U.S. PRESIDENT'S MALARIA INITIATIVE

PMI VECTORLINK
DEMOCRATIC REPUBLIC OF CONGO
ENTOMOLOGICAL MONITORING ANNUAL REPORT

JANUARY – DECEMBER 2019

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ACRONYMS

CDC	Centers for Disease Control and Prevention
DRC	Democratic Republic of Congo
EIR	Entomological Inoculation Rate
ELISA	Enzyme-linked Immunosorbent Assay
HBR	Human Biting Rate
HLC	Human Landing Catch
INRB	*Institut National de Recherche Biomédicale*/National Institute of Biomedical Research
ITN	Insecticide-treated nets
kdr	Knockdown Resistance
NMCP	National Malaria Control Program
PBO	Piperonyl Butoxide
PCR	Polymerase Chain Reaction
PMI	President's Malaria Initiative
PSC	Pyrethrum Spray Catch
SR	Sporozoite Rate
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 2019. Activities took place in 13 sentinel sites distributed nationwide. The project did monthly longitudinal monitoring of malaria vector biting rates, resting densities, and entomological inoculation rates (EIRs) in three sites (Kabondo, Lodja, and Kalemie). Indoor resting densities used pyrethrum spray catch (PSC) collections, and human landing catch (HLC) was done indoors and outdoors to determine biting rates. To inform the National Malaria Control Program's (NMCP's) choice of insecticide for future insecticide-treated nets (ITN) distribution campaigns, insecticide susceptibility tests were conducted in 10 other sites. Resistance intensity bioassays using *Anopheles gambiae* s.l. were conducted with permethrin, deltamethrin, and alpha-cypermethrin at 1, 5, and 10 times the diagnostic concentration, according to World Health Organization (WHO) protocols. The project did piperonyl butoxide (PBO) synergist bioassays with pyrethroids, and it did bottle bioassays to determine susceptibility status to the new insecticide chlorfenapyr.

An. gambiae s.l. was found to be the predominant malaria vector throughout the year in all three longitudinal surveillance sites. Its biting rates were particularly high in Kabondo and Lodja throughout the year, and much lower in Kalemie. In Kabondo, the mean *An. gambiae* s.l. biting rate was 18 bites per person per night indoors and 20 outdoors, with a malaria sporozoite rate of 3.2% (76/2,391). This equates to an annual EIR of 186 infectious bites per person in Kabondo. In Lodja, the mean sporozoite rate was slightly lower, 1.5% (36/2,400), but the annual EIR was still high at 103 infectious bites per person per year. In Kalemie, the mean *An. gambiae* s.l. biting rate was 3 bites per person per night indoors, with a sporozoite rate of 0.5% (2/416), giving a much lower EIR of 3.8 infectious bites per person per year. These results highlight that there is an extremely high year-round malaria transmission risk in Kabondo and Lodja. They also show that there is heterogeneity across the country, with Kalemie in Eastern DRC having a relatively low transmission risk.

In Kalemie, 60% of *Anopheles gambiae* s.l. captured were caught by PSC (with 40% captured by HLC), while PSC accounted for only 4% of the total catch in Lodja and 12% in Kabondo. This appears to indicate that *An. gambiae* s.l. exit houses early in the morning, before dawn, in Lodja and Kabondo, but rest indoors for longer in Kalemie.

Insecticide susceptibility tests showed that pyrethroid resistance is widespread. In all sites, *An. gambiae* s.l. were resistant to permethrin and alpha-cypermethrin, with resistance to deltamethrin in eight of 10 sites. Resistance intensity varied by site and by insecticide, but it was usually moderate or high. For example, permethrin resistance intensity was low in two sites, Mikalayi and Tshikaji; moderate in three sites, Kingasani, Binza-Meteo, and Muanda; and high (<98% mortality at ×10 dose) in five sites, Kimpese, Aketi, Buta, Pawa, and Nebobongo. 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 such as synergists or mixtures for future net distribution campaigns.

In seven of 10 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 all 10 sites. There was a significant increase in mortality with deltamethrin following pre-exposure to PBO 4% in in all 10 sites; increases in mortality were particularly large in Buta, Aketi, Kimpese, Nebobongo, and Pawa. 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 10 sites; that said, the increased cost of those nets may be prohibitive.

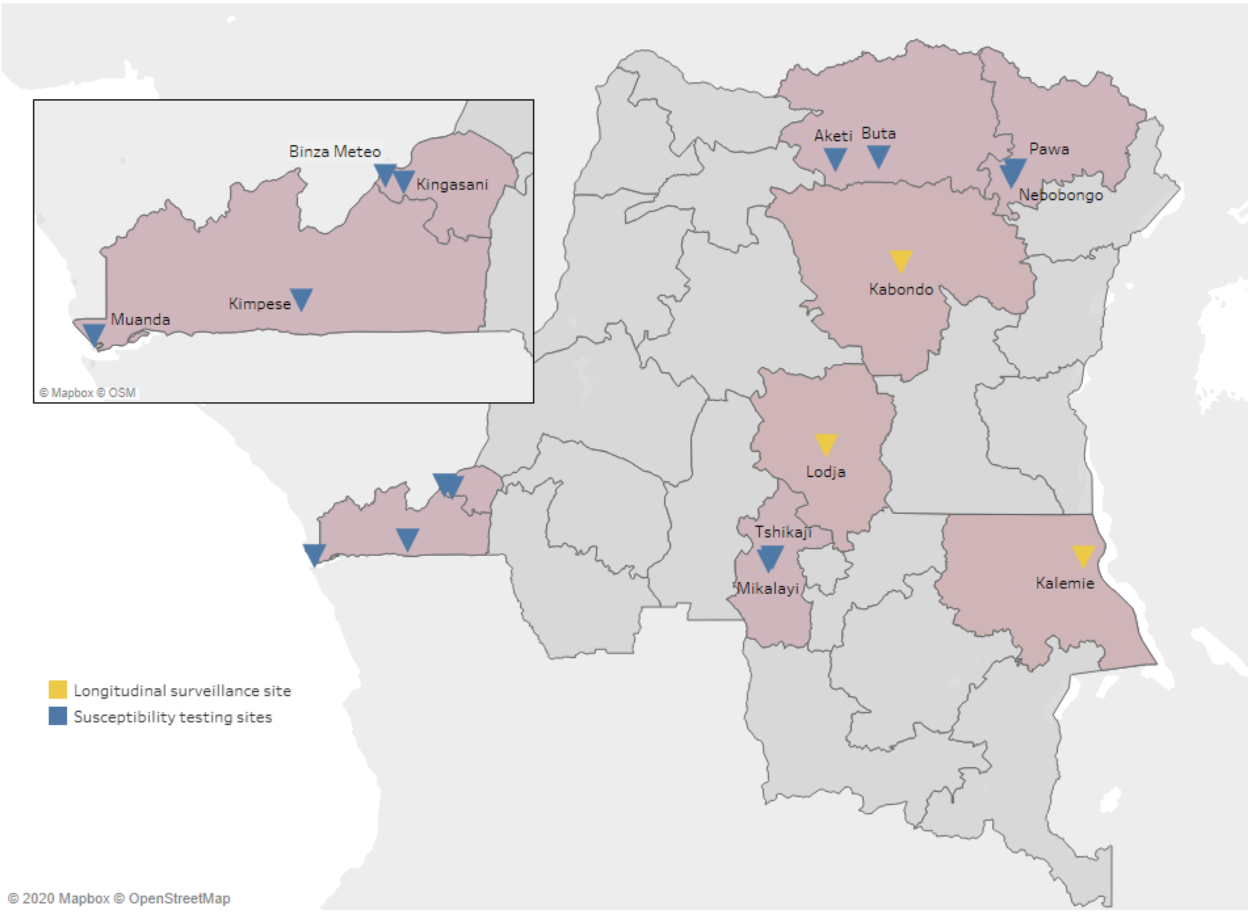
To prevent delays in entomological laboratory analysis and to build local capacity, PMI VectorLink DRC bought equipment for polymerase chain reaction and ELISA (Enzyme-linked Immunosorbent Assay) testing for the National Institute of Biomedical Research (*Institut National de Recherche Biomédicale*). The dedicated entomology molecular laboratory should be established by mid-2020.

1. Methodology

1.1 Study Area

This report details the results of entomological monitoring activities that the President's Malaria Initiative VectorLink Project conducted in 13 sites in the Democratic Republic of Congo (DRC) from January to December 2019 (Figure 1).

Figure 1. Map Showing Location Of Sentinel Sites For Entomological Monitoring In 2019



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

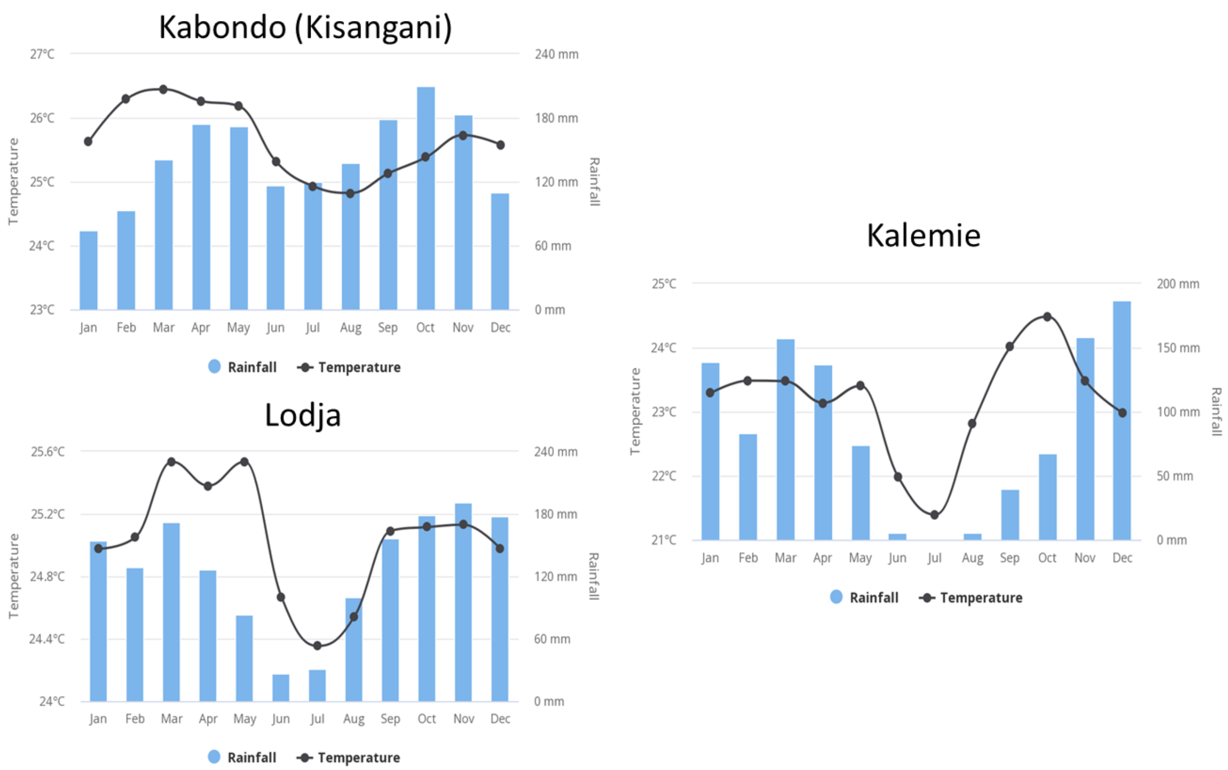
TABLE 1. SCHEDULE FOR 2019 ENTOMOLOGICAL ACTIVITIES

Activity	Purpose	Sites	Timeline	Frequency	Status
Vector susceptibility and intensity of resistance	Determine vector susceptibility to four insecticides and the importance of metabolic pyrethroid resistance mechanisms through bioassays with the synergist PBO	Kinshasa, Binza-Meteo, Kimpese, Muanda, Mikalayi, Tshikaji, Pawa, Nebobongo, Aketi, and Buta	May–September 2019	10 sites, 1 time per site	Completed
Monthly species composition, biting rate and times, inoculation rate	To gather more-detailed annual information on malaria vector dynamics and behavior	Kabondo, Lodja, and Kalemie	January–December 2019	3 sites, every month	Completed
ITN cone bioassays	To determine the bio-efficacy of 84 DawaPlus 2.0 in laboratory in INRB	Lodja, Mbuji-Mayi, Bukavu, Kalemie, Kolwezi, Kinshasa, Kananga	May 2019	7 sites, 1 time per site	Completed and activity report approved
Molecular assays	To identify mosquito species of the *An. gambiae* s.l. species complex, mechanisms of *kdr*, and sporozoite rates	Kabondo, Lodja, Kalemie, Kinshasa, Binza-Meteo, Kimpese, Muanda, Mikalayi, Tshikaji, Pawa, Nebobongo, Aketi, and Buta	April–December 2019	11 sites	Completed for sporozoite ELISA; PCR will be reported by May 1, 2020
Sud Ubangi ITN monitoring	To collect baseline data on the density of vectors and the susceptibility of *An. gambiae* s.l. to deltamethrin with and without PBO. To conduct baseline ITN bioassay testing of PBO and pyrethroid nets using locally collected *An. gambiae* s.l. from Gemena.	Sud Ubangi in 8 health zones	Scheduled for September 2019.	8 sites, 1 time per site	Completed in January 2020 so results to be included in Annual Report.

Note: ELISA=Enzyme-linked Immunosorbent Assay, INRB=National Institute of Biomedical Research (*Institut National de Recherche Biomédicale*), ITN=insecticide-treated nets, *kdr*= knockdown resistance, PBO=piperonyl butoxide, PCR=polymerase chain reaction

Figure 2 shows rainfall and mean temperature for the three longitudinal monitoring sites. Lodja and Kalemie both have a dry season in the middle of the year that lasts approximately four months in Lodja and six months in Kalemie; the mean temperature is lower during this period. Kabondo has considerable rainfall for most of the year, with peaks in April/May and September/October. Temperature there is quite stable year round, with a mean of 25-27°C, providing perfect conditions for mosquito survival.

FIGURE 2. AVERAGE MONTHLY TEMPERATURE AND RAINFALL FOR 1991-2016 FOR THREE SITES USED FOR LONGITUDINAL ENTOMOLOGICAL MONITORING BY HLC AND PSC



Source: Climatic Research Unit of University of East Anglia

Mosquitoes were collected monthly using Human Landing Catches and pyrethrum spray catches in Kabondo, Lodja, and Kalemie. See Table 2 for a summary of collection methods.

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	10 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 during four consecutive nights in two different houses each night, with one person placed indoors and another placed outdoors in each selected house. Collectors rotated indoors and outdoors every hour. Houses were sampled in different villages each month to get a representative sample of the areas and HLCs were performed in two different houses over four consecutive nights.

All *Anopheles* mosquitoes collected by the HLCs 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 in the same areas as 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 properly labeled, 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 was conducted in 10 sentinel sites. Two sentinel sites per province were selected in coordination with the National Malaria Control Program (NMCP). These are listed in Table 2. Provinces were chosen based on ITN distribution campaigns scheduled for 2020 and 2021, in order to obtain insecticide susceptibility data to inform ITN procurement decisions. INRB entomologists traveled to each site to collect larvae and pupae, which were reared to adulthood before the susceptibility and resistance intensity tests were conducted. In addition to testing at the diagnostic dose, World Health Organization (WHO) intensity bioassays were also conducted, by testing pyrethroid papers treated with 5 and 10 times the diagnostic dose.

The insecticides tested in 2019 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%
- Chlorfenapyr 100µg/bottle (chlorfenapyr was tested in 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 (USM-Malaysia). Exposure tests were accompanied by negative control tests, in which mosquitoes were exposed to filter papers impregnated with oil or solvent. Testing was done 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 and deltamethrin with pre-exposure to PBO over 60 minutes to determine the change in mortality rates with PBO exposure.

CDC bottle bioassays were completed in all sites to determine the susceptibility status of *An. gambiae* s.l. populations to chlorfenapyr. PMI VectorLink indicated that 100µg/bottle is considered the interim diagnostic concentration. Four replicates of at least 20 *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 mosquitoes were still in the bottle. After 60 minutes of exposure, 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.

TABLE 2. SITES FOR INSECTICIDE RESISTANCE MONITORING IN 2019

Province	Updated Sentinel Sites	Year Added	Next ITN Distribution
Kinshasa	Kingasani	2014	Jan – Jun 2020
	Binza Méteo	2019	
Haut-Uele	Pawa	2016	Jul – Dec 2020
	Nebobongo	2019	
Bas-Uele	Buta	2019	Jul – Dec 2020
	Aketi		
Kongo Central	Kimpese (*An. funestus*)	2016	Jan – Jun 2020
	Muanda	2019	
Kasai Central	Mikalayi	2014	Jan – Jun 2020
	Tshikaji	2019 (previously monitored in 2014/15)	

1.5 MOLECULAR ANALYSIS

Molecular analyses were conducted in the molecular laboratory of the INRB Parasitology Department, Kinshasa. 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*
	Martinez-Torres et al. (1998)	Voltage gated sodium channel mutation L1014F presence: Monitored pyrethroid target site resistance mechanism frequency

Testing of sporozoite infection rate using ELISA was completed for all 2019 samples and is reported below in Section 3.6. PCR analysis for species identification and resistance mechanism detection is ongoing and will be shared by May 1, 2020. Delays in PCR analysis were primarily due to the infrequent access to the small PCR laboratory which is shared among several projects at INRB. This is being addressed by establishing a dedicated entomology PCR laboratory, which should be functional by July 2020. ELISA tests were conducted on a subsample of *Anopheles* collected through HLCs in Kabondo, Lodja, and Kalemie (targeted at a sample of 200 per month, or 2,400 total).

1.6 Data Analysis

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 the number of nights in the month

2. RESULTS

2.1 MALARIA VECTOR SPECIES COMPOSITION

Over the study period of January to December 2019, a total of 11,947 *Anopheles* were collected from the three routine monitoring sites through monthly HLC and PSC. Only three *Anopheles* species (*An. gambiae* s.l., *An. funestus* s.l., and *An. paludis*) were collected, with *An. gambiae* s.l. being the most common in all three sites. The species composition is presented by site and by collection method in Figures 3–5.

In Kabondo, the abundance of *Anopheles* species was greater (88%: 3,753/4,274) from HLC than from PSC (12%: 521/4,274). The same tendency was observed in Lodja, where 96% (6,319/6,601) of *Anopheles* species were collected by HLC and only 4% (282/6,601) by PSC. In Kalemie, 60% (646/1,072) of *Anopheles* were sampled by PSC compared with only 40% (426/1,072) by HLC. Details on the indoor resting densities and biting rates are presented in Sections 3.2 and 3.3. See Tables A1–A3 in the annex for monthly details on species composition and abundance. Molecular species identification is ongoing and is not presented in this report. *An. paludis* was relatively abundant in Lodja, particularly from outdoor HLC, but was not collected in Kabondo, and Kalemie.

FIGURE 3. SPECIES COMPOSITION OF *ANOPHELES* CAPTURED BY PSC AND HLC (INDOORS AND OUTDOORS) IN KABONDO FROM MONTHLY COLLECTIONS, JANUARY–DECEMBER 2019

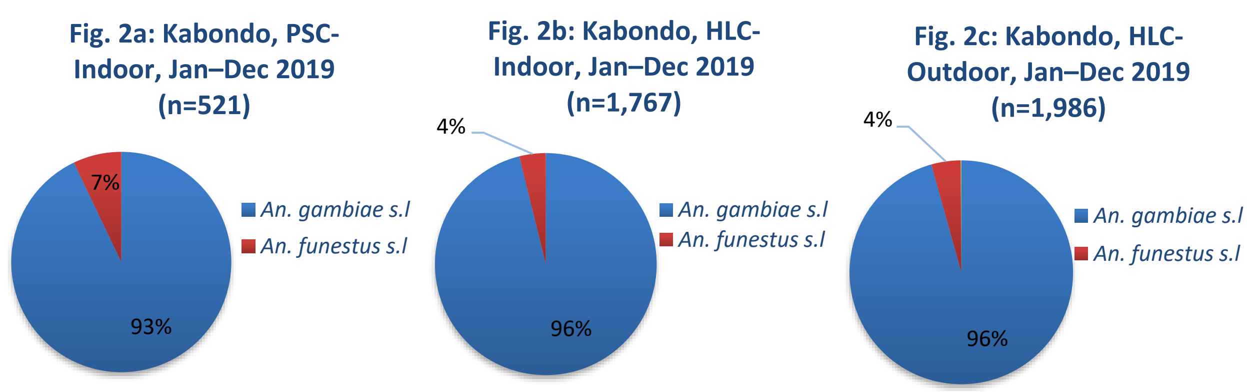


FIGURE 4. SPECIES COMPOSITION OF *ANOPHELES* CAPTURED BY PSC AND HLC (INDOORS AND OUTDOORS) IN LODJA FROM MONTHLY COLLECTIONS, JANUARY–DECEMBER 2019

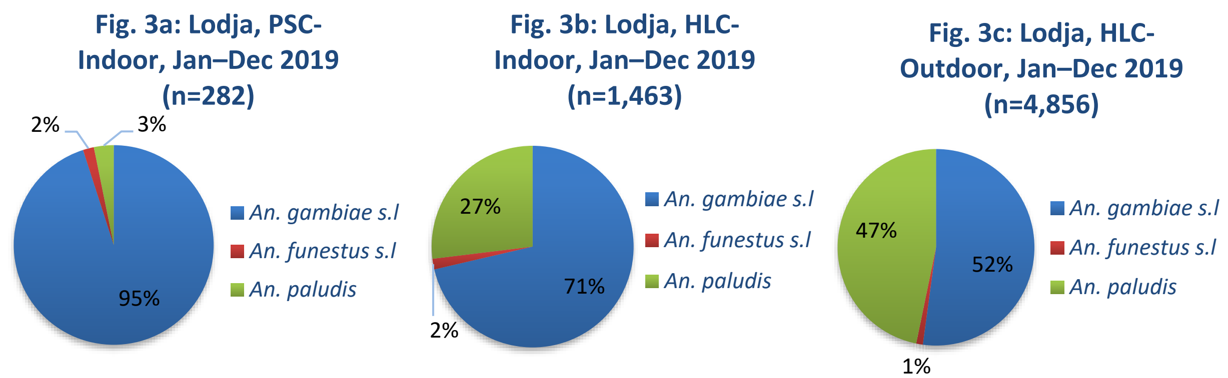
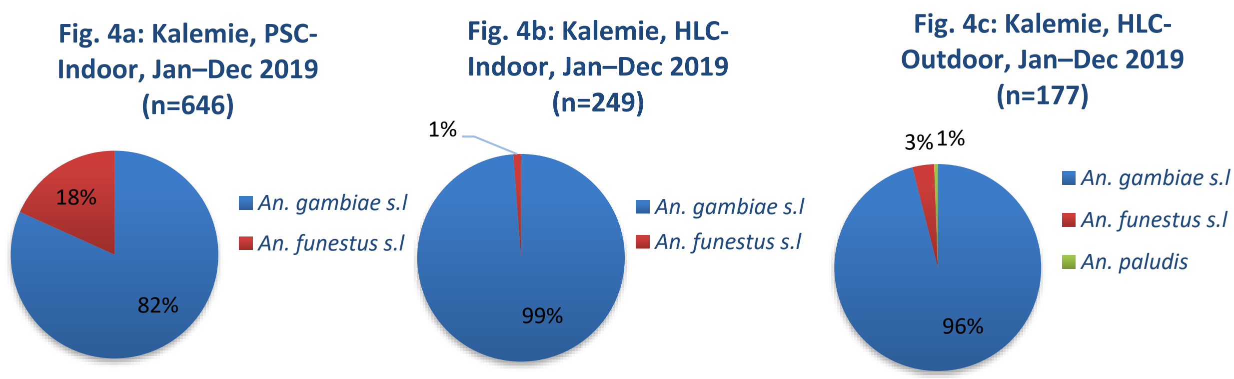


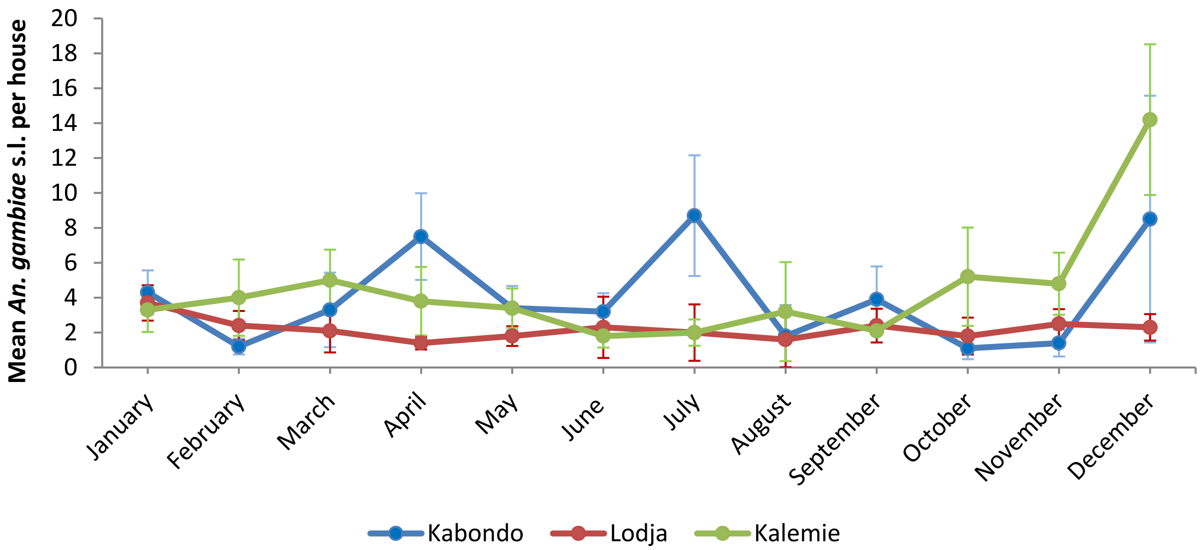
FIGURE 5. SPECIES COMPOSITION OF ANOPHELES CAPTURED BY PSC AND HLC (INDOORS AND OUTDOORS) IN KALEMIE FROM MONTHLY COLLECTIONS, JANUARY–DECEMBER 2019



2.2 MALARIA INDOOR VECTOR RESTING DENSITY (BY PSC)

Figure 6 shows the mean indoor resting density of *An. gambiae* s.l. (the predominant species in all sites) per house per day, collected by PSC. In all three sites, the indoor resting density was fairly stable throughout the year, with the biggest peak in December for Kalemie and Kabondo. In Lodja, the mean indoor resting density was less than four *An. gambiae* s.l. per house per day for the entire reporting period (January–December 2019).

FIGURE 6. MEAN INDOOR RESTING DENSITY PER HOUSE PER DAY OF *AN. GAMBIAE* S.L. CAPTURED BY PSC IN KABONDO, LODJA, AND KALEMIE FROM MONTHLY COLLECTIONS, JANUARY–DECEMBER 2019



The abdominal status of malaria vectors collected indoors by PSC between January and December 2019 is presented in Table 4. The majority of *Anopheles* collected were blood-fed in Kabondo and Kalemie. In Lodja, there was a similar proportion of blood-fed and unfed.

TABLE 4. ABDOMINAL STATUS OF *ANOPHELES* COLLECTED BY INDOOR PSC IN KABONDO, LODJA, AND KALEMIE, JANUARY–DECEMBER 2019

Kabondo					
Species	**Unfed**	**Blood-fed**	**Half-gravid**	**Gravid**	**Total**
An. gambiae s.l.	115 (24%)	366 (76%)	3 (1%)	0 (0%)	484 (100%)
An. funestus s.l.	10 (27%)	27 (73%)	0 (0%)	0 (0%)	37 (100%)
Total *Anopheles*	**125 (24%)**	**393 (75%)**	**3 (1%)**	0 (0%)	**521 (100%)**
Lodja					
An. gambiae s.l.	154 (57%)	115 (43%)	0 (0%)	0 (0%)	268 (100%)
An. funestus s.l.	0 (0%)	5 (100%)	0 (0%)	0 (0%)	5 (100%)
An. paludis	0 (0%)	9 (100%)	0 (0%)	0 (0%)	9 (100%)
Total *Anopheles*	**154 (55%)**	**128 (45%)**	**0 (0%)**	**0 (0%)**	**282 (100%)**
Kalemie					
An. gambiae s.l.	58 (11%)	460 (87%)	8 (2%)	2 (0%)	528 (100%)
An. funestus s.l.	9 (8%)	105 (89%)	4 (3%)	0 (0%)	118 (100%)
Total *Anopheles*	**67 (11%)**	**565 (87%)**	**12 (2%)**	**2 (0%)**	**646 (100%)**

2.3 MALARIA VECTOR HUMAN BITING RATES (BY HLC)

Figures 7–9 show the mean monthly biting rate per person per night in Kabondo, Lodja, and Kalemie, by vector species (further details are in the Annex, Tables A4–A6). *An. gambiae* s.l. biting rates were particularly high in Kabondo, with a mean over the 12-month period of 18 bites per person per night indoors and 20 outdoors. *An. gambiae* s.l. biting rates in Kabondo were >10 bites per person per night for 11 months of the year, with two peaks recorded from June to August and November to January (Figure 7). *An. funestus* s.l. was also present, albeit at very low densities (<3 bites per person per night) year-round in Kabondo. The mean *An. gambiae* s.l. biting rates were consistent year round in Lodja, with no clear seasonality. The one exception was April 2019, but this decrease was attributed to heavy rainfall during the collection time. Over the 12 month period, the mean biting rate in Lodja was 11 bites per person per night indoors and 23 bites outdoors (Figure 8). In Kalemie, the biting rates were generally much lower than in Kabondo and Lodja, with a mean *An. gambiae* s.l. biting rate over the 12-month period of 3 bites per person per night indoors and 2 bites outdoors (Figure 9).

FIGURE 7. MEAN MONTHLY INDOOR AND OUTDOOR *AN. GAMBIAE* S.L. AND *AN. FUNESTUS* S.L. BITING RATE IN KABONDO, JANUARY–DECEMBER 2019 (N=1,699 INDOORS, N=1,900 OUTDOORS FOR *AN. GAMBIAE* S.L. AND N=68 INDOORS, N=84 OUTDOORS FOR *AN. FUNESTUS* S.L.)

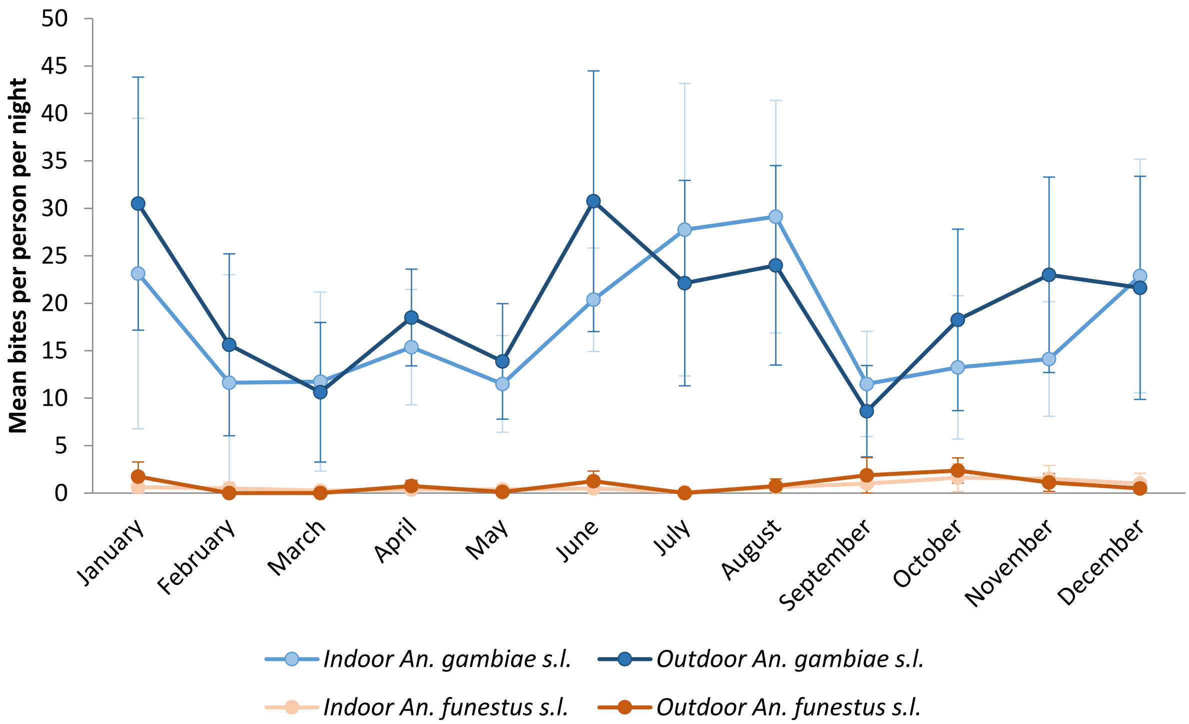


FIGURE 8. MONTHLY INDOOR AND OUTDOOR *AN. GAMBIAE* S.L. BITING RATE IN LODJA, JANUARY–DECEMBER 2019 (N=1,044 INDOORS, N=2,255 OUTDOORS)

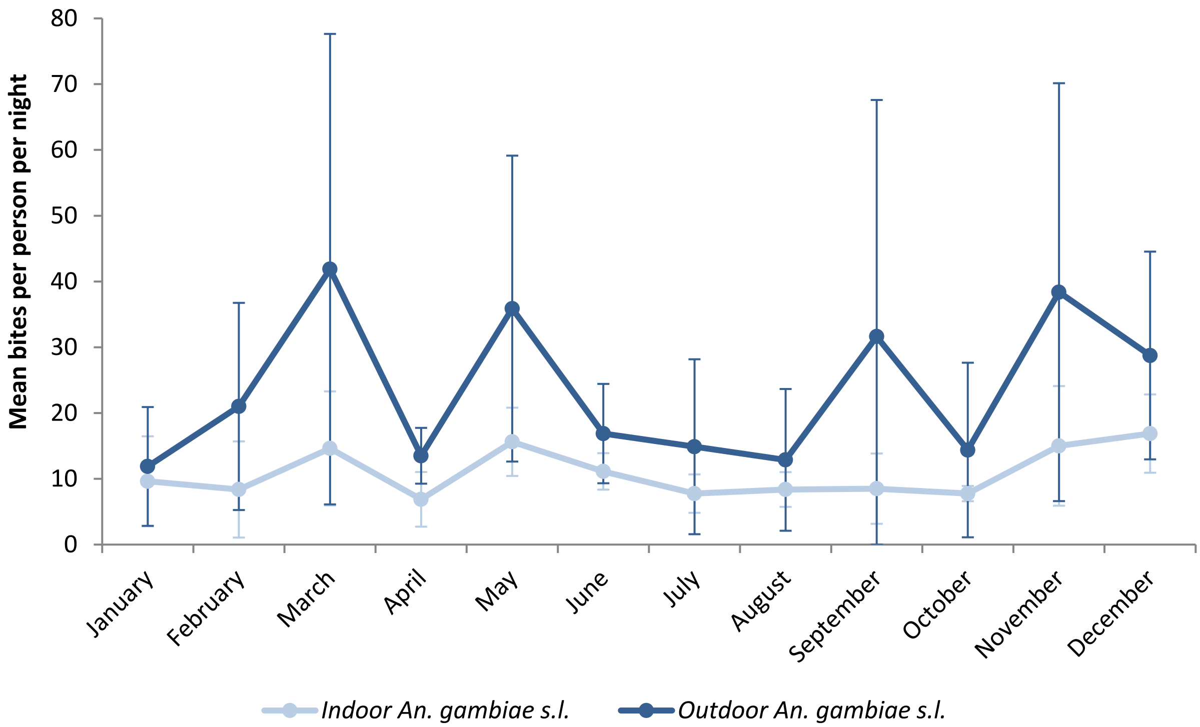
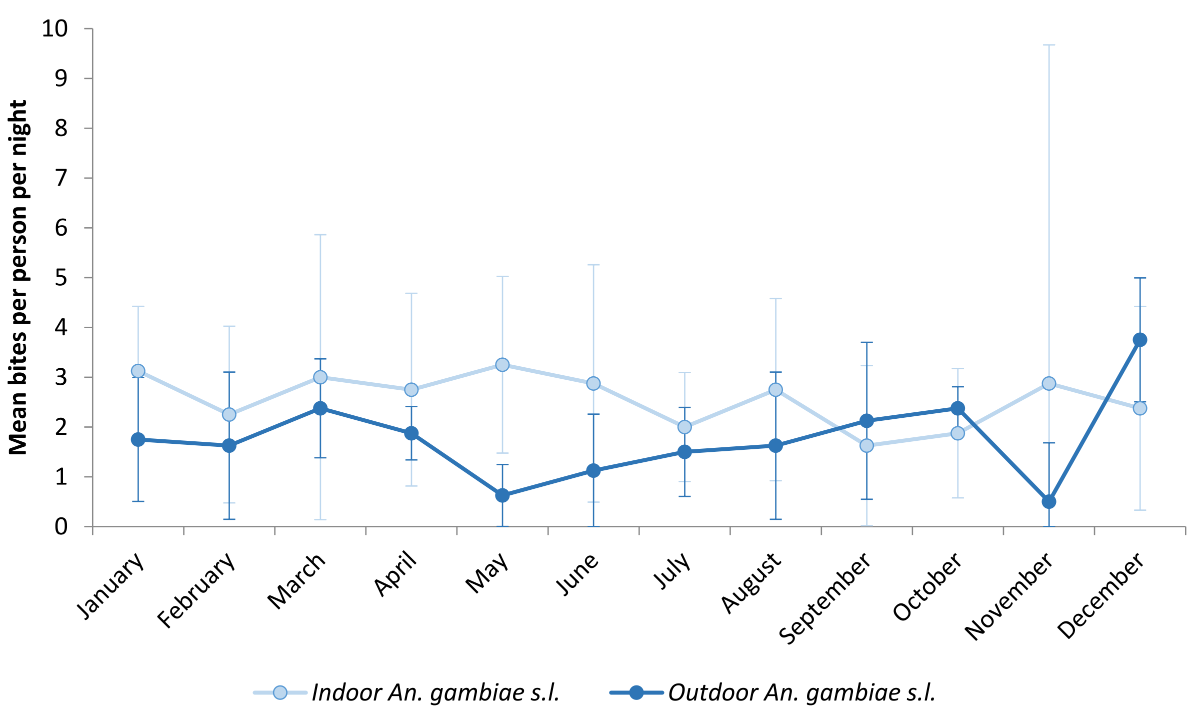


FIGURE 9. MEAN MONTHLY INDOOR AND OUTDOOR *AN. GAMBIAE* S.L. BITING RATE IN KALEMIE, JANUARY–DECEMBER 2019 (N=246 INDOORS, N=170 OUTDOORS)



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

In general, the peak period of *An. gambiae* 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 10–12). Biting rates in Lodja were substantially greater outdoors than indoors, particularly between 9 p.m. and 2 a.m.

Figure 10. Mean *An. gambiae* s.l. Hourly Biting Rates in Kabondo, January–December 2019 (n=1,699 indoors, n=1,900 outdoors)

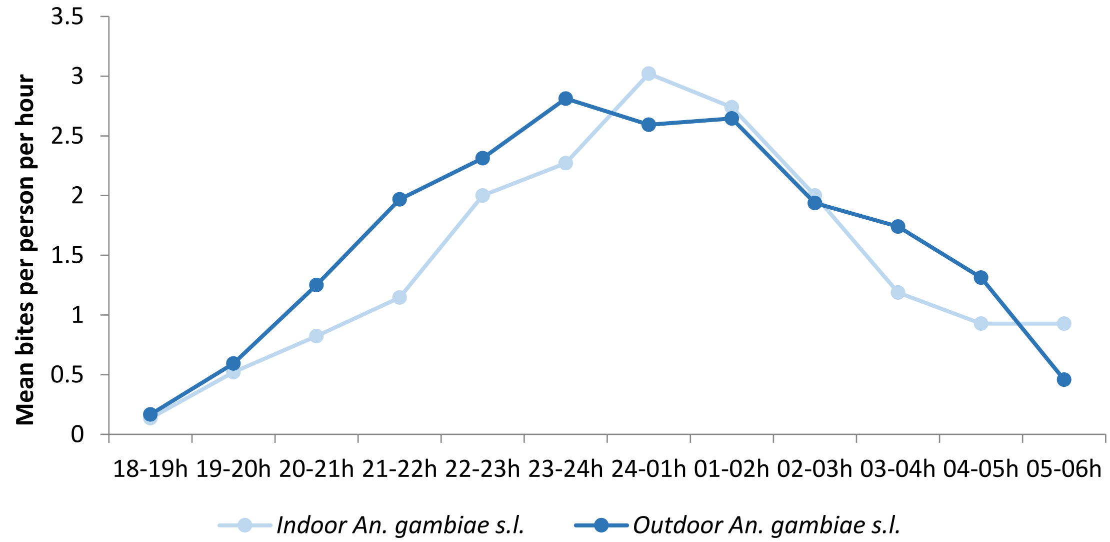


Figure 11. Mean *An. gambiae* s.l. Hourly Biting Rates in Lodja, January–December 2019 (n=1,044 indoors, n=2,251 outdoors).

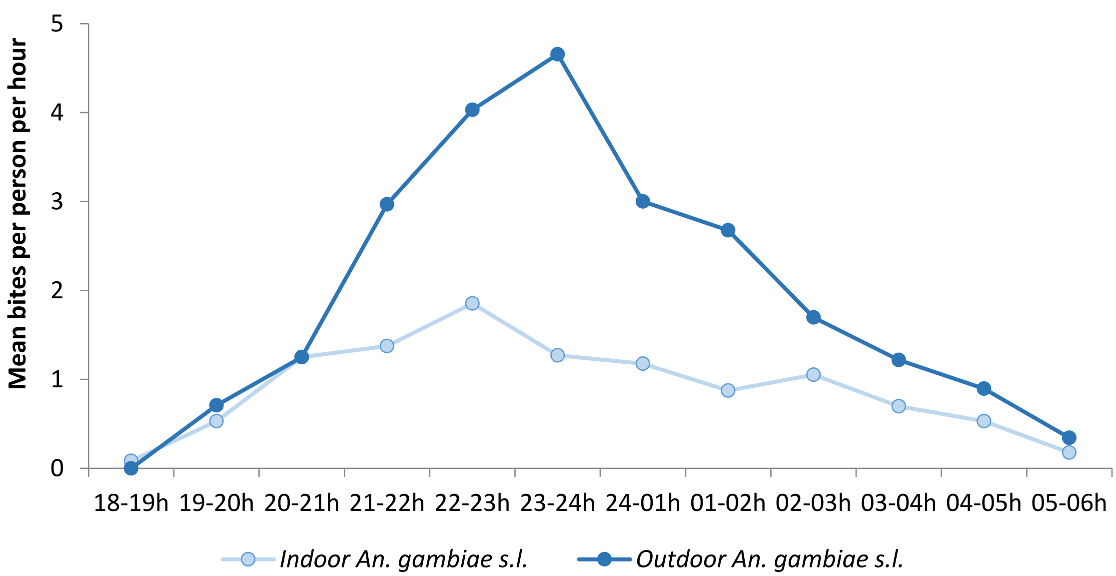
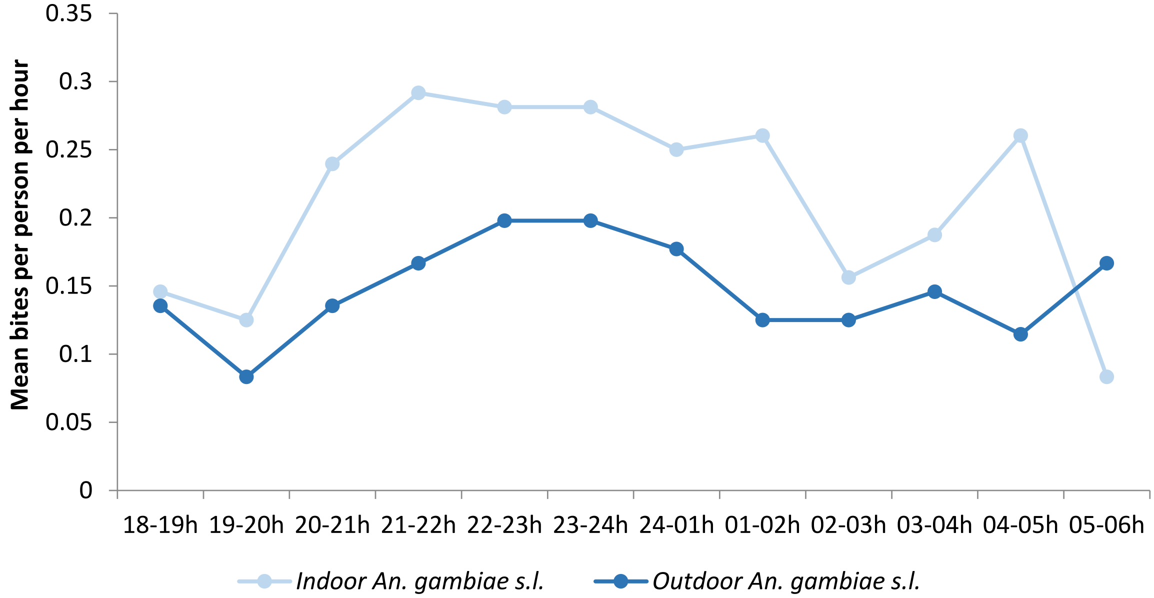


FIGURE 12. MEAN *AN. GAMBIAE* S.L. HOURLY BITING RATES IN KALEMIE, JANUARY–DECEMBER 2019 (N=246 INDOORS, N=170 OUTDOORS).



2.5 INSECTICIDE SUSCEPTIBILITY, PBO SYNERGIST, AND RESISTANCE INTENSITY

WHO insecticide susceptibility and resistance intensity tests were completed with *An. gambiae* s.l. populations that were collected as larvae in all 10 sites. Figure 13 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, Mikalayi and Tshikaji; moderate (<98% mortality at ×5 dose) in three sites, Kingasani, Binza-Meteo, and Muanda; and high (<98% mortality at ×10 dose) in five sites, Kimpese, Aketi, Buta, Pawa, and Nebobongo. Resistance to deltamethrin was recorded in all sites, except Kingasani and Muanda where possible resistance was observed (90-97% mortality) (Figure 14). The intensity of deltamethrin resistance was low in one site, Binza-Meteo; moderate in two sites, Buta and Pawa; and high in five sites, Kimpese, Mikalayi, Tshikaji, Aketi, and Nebobongo. Resistance to alpha-cypermethrin was also observed in all sites, except Mikalayi where possible resistance was observed (Figure 15). The intensity of alpha-cypermethrin resistance was low in one site, Muanda; moderate in four sites, Kingasani, Binza-Meteo, Nebobongo, and Kimpese; and high in four sites, Pawa, Tshikaji, Aketi, and Buta. 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 (x1 dose) following pre-exposure to PBO 4% in WHO tube tests showed an increase in mortality compared with permethrin alone in seven of 10 sites (Figure 16), with no increase in mortality in Aketi, Kimpese, and Mikalayi. Despite an increase in mortality after pre-exposure to PBO, mortality was still <90% in all 10 sites. There was a significant increase in mortality with deltamethrin (x1 dose) following pre-exposure to PBO 4% in WHO tube tests in all 10 sites. There were particularly large increases in mortality in Buta, Aketi, Kimpese, Nebobongo, and Pawa (Figure 17).

FIGURE 13. PERCENTAGE MORTALITY OF *AN. GAMBIAE* S.L. AFTER EXPOSURE TO PERMETHRIN AT ×1, ×5, AND ×10 TIMES THE DIAGNOSTIC CONCENTRATION IN WHO TUBE TESTS IN 10 SITES

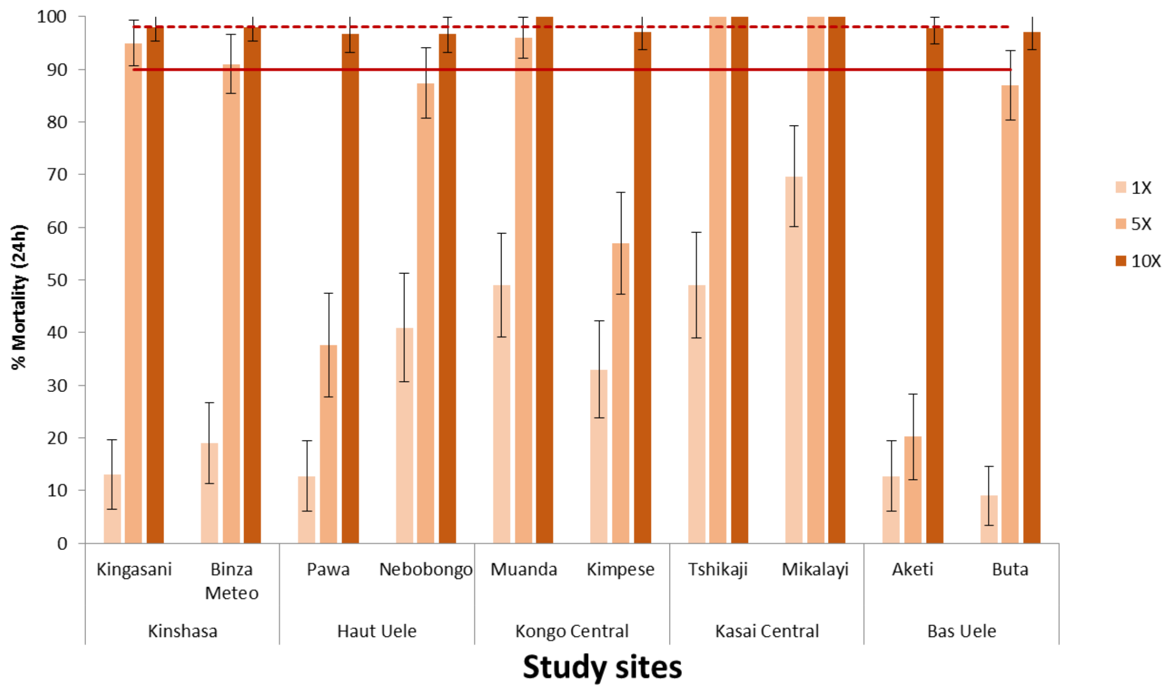


FIGURE 14. PERCENTAGE MORTALITY OF *AN. GAMBIAE* S.L. AFTER EXPOSURE TO DELTAMETHRIN AT ×1, ×5, AND ×10 THE DIAGNOSTIC CONCENTRATION IN WHO TUBE TESTS IN 10 SITES

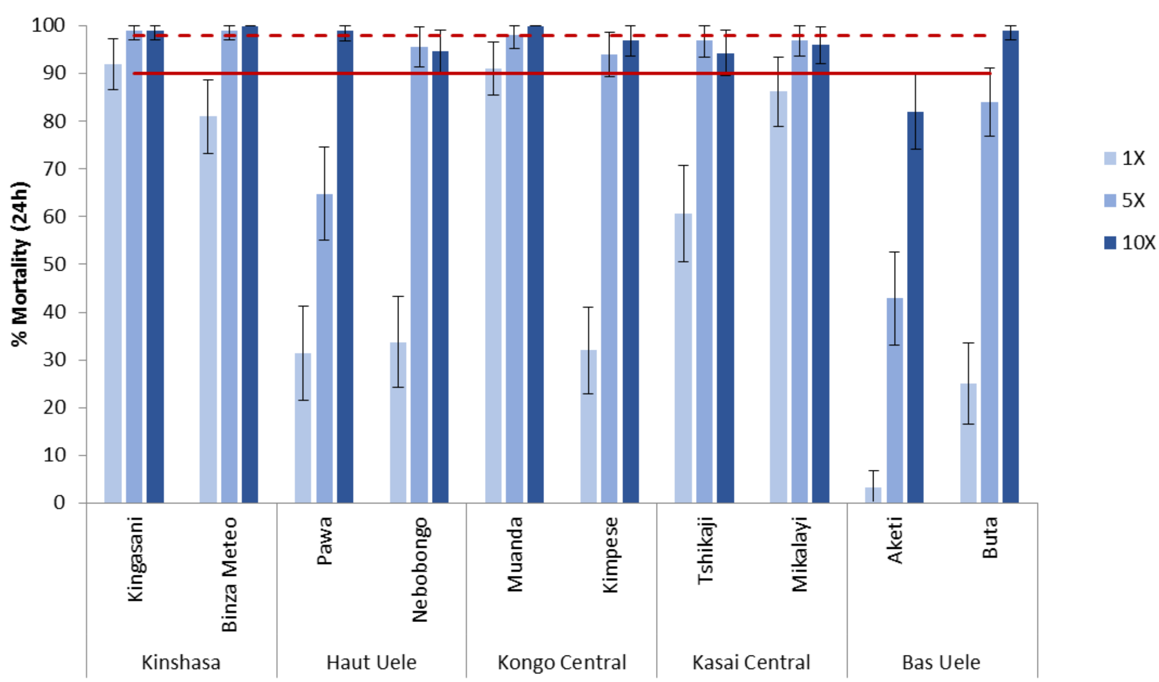


FIGURE 15. PERCENTAGE MORTALITY OF *AN. GAMBIAE* S.L. AFTER EXPOSURE TO ALPHA-CYPERMETHRIN AT ×1, ×5, AND ×10 THE DIAGNOSTIC CONCENTRATION IN WHO TUBE TESTS IN 10 SITES

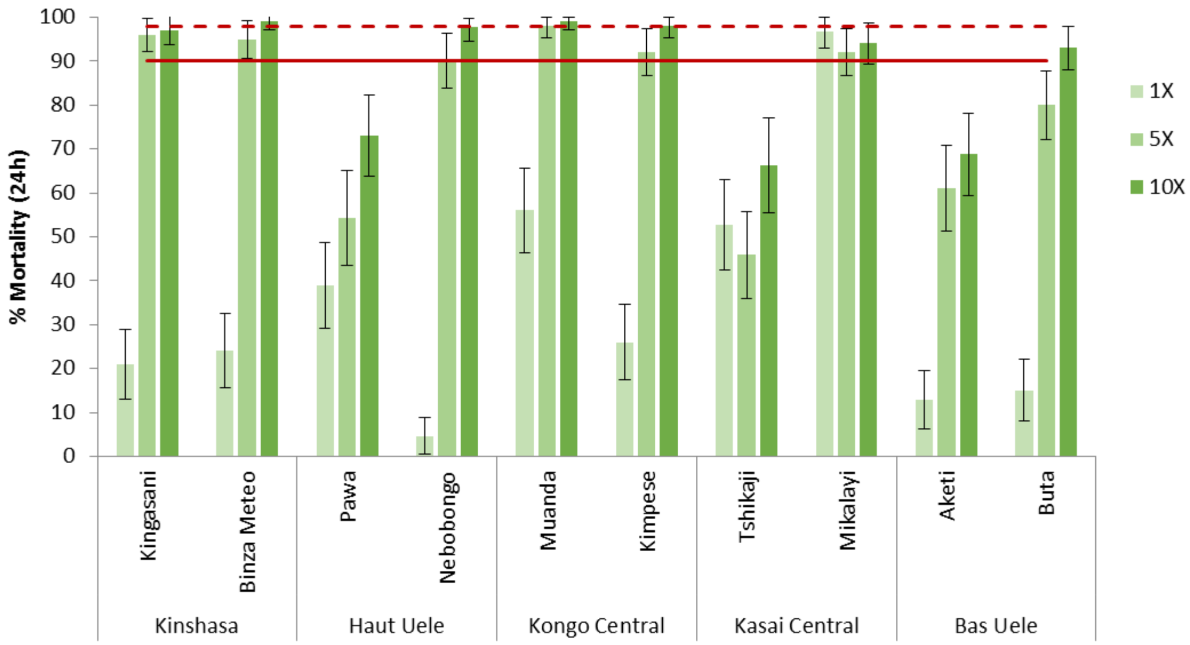
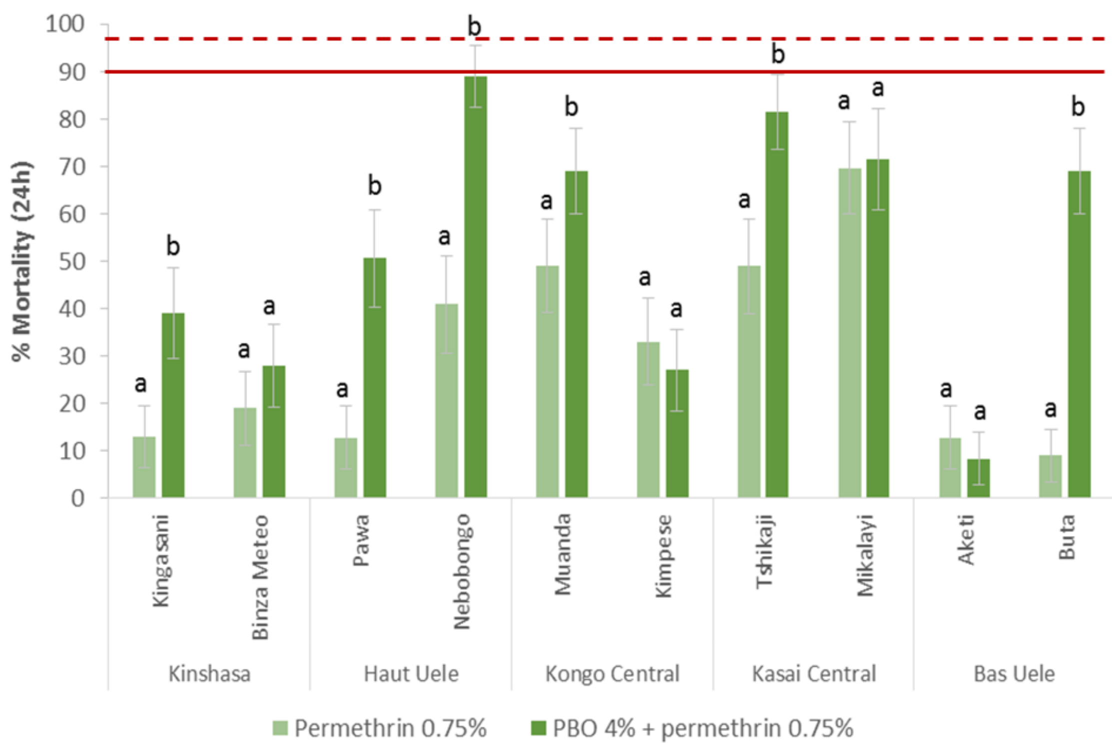
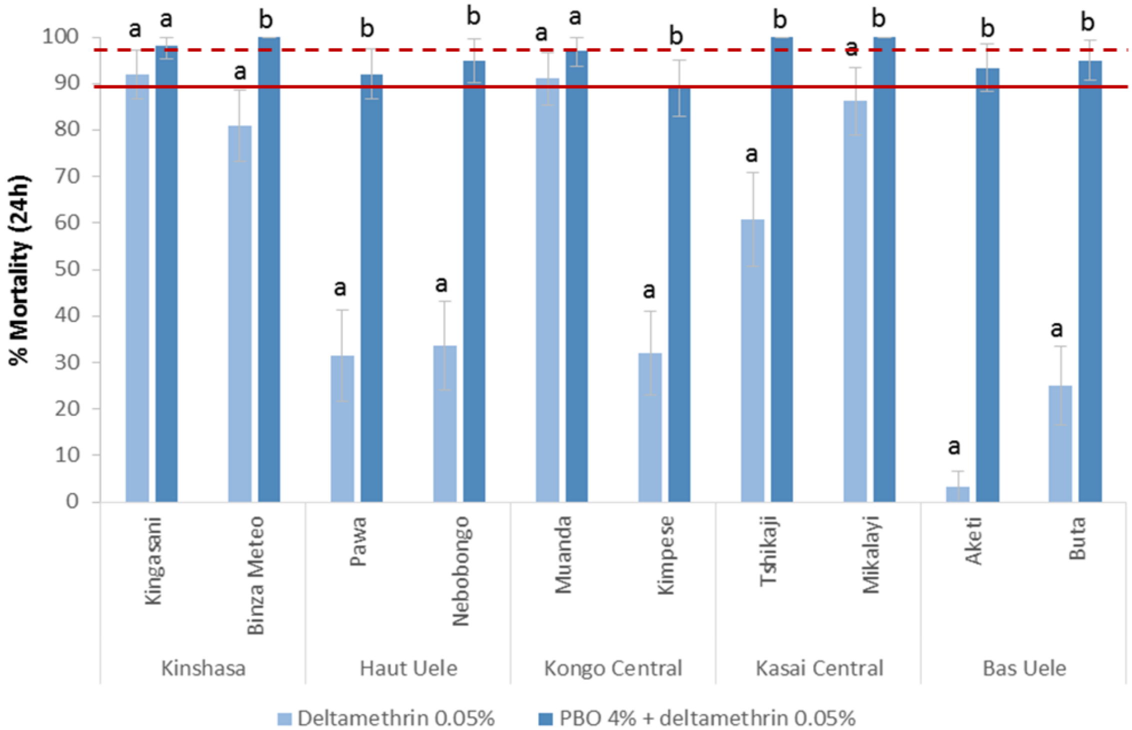


FIGURE 16. PERCENTAGE MORTALITY OF *AN. GAMBIAE* S.L. AFTER PRE-EXPOSURE TO PBO FOLLOWED BY PERMETHRIN AT THE DIAGNOSTIC CONCENTRATION IN WHO TUBE TESTS IN 10 SITES



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 17. PERCENTAGE MORTALITY OF *AN. GAMBIAE* S.L. AFTER PRE-EXPOSURE TO PBO FOLLOWED BY DELTAMETHRIN AT THE DIAGNOSTIC CONCENTRATION IN WHO TUBE TESTS IN 10 SITES



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.

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 10 sites within 24h of exposure.

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 of mosquitoes were met, with the exception of Kalemie due to an insufficient number of specimens collected through HLC (all 416 collected 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 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 2019	Total *An. funestus* s.l. Tested	% Sporozoite Rate (positive/ tested)
Kabondo	3,599	2,391/2,400 (200 per month)	3.2% (76/2,391)	152	145	2.8% (4/145)
Lodja	3,295	2400/2400 (200 per month)	1.5% (36/2,400)	69	63	3.2% (2/63)
Kalemie	416	416/2,400 (all collected)	0.5% (2/416)	9*	0	n/a
Total	7,310	5,207	2.2% (114/5,207	230	208	2.9% (6/208)

*Not enough *An. funestus* s.l. captured in Kalemie for testing.

The mean *An. gambiae* s.l. infection rate over 12 months was 3.2% (95% CI; 2.5-3.9) in Kabondo, 1.5% (95% CI; 1.0-2.0) in Lodja, and 0.5% (95% CI; 0.1-1.0%) in Kalemie. The mean *An. funestus* s.l. sporozoite rate was 2.8% (95% CI; 0.1-5.4) in Kabondo and 3.2% in Lodja (95% CI; 0.1-7.5), while in Kalemie, no *An. funestus* were tested (Table 5). The monthly *An. gambiae* s.l. sporozoite rate for Kabondo and Lodja from HLCs is presented in Figures 18 and 19. There is no figure presented for Kalemie due to the low number of sporozoite positive *An. gambiae* s.l. collected. Although there appeared to be peaks of infection (e.g., Kabondo in May and September) the confidence intervals are quite large as only 200 mosquitoes were tested per month and it is not possible to clearly determine any seasonality in infection rate.

FIGURE 18. MONTHLY *P. FALCIPARUM* SPOROZOITE RATE OF *AN. GAMBIAE* S.L. COLLECTED BY HLC IN KABONDO IN 2019

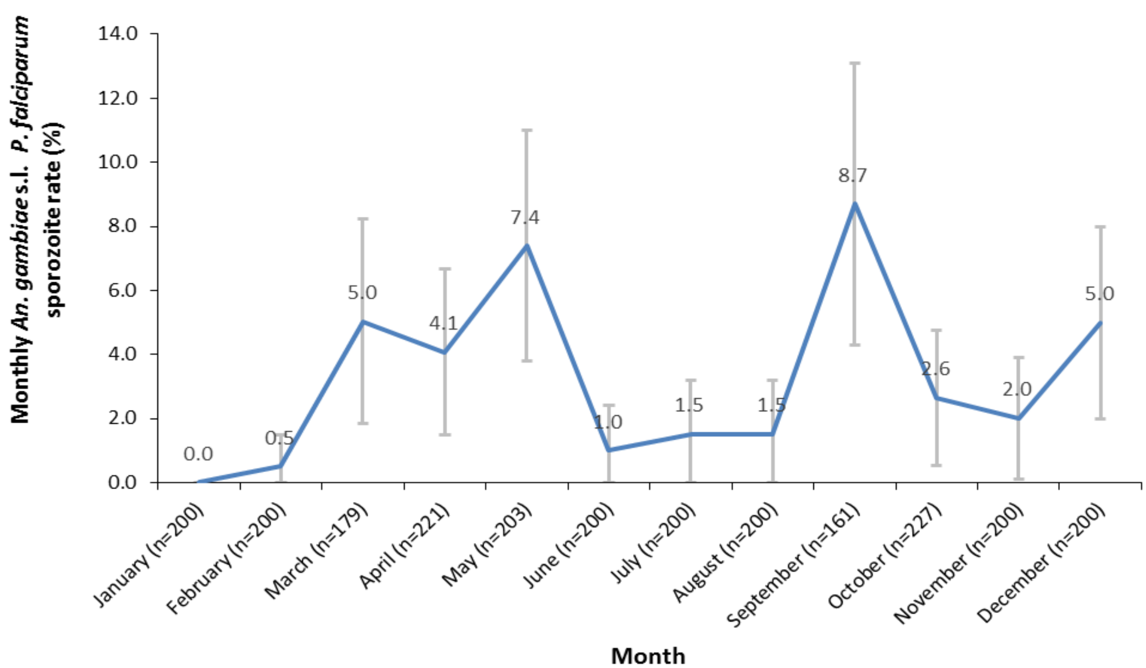
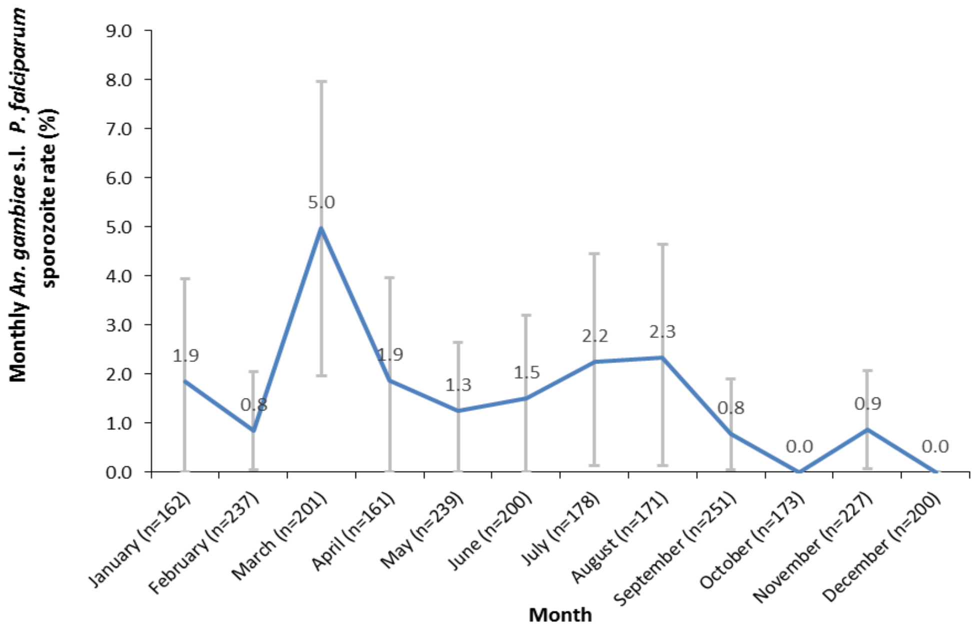


FIGURE 19. MONTHLY *P. FALCIPARUM* SPOROZOITE RATE OF AN. GAMBIAE S.L. COLLECTED BY HLC IN LODJA IN 2019



2.7 ENTOMOLOGICAL INOCULATION RATE

The combined indoor and outdoor monthly EIRs for *An. gambiae* s.l. in Kabondo and Lodja for 2019 are summarized in Tables 6 and 7. Addition of the monthly EIR gave an annual EIR of 186 infectious bites per person per year for Kabondo and 103 infectious bites per person per year for Lodja. Sporozoite positive *An. gambiae* s.l. were detected in Kalemie only in December 2019, giving an annual EIR of 3.8 infectious bites per person.

As the monthly sporozoite rate has a wide confidence interval, an alternative way to calculate the annual EIR is to multiply the mean sporozoite rate by the mean biting rate. This approach would give an annual EIR of 218 infectious bites per person per year for Kabondo ((3,599 bites/192 trap nights × 365 nights) × 3.18% SR) and 94 infectious bites per person for Lodja ((3,295 bites/192 trap nights × 365 nights) × 1.50% SR). For Kalemie, the annual EIR would be 4 infectious bites per person per year ((416 bites/192 trap nights) × 365 nights) × 0.50% SR).

Overall, these results demonstrate the extremely high malaria transmission risk faced year round in Kabondo and Lodja, despite the use of pyrethroid ITNs. However, in Kalemie, the malaria transmission risk was relatively low.

TABLE 6. MONTHLY *AN. GAMBIAE* S.L. EIR IN KABONDO, JANUARY–DECEMBER 2019

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	D
Total *An. gambiae* s.l. (HLC) collected	429	218	179	271	203	409	399	425	161	252	297	3
HLC trap-nights (indoors + outdoors)	16	16	16	16	16	16	16	16	16	16	16	1
HBR per night	27	14	11	17	13	26	25	27	10	16	19	2
Total *An. gambiae* s.l. tested by ELISA	200	200	179	221	203	200	200	200	161	227	200	2
Sporozoite rate (%)	0	0.50	5.03	4.07	7.39	1.00	1.50	1.50	8.70	2.64	2.00	5
EIR per night	0	0.07	0.56	0.69	0.94	0.26	0.37	0.40	0.88	0.42	0.37	1
EIR per month*	0	1.91	17.44	20.68	29.07	7.67	11.60	12.35	26.26	12.89	11.14	34
Kabondo 12 month EIR Jan–Dec 2019 = 186 infectious bites per person												

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

TABLE 7. MONTHLY *AN. GAMBIAE* S.L. EIR IN LODJA, JANUARY–DECEMBER 2019

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	D
Total *An. gambiae* s.l. (HLC) collected	172	235	452	163	412	224	177	170	321	177	427	3
HLC trap-nights (indoors + outdoors)	16	16	16	16	16	16	16	16	16	16	16	1
HBR per night	11	15	28	10	26	14	11	11	20	11	27	2
Total *An. gambiae* s.l. tested by ELISA	162	237	201	161	239	200	178	171	251	173	227	2
Sporozoite rate (%)	1.85	0.84	4.98	1.86	1.26	1.50	2.25	2.34	0.80	0.00	0.88	0.
EIR per night	0.20	0.12	1.41	0.19	0.32	0.21	0.25	0.25	0.16	0.00	0.23	0.
EIR per month*	6.17	3.45	43.61	5.68	10.06	6.30	7.72	7.71	4.82	0.00	7.05	0.
Lodja 12 month EIR Jan–Dec 2019 = 103 infectious bites per person												

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

3. Capacity Building

Conferences and meetings	VectorLink DRC supported the attendance of INRB staff, Prof Francis Watsenga at the Pan-African Mosquito Control Association conference held in Yaounde on September 23-26, 2019. VectorLink DRC data was presented entitled "Increasing intensity of pyrethroid resistance in *An. gambiae* s.l. and the implication for vector control in Democratic Republic of Congo." The VectorLink DRC Technical Director attended the Global PMI VectorLink conference held in Kigali on March 25-28, 2019. The project supported the DRC NMCP in organizing the annual national malaria scientific conference held in Kinshasa on April 29-30, 2019. It paid venue costs (including meals and coffee breaks), airfares, and hotel costs for eight participants including field entomological supervisors from Kabondo, Kapolowe, Kimpese, Tanganyika, and Lodja and researchers from Bandundu and Bukavu (Lwiro Center). The project also held a symposium entitled "*Surveillance entomologique en RDC de 2013 à 2018*," chaired by CDC's Dr. Seth Irish and colleagues. VectorLink DRC financially and technically supported the NMCP in planning and holding the first national meeting of the Vector Control Working Group (*Groupe de Travail Lutte Anti-Vectorielle* (GTLAV)) at Carita Congo Hotel on August 19, 2019. 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. The terms of reference for the working group were set during this first meeting.
Scientific support – short-term technical assistance in DRC from Richard Oxborough (PMI VectorLink). Technical, financial, and contractual support from Tiffany Clark (PMI VectorLink). **Technical support from Rodrigue Agossa (PMI VectorLink)**	The technical team conducted a short-term technical assistance trip in August 2019. The technical team met with PMI VectorLink DRC, NMCP, USAID, USAID Integrated Health Program, and INRB to discuss priorities for 2019-2020 work plan activities and challenges. The technical team shared the objectives of the Sud Ubangi study with the local authorities and gave guidance to supervisors for mosquito species identification and susceptibility testing with chlorfenapyr and synergist PBO. 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. He was also involved in the preparation of the training materials used for training the field staff in Sud Ubangi. Rodrigue worked with INRB technicians in October 2019 to establish a collection of pinned *An. gambiae* s.l., fresh laboratory susceptible mosquitoes. Pinning will continue this year with fresh *An. funestus* s.l., *An. pharoensis*, *An. coustani*, *An. paludis*, *An. moucheti* and any other *Anopheles* species that are collected.

Staffing	The project hired a part-time Financial and Administration Manager and a driver, who started on January 8, 2020.

4. Discussion

The climate of Kabondo and Lodja provinces 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 these two provinces throughout the year, but were much lower in Kalemie. The annual EIR of 186 infectious bites per person in Kabondo and 103 infectious bites per person per year in Lodja highlight the extremely high year-round malaria transmission risk in these provinces, but the relatively low transmission risk (EIR of 3.8) in Kalemie in eastern DRC shows that there is heterogeneity across the country. It is clear that, in high transmission areas, multiple interventions 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 and alpha-cypermethrin, and there was resistance in eight of 10 sites to deltamethrin. 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 that use synergists or mixtures for future net distribution campaigns.

Bioassays with permethrin following pre-exposure to PBO 4% in WHO tube tests showed an increase in mortality compared with permethrin alone in seven of 10 sites. Despite an increase in mortality after pre-exposure to PBO, mortality was still <90% in all 10 sites. There was a significant increase in mortality with deltamethrin following pre-exposure to PBO 4% in WHO tube tests in all 10 sites. There were particularly large increases in mortality in Buta, Aketi, Kimpese, Nebobongo, and Pawa. The general increase in mortality when a PBO synergist was used indicates that ITNs containing PBO may provide greater control, although susceptibility was not fully restored. A better option may be Interceptor G2 ITNs, as susceptibility to chlorfenapyr was recorded in all 10 sites, although the increased cost may be prohibitive. In 2020, the effectiveness of ITNs containing PBO are being tested in Sud Ubangi through entomological data collection and interpretation.

5. ANNEX

TABLE A1. MONTHLY SPECIES COMPOSITION AND ABUNDANCE OF *ANOPHELES* COLLECTED IN KABONDO BY PSC AND HLC, JANUARY –DECEMBER 2019

Kabondo- January–December, 2019					
Month	**Species**	**PSC Indoors**	**HLC Indoors**	**HLC Outdoors**	**Total**
January	*An. gambiae* s.l.	43 (100%)	185 (97%)	244 (95%)	472 (96%)
	An. funestus s.l.	0 (0%)	5 (3%)	14 (5%)	19 (4%)
	Sub-total	**43 (100%)**	**190 (100%)**	**258 (100%)**	**491 (100%)**
February	*An. gambiae* s.l.	12 (92%)	93 (96%)	125 (100%)	230 (98%)
	An. funestus s.l.	1 (8%)	4 (4%)	0 (0%)	5 (2%)
	Sub-total	**13 (100%)**	**97 (100%)**	**125 (100%)**	**235 (100%)**
March	*An. gambiae* s.l.	33 (97%)	94 (98%)	85 (100%)	212 (99%)
	An. funestus s.l.	1 (3%)	2 (2%)	0 (0%)	3 (1%)
	Sub-total	**34 (100%)**	**96 (100%)**	**85 (100%)**	**215 (100%)**
April	*An. gambiae* s.l.	75 (96%)	123 (98%)	148 (95%)	346 (96%)
	An. funestus s.l.	3 (4%)	3 (2%)	6 (4%)	12 (3%)
	An. paludis s.l.	0 (0%)	0 (0%)	1 (1%)	1 (0%)
	Sub-total	**78 (100%)**	**126 (100%)**	**155 (100%)**	**359 (100%)**
May	*An. gambiae* s.l.	34 (100%)	92 (97%)	111 (99%)	237 (98%)
	An. funestus s.l.	0 (0%)	3 (3%)	1 (1%)	4 (2%)
	Sub-total	**34 (100%)**	**95 (100%)**	**112 (100%)**	**241 (100%)**
June	*An. gambiae* s.l.	32 (89%)	163 (98%)	246 (96%)	441 (96%)
	An. funestus s.l.	4 (11%)	4 (2%)	10 (4%)	18 (4%)
	Sub-total	**36 (100%)**	**167 (100%)**	**256 (100%)**	**459 (100%)**
July	*An. gambiae* s.l.	87 (97%)	222 (100%)	177 (100%)	486 (99%)
	An. funestus s.l.	3 (3%)	1 (0%)	0 (0%)	4 (1%)
	Sub-total	**90 (100%)**	**223 (100%)**	**177 (100%)**	**490 (100%)**
August	*An. gambiae* s.l.	19 (79%)	233 (98%)	192 (97%)	444 (97%)
	An. funestus s.l.	5 (21%)	5 (2%)	6 (3%)	16 (3%)
	Sub-total	**24 (100%)**	**238 (100%)**	**198 (100%)**	**460 (100%)**
September	*An. gambiae* s.l.	39 (80%)	92 (92%)	69 (82%)	200 (86%)
	An. funestus s.l.	10 (20%)	8 (8%)	15 (18%)	33 (14%)
	Sub-total	**49 (100%)**	**100 (100%)**	**84 (100%)**	**233 (100%)**
October	*An. gambiae* s.l.	11 (73%)	106 (89%)	146 (88%)	263 (88%)
	An. funestus s.l.	4 (27%)	13 (11%)	19 (12%)	36 (12%)
	Sub-total	**15 (100%)**	**119 (100%)**	**165 (100%)**	**299 (100%)**
November	*An. gambiae* s.l.	14 (88%)	113 (90%)	184 (95%)	311 (93%)

	An. funestus s.l.	2 (13%)	12 (10%)	9 (5%)	23 (7%)
	Sub-total	**16 (100%)**	**125 (100%)**	**193 (100%)**	**334 (100%)**
December	*An. gambiae* s.l.	85 (96%)	183 (96%)	173 (97%)	441 (96%)
	An. funestus s.l.	4 (4%)	8 (4%)	4 (4%)	16 (3%)
	An. paludis	0 (0%)	0 (0%)	1 (0%)	1 (0%)
	Sub-total	**89 (100%)**	**191 (100%)**	**178 (100%)**	**458 (100%)**
Kabondo 2019 annual summary		**PSC Indoors**	**HLC Indoors**	**HLC Outdoors**	**Total**
	An. gambiae s.l.	484 (93%)	1,699 (96%)	1,900 (96%)	4,083 (96%)
	An. funestus s.l.	37 (7%)	68 (4%)	84 (4%)	189 (4%)
	An. paludis	0 (0%)	0 (0%)	2 (0%)	2 (0%)
Total *Anopheles*		**521 (100%)**	**1,767 (100%)**	**1,986 (100%)**	**4,274 (100%)**

TABLE A2. MONTHLY SPECIES COMPOSITION AND ABUNDANCE OF *ANOPHELES* COLLECTED IN LODJA BY PSC AND HLC, JANUARY–DECEMBER 2019

Lodja- January–December, 2019					
Month	**Species**	**PSC Indoors**	**HLC Indoors**	**HLC Outdoors**	Total
January	*An. gambiae* s.l.	37 (90%)	77 (41%)	95 (24%)	209 (33%)
	An. funestus s.l.	0 (0%)	1 (1%)	1 (0%)	2 (0%)
	An. paludis	4 (10%)	108 (58%)	305 (76%)	417 (66%)
		41 (100%)	**186 (100%)**	**401 (100%)**	**628 (100%)**
February	*An. gambiae* s.l.	24 (89%)	67 (58%)	168 (46%)	259 (51%)
	An. funestus s.l.	0 (0%)	7 (6%)	3 (1%)	10 (2%)
	An. paludis	3 (11%)	42 (36%)	198 (54%)	243 (47%)
		27 (100%)	**116 (100%)**	**369 (100%)**	**512 (100%)**
March	*An. gambiae* s.l.	21 (100%)	117 (82%)	335 (69%)	473 (73%)
	An. funestus s.l.	0 (0%)	4 (3%)	13 (3%)	17 (3%)
	An. paludis	0 (0%)	21 (15%)	135 (28%)	156 (24%)
		21 (100%)	**142 (100%)**	**483 (100%)**	**646 (100%)**
April	*An. gambiae* s.l.	14 (93%)	55 (60%)	108 (46%)	177 (52%)
	An. funestus s.l.	1 (7%)	0 (0%)	0 (0%)	1 (0%)
	An. paludis	0 (0%)	36 (40%)	125 (54%)	161 (47%)
		15 (100%)	**91 (100%)**	**233 (100%)**	**339 (100%)**
May	*An. gambiae* s.l.	18 (100%)	125 (81%)	287 (76%)	430 (78%)
	An. funestus s.l.	0 (0%)	2 (1%)	2 (1%)	4 (1%)
	An. paludis	0 (0%)	27 (18%)	90 (24%)	117 (21%)
		18 (100%)	**154 (100%)**	**379 (100%)**	**551 (100%)**
June	*An. gambiae* s.l.	23 (100%)	89 (69%)	135 (36%)	247 (47%)
	An. funestus s.l.	0 (0%)	1 (1%)	1 (0%)	2 (0%)
	An. paludis	0 (0%)	39 (30%)	235 (63%)	274 (25%)
		23 (100%)	**129 (100%)**	**371 (100%)**	**523 (100%)**

July	*An. gambiae* s.l.	20 (95%)	62 (74%)	115 (25%)	197 (35%)
	An. funestus s.l.	1 (5%)	2 (2%)	6 (1%)	9 (2%)
	An. paludis	0 (0%)	20 (24%)	331 (73%)	351 (63%)
		21 (100%)	**84 (100%)**	**452 (100%)**	**557 (100%)**
August	*An. gambiae* s.l.	16 (100%)	67 (86%)	103 (36%)	186 (49%)
	An. funestus s.l.	0 (0%)	0 (0%)	2 (1%)	2 (1%)
	An. paludis	0 (0%)	11 (14%)	183 (64%)	194 (51%)
		16 (100%)	**78 (100%)**	**288 (100%)**	**382 (100%)**
September	*An. gambiae* s.l.	29 (100%)	68 (69%)	253 (40%)	350 (46%)
	An. funestus s.l.	0 (0%)	1 (1%)	11 (2%)	12 (2%)
	An. paludis	0 (0%)	30 (30%)	365 (58%)	395 (52%)
		29 (100%)	**99 (100%)**	**629 (100%)**	**757 (100%)**
October	*An. gambiae* s.l.	18 (86%)	62 (76%)	115 (25%)	195 (35%)
	An. funestus s.l.	3 (14%)	2 (0%)	2 (0%)	7 (1%)
	An. paludis	0 (0%)	18 (22%)	334 (74%)	352 (64%)
		21 (100%)	**82 (100%)**	**451 (100%)**	**554 (100%)**
November	*An. gambiae* s.l.	25 (93%)	120 (81%)	307 (66%)	452 (70%)
	An. funestus s.l.	0 (0%)	2 (1%)	2 (0%)	4 (1%)
	An. paludis	2 (7%)	27 (18%)	157 (34%)	186 (29%)
		27 (100%)	**149 (100%)**	**466 (100%)**	**642 (100%)**
December	*An. gambiae* s.l.	23 (100%)	135 (88%)	230 (69%)	388 (76%)
	An. funestus s.l.	0 (0%)	3 (2%)	1 (0%)	4 (1%)
	An. paludis	0 (0%)	15 (10%)	103 (31%)	118 (23%)
		23 (100%)	**153 (100%)**	**334 (100%)**	**510 (100%)**
Lodja 2019 annual summary		**PSC Indoors**	**HLC Indoors**	**HLC Outdoors**	**Total**
	An. gambiae s.l.	268 (95%)	1,044 (71%)	2,251 (46%)	3,563 (54%)
	An. funestus s.l.	5 (2%)	25 (2%)	44 (1%)	74 (1%)
	An. paludis	9 (3%)	394 (27%)	2,561 (53%)	2,964 (45%)
Total *Anopheles*		**282 (100%)**	**1,463 (100%)**	**4,856 (100%)**	**6,601 (100%)**

TABLE A3. MONTHLY SPECIES COMPOSITION AND ABUNDANCE OF *ANOPHELINAE* COLLECTED IN KALEMIE BY PSC AND HLC, JANUARY–DECEMBER 2019

Kalemie- January–December, 2019					
Month	**Species**	**PSC Indoors**	**HLC Indoors**	**HLC Outdoors**	**Total**
January	*An. gambiae* s.l.	33 (83%)	25 (100%)	14 (100%)	72 (91%)
	An. funestus s.l.	7 (18%)	0 (0%)	0 (0%)	7 (9%)
	Sub-total	**40** (100%)	**25** (100%)	**14** (100%)	**79** (100%)
February	*An. gambiae* s.l.	40 (93%)	18 (100%)	13 (100%)	71 (96%)
	An. funestus s.l.	3 (7%)	0 (0%)	0 (0%)	3 (4%)
	Sub-total	**43 (100%)**	**18 (100%)**	**13 (100%)**	**74 (100%)**
March	*An. gambiae* s.l.	50 (83%)	24 (100%)	19 (100%)	93 (90%)
	An. funestus s.l.	10 (17%)	0 (0%)	0 (0%)	10 (10%)
	Sub-total	**60 (100%)**	**24 (100%)**	**19 (100%)**	**103 (100%)**
April	*An. gambiae* s.l.	38 (83%)	22 (100%)	15 (100%)	75 (90%)
	An. funestus s.l.	8 (17%)	0 (0%)	0 (0%)	8 (10%)
	Sub-total	**46 (100%)**	**22 (100%)**	**15 (100%)**	**83 (100%)**
May	*An. gambiae* s.l.	34 (97%)	26 (100%)	5 (100%)	65 (98%)
	An. funestus s.l.	1 (3%)	0 (0%)	0 (0%)	1 (2%)
	Sub-total	**35 (100%)**	**26 (100%)**	**5 (100%)**	**66 (100%)**
June	*An. gambiae* s.l.	18 (82%)	23 (100%)	9 (100%)	50 (93%)
	An. funestus s.l.	4 (18%)	0 (0%)	0 (0%)	4 (7%)
	Sub-total	**22 (100%)**	**23 (100%)**	**9 (100%)**	**54 (100%)**
July	*An. gambiae* s.l.	20 (87%)	16 (100%)	12 (100%)	48 (94%)
	An. funestus s.l.	3 (13%)	0 (0%)	0 (0%)	3 (6%)
	Sub-total	**23 (100%)**	**16 (100%)**	**12 (100%)**	**51 (100%)**
August	*An. gambiae* s.l.	32 (65%)	22 (100%)	13 (100%)	67 (80%)
	An. funestus s.l.	17 (35%)	0 (0%)	0 (0%)	17 (20%)
	Sub-total	**49 (100%)**	**22 (100%)**	**13 (100%)**	**84 (100%)**
September	*An. gambiae* s.l.	21 (47%)	13 (93%)	17 (100%)	51 (67%)
	An. funestus s.l.	24 (53%)	1 (7%)	0 (0%)	25 (33%)
	Sub-total	**45 (100%)**	**14 (100%)**	**17 (100%)**	**76 (100%)**
October	*An. gambiae* s.l.	52 (83%)	15 (100%)	19 (95%)	86 (88%)
	An. funestus s.l.	11 (17%)	0 (0%)	1 (5%)	12 (12%)
	Sub-total	**63 (100%)**	**15 (100%)**	**20 (100%)**	**98 (100%)**
November	*An. gambiae* s.l.	48 (91%)	23 (96%)	4 (80%)	75 (91%)
	An. funestus s.l.	5 (9%)	1 (4%)	1 (20%)	7 (9%)
	Sub-total	**53 (100%)**	**24 (100%)**	**5 (100%)**	**82 (100%)**
December	*An. gambiae* s.l.	142 (85%)	19 (95%)	30 (86%)	191 (86%)
	An. funestus s.l.	25 (15%)	1 (5%)	4 (11%)	30 (14%)
	An. paludis	0 (0%)	0 (0%)	1 (3%)	1 (0%)
	Sub-total	**167 (100%)**	**20 (100%)**	**35 (100%)**	**222 (100%)**

Kalemie 2019 annual summary		PSC Indoors	HLC Indoors	HLC Outdoors	Total
	An. gambiae s.l.	528 (82%)	246 (99%)	170 (96%)	944 (88%)
	An. funestus s.l.	118 (18%)	3 (1%)	6 (3%)	127 (12%)
	An. paludis	0 (0%)	0 (0%)	1 (1%)	1 (0%)
Total *Anopheles*		**646 (100%)**	**249 (100%)**	**177 (100%)**	**1,072 (100%)**

TABLE A4. MONTHLY HBR OF MALARIA VECTORS COLLECTED INDOORS AND OUTDOORS BY HLC IN KABONDO, JANUARY–DECEMBER 2019

Kabondo- January–December 2019															
Species	Location	Variables	Jan	Feb	Mar	Apr	May	Jun	July	Aug	Sep	Oct	Nov	Dec	2019 total
An. gambiae s.l.	Indoor	Total collected	185	93	94	123	92	163	222	233	92	106	113	183	1,699
		Nbr person-nights	8	8	8	8	8	8	8	8	8	8	8	8	96
		HBR/night	**23**	**12**	**12**	**15**	**12**	**20**	**28**	**29**	**12**	**13**	**14**	**23**	**18**
	Outdoor	Total collected	244	125	85	148	111	246	177	192	69	146	184	173	1,900
		Nbr person-nights	8	8	8	8	8	8	8	8	8	8	8	8	96
		HBR/night	**31**	**16**	**11**	**19**	**14**	**31**	**22**	**24**	**9**	**18**	**23**	**22**	**20**
An. funestus s.l.	Indoor	Total collected	5	4	2	3	3	4	1	5	8	13	12	8	68
		Nbr person-nights	8	8	8	8	8	8	8	8	8	8	8	8	96
		HBR/night	**1**	**1**	**0**	**0**	**0**	**1**	**0**	**1**	**1**	**2**	**2**	**1**	**1**
	Outdoor	Total collected	14	0	0	6	1	10	0	6	15	19	9	4	84
		Nbr person-nights	8	8	8	8	8	8	8	8	8	8	8	8	88
		HBR/night	**2**	**0**	**0**	**1**	**0**	**1**	**0**	**1**	**2**	**2**	**1**	**1**	**1**
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	80
		HBR/night	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	Outdoor	Total collected	0	0	0	1	0	0	0	0	0	0	0	1	2
		Nbr person-nights	8	8	8	8	8	8	8	8	8	8	8	8	80
		HBR/night	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**

TABLE A5. MONTHLY HBR OF MALARIA VECTORS COLLECTED INDOORS AND OUTDOORS BY HLC IN LODJA, JANUARY–DECEMBER 2019

Lodja- January–December 2019															
Species	Location	Variables	Jan	Feb	Mar	Apr	May	Jun	July	Aug	Sep	Oct	Nov	Dec	2019 total
An. gambiae s.l.	Indoor	Total collected	77	67	117	55	125	89	62	67	68	62	120	135	1,044
		Nbr person-nights	8	8	8	8	8	8	8	8	8	8	8	8	96
		HBR/night	**10**	**8**	**15**	**7**	**16**	**11**	**8**	**8**	**9**	**8**	**15**	**17**	**11**
	Outdoor	Total collected	95	168	335	108	287	135	115	103	253	115	307	230	2,251
		Nbr person-nights	8	8	8	8	8	8	8	8	8	8	8	8	96
		HBR/night	**12**	**21**	**42**	**14**	**36**	**17**	**14**	**13**	**32**	**14**	**38**	**29**	**23**
An. funestus s.l.	Indoor	Total collected	1	7	4	0	2	1	2	0	1	2	2	3	25
		Nbr person-nights	8	8	8	8	8	8	8	8	8	8	8	8	96
		HBR/night	**0**	**1**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	Outdoor	Total collected	1	3	13	0	2	1	6	2	11	2	2	1	44
		Nbr person-nights	8	8	8	8	8	8	8	8	8	8	8	8	96
		HBR/night	**0**	**0**	**2**	**0**	**0**	**0**	**1**	**0**	**1**	**0**	**0**	**0**	**0**
An. paludis	Indoor	Total collected	108	42	21	36	27	39	20	11	30	18	27	15	394
		Nbr person-nights	8	8	8	8	8	8	8	8	8	8	8	8	96
		HBR/night	**14**	**5**	**3**	**5**	**3**	**5**	**3**	**1**	**4**	**2**	**3**	**2**	**4**
	Outdoor	Total collected	305	198	135	125	90	235	331	183	365	334	157	103	2,561
		Nbr person-nights	8	8	8	8	8	8	8	8	8	8	8	8	96
		HBR/night	**38**	**25**	**17**	**16**	**11**	**29**	**41**	**23**	**46**	**42**	**20**	**13**	**27**

TABLE A6. MONTHLY HBR OF MALARIA VECTORS COLLECTED INDOORS AND OUTDOORS BY HLC IN KALEMIE, JANUARY–DECEMBER 2019

Kalemie- January–December 2019															
Species	Location	Variables	Jan	Feb	Mar	Apr	May	Jun	July	Aug	Sep	Oct	Nov	Dec	2019 total
An. gambiae s.l.	Indoor	Total collected	25	18	24	22	26	23	16	22	13	15	23	19	246
		Nbr person-nights	8	8	8	8	8	8	8	8	8	8	8	8	96
		HBR/night	**3**	**2**	**3**	**3**	**3**	**3**	**2**	**3**	**2**	**2**	**3**	**2**	**3**
	Outdoor	Total collected	14	13	19	15	5	9	12	13	17	19	4	30	170
		Nbr person-nights	8	8	8	8	8	8	8	8	8	8	8	8	96
		HBR/night	**2**	**2**	**2**	**2**	**1**	**1**	**2**	**2**	**2**	**2**	**1**	**4**	**2**
An. funestus s.l.	Indoor	Total collected	0	0	0	0	0	0	0	0	1	0	1	1	3
		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	1	4	6
		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**	**1**	**0**
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	0	0	0	0	0	0	1	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**