and is likely to contribute to pyrethroid resistance together with other metabolic mechanisms that were implicated in PBO bioassays.

Bioassays with permethrin following pre-exposure to PBO 4% in WHO tube tests showed an increase in mortality compared with permethrin alone in seven out of the 13 sites. Despite an increase in mortality after pre-exposure to PBO, mortality was still <90% in six sites. There was a significant increase in mortality with deltamethrin and alpha-cypermethrin following pre-exposure to PBO 4% in all 13 sites. Increases in

deltamethrin mortality were particularly large in Katana, Kingasani, Mweneditu, Dibindi, and Mbandaka. Despite an increase in mortality after pre-exposure to PBO, alpha-cypermethrin mortality was still <90% in five sites (Inongo, Mikalayi, Kapolowe, Lodja, and Lisala). Although susceptibility was not fully restored, the general increase in mortality when a PBO synergist was used indicates that ITNs containing PBO may provide greater control. A better option may be Interceptor G2 ITNs, as susceptibility to chlorfenapyr was recorded in all six sites. However, the increased cost may be prohibitive. The results of PBO ITN durability studies being conducted by VectorLink over three years in Sud Ubangi and Tanganyika provinces will be particularly informative for the NMCP and stakeholders.

5. ANNEX

Table 10: Monthly HBR of Malaria Vectors Collected Indoors and Outdoors by HLC in Lodja.

Lodja, January–December 2020															
Species	Location	Variables	Jan	Feb	Mar	Apr	May	Jun	July	Aug	Sep	Oct	Nov	Dec	2020 total
An. gambiae s.l.	Indoor	Total collected	264	118	123	130	128	107	128	91	121	129	207	90	1,636
		Nbr person-nights	8	8	8	8	8	8	8	8	8	8	8	8	96
		HBR/night	**33**	**15**	**15**	**16**	**16**	**13**	**16**	**11**	**15**	**16**	**26**	**11**	**17**
	Outdoor	Total collected	487	265	192	256	239	208	218	209	263	401	357	272	3,367
		Nbr person-nights	8	8	8	8	8	8	8	8	8	8	8	8	96
		HBR/night	**61**	**33**	**24**	**32**	**30**	**26**	**27**	**26**	**33**	**50**	**45**	**34**	**35**
An. funestus s.l.	Indoor	Total collected	2	1	2	0	0	0	0	0	0	0	0	0	5
		Nbr person-nights	8	8	8	8	8	8	8	8	8	8	8	8	96
		HBR/night	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	Outdoor	Total collected	1	0	3	2	0	2	0	0	0	1	0	2	11
		Nbr person-nights	8	8	8	8	8	8	8	8	8	8	8	8	96
		HBR/night	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
An. paludis	Indoor	Total collected	25	43	20	18	8	12	41	32	50	15	3	11	278
		Nbr person-nights	8	8	8	8	8	8	8	8	8	8	8	8	96
		HBR/night	**3**	**5**	**3**	**2**	**1**	**2**	**5**	**4**	**6**	**2**	**0**	**1**	**3**
	Outdoor	Total collected	113	149	120	81	25	53	132	168	202	51	31	59	**1,184**
		Nbr person-nights	8	8	8	8	8	8	8	8	8	8	8	8	96
		HBR/night	**14**	**19**	**15**	**10**	**3**	**7**	**17**	**21**	**25**	**6**	**4**	**7**	**12**

Table 11: Monthly HBR of Malaria Vectors Collected Indoors and Outdoors by HLC in Kimpese.

Kimpese, January–December 2020														
Species	**Location**	**Variables**	**Jan**	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**July**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**	**2020 total**
An. gambiae s.l.	Indoor	Total collected	73	102	105	18	11	1	5	0	0	0	37	109	461
		Nbr person-nights	8	8	8	8	8	8	8	8	8	8	8	8	96
		HBR/night	**9**	**13**	**13**	**2**	**1**	**0**	**1**	**0**	**0**	**0**	**5**	**14**	**5**
	Outdoor	Total collected	88	84	178	20	15	2	9	0	0	0	127	140	663
		Nbr person-nights	8	8	8	8	8	8	8	8	8	8	8	8	96
		HBR/night	**11**	**11**	**22**	**3**	**2**	**0**	**1**	**0**	**0**	**0**	**16**	**18**	**7**
An. funestus s.l.	Indoor	Total collected	54	160	79	73	351	222	262	20	253	171	99	231	1,975
		Nbr person-nights	8	8	8	8	8	8	8	8	8	8	8	8	96
		HBR/night	**7**	**20**	**10**	**9**	**44**	**28**	**33**	**3**	**32**	**21**	**12**	**29**	**21**
	Outdoor	Total collected	82	93	157	76	264	333	311	158	122	162	139	219	2,116
		Nbr person-nights	8	8	8	8	8	8	8	8	8	8	8	8	96
		HBR/night	**10**	**12**	**20**	**10**	**33**	**42**	**39**	**20**	**15**	**20**	**17**	**27**	**22**
An. paludis	Indoor	Total collected	0	0	0	0	0	0	0	0	0	0	0	0	0
		Nbr person-nights	8	8	8	8	8	8	8	8	8	8	8	8	96
		HBR/night	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	Outdoor	Total collected	0	0	0	0	0	1	0	0	0	0	0	0	1
		Nbr person-nights	8	8	8	8	8	8	8	8	8	8	8	8	96
		HBR/night	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**

Table 12: Monthly HBR of Malaria Vectors Collected Indoors and Outdoors by HLC in Inongo.

Inongo, January–December 2020															
Species	**Location**	**Variables**	**Jan**	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**July**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**	**2020 total**
An. gambiae s.l.	Indoor	Total collected	1	7	22	11	6	4	0	0	0	1	0	0	52
		Nbr person-nights	8	8	8	8	8	8	8	8	8	8	8	8	96
		HBR/night	**0**	**1**	**3**	**1**	**1**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**1**
	Outdoor	Total collected	0	8	25	14	9	1	0	1	0	2	1	0	61
		Nbr person-nights	8	8	8	8	8	8	8	8	8	8	8	8	96
		HBR/night	**0**	**1**	**3**	**2**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**1**
An. funestus s.l.	Indoor	Total collected	0	0	1	0	0	0	0	0	0	0	0	0	1
		Nbr person-nights	8	8	8	8	8	8	8	8	8	8	8	8	96
		HBR/night	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	Outdoor	Total collected	0	0	0	0	0	0	0	0	0	1	0	0	1
		Nbr person-nights	8	8	8	8	8	8	8	8	8	8	8	8	96
		HBR/night	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
An. paludis	Indoor	Total collected	48	35	53	56	109	101	91	186	121	104	86	116	1,106
		Nbr person-nights	8	8	8	8	8	8	8	8	8	8	8	8	96
		HBR/night	**6**	**4**	**7**	**7**	**14**	**13**	**11**	**23**	**15**	**13**	**11**	**15**	**12**
	Outdoor	Total collected	43	38	59	58	77	105	105	127	41	89	81	86	909
		Nbr person-nights	8	8	8	8	8	8	8	8	8	8	8	8	96
		HBR/night	**5**	**5**	**7**	**7**	**10**	**13**	**13**	**16**	**5**	**11**	**10**	**11**	**9**

Figure 31: Gel Image from INRB Testing for the Vgsc-L1014F Mutation in *An. gambiae* s.l. Showing the Presence of RR Resistant Samples.