USAID
FROM THE AMERICAN PEOPLE

U.S. PRESIDENT'S MALARIA INITIATIVE

U.S. PRESIDENT'S MALARIA INITIATIVE
vectorlink
INTELLIGENT › INNOVATIVE › INTEGRATED

THE PMI VECTORLINK PROJECT CAMEROON

ANNUAL ENTOMOLOGY REPORT

OCTOBER 2020 – SEPTEMBER 2021

Recommended Citation: The President's Malaria Initiative (PMI)/VectorLink Project. *The PMI VectorLink Project Cameroon Annual Entomology Report (October 2019-September 2020).* Rockville, MD. The PMI VectorLink Project, Abt Associates.

Contract: AID-OAA-I-17-00008

Task Order: AID-OAA-TO-17-00027

Submitted to: United States Agency for International Development/PMI

Submitted on: December 23, 2021

Approved on: February 14, 2022

Abt Associates | 6130 Executive Blvd | Rockville, Maryland 20852
T. 301.347.5000
abtassociates.com

THE PMI VECTORLINK PROJECT
CAMEROON
ANNUAL ENTOMOLOGY REPORT
OCTOBER 2020 – SEPTEMBER 2021

CONTENTS

LIST OF TABLES

LIST OF FIGURES

ACRONYMS

BTC	Biotechnology Center
CDC	(U.S.) Centers for Disease Control and Prevention
CRID	Center for Research in Infectious Diseases
EIR	Entomological Inoculation Rate
ELISA	Enzyme-Linked Immunosorbent Assay
HBI	Human Blood Index
HBR	Human Biting Rate
HLC	Human Landing Catch
ITN	Insecticide-Treated Net
kdr	Knock Down Resistance
LT	Light Trap
NMCP	National Malaria Control Program
OCEAC	Organization for the Coordination of Endemic Diseases Control in Central Africa
PBO	Piperonyl Butoxide
PCR	Polymerase Chain Reaction
PMI	(U.S.) President's Malaria Initiative
PSC	Pyrethrum Spray Catch
RFLP	Restriction Fragment Length Polymorphism
SOP	Standard Operating Procedure
USAID	United States Agency for International Development
WHO	World Health Organization

EXECUTIVE SUMMARY

From October 2020 to September 2021, the U.S. President's Malaria Initiative (PMI) VectorLink Project conducted malaria vector surveillance in five sentinel sites (Gounougou, Simatou, Mangoum, Nyabessang, Bonabéri) and insecticide resistance monitoring in 10 additional sites (Bertoua, Djohong, Garoua, Gazawa, Mada, Mogode, Ndelele, Ngaoundere, Njombe, and Touboro) in Cameroon. In the five longitudinal monitoring sites, adult mosquitos were collected monthly from October 2020 to April 2021 and every other month from April to September 2021, for a total of 10 collection efforts at each site during the reporting period. Susceptibility testing was conducted once in either August or September 2021 in the insecticide monitoring sites.

Four collection methods were used: human landing catches (HLCs), pyrethrum spray catches (PSCs), U.S. Centers for Disease Control and Prevention (CDC) Light Traps (LTs) and Prokopack/mouth aspirators. HLCs, PSCs, and CDC LTs were used to collect adult mosquitoes in households indoors and outdoors and assess vector composition, human biting rate (HBR), endophagic index, indoor resting density, parity rate, human blood index (HBI), infection rate, and entomological inoculation rate (EIR). The team began using Prokopack/mouth aspirators in July 2021 to conduct outdoor collections to assess species composition and mosquito resting behavior at the five sentinel sites. In addition, insecticide susceptibility was performed at the 10 sites using pyrethroid (alpha-cypermethrin, deltamethrin, and permethrin), organophosphate (pirimiphos-methyl), carbamate (bendiocarb), neonicotinoid (clothianidin) and pyrrole (chlorfenapyr) insecticides. When pyrethroid resistance was confirmed, intensity of resistance, and synergist assays with piperonyl butoxide (PBO) were conducted.

A total of 12 *Anopheles* species and species groups were recorded across all collection methods and sites (*An. gambiae* s.l., *An. funestus* s.l., *An. pharoensis*, *An. paludis*, *An. moucheti*, *An. demeilloni*, *An. ziemanni*, *An. nili*, *An. marshallii*, *An. multicinctus*, *An. rufipes, An. squamosus*)—seven of which are involved in malaria transmission (*An. gambiae* s.l., *An. funestus* s.l., *An. nili*, *An. moucheti*, *An. pharoensis*, *An. ziemanni*, and *An. paludis).* Further identification of the *An. gambiae* complex and *An. funestus* group using polymerase chain reaction (PCR) revealed the presence of three *An. gambiae* complex species: *An. gambiae* s.s. (23.1%), *An. coluzzii* (71.2%), and *An. arabiensis* (5.6%). Two species of the *An. funestus* group were identified in Gounougou: *An. funestus* s.s. (94.0%) and *An. leesoni* (6.0%) while *An. funestus* s.s. (100%) was found in Simatou.

The mean HBR, estimated by HLCs, of *An. gambiae* s.l. ranged from 1.2 bites/person/night (b/p/n) in Nyabessang, where seasonal rainfall and farming create temporary breeding sites, to 25.4 b/p/n in Simatou, where widespread rice cultivation enables permanent suitable vector breeding habitats. Early morning biting was observed for *An. gambiae* s.l., particularly in the Northern sites of Gounougou and Simatou where biting occurred until 8 a.m. Monthly indoor resting density, estimated by PSCs, of *Anopheles* varied across sites from 0.0 (multiple sites and months) to 119.0 females/room/night (Simatou in July 2021), while the mean parity rate across sites was 75.0% for *An. gambiae* s.l. and 91.6% for *An. funestus* s.l. The HBI of *An. gambiae* s.l. was 65.0% in Gounougou and 59.3% in Simatou. Fewer *An. gambiae* s.l. were tested from Mangoum, Nyabessang, and Bonabéri than Gounougou and Simatou, but all three sites recorded an HBI of 100%. The endophagic indexes of *An. gambiae* s.l. were 0.49 in Gounougou and 0.51 in Simatou, indicating that *An. gambiae* s.l. bite equally indoors and outdoors in these two sites. Endophagic indexes recorded in Mangoum and Bonabéri (0.46 and 0.31, respectively) suggest that *An. gambiae* s.l. bite more outdoors in these two sites. The mean monthly EIR was 20.5 infected bites/person/month (ib/p/m) in Gounougou, 8.8 ib/p/m in Simatou, 26.4 ib/p/m in Mangoum, 13.8 ib/p/m in Nyabessang, and 9.7 ib/p/m in Bonabéri.

Resistance of *An. gambiae* s.l. to the diagnostic dose of all pyrethroids was recorded in all 10 sites tested. Resistance to bendiocarb was observed in six sites (Bertoua, Djohong, Garoua, Ndelele, Ngaoundere, and Njombe) and to pirimiphos-methyl in seven of the sites. Susceptibility to pirimiphos-methyl was not observed

in any of the sites. High pyrethroid resistance was observed at all sites and across the three pyrethroids tested except at Bertoua, where moderate permethrin resistance was found. Pre-exposure of mosquitoes to PBO substantially increased the mortality of *An. gambiae* s.l. but did not restore full susceptibility in most sites surveyed, except in Bertoua and Mogode with deltamethrin.

In all 10 sites, *An. gambiae* s.l. were susceptible to clothianidin (2%) tested using WHO test kits; six sites recorded susceptibility to clothianidin (4 µg/bottle) using CDC bottle assay. Susceptibility to chlorfenapyr (200 µg/bottle) was recorded at all sites except Ngaoundere. Furthermore, target site knockdown resistance (*Kdr*) west and east (*kdr*-e and *kdr*-w), *Ace*-1, and N1575Y were found to be involved in the insecticide resistance of the vectors of the different sites. Additional resistance markers (CYP6P5, CY6M7, CYP6P9a, and CYP6P9b) were found within *An. funestus* s.l. from Touboro.

These entomological monitoring data will guide Cameroon's National Malaria Control Program (NMCP) on options for vector control tool selection during the implementation of the insecticide resistance management plan (IRMP) and with the targeted distribution of PBO insecticide-treated nets (ITNs) and dual active ingredient ITNs in the country.

1. INTRODUCTION

Malaria remains a public health problem in Cameroon and is one of the main causes of morbidity and mortality with nearly three million cases and 4,121 deaths recorded by health facilities in 2020 [National Malaria Control Program (NMCP), 2019]. Cameroon is among 10 countries in sub-Saharan Africa with the highest burden of malaria; it accounted for approximately 4% of global malaria cases in 2017 (WHO, 2018). Children under five years of age are disproportionately at risk, accounting for around 32% of malaria cases and 64% of deaths. The morbidity among pregnant women increased from 12.7% in 2013 to 22.5% in 2020 (NSP 2019-2023, NMCP, 2021). In an effort to reduce the malaria burden in the country, the Ministry of Public Health and its partners are implementing high-impact interventions, including i) the free distribution of insecticide-treated nets (ITNs) during national mass campaigns and antenatal consultations to pregnant women, ii) intermittent preventive treatment to pregnant women during antenatal consultations, iii) seasonal chemoprophylaxis of malaria in children between three and 59 months, and iv) free treatment of uncomplicated and severe malaria in children under five years old. Cameroon's National Strategic Plan 2019-2023 includes plans to expand continuous distribution of ITNs to pregnant women and children through the Expanded Programme for Immunization and preschool consultations, but these have yet to be operationalized.

In September 2017, Abt Associates was awarded the five-year U.S. President's Malaria Initiative (PMI) VectorLink Project to conduct entomological surveillance in Cameroon. Since October 2018, PMI VectorLink Cameroon has carried out entomological monitoring including vector surveillance and insecticide resistance monitoring in five sentinel sites located in various regions and representing different ecologies in the country. In 2021, the project shifted insecticide resistance monitoring to 10 new sites in order to generate data needed for the strategic deployment of vector control interventions. VectorLink supports the NMCP and three local research institutions—the Biotechnology Center (BTC), the Center for Research in Infectious Diseases (CRID), and the Organization for the Coordination of Endemic Diseases Control in Central Africa (OCEAC)—to conduct longitudinal surveillance and insecticide resistance monitoring.

2. METHODS

2.1 STUDY SITES

From October 2020 to September 2021, VectorLink Cameroon conducted entomological vector surveillance using human landing catches (HLCs), pyrethrum spray catches (PSCs), U.S. Centers for Disease Control and Prevention (CDC) light traps (LTs), and Prokopack and mouth aspirators for outdoor resting collections in five sentinel sites—Gounougou, Simatou, Mangoum, Nyabessang, and Bonabéri (where entomological vector surveillance has been conducted since 2018)—and insecticide resistance monitoring in 10 newly selected sites (Bertoua, Djohong, Garoua, Gazawa, Mada, Mogode, Ndelele, Ngaoundere, Njombe, and Touboro) (Figure 1 and Table 1).

In all five vector surveillance sites, adult mosquito collections were conducted every month from October 2020 to April 2021 and every other month from May 2021 to September 2021, for a total of 10 collection efforts during the reporting period. Insecticide resistance monitoring was conducted once per site either in August or September 2021 at the 10 sites, coinciding with the rainy season.

The ecology varies greatly by site. Gounougou and Simatou are in the dry savannah and Sahelian zones of the North and Far North regions, Mangoum is in the wet savannah zone of the West region, Nyabessang is in the rainforest area of the South region, and Bonabéri is in the coastal zone of the Littoral region. The insecticide resistance monitoring sites selected across the country range from the forest, wet and humid Littoral regions to the dry Far North regions.

Figure 1: Map of VectorLink Cameroon Sentinel Sites

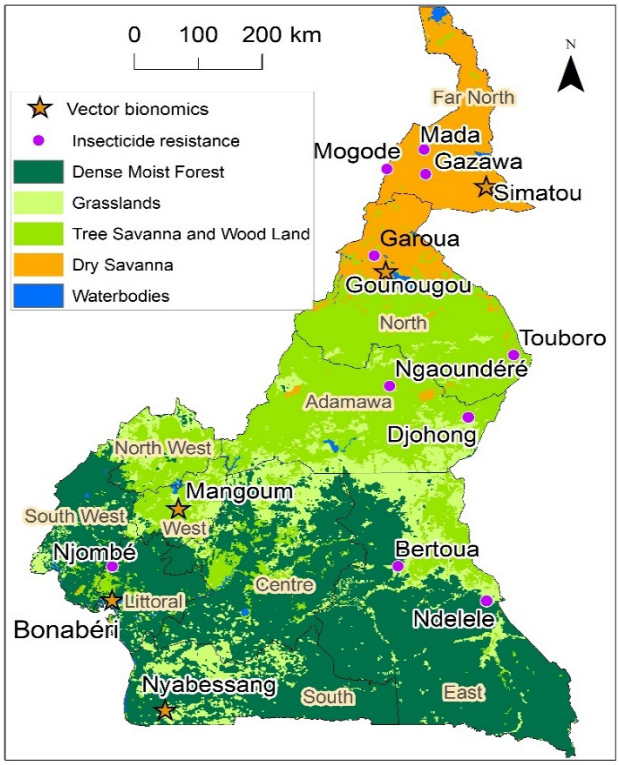


Table 1: Districts and Sites for Entomological Monitoring

Region	District	Site	HLC	CDC LTs	PSC	Prokopack/ Mouth Aspiration	Insecticide Susceptibility Testing
Far North	Maga	Simatou	×	×	×	×	
	Mogode	Mogode					×
	Gazawa	Gazawa					×
	Mada	Mada					×
North	Lagdo	Gounougou	×	×	×	×	
	Garoua	Garoua					×
	Touboro	Touboro					×
Adamawa	Ngaoundere	Ngaoundere					×
	Tibati	Djohong					×
East	Bertoua	Bertoua					×
	Yokadouma	Ndelele					×
Littoral	Bonassama	Bonabéri	×	×	×	×	
	Loum	Njombe					×
South	Ambam	Nyabessang	×	×	×	×	
West	Foumbot	Mangoum	×	×	×	×	

2.2 LONGITUDINAL MONITORING OF MALARIA VECTORS

VectorLink Cameroon collected adult mosquitoes using HLCs, PSCs, and CDC LTs in all sentinel sites following the PMI VectorLink Standard Operating Procedures (SOPs)[1]. For each collection method, the same houses were used each month for collections and 10 collection efforts were completed in each site from October 2020 to September 2021. In addition, in July 2021, the team began using Prokopack and mouth aspirators for outdoor resting collections in the five sentinel sites. A total of two collection efforts (July and September 2021) were done during the reporting period.

Table 2 provides additional information on mosquito collection methods used and Table 3 summarizes the indicators calculated based on the number of mosquitoes captured through each collection method.

Table 2: Adult Mosquito Collection Methods for Vector Surveillance

Collection Method	Time	Collection Location	Frequency	Sample
HLCs	6:00 p.m.–8:00 a.m.	Indoors and outdoors	Two nights per site	Three houses per site (same houses every month)
PSCs	6:00 a.m.–8:00 a.m.	Indoors	Two days per site	Twenty houses per site (the same houses most of the time)
CDC LTs	6:00 p.m.–6:00 a.m.	Indoors (baited) and outdoors (no bait)	Two nights per site	Four houses per site (same houses every month)
Prokopack/Mouth Aspiration	6:00 a.m.–8:00 a.m.	Outdoors	Two days per site	Three shelters[2] per site (same shelters every month)

[1]Complete SOPs can be found here: https://pmivectorlink.org/resources/tools-and-innovations/
[2] Sampled shelters included tree holes, cow pens, clay pots, and uninhabited houses.

Table 3: Vector Surveillance Indicators by Collection Method

Collection Method	Indicator	Definition
HLC	Human Biting Rate	Mean number of bites per person per night
	Peak biting time	Hour of highest human biting rate
	Parity Rate	Percentage of parous mosquitoes / total dissected
	Exophagic Rate	Proportion of mosquitoes biting outdoors
	Endophagic Rate	Proportion of mosquitoes biting indoors
PSC	Indoor Resting Density	Mean number of mosquitoes per room per day
	% of fed females	Number of fed mosquitoes / total collected by PSC
	Human Blood Index	Number of female mosquitoes that have taken human bloodmeal / total female mosquitoes with bloodmeal
CDC LT	Indoor/Outdoor Density	Mean number of mosquitoes collected indoors or outdoors per trap per night
	Parity Rate	Percentage of parous mosquitoes / total dissected
Prokopack/Mouth Aspiration	% of fed females	Number of fed female mosquitoes / total collected by Prokopack/Mouth Aspirator
	Human Blood Index	Number of female mosquitoes that have taken human bloodmeal / total female mosquitoes with bloodmeal

2.2.1 Human Landing Catches

HLCs were performed indoors and outdoors from 6:00 p.m. to 8:00 a.m. in three houses for two consecutive nights per collection effort to collect adult mosquitoes landing on human baits. Four collectors were used (two indoors and two outdoors) from October 2020 to April 2021 before switching to one collector indoors and one outdoors between May and September 2021. The impetus for the change was to standardize methods across PMI VectorLink country programs. With legs exposed to attract host-seeking mosquitoes, the human baits serving as mosquito collectors were seated about 1.5-2 meters from each other indoors and outdoors when there were two in each position. The two teams of collectors worked in two shifts—6:00 p.m. to 12:00 a.m. and 12:00 a.m. to 8:00 a.m. The collectors swapped positions (indoor and outdoor) every hour. The doors of the houses were kept closed when collections were underway. The collectors used flashlights and hemolysis tubes to collect mosquitoes that landed on their legs before the mosquitoes could bite. The tubes were covered with cotton after individual collection of mosquitoes. The teams transferred the mosquitoes hourly to custom-made bags for a total of 14 hours. Mosquitoes collected were then identified morphologically.

2.2.2 Pyrethrum Spray Catches

PSCs were carried out during morning hours, between 6:00 a.m. and 8:00 a.m. for two consecutive days in 20 sleeping rooms in 20 different houses. White cloth sheets were placed on the floor from wall to wall in sampled rooms. After closing the windows and doors and covering or removing drinking water and food items, the rooms were sprayed with a commercial pyrethroid + piperonyl butoxide (PBO) insecticide. For houses with open eaves, collectors sprayed from the outside through the eaves before entering and spraying indoors. Ten minutes after spraying, all mosquitoes knocked down by the insecticide were collected using the white sheets. The mosquitoes were kept in petri dishes and then sorted by species using an identification key (Coetzee, 2020). The abdominal status of all female *Anopheles* was determined, and individuals were sorted into four categories

(unfed, blood-fed, half-gravid, and gravid) and kept individually in labeled Eppendorf tubes containing silica gel for blood meal analysis to calculate HBI.

2.2.3 CDC Light Traps

CDC LTs (one indoors and one outdoors) were installed for two consecutive nights in four houses (eight traps per night) in each site at each collection period between 6:00 p.m. and 6:00 a.m. Both indoor and outdoor traps were suspended 1.5 meters above the ground. Indoors, the trap was installed near the feet of the sleeper in a bedroom used for sleeping by at least one household member and with at least one treated mosquito net in use (typically originated by the different ITN distribution channels). Outdoors, the trap was set un-baited about 5-10 meters from the house of collection. Two volunteers were recruited to check on the traps hourly during collection nights to ensure the trap is functioning. The next morning, collected *Anopheles* were identified and the ovaries of subsamples of unfed *Anopheles* that were still alive were dissected.

2.2.4 Prokopack and Mouth Aspirators

Outdoor collections were carried out using Prokopack or mouth aspirators from 6 a.m. to 8 a.m. (two days per collection effort) in three shelters (such as tree holes, abandoned houses, clay pots, or cow pens) per sentinel site. Mosquitoes were subsequently identified, labelled, and preserved individually in Eppendorf tubes containing silica gel according to their state of repletion.

2.2.5 Identification of Malaria Vectors

All mosquitoes were identified morphologically using identification keys (Coetzee, 2020). All *Anopheles* specimens collected were labelled and stored individually over silica gel in Eppendorf tubes for further processing. Subsamples of monthly collected mosquitoes were sent to CRID for molecular analysis.

2.2.6 PCR Identification of Members of *An. gambiae* Complex and *An. funestus* Group

Polymerase chain reaction (PCR) assays were carried out on mosquito samples collected to identify members of the *An. gambiae* complex and *An. funestus* group at CRID, Yaoundé. PCR was conducted on approximately 100 *An. gambiae* s.l. and 25 *An. funestus* s.l. per month using the *An. gambiae* species-specific single interspersed element PCR (Santolamazza *et al.* 2008). In coastal sites where other species, such as *An. melas* are present, the team used the PCR-Restriction Fragment Length Polymorphism (RFLP) protocol described by Fanello *et al.*, 2002. Mosquitoes belonging to the *An. funestus* group were determined using a multiplex PCR with addition of the *An. rivulorum-like* primers. gDNA from 17 randomly selected *An. funestus* s.l. mosquitoes were processed per each location period per month as described by Koekemoer *et al,* 2002. All PCR products were run via electrophoresis through a 1.5% agarose gel with Midori Green® (Gene flow, UK) and visualized using ultraviolet light.

2.2.7 Blood Meal Assays

The source of the blood contained in the abdomen of resting mosquitoes collected by indoor PSCs and outdoor Prokopack collections was determined using direct Enzyme-linked Immunosorbent Assays (ELISAs) as described by Beier *et al*, 1988. This technique simultaneously allows the identification of human, cow, pig chicken, pig, horse, and dog blood and all of these target species were used in the analysis. Peroxidase conjugated anti-bodies, as well as animal heterologous serum, were obtained from Sigma (www.sigmaaldrich.com). After manipulation, absorbance at 414 nm were determined with an ELISA plate reader. Samples were considered positive if absorbance values exceeded the mean plus three times the standard deviation of four negative controls, represented by unfed mosquitoes.

2.2.8 Determination of Parity Rate

To determine parity rate, the team dissected ovaries about 20% of the total of randomly selected hourly, unfed, female *Anopheles* collected using HLCs (indoors and outdoors) and CDC LTs. Ovary dissection was done each

month for seven months and every other month for the remaining three months. The ovary status of the dissected mosquitoes was determined following the methods described by Detinova 1962, Detinova and Gillies 1964. All *Anopheles* and the carcasses of the dissected *Anopheles* were individually stored in labeled Eppendorf tubes containing silica gel. Mean parity rate was determined by dividing the number of parous females by the total number dissected and confirmed by observing the degree of coiling by the ovarian tracheoles [World Health Organization (WHO), 2013].

2.2.9 *Plasmodium* Sporozoite Detection

To estimate the *Plasmodium* infection rate in the mosquito population, CRID performed ELISAs for sporozoite antigen on a proportion of randomly-selected mosquitoes collected from the field through HLC method. An ELISA-circumsporozoite protein method described by Burkot *et al.* (1984), and modified by Wirtz *et al.* (1987), was used for sporozoite detection in the head and thorax of mosquitoes. This method uses a monoclonal antibody that recognizes a repetitive epitope on the circumsporozoite protein of *P. falciparum*. *Plasmodium falciparum* sporozoite ELISA reagent kits (MRA-890) were obtained from BEI Resources (NIAID, NIH, USA). Lyophilized *P. falciparum* monoclonal antibody was reconstituted prior to utilization using glycerol-water solution to achieve a final concentration of 0.5 mg/ml. Similarly, all reagents including phenol red, 1X Phosphate Buffered Saline, Blocking Buffer, grinding buffer, 1X Phosphate Buffered Saline-Tween wash solution were prepared before starting the manipulation, following the product information sheet provided with the MR4-890 kit. Diluted *P. falciparum* sporozoite proteins supplied by CDC (Atlanta, USA) were used as positive controls, while ground male mosquitoes were used as negative controls. Determination of positive samples was done after reading optical densities at 405 nm on an ELISA plate reader (Biotek ELx800, Swindon, UK). Positive samples were determined by optical density readings two times greater than the negative controls and were tested a second time for validation. In addition, since May 2021, all positive samples were boiled and retested for confirmation and to detect false positives.

2.3 Insecticide Resistance Monitoring

2.3.1 Susceptibility Tests of *An. gambiae* s.l.

From August and September 2021, the team completed insecticide resistance monitoring in 10 sites (Bertoua, Djohong, Garoua, Gazawa, Mada, Mogode, Ndelele, Ngaoundere, Njombe, and Touboro). *An. gambiae* s.l. larvae and pupae were collected at each site from different larval habitats, pooled, and reared to adulthood in the field laboratory. Insecticide susceptibility tests were conducted on two- to five-day old adult females using WHO tube tests. CDC bottle assays were used to test the susceptibility to chlorfenapyr and clothianidin. For each WHO susceptibility test and CDC bottle assay, two control groups of 20-25 female *An. gambiae* s.l. were used and tested similarly using paper impregnated with either silicone oil for pyrethroid or olive oil for organophosphate/carbamate controls for the WHO tube test. Bottles coated with acetone alone or acetone + Mero were used for the CDC bottle assays for chlorfenapyr and clothianidin bottle tests, respectively.

The diagnostic concentrations of permethrin (0.75%), deltamethrin (0.05%), alpha-cypermethrin (0.05%), bendiocarb (0.1%), and pirimiphos-methyl (0.25%) were tested in all sites. Resistance was defined following the WHO criteria, with less than 90% mortality indicating confirmed resistance, between 90-97% mortality indicating possible resistance, and greater than 98% indicating susceptibility. When insecticide resistance was confirmed, resistance intensity (high, moderate, and low) was also tested at five- and 10-times the diagnostic concentration of permethrin, deltamethrin, and alpha-cypermethrin.

Synergist assays with PBO were conducted for deltamethrin, permethrin, and alpha-cypermethrin according to the WHO tube test protocol to determine the involvement of P450s in pyrethroid resistance. A high percentage mortality and/or reversal of susceptibility using PBO indicated probable involvement of enzyme activities such as P450s in this insecticide resistance mechanism.

Clothianidin-impregnated papers were treated locally at the dose of 2% using a protocol designed by VectorLink and the susceptibility testing was done as described above with a seven-day delay mortality recording. CDC bottles were treated with chlorfenapyr (100 µg/bottle and 200 µg/bottle) while clothianidin was coated at 4 µg/bottle. The mosquitoes were exposed for one hour and the mortality was recorded up to three days for chlorfenapyr and 24 hours for clothianidin. All tests, paper impregnation, and coating of bottles were conducted following PMI VectorLink SOPs.

2.3.2 Detection of Resistance Mechanisms

After exposure to insecticides, a subsample of 100 randomly selected mosquitoes per site (dead and alive) were morphologically identified and resistance mechanisms determined using PCR.

2.3.2.1 Target Site Resistance Mechanisms

Anopheles gambiae s.l. mosquitoes were examined for the presence of sodium channel mutations *kdr* alleles "west" and "east" (L1014F and L1014S) using relevant PCR protocols described by Martinez-Torres *et al.*, 1998 and Ranson *et al.,* 2000 already optimized by CRID. The different amplicons were run on a 2% agarose gel and visualized under UV light, allowing the definition of the genetic profile of each mosquito sample (*kdr-w*, *kdr-e* or no *kdr*) from the size of the amplicons observed. The presence of the additional *kdr* allele N1575I (shown to increase resistance in the presence of L1014F in *An. gambiae* s.l.) were monitored using a TaqMan assay (Jones *et al.*, 2012). To assess the direct involvement of *kdr* in pyrethroid/DDT resistance, the team genotyped a set of mosquitoes (50 dead and 50 alive) tested for susceptibility.

The *Ace*-1 gene mutation was detected by PCR using the protocol of Weill *et al.,* 2004. Extracted DNA was amplified by PCR with Ex3AGdir and Ex3AGrev oligonucleotide primers. The PCR amplification products were analyzed by electrophoresis onto a 2% agarose gel and visualized under UV light. The two primers produced a 403 bp fragment, which is undigested by AluI for susceptible homozygous mosquitoes (SS) and cut into two fragments (253 bp and 150 bp) for homozygous resistant (RR). Heterozygous individuals (RS) display a combined pattern.

2.3.2.2 Metabolic Resistance Enzyme Detection

The gene expression patterns of key detoxification genes which have previously been detected as over-expressed in populations of malaria vectors in Cameroon were assessed for *An. funestus* s.l. Five cytochrome P450s and one GST genes were assessed using qRT-PCR. The selected genes were CYP6P9a, CYP6P9b, CYP6M7, CYP325A, CYP6P5, and GSTe2 (Riveron *et al.*, 2014). RNA was extracted from three biological replicates (pool of 15 specimens) of resistant *Anopheles* mosquitoes (R) and that of control unexposed (C), and the fully susceptible laboratory strain of the respective species (S) (*An. funestus* s.s.). The relative expression and fold change of each target gene in R and C relative to S was calculated according to the 2-ΔΔCT method, incorporating PCR efficiency after normalization with the housekeeping genes RSP7 (ribosomal protein S7, AGAP010592).

3. RESULTS

3.1 LONGITUDINAL MONITORING

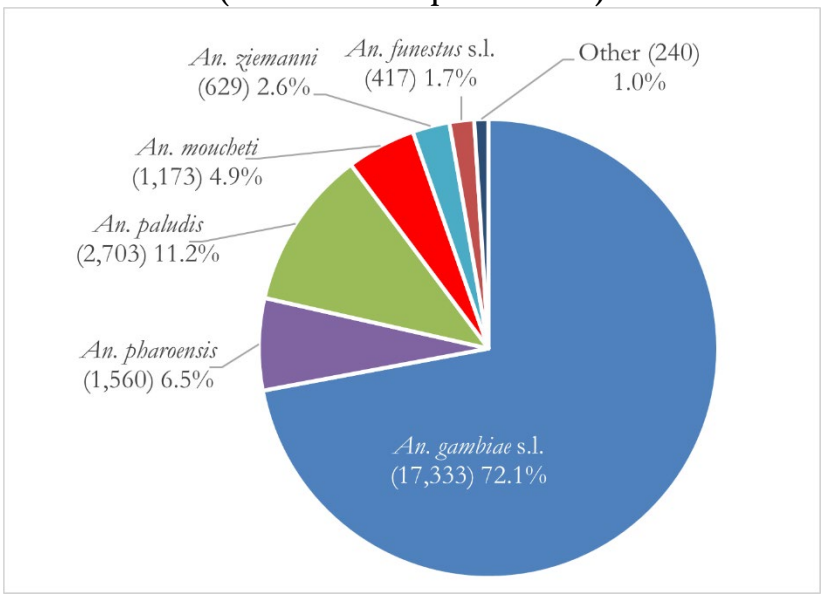
From October 2020 to September 2021, VectorLink Cameroon collected 40,729 *Anopheles* mosquitoes across five sentinel sites (Gounougou, Simatou, Mangoum, Nyabessang, and Bonabéri) using all four collection methods.

3.1.1 SPECIES COMPOSITION OF MOSQUITOES COLLECTED BY HLCS, CDC LTS, PSCS, AND PROKOPACK ACROSS ALL SITES

A total of 24,055 *Anopheles* mosquitoes were collected by HLCs alone across the five sentinel sites. Twelve species were identified, with *An. gambiae* s.l. being the predominant species (72.1%), followed by *An. paludis* (11.2%), *An. pharoensis* (6.5%), and *An. moucheti* (4.9%) (Figure 2 and Table A1 in Annex A). *An. gambiae* s.l. were collected by HLCs at all five sentinel sites while *An. moucheti* and *An. nili* were only found at Nyabessang, which is surrounded by large rivers that offer suitable breeding sites for these two species.

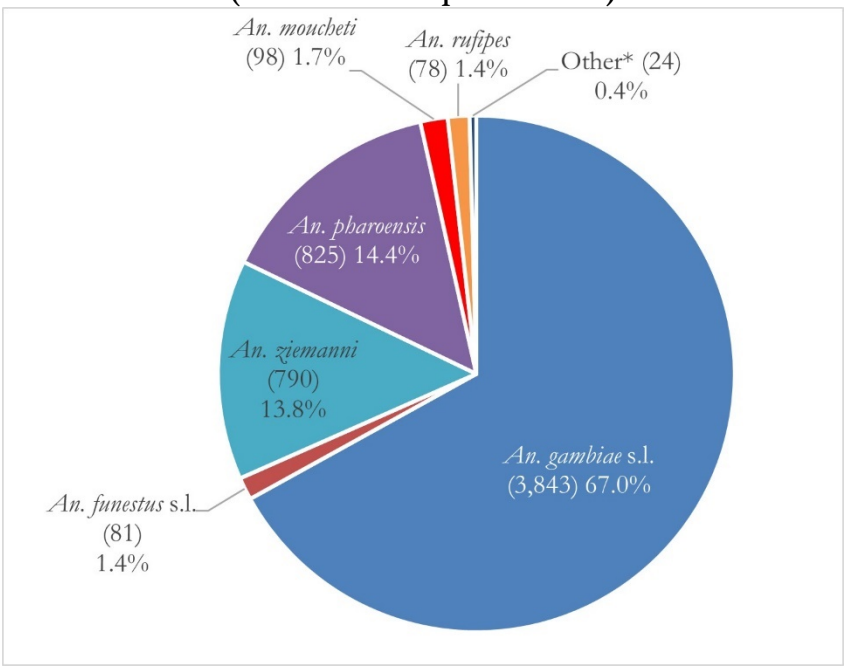
A total of 5,739 mosquitoes, including 3,843 *Anopheles* mosquitoes belonging to nine species, were caught using CDC LTs. *An. gambiae* s.l. (67.0%), *An. ziemanni* (13.8%), and *An. pharoensis* (14.4%) were the most abundant (Figure 3 and Table A2, Annex A). PSC collections across the five sites yielded a total of 9,930 *Anopheles* mosquitoes belonging to nine different species. *An. gambiae* s.l. (92.7%) and *An. funestus* s.l. (5.3%) were the predominant species collected (Figure 4 and Table A3, Annex A). Using Prokopack/mouth aspirators, teams collected 1,005 *Anopheles* mosquitoes belonging to six different species. *An. gambiae* s.l. (97.2%) was the predominant species collected (Figure 5 and Table A4, Annex A).

Figure 2: Species Composition of *Anopheles* Mosquitoes Collected Across All Sites Using HLCs (October 2020-September 2021)



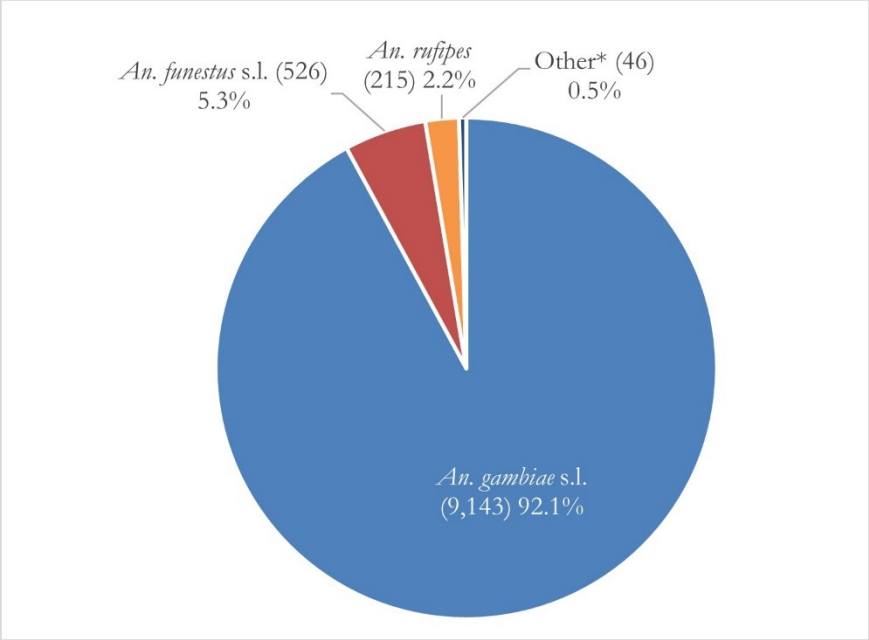
Other: An. rufipes* (29) *An. nili* (136), *An. marshallii* (26), *An. multicinctus* (34), *An. demeilloni* (10), and *An. squamosus* (5).

Figure 3: Species Composition of *Anopheles* Mosquitoes Collected Across All Sites Using CDC LTs (October 2020-September 2021)



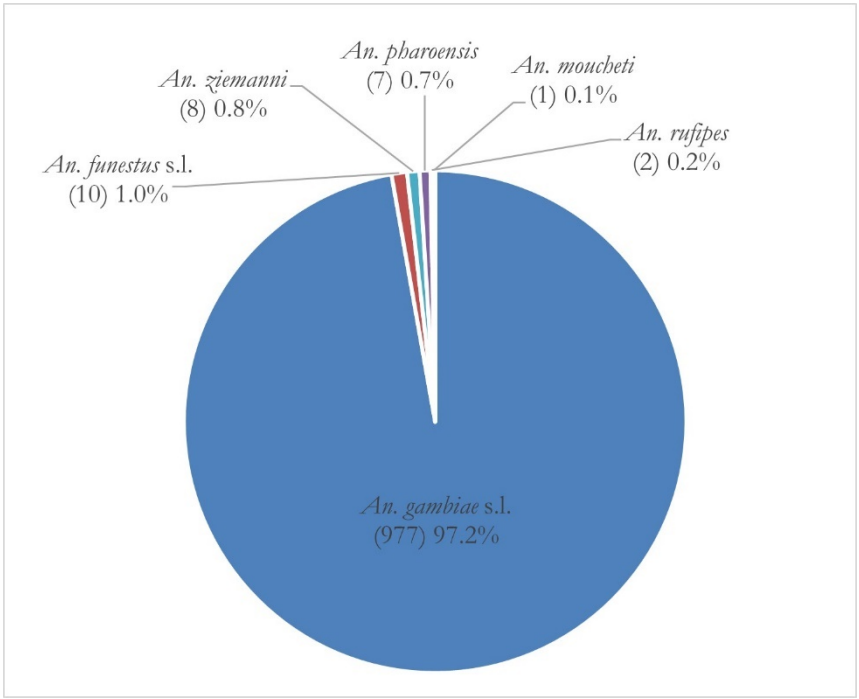
*Other: *An. paludis* (19), *An. nili* (3), and *An. multicinctus* (2).

Figure 4: Species Composition of *Anopheles* Mosquitoes Collected Across All Sites Using PSCs (October 2020-September 2021)



*Other: *An. moucheti* (19), *An. paludis* (9), *An. squamosus* (5), *An. pharoensis* (4), *An. multicinctus* (7), and *An. ziemanni* (2).

Figure 5: Species Composition of *Anopheles* Mosquitoes Collected Across All Sites Using Prokopack and Mouth Aspirators (July and September 2021)



3.1.2 Species Composition of Mosquitoes Collected by HLCs, CDC LTs, PSCs, and Prokopack by Site

3.1.2.1 Gounougou

In Gounougou, 5,275 *Anopheles* mosquitos were collected using HLCs. *An. gambiae* s.l. (89.8%) was the most abundant (Figure 6). *An. gambiae* s.l. constituted 90.8% of those collected by CDC LTs, 86.3% of those collected by PSCs, and 95.8% of those collected using Prokopack (Figures 7, 8, and 9).

Figure 6: Species Composition of *Anopheles* Mosquitoes Collected in Gounougou Using HLCs

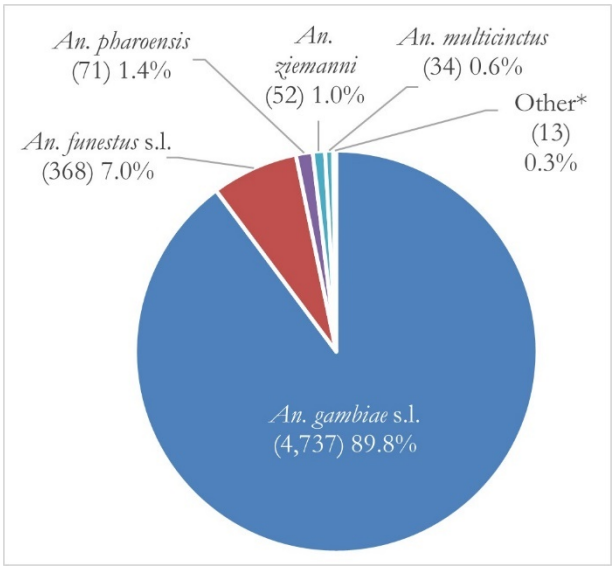
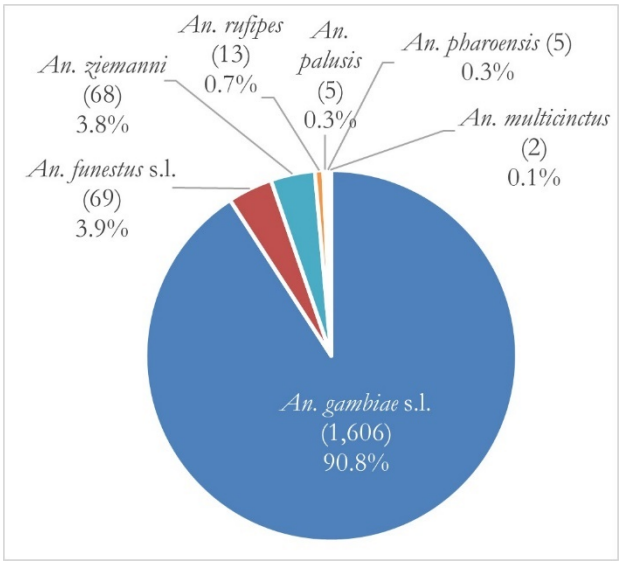


Figure 7: Species Composition of *Anopheles* Mosquitoes Collected in Gounougou Using CDC LTs



*Other: *An. paludis* (7), *An. squamosus* (5), and *An. rufipes* (1).

Figure 8: Species Composition of *Anopheles* Mosquitoes Collected in Gounougou Using PSCs

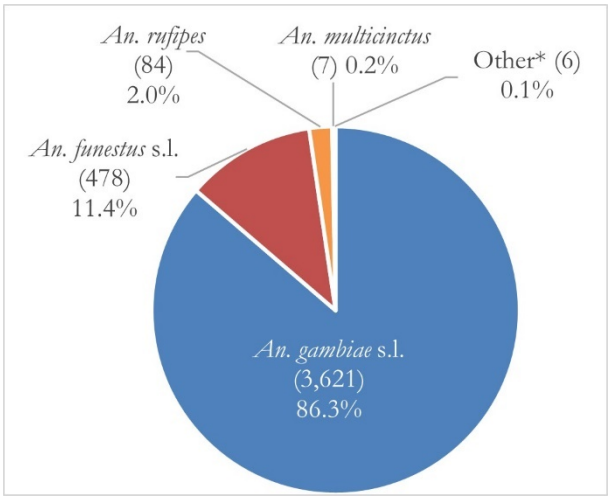
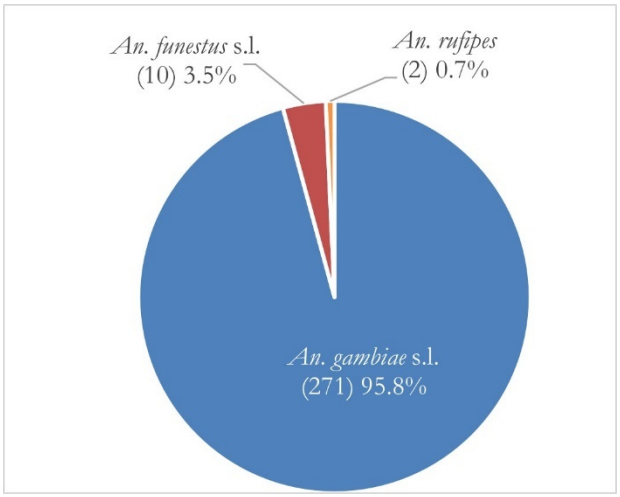


Figure 9: Species Composition of *Anopheles* Mosquitoes Collected in Gounougou Using Prokopack



*Other: *An. squamosus* (5) and *An. ziemanni* (1).

3.1.2.2 SIMATOU

In Simatou, *An. gambiae* s.l. represented 71.3% (5,174) of the 7,260 total *Anopheles* species collected using HLCs from October 2020 to September 2021. *An. pharoensis* (1489, 20.5%) were also collected (Figure 10). A total of 2,513, 5,238, and 719 *Anopheles* mosquitos were collected in Simatou through CDC LTs, PSCs, and Prokopack, respectively. For the three methods, *An. gambiae* s.l. was the main vector collected, representing 36.0% (n=905) of the total vectors collected for CDC LTs, 96.5% (n=5,054) for PSCs, and 97.9% (n=704) for Prokopack (Figures 11, 12 and 13).

Figure 10: Species Composition of *Anopheles* Mosquitoes Collected in Simatou Using HLCs

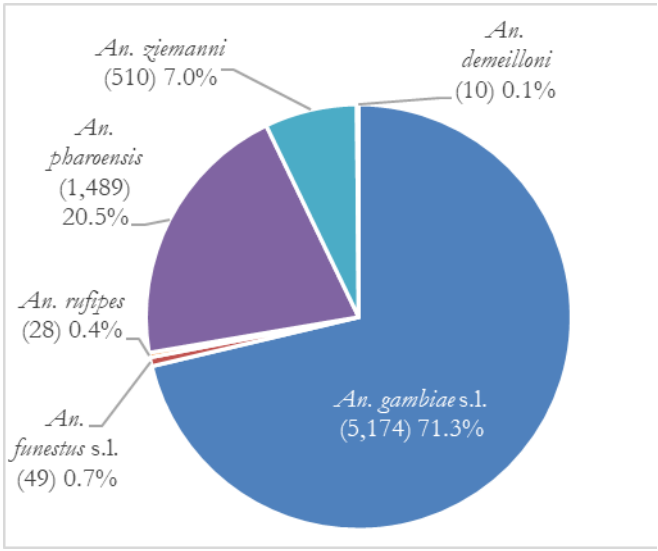


Figure 11: Species Composition of *Anopheles* Mosquitoes Collected in Simatou Using CDC LTs

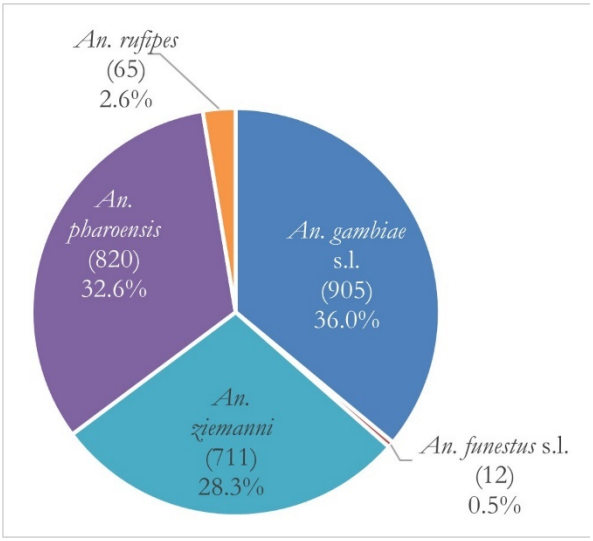


Figure 12: Species Composition of *Anopheles* Mosquitoes Collected in Simatou Using PSCs

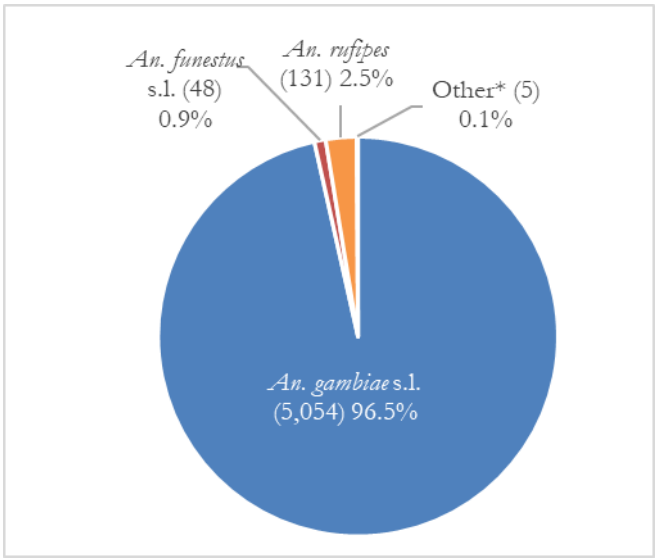
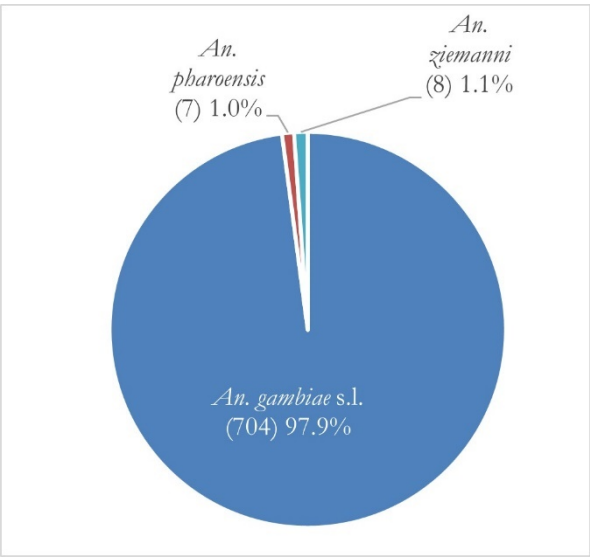


Figure 13: Species Composition of *Anopheles* Mosquitoes Collected in Simatou Using Prokopack



Other*: *An. pharoensis* (4) and *An. ziemanni* (1).

3.1.2.3 MANGOUM

Between October 2020 and September 2021, *An. gambiae* s.l. and *An. ziemanni* were the only *Anopheles* species collected using HLCs and CDC LTs in Mangoum (Figure 14-15). Only *An. gambiae* s.l. were collected using PSCs (Figure 16). A single *An. gambiae* s.l. mosquito was collected using Prokopack. Overall, *An. gambiae* s.l. represented more than 98% of the total *Anopheles* mosquitoes collected using the four methods in this site.

Figure 14: Species Composition of *Anopheles* Mosquitoes Collected in Mangoum Using HLCs

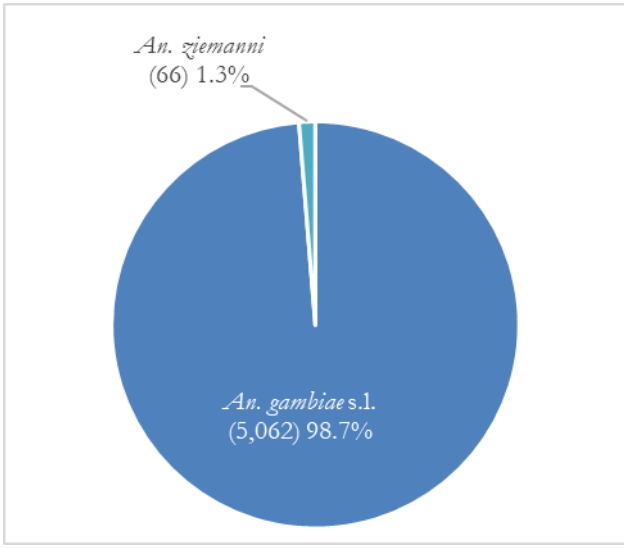


Figure 15: Species Composition of *Anopheles* Mosquitoes Collected in Mangoum Using CDC LTs

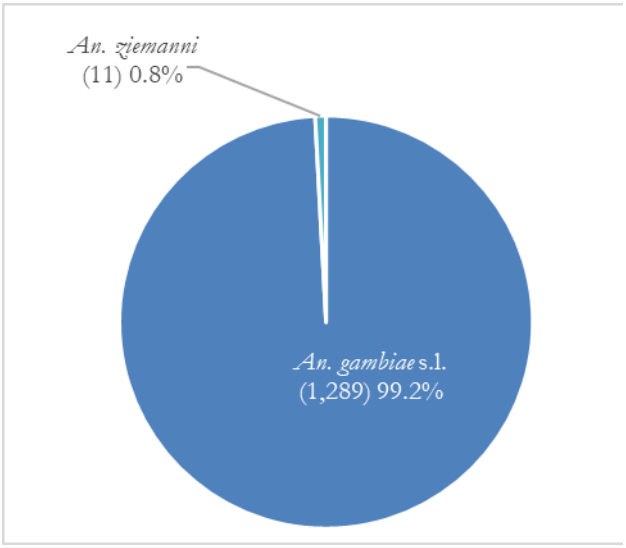
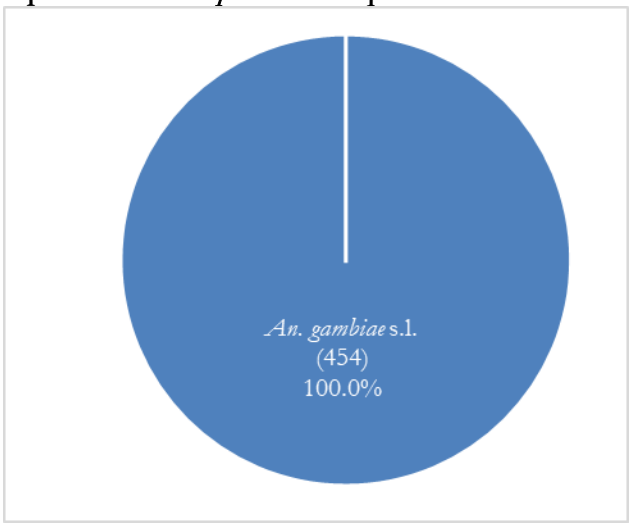


Figure 16: Species Composition of *Anopheles* Mosquitoes Collected in Mangoum Using PSCs



3.1.2.5 NYABESSANG

Nyabessang is the only site where *An. moucheti* and *An. paludis* were predominantly collected using HLCs, CDC LTs, and PSCs (Figure 17-19). No *An. nili* were collected using PSCs or Prokopacks, only in HLCs and CDC LTs. Only two individual *Anopheles* mosquitoes were collected outdoor using Prokopacks—one *An. gambiae* s.l. and one *An. moucheti*.

Figure 17: Species Composition of *Anopheles* Mosquitoes Collected in Nyabessang Using HLCs

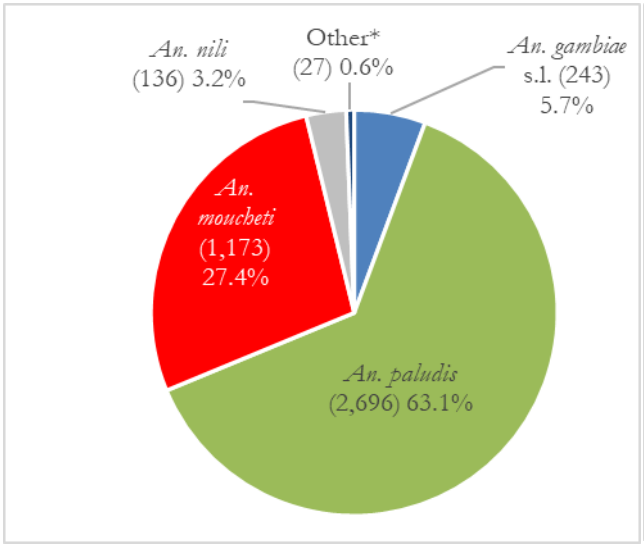
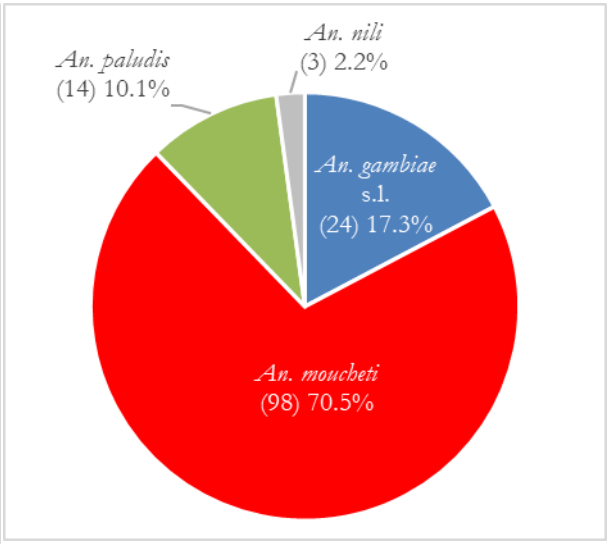
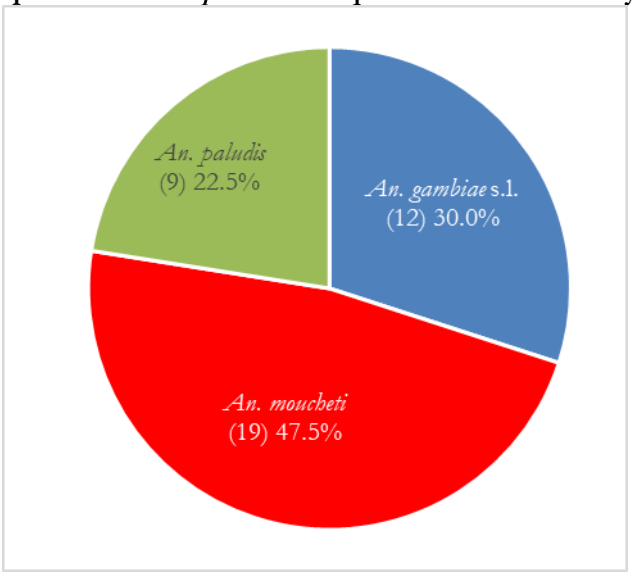


Figure 18: Species Composition of *Anopheles* Mosquitoes Collected in Nyabessang Using CDC LTs



*Other: *An. marshallii* (26) and *An. ziemanni* (1).

Figure 19: Species Composition of *Anopheles* Mosquitoes Collected in Nyabessang Using PSCs



3.1.2.6 Bonabéri

Bonabéri recorded the fewest number of *Anopheles* mosquitoes collected among the five sites. *An. gambiae* s.l. represented the only *Anopheles* species collected using the three methods (Figures 20-21). In addition, two *An. gambiae* s.l. were collected using PSCs and one was collected via Prokopack.

Figure 20: Species Composition of *Anopheles* Mosquitoes Collected in Bonabéri Using HLCs

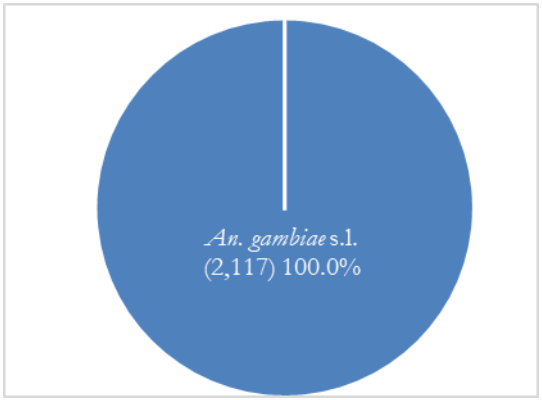
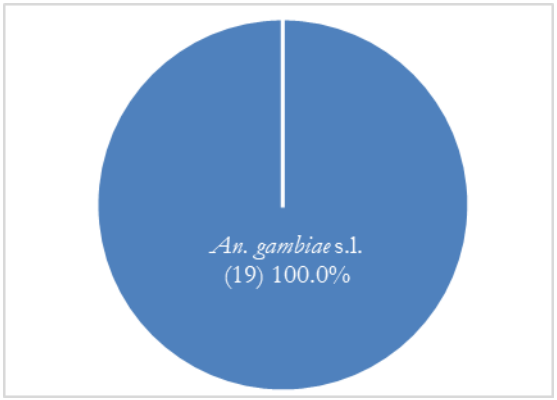


Figure 21: Species Composition of *Anopheles* Mosquitoes Collected in Bonabéri Using CDC LTs



3.1.3 Species Composition of *An. gambiae* Complex and *An. funestus* Group

Across the five sites, a total of 5,344 *An. gambiae* s.l. and *An. funestus* s.l. mosquitoes collected between October 2020-September 2021 were sent to CRID for analysis. Out of the total, 5,096 (95.4%) were molecularly identified, while 248 (4.6%) did not amplify. Table A5 in Annex A provides a breakdown by site.

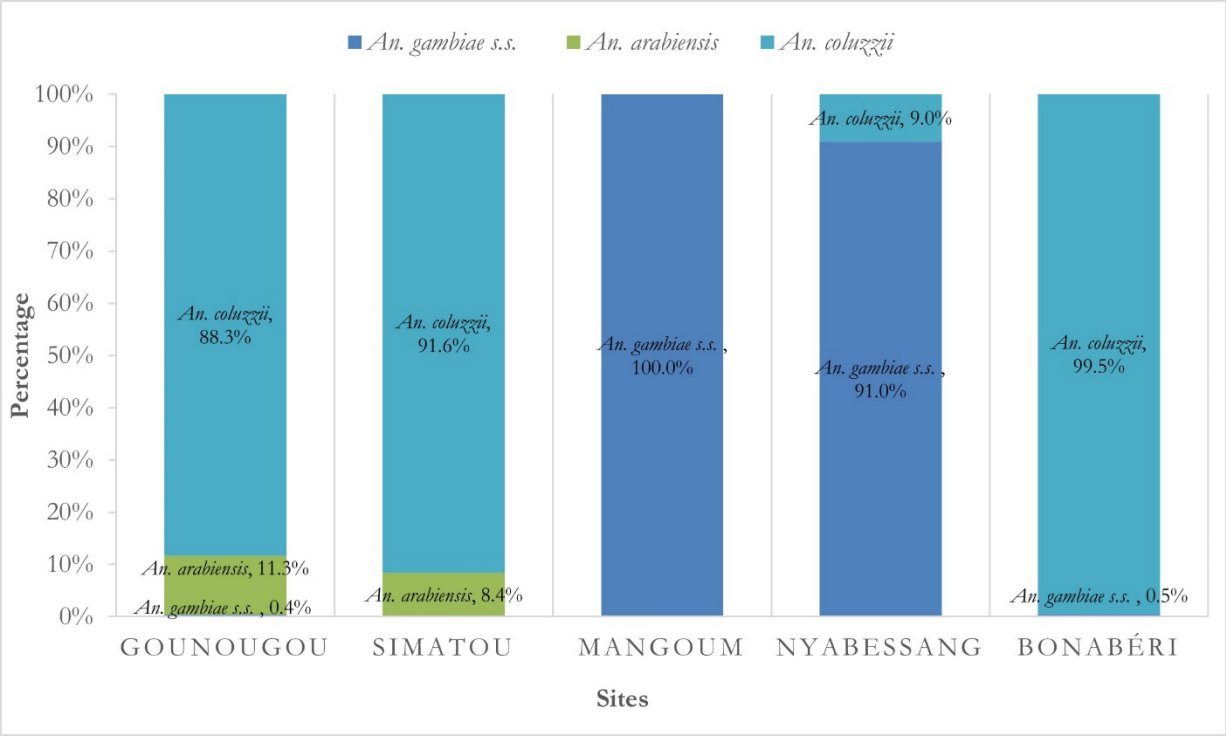
A total of 2,428 *An. gambiae* s.l. and 261 *An. funestus* s.l. were tested by PCR for molecular identification of the sub-species of each complex (Table A6, Annex A).

3.1.3.1 *An. gambiae* Complex

Out of a total of 2,428 *An. gambiae* s.l. mosquitoes analyzed across the five sites, three species from the *An. gambiae* complex were identified: *An. gambiae* s.s. (n=601, 24.8%), *An. coluzzii* (n=1693, 69.7%), and *An. arabiensis* (n=134, 5.5%). In total, 489 from Gounougou, 866 *An. gambiae* s.l. from Simatou, 509 from Mangoum, 95 from Nyabessang, and 469 from Bonabéri underwent species identification by PCR. *An. gambiae* s.s., *An. coluzzii,* and *An. arabiensis* were found in Gounougou, while *An. gambiae* s.s. and *An. coluzzii* were recorded in Nyabessang and Bonabéri. *An. coluzzii* constituted the main vector in Simatou (91.6%), Gounougou (88.3%), and Bonabéri (99.5%), in contrast to Mangoum where *An. gambiae* s.s. was 100% of the population tested (Figure 22 and Table A6, Annex A).

Figure 20 is a pie chart depicting the species composition of samples collected in Bonabéri from October 2020 to September 2021 using HLCs. 100 percent (2,117) of the *Anopheles* collected were *An. gambiae* s.l.

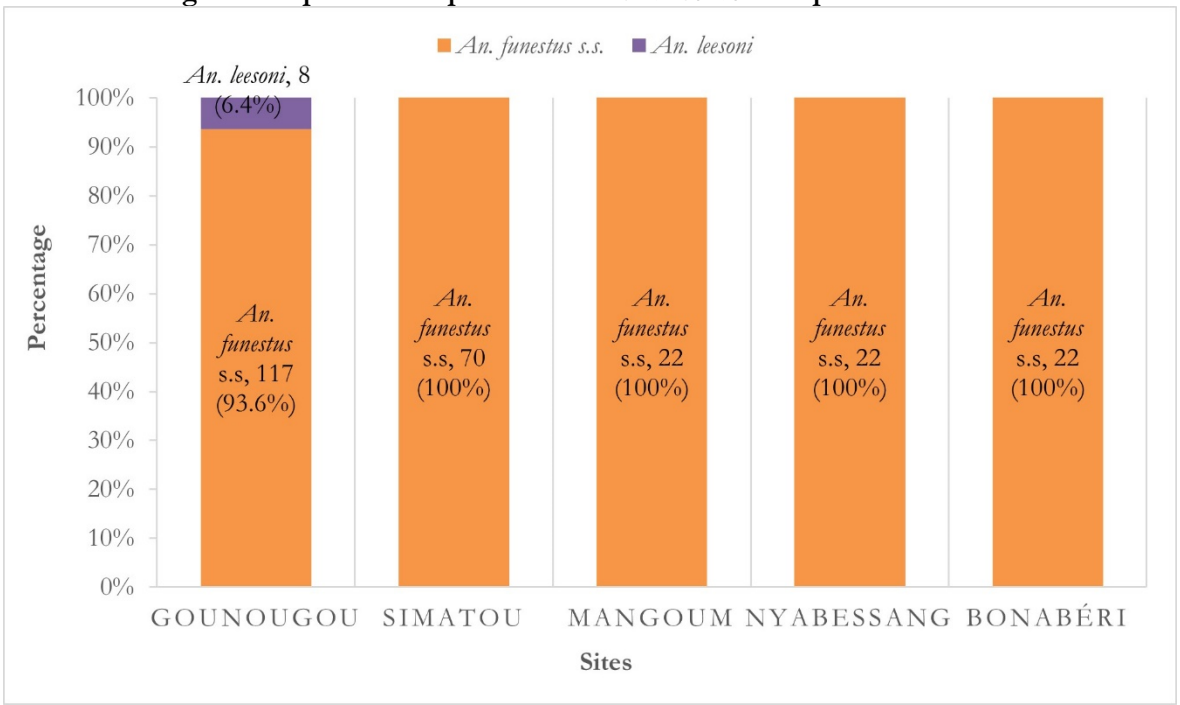
Figure 22: Species Composition of *An. gambiae* Complex Collected Across All Sites



3.1.3.2 *An. funestus* Group

A total of 261 *An. funestus* s.l. (125 from Gounougou, 70 from Simatou, and 22 from Mangoum, Nyabessang, and Bonabéri each for a total of 66) were molecularly identified. Two subspecies of the *An. funestus* group were found in Gounougou: *An. funestus* s.s. (93.6%) and *An. leesoni* (6.4%). *An. funestus* s.s. was the only species found in the other four sites (Figure 23 and Table A6, Annex A).

Figure 23: Species Composition of *An. funestus* Group Across All Sites



3.1.4 Human Biting Rate and Seasonal Variation of *An. gambiae* s.l.

3.1.4.1 Human Biting Rate of *An. gambiae* s.l. in All Sites

The mean HBR of *An. gambiae* s.l. varied between October 2020 and September 2021 with the highest rate recorded in Simatou (241.3 bites per person per night, or b/p/n) and the lowest in Nyabessang (0.17 b/p/n), both in July 2021 (Figure 24 and Annex B).

The mean HBR of *An. gambiae* s.l. in Gounougou was 23.2 b/p/n (22.6 indoors and 23.7 outdoors), 25.3 b/p/n (26.1 indoors, 24.6 outdoors) in Simatou, 24.8 b/p/n (22.7 indoors, 26.9 outdoors) in Mangoum, 1.2 b/p/n (1.4 indoors and 1.0 outdoors) in Nyabessang where *An. gambiae* s.l. was not the main vector, and 10.4 b/p/n (6.5 indoors, 14.3 outdoors) in Bonabéri where *An. gambiae* s.l. was the only *Anopheles* collected (Annex B, Table B9).

The highest biting by *An. gambiae* s.l. was observed between 11 p.m. and 5 a.m. in all sites though the peak varied from site to site, both indoors and outdoors. Three sites recorded bimodal peaks: Mangoum, where biting peaked between 11 p.m.-12 a.m. and again 2-3 a.m., Gounougou, where peaks occurred between 12-1 a.m., and 3-4 a.m., and Bonabéri, where the first peak occurred between 1-2 a.m. and the second between 3-4 a.m. In most sites, *An. gambiae* s.l. continued to bite until 8 a.m. both indoors and outdoors; the exceptions were Bonabéri and Nyabessang where biting outdoors dropped off at 7 a.m. (Figure 25).

The endophagic index of *An. gambiae* s.l. was 0.49 in Gounougou, 0.51 in Simatou, 0.46 in Mangoum, 0.58 in Nyabessang, and 0.31 in Bonabéri, indicating that *An. gambiae* s.l. bite almost equally indoors and outdoors in Gounougou and Simatou, indoor biting predominates in Nyabessang, and biting occurs more outdoors than indoors in Mangoum and Bonabéri (Annex B, Table B6-B10).

Figure 24: Mean Monthly Human Biting Rate of *An. gambiae* s.l. in All Sites (October 2020-September 2021)

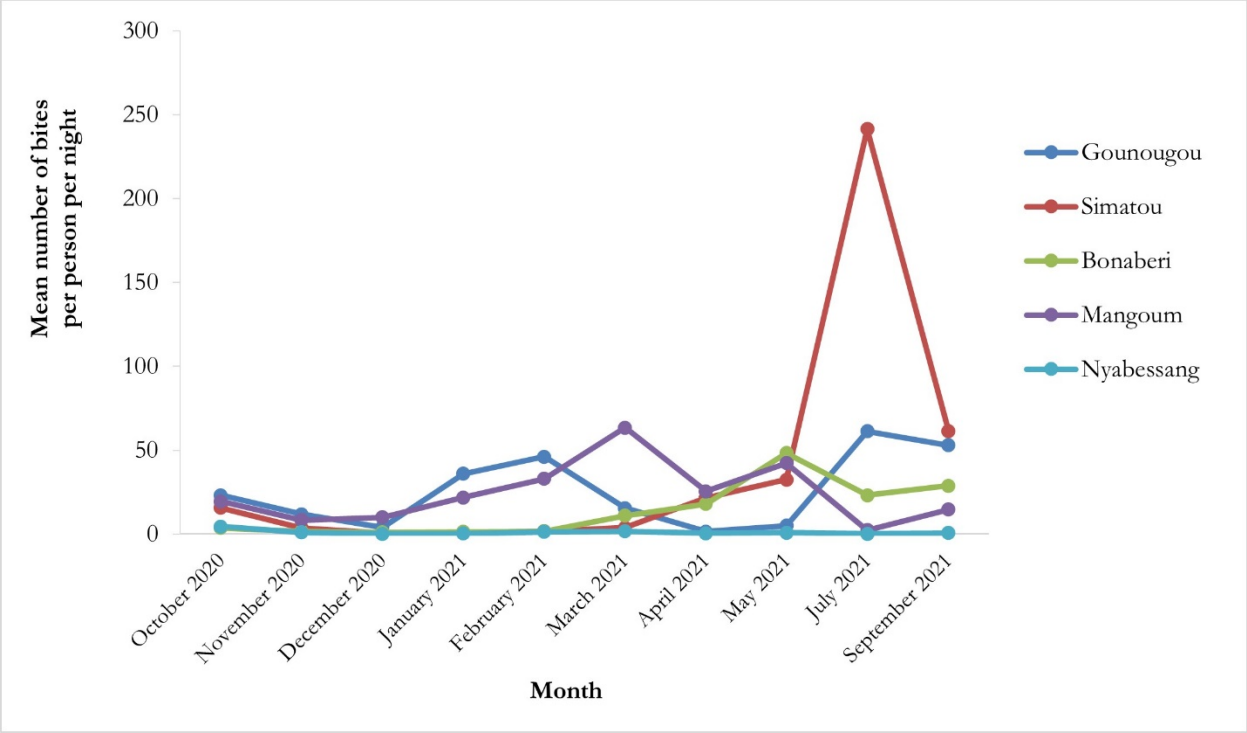
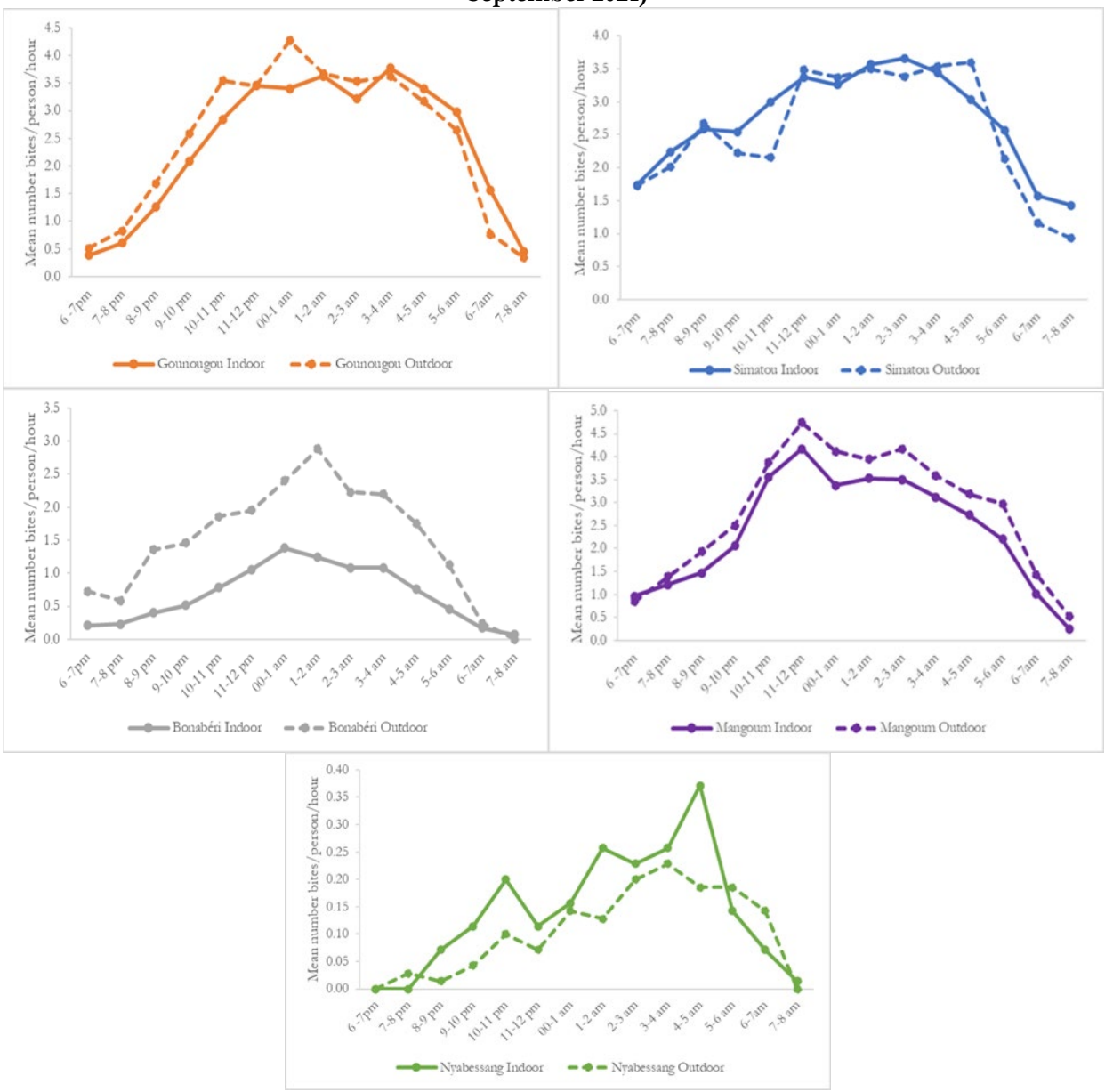


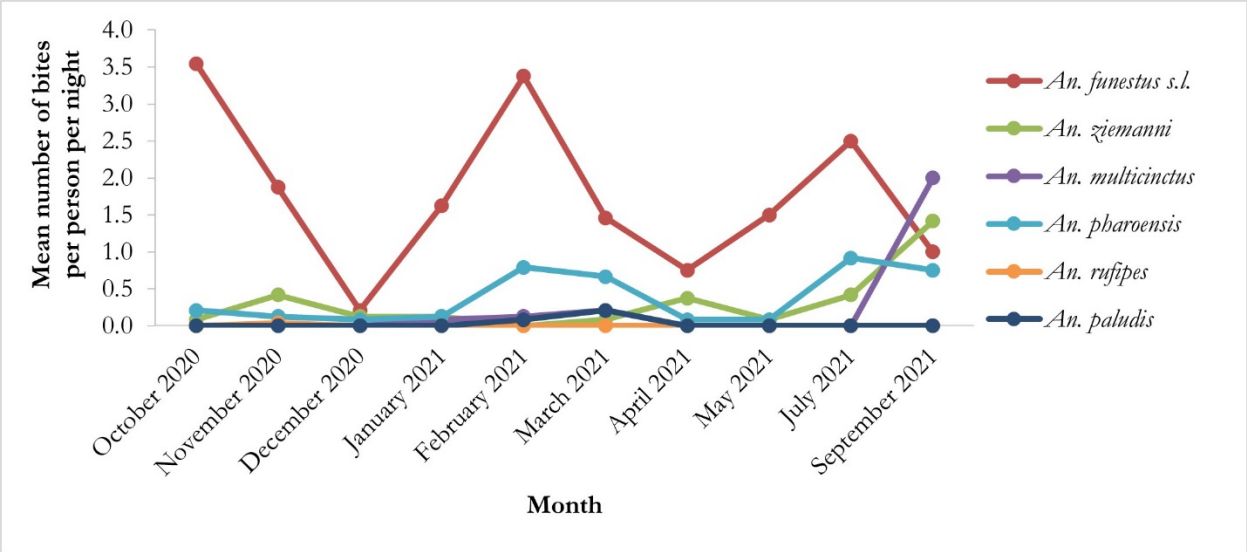
Figure 25: Mean Indoor and Outdoor Hourly Biting of *An. gambiae* s.l. in All Sites (October 2020-September 2021)



3.1.4.2 Human Biting Rate of Other *Anopheles* Species Collected in Gounougou

The mean HBR of *An. funestus* s.l. was 1.8 b/p/n (1.9 b/p/n indoors and 1.6 b/p/n outdoors). (Table B1, Annex B). The HBR varied monthly, and the highest peaks were observed in February (when rice fields typically are filled with water and create many breeding sites) and in July, during the rainy season (Figure 26). The endophagic index was 0.55, indicating that *An. funestus* s.l. in this area likely bite more indoors than outdoors (Table B2, Annex B). The other *Anopheles* with perennial biting were *An. ziemanni*, *An. multicinctus*, *An. pharoensis*, *An. rufipes,* and *An. paludis*.

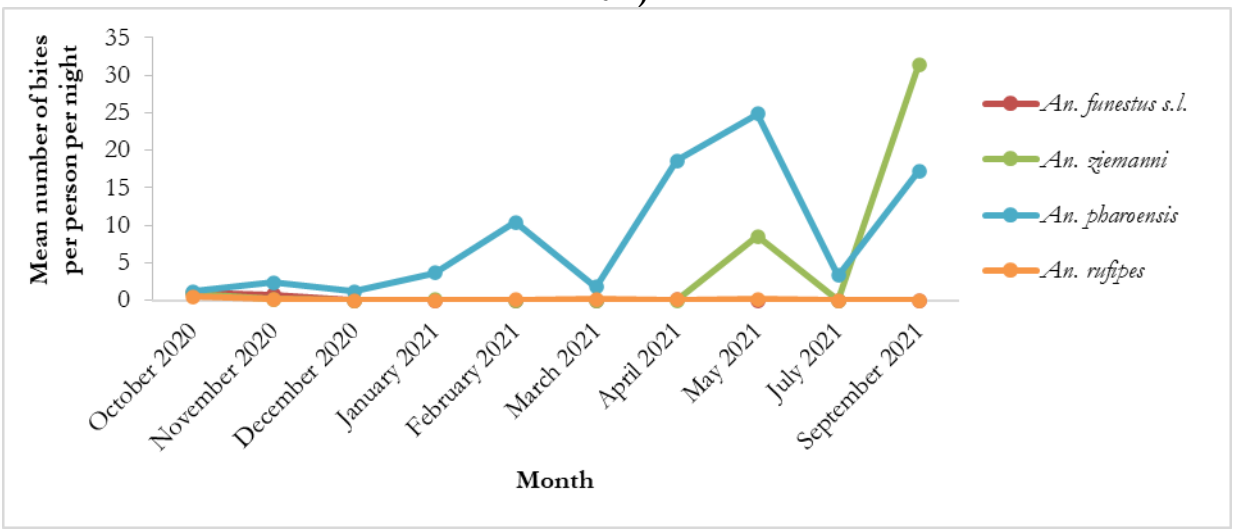
Figure 26: Human Biting Rate of Other *Anopheles* Collected in Gounougou (October 2020-September 2021)



3.1.4.3 Human Biting Rate of Other *Anopheles* Species Collected in Simatou

In Simatou, the mean HBR for *An. funestus* s.l. was 0.24 b/p/n (0.3 indoors, 0.2 outdoors) (Table B3, Annex B). Seasonal variation of the HBR was also observed in this site for all secondary *Anopheles* vector species (Figure 27), with the highest HBR observed in May 2021 for *An. pharoensis* (24.8 b/p/n) and in September 2021 for *An. ziemanni* (31.4 b/p/n) (Table B4, Annex B).

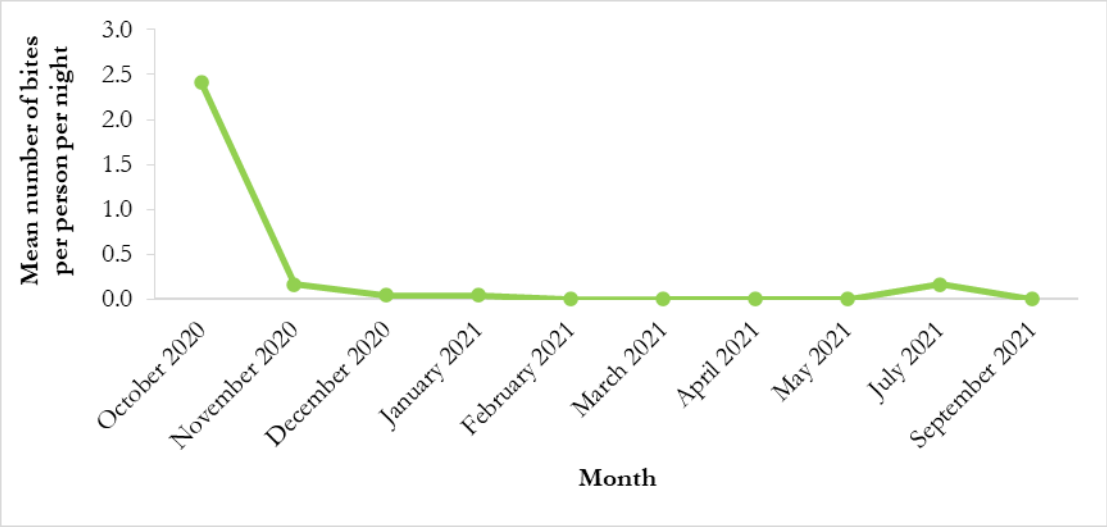
Figure 27: Human Biting Rate of Other *Anopheles* Collected in Simatou (October 2020-September 2021)



3.1.4.4 Human Biting Rate of Other *Anopheles* Species Collected in Mangoum

An. ziemanni was the only additional species collected in Mangoum after *An. gambiae* s.l. and recorded a mean HBR of 0.3 b/p/n (0.07 b/p/n indoors, 0.6 b/p/n outdoors) (Figure 28 and Table B5 in Annex B).

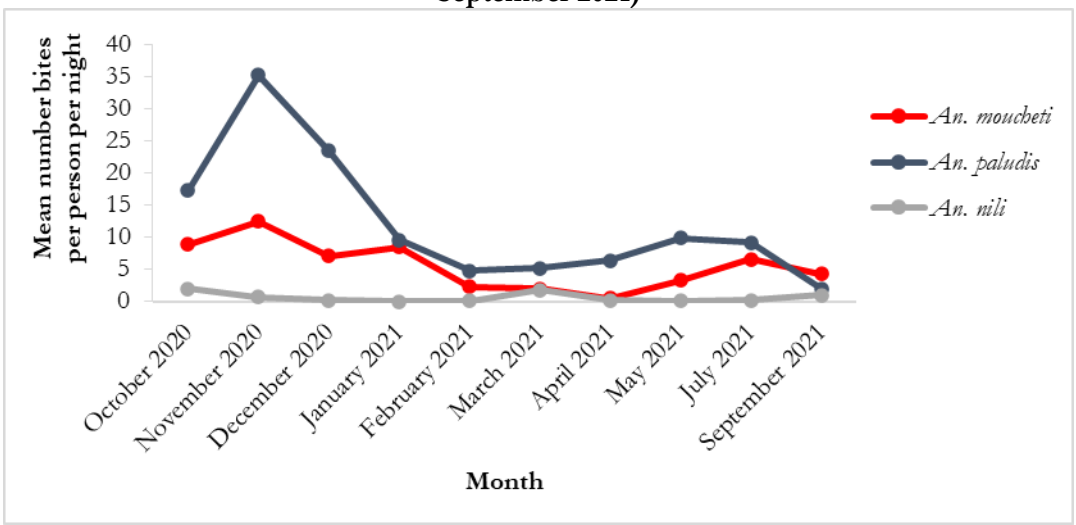
Figure 28: Human Biting Rate of *An. ziemanni* Collected in Mangoum (October 2020-September 2021)



3.1.4.5 Human Biting Rate of Other *Anopheles* Species Collected in Nyabessang

In Nyabessang, *An. paludis* and *An. moucheti* were found at higher densities than *An. gambiae* s.l. The average HBR was 13.2 b/p/n for *An. paludis* (10.4 indoors, 15.0 outdoors) and 7.7 b/p/n for *An. moucheti* (9.3 indoors, 6.1 outdoors). *An. nili* represented the third other *Anopheles* species with a mean HBR of 2.1 b/p/n for (8.8 indoors, 17.6 outdoors) (Figure 29 and Table B7 in Annex B). The endophagic index was 0.5 for *An. moucheti* and 0.3 for *An. paludis* (Table B8, Annex B).

Figure 29: Human Biting Rate of Other *Anopheles* Collected in Nyabessang (October 2020-September 2021)



3.1.5 Indoor Resting Density

The mean *An. gambiae* s.l. density per room varied between 0.0 and 119.7 females per room per night, which was found in Simatou in July 2021. No *An. gambiae* s.l. were collected in Bonabéri using PSCs. Table 4 illustrates the trend in each site from October 2020 to September 2021.

Table 4: Indoor Resting Density of *An. gambiae* s.l. Across Sites (October 2020-September 2021)

	Month									
Sites	**Oct-20**	**Nov-20**	**Dec-20**	**Jan-21**	**Feb-21**	**Mar-21**	**Apr-21**	**May-21**	**Jul-21**	**Sep-21**
Gounougou	13.9	7.4	4.4	13.2	19.5	19.6	1.9	6.0	75.8	19.7
Simatou	12.7	6.4	1.8	7.7	5.0	13.0	29.5	11.3	119.7	45.8
Mangoum	0.8	0.4	1.8	4.3	2.3	4.6	2.6	2.1	0.5	3.6
Nyabessang	0.2	0.2	0.0	0.0	0.0	0.2	0.1	0.0	0.0	0.0
Bonabéri	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0

3.1.6 Host Preference

A total of 1,152 blood-fed *Anopheles* mosquitoes including 1,054 *An. gambiae* s.l. collected using PSCs in Gounougou, Simatou, Mangoum, Nyabessang, and Bonabéri were analyzed using ELISAs. The average overall human blood index (HBI) was 61.1%. The HBI of *An. gambiae* s.l. was 65.0% in Gounougou, 59.3% in Simatou, and 100.0% in Mangoum, Nyabessang, and Bonabéri, where fewer numbers were recorded compared to Gounougou and Simatou (Table C1, Annex C).

3.1.7 Parity

The ovaries of 4,113 *Anopheles* mosquitoes were dissected including 3,982 *An. gambiae* s.l. and 131 *An. funestus* s.l. The average parity rate was 75.0% for *An. gambiae* s.l. and 91.6% for *An. funestus* s.l. The parity rate varied across sites (Annex C). Across sites, the parity rates of *An. gambiae* s.l. recorded over the reporting period were similar indoors and outdoors, except in Bonabéri where higher parity was observed indoors than outdoors between November 2020 and April 2021. The lowest parity rates were recorded in Mangoum (Figure 30).

Figure 30: Indoor and Outdoor Parity Rates of *An. gambiae* s.l. Across Sites (October 2020-September 2021)

3.1.8 Entomological Inoculation Rate per Site Using HLCs

A total of 6,319 *Anopheles* mosquitoes were tested by ELISA and 142 were found with circumsporozoite antigen of *Plasmodium falciparum*. The average infection rate of the main vector *An. gambiae* s.l. was 2.6% (Table C3, Annex C). The infection rates of *An. gambiae* s.l. were as follows: Gounougou (2.6%), Simatou (1.1%), Mangoum (3.4%), Nyabessang (0.8%), and Bonabéri (3.0%).

Seven *Anopheles* species collected were found to be positive for *P. falciparum*: *An. gambiae* s.l., *An. funestus* s.l., *An. nili*, *An. moucheti*, *An. paludis*, *An. pharoensis*, and *An. ziemanni*. The infection rate was 2.6% for *An. gambiae* s.l., 4.3% for *An. funestus* s.l., 3.1% for *An. nili*, 0.6% for *An. ziemanni*, 1.9% for *An. moucheti*, 0.3% for *An. pharoensis* and 0.9% for *An. paludis* (Annex C, Table C3). The total EIRs across sites were: 20.5 infected bites per person per month (ib/p/m) in Gounougou, 8.8 ib/p/m in Simatou, 26.4 ib/p/m in Mangoum, 13.8 ib/p/m in Nyabessang, and 9.7 ib/p/m in Bonabéri (Figure 31 and Table 5).

Figure 31: Mean Monthly Entomological Inoculation Rate Across Sites

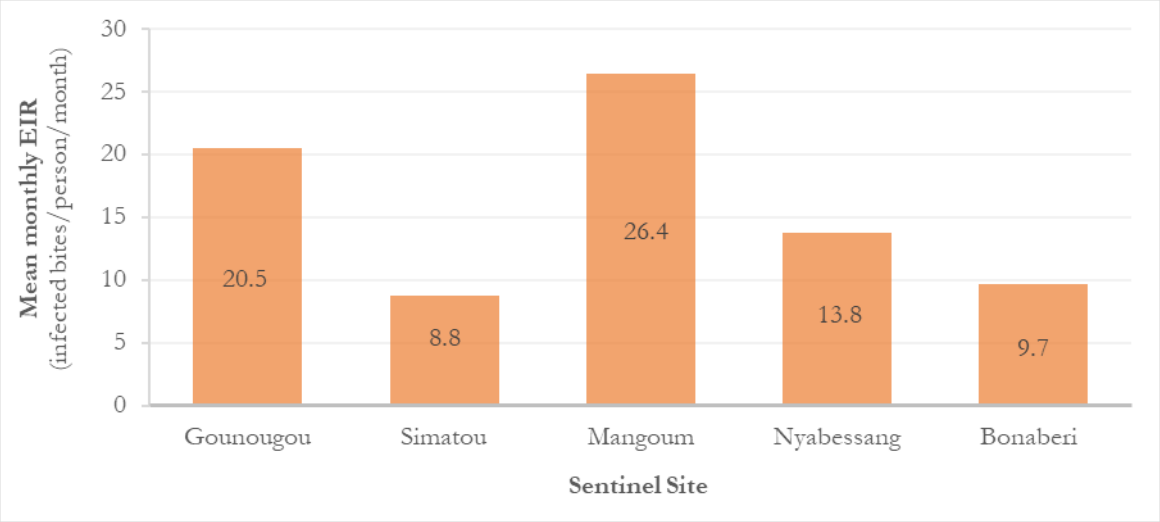


Table 5: Entomological Inoculation Rates Across Sites and Species

Sentinel Site	Species	HBR	Infection Rate	EIR (infected bites/person/night)	Monthly EIR (infected bites/person/month)
Gounougou	*An. gambiae* s.l.	23.22	0.03	0.60	18.1
	An. funestus s.l.	1.80	0.04	0.08	2.3
	Total EIR	**25.02**	**0.03**	**0.68**	**20.5**
Simatou	*An. gambiae* s.l.	25.36	0.01	0.28	8.4
	An. pharoensis	7.30	0.01	0.03	0.9
	Total EIR	**32.66**	**0.01**	**0.29**	**8.8**
Mangoum	*An. gambiae* s.l.	24.81	0.03	0.84	25.3
	An. ziemanni	0.32	0.05	0.02	0.5
	Total EIR	**25.13**	**0.04**	**0.88**	**26.4**
Nyabessang	*An. gambiae* s.l.	1.19	0.01	0.01	0.3
	An. moucheti	5.75	0.02	0.11	3.3
	An. paludis	13.22	0.01	0.12	3.6
	An. nili	0.67	0.03	0.02	0.6
	Total EIR	**20.83**	**0.02**	**0.46**	**13.8**
Bonabéri	*An. gambiae* s.l.	10.38	0.03	0.31	9.3
	Total EIR	10.38	0.03	0.32	**9.7**

3.2 INSECTICIDE RESISTANCE MONITORING

3.2.1 SUSCEPTIBILITY STATUS OF *AN. GAMBIAE* S.L.

Anopheles gambiae s.l. from 10 sites were tested for insecticide resistance during the reporting period. Testing was conducted in Bertoua, Djohong, Ndelele, Ngaoundere, and Njombe in August 2021, while testing in Garoua, Gazawa, Mada, Mogode, and Touboro took place in September 2021. Figures 32-37 below show the resistance status of *An. gambiae* s.l. to the different pyrethroid, carbamate, organophosphate, neonicotinoid, and pyrrole classes of insecticide tested at each site (Annex D).

Resistance was observed to the diagnostic doses of all pyrethroids and pirimiphos-methyl in all sites. Resistance to bendiocarb was observed in six sites (Bertoua, Djohong, Garoua, Ndelele, Ngaoundere, and Njombe) (Figure 32-33). Pre-exposure of mosquitoes to PBO before deltamethrin, permethrin, or alpha-cypermethrin partially increased the mortality of *An. gambiae* s.l. but did not reach full susceptibility in sites surveyed except in Bertoua with deltamethrin (Figure 32). High pyrethroid resistance intensity (less than 98% mortality at 10x the diagnostic dose) to deltamethrin, permethrin, and alpha-cypermethrin was observed in nine of the 10 sites (Bertoua being the exception). Moderate resistance (below 98% mortality at 5x or greater than 98% at 10x the diagnostic dose) was observed at Bertoua with permethrin (Figure 34).

Susceptibility of *An. gambiae* s.l. to clothianidin (2%) was observed in all 10 sites (Figure 35). Susceptibility to clothianidin (4 µg/bottle) was found in six sites while probable resistance was noted in Djohong (94.8%) and Njombe (96%) and resistance in Ngaoundere (66.2%) and Touboro (37.5%) (Figure 36). *An. gambiae* s.l. was also susceptible to chlorfenapyr (200 µg/bottle), in all sites except Ngaoundere (Figure 37).

[Note: The red and green lines in Figures 32-38 represent the resistance and susceptibility thresholds, respectively.]

Figure 32: Insecticide Susceptibility and Effect of PBO Across Sites in 2021

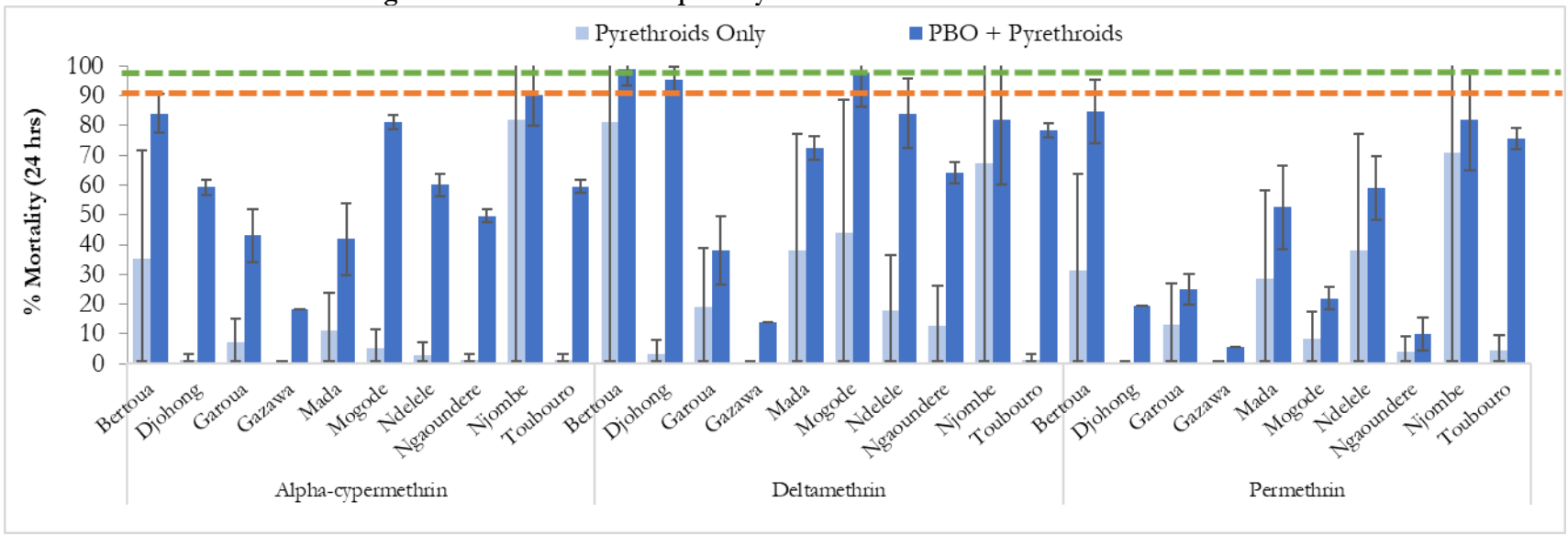


Figure 33: Susceptibility to Bendiocarb and Pirimiphos-Methyl Across Sites in 2021

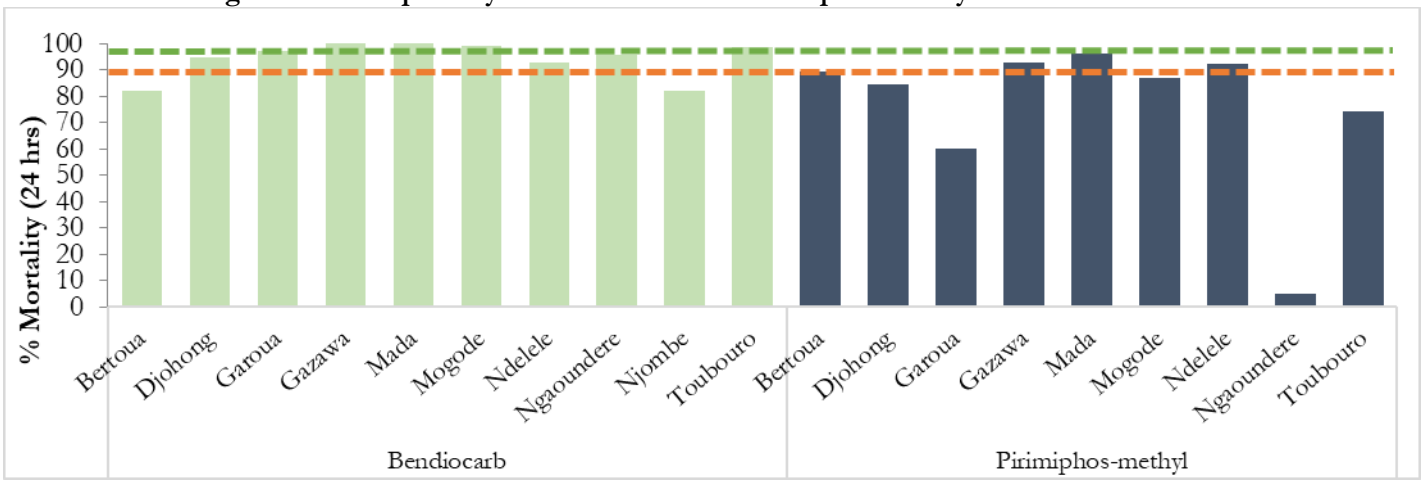


Figure 34: Resistance Intensity to Insecticides Across Sites in 2021

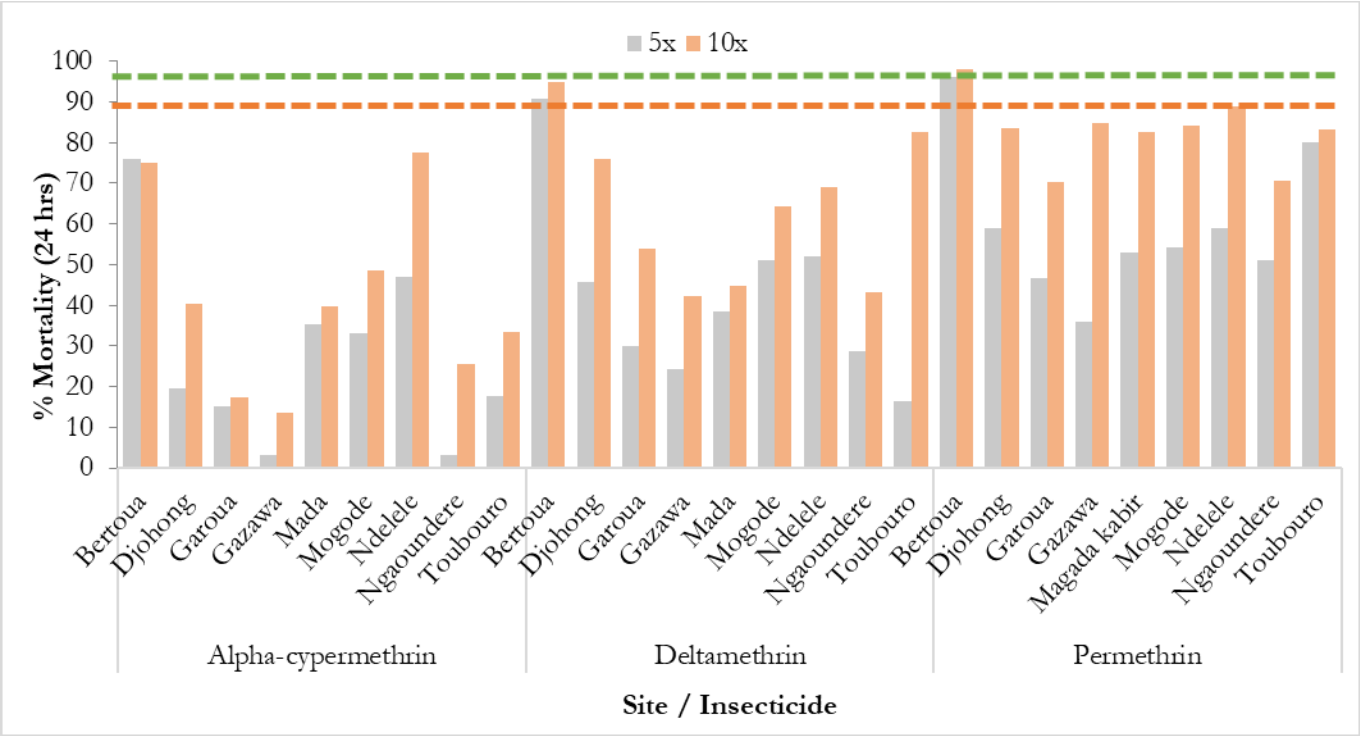


Figure 35: Susceptibility of *An. gambiae* s.l. to Clothianidin 2% Using WHO Susceptibility Test at All Sites in 2021

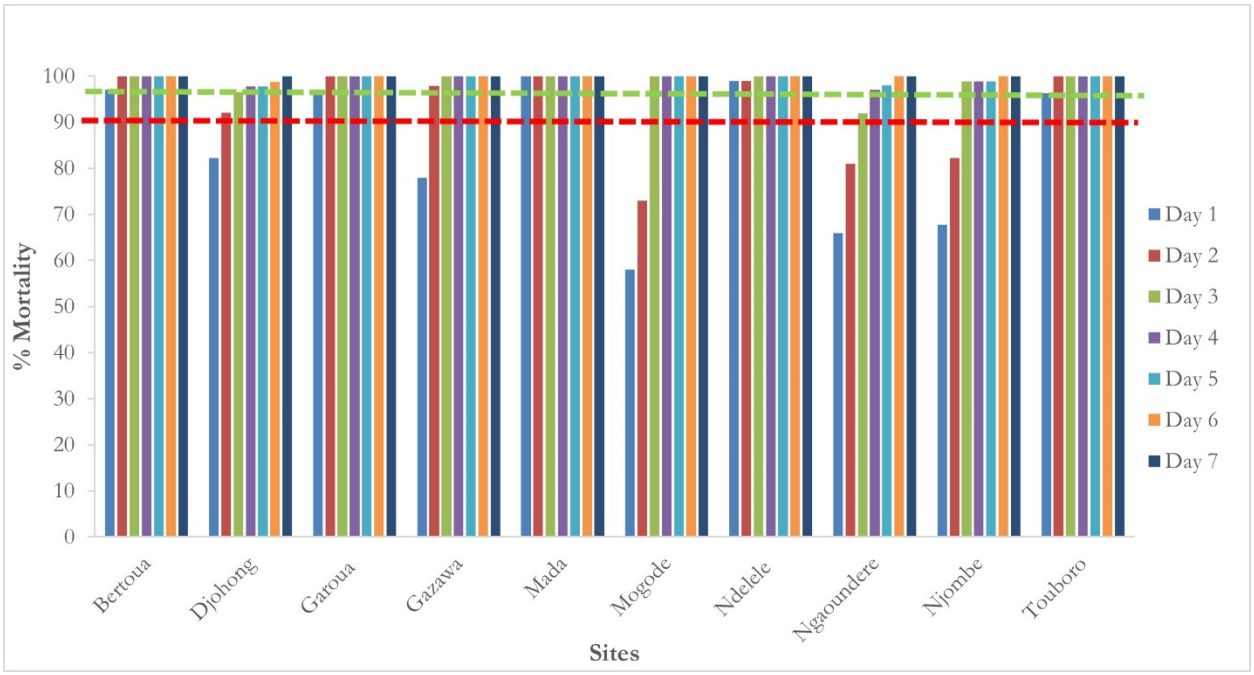


Figure 36: Susceptibility of *An. gambiae* s.l. to Clothianidin (4 µg/bottle) Using CDC Bottle Assay at All Sites in 2021

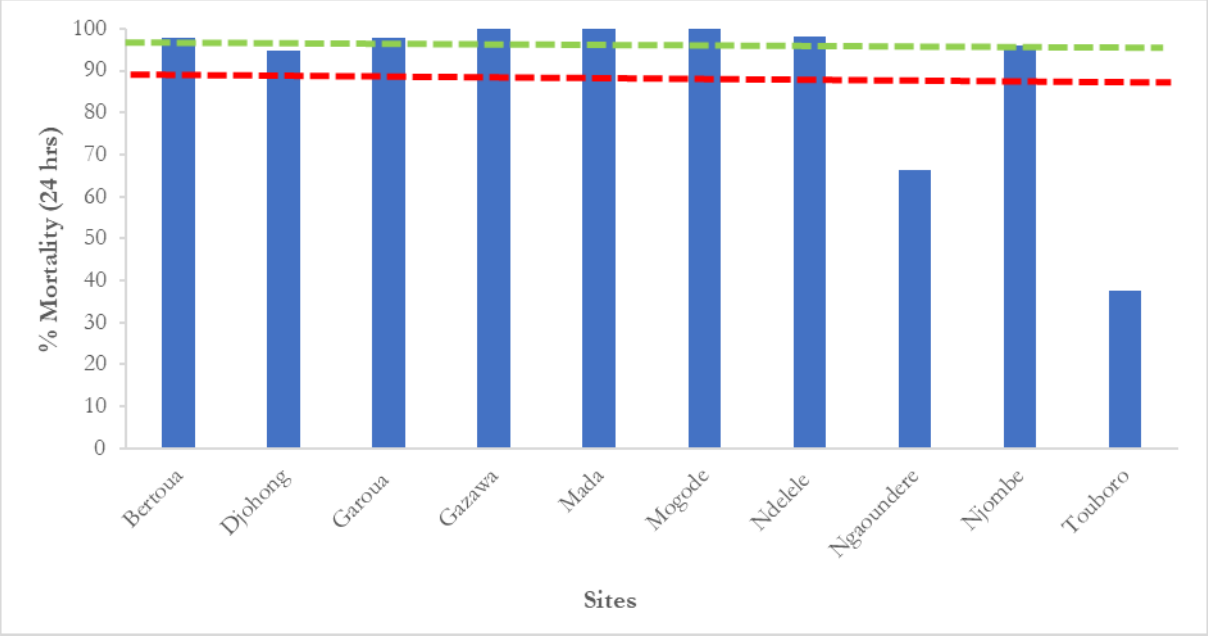
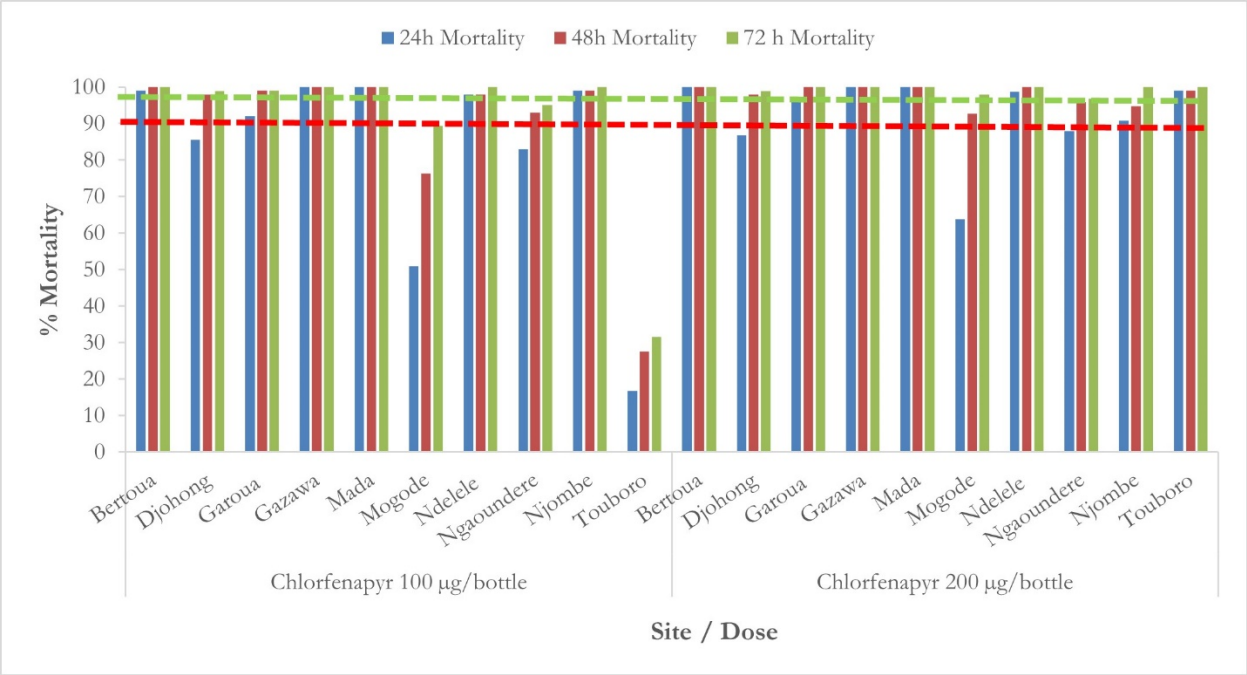


Figure 37: Susceptibility of *An. gambiae* s.l. to Chlorfenapyr (100 & 200 µg/bottle) Using CDC Bottle Assay at All Sites in 2021

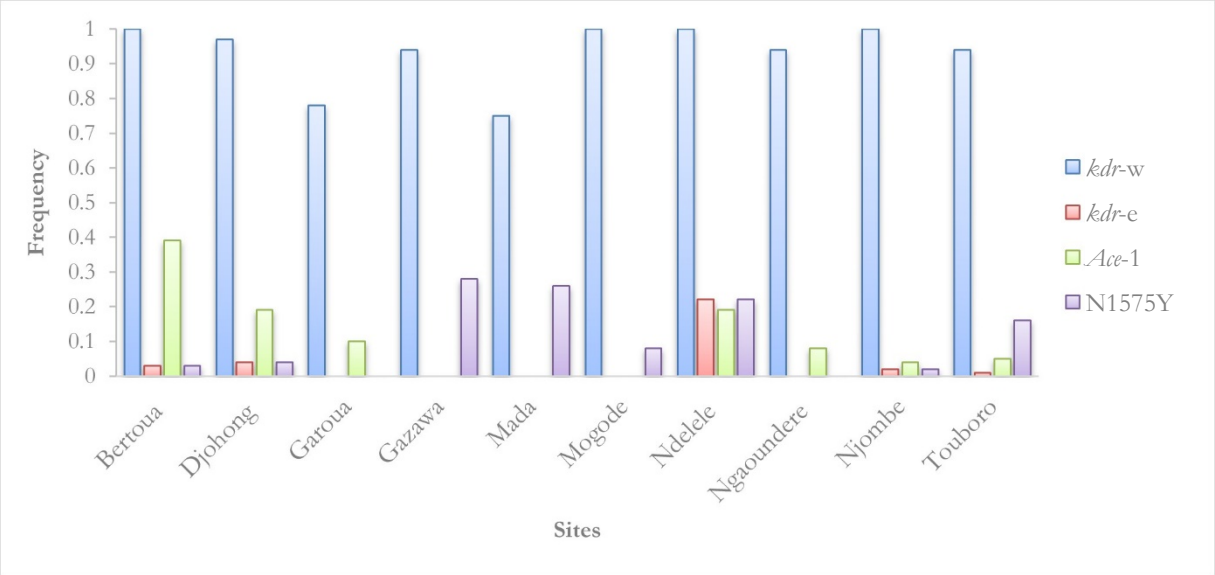


3.2.2 Insecticide Resistance Mechanisms

3.2.2.1 Target Site Resistance

Phenotypic insecticide resistance in mosquitoes can be related to target site mutations. Among them, resistance to pyrethroids and DDT is described as a substitution of amino acid leucine to either phenylalanine (L1014F, referred as *kdr*-west) or serine (L1014S, referred as *kdr*-east) at the position 1014 in the sodium channel gate. The N1575Y represents and additional mutation involved in the *kdr* mutation. For organophosphate and carbamate insecticides, the target site mechanism, known as *Ace*-1 (G296S), is a substitution of the amino acid glycine to serine at position 119. Four gene mutations (*kdr*-w, *kdr*-e, N1575Y, *Ace*-1) were detected in *An. gambiae* s.l. from four of the 10 sites (Figure 38). The *kdr*-w mutation was present in all sites with high frequency (100% resistance allele) in four sites (Bertoua, Mogode, Ndelele, and Njombe). In contrast, *kdr*-e was only found in five sites (Bertoua, Djohong, Ndelele, Njombe, and Touboro); the highest allele frequency was observed in Ndelele (22%). The *Ace*-1 mutation was found in all sites except Gazawa, Mada, and Mogode, while the N1575Y mutation was detected in all sites except Njombe and Ngaoundere, with the highest frequencies recorded in Gazawa (28%), Ndelele (22%), and Touboro (16%).

Figure 38: Frequency of Target Site Mechanisms Involved in Insecticide Resistance of *An. gambiae* s.l.

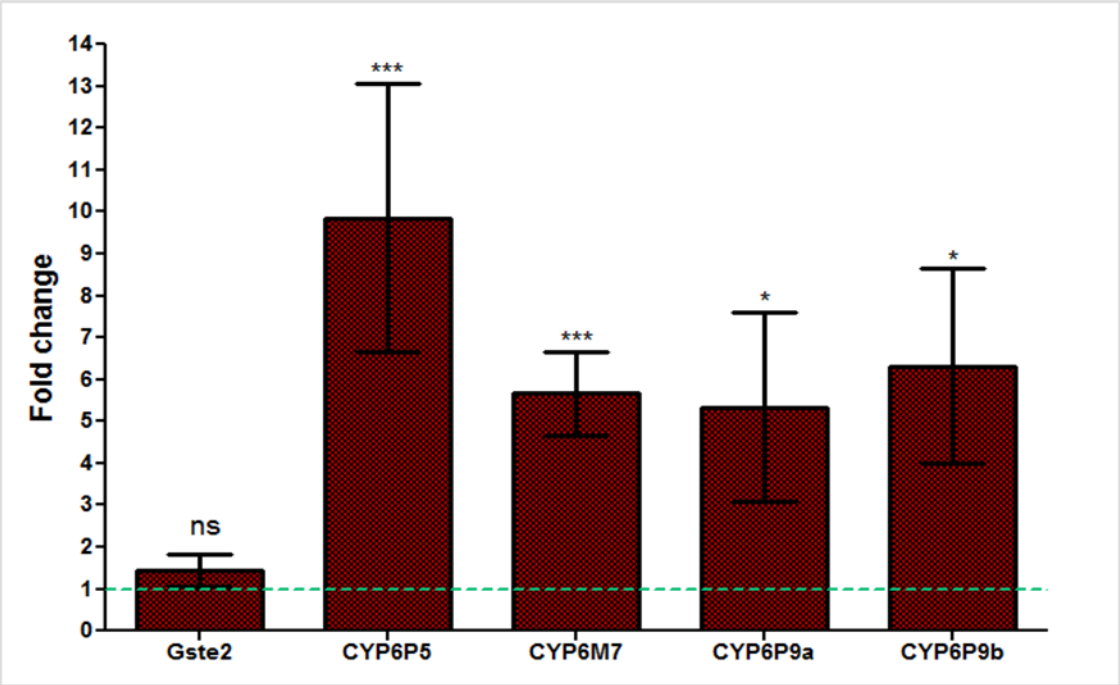


3.2.2.2 Metabolic Resistance

Metabolic resistance represents the production of enzymes by the mosquitoes to decrease the insecticidal effect of the pyrethroid insecticides. Different enzymes are involved in the metabolic resistance and are referred to mono-oxygenase (the CY group), esterases, or Glutation-S-Transferases (GSTe2). The metabolic resistance is expressed as a number fold change of the mosquito population tested (Figure 39).

qPCR analyses were performed to assess the level of expression of a set of genes known to be involved in the metabolic resistance in *An. funestus* s.l. mosquito species. This included genes such as GSTe2, CYP6P5, CYP6M7, CYP6P9a, and CYP6P9b. Analyses were done only on samples collected from Touboro. For this purpose, the level of expression of these genes were compared between field collected mosquitoes and a fully susceptible *An. funestus* lab strain (FANG). Results from these analyses are presented in Figure 38 and Table 6 below.

Figure 39: Frequency of *An. funestus* s.l. Fold Changes at Touboro



Note: The green dotted line represents the intercept corresponding to the level of expression of the gene in the susceptible lab strain.

Table 6: Frequency of An. funestus s.l. Fold Changes for Various Genes at Touboro

Gene name	Fold change	Confidence interval	P-value
Gste2	1.43	1.04 - 1.82	0.3
CYP6P5	9.85	6.64 - 13.06	0.0061
CYP6M7	5.67	4.67 - 6.66	0.001
CYP6P9a	5.34	3.08 - 7.6	0.0198
CYP6P9b	6.32	3.99 - 8.65	0.0113

Figure 38 shows that level of expression of four genes is significantly higher in *An. funestus* s.l. from Touboro than the fully susceptible lab strain—the expression of CYP6P5 is almost 10-fold higher in *An. funestus* s.l. than the lab strain, CYP6M7 almost six-fold higher, CYP6P9a five-fold higher, and CYP6P9b six-fold greater. However, the level of expression of the GSTe2 is not significantly different between field and full susceptible lab *An. funestus* s.s. strains.

4. Discussion and Conclusions

VectorLink Cameroon conducted longitudinal vector surveillance data collection monthly from October 2020 to April 2021 and every other month from April to September 2021, for a total of 10 collection efforts at each of the five selected sentinel sites during the reporting period. The high diversity of *Anopheles* species recorded previously across all sites continued in 2020-2021. *An. gambiae* s.l., *An. pharoensis,* and *An. funestus* s.s. were still the most abundant *Anopheles* and were collected through all collection methods and in all sites, except Bonabéri, where only *An. gambiae* s.l. was collected using HLC. *An. moucheti* and *An. nili* were collected only at Nyabessang. *An. gambiae* s.l. were collected in all sites at variable proportions depending on the collection method. Furthermore, all three sub-species of *An. gambiae* complex (*An. gambiae* s.s., *An. arabiensis,* and *An. coluzzii*) found in 2018/2019 and 2019/2020 were observed once again in 2020/2021, with *An. coluzzii* being the main species of the complex found in Gounougou, Simatou, and Bonabéri. All *An. gambiae* complex specimens collected in Mangoum and 91% of those from Nyabessang were identified to be *An. gambiae* s.s. As in previous years of collections, *An. arabiensis* was recorded only in the northern sites of Gounougou and Simatou but at a very low frequency. The northern *Anopheles* species composition confirms that *An. arabiensis* is species of the complex often found in drier areas as described in several studies (White 1974, Lindsay et al. 1998, Coetzee et al. 2000). For the *An. funestus* group, *An. funestus* s.s. and *An. leesoni* represented the two sub-species collected, with *An. leesoni* found only at Gounougou in the North and about 6% of the total number of *An. funestus* s.l. samples collected.

The *Anopheles* species composition given the geographical location of each site should be considered during vector control strategy selection and planning. The same vectors and *Anopheles* populations were observed in the same areas at approximately equal proportions as 2020 and earlier. Though *An. gambiae* s.l. represented the overall main vector of the country, other vectors such as *An. moucheti* and *An. paludis* (collected in large numbers compared to *An. gambiae* s.l. at Nyabessang in the southern part of the country) require deeper investigation in terms of susceptibility to insecticides used in vector control tools.

The highest densities of vectors were observed in different months of the year, with *An. gambiae* s.l. peaking in July in the northern sites of Gounougou and Simatou and between March and May in Mangoum and Bonabéri. These peaks are likely due to the different geographical positions and rainy seasons within the country—the rainy season occurs from May-October in Gounougou and from July-October in Simatou, while the southern part of the country (where Mangoum, Nyabessang, and Bonabéri sites are located) experiences two rainy seasons (March-June and July-September). Furthermore, Gounougou and Simatou cultivate rice during the rainy season. Nyabessang recorded its highest density of main vectors (*An. paludis* and *An. moucheti*) around the end of year in October-November. While *An. gambiae* s.l. peaks were observed during the high precipitation season, the other vectors in Nyabessang were collected in larger numbers at the end of the rainy season. This constitutes a worrying situation where the other *Anopheles* species contribute to malaria transmission as alternative vectors.

Furthermore, *An. gambiae* s.l. showed a similar biting pattern in all sites with peak biting hours recorded between 11 p.m. and 5 a.m. at all sites while biting almost similarly indoors and outdoors except in Bonabéri. However, the resting behavior of the vectors will need further investigation to enable conclusions on the probable resting locations. The outdoor collections conducted for only two months yielded higher number of *Anopheles* only in Gounougou and Simatou. The other three sites recorded fewer mosquitoes, which may be caused by the ecological location of the sites. As described earlier in the report, the southern sites are in the wetter dense forest and grassland zones of the country while Gounougou and Simatou are in the dry Savanna zone.

High parity rates were observed across all sites, both indoors and outdoors, and throughout the year except in Mangoum between February and May 2021. This showed that the vectors live longer, enabling the completion

of the sporogony cycle to become infectious. Parity is considered as a key parameter of malaria transmission as the older the population, the higher the expected number of sporozoite positive vectors. Additionally, a low parity rate of the *Anopheles* vectors suggests that the vector control intervention being implemented is effective.

Plasmodium falciparum sporozoites were detected in seven *Anopheles* species: *An. gambiae* s.l. *An. funestus* s.l., *An. ziemanni*, *An. moucheti*, *An. nili, An. pharoensis,* and *An. paludis*. The monthly EIR of the main vector, *An. gambiae* s.l., ranged from 0.29 ib/p/m in Nyabessang to 25.30 ib/p/m in Mangoum, while *An. paludis* and *An. moucheti* represented the main *P. falciparum* sporozoite carriers of Nyabessang. With this diversity of vectors, the vectorial capacity and highest transmission period of each *Anopheles* will need to be investigated to enable appropriate vector control strategy decision making and timing.

The selection of 10 new sites for insecticide resistance monitoring contributes to the expansion of data collected across the country to continue to guide NMCP on appropriate and strategic deployment of vector control tools. Similar to data collected by VectorLink in 2020 from Gounougou, Simatou, Mangoum, Nyabessang, and Bonabéri, high pyrethroid resistance was observed in all 10 sites surveyed. Moreover, mortalities were substantially and partially increased when the mosquitoes were pre-exposed to PBO, with reversal to susceptibility to deltamethrin observed in Bertoua and Mogode. The distribution of Olyset net® Plus ITNs in Bertoua during the 2019 mass campaign may have contributed to this result. The trend yielded showed the probable involvement of oxidase enzymes, even though high frequency of the *kdr*-west was recorded at all 10 sites and almost fixed in about eight sites (frequency between 0.9 and 1). Other target sites resistance markers were recorded at all sites. Additionally, susceptibility to chlorfenapyr at 100 µg/bottle was recorded at seven sites and 200 µg/bottle at nine of the 10 sites surveyed. These data can contribute to or confirm ITN selection decisions by the NMCP for the upcoming mass ITN distribution campaign in the country.

As the new selected insecticide resistance monitoring sites were particularly within agricultural settings, the intense use of pesticides and insecticides may be contributing to the resistance observed against carbamate and organophosphates, with resistance to pirimiphos-methyl observed at nine sites and resistance to bendiocarb at six sites. It is known that most of the pesticides and insecticides used in agriculture are mixtures of all classes of insecticides. Therefore, a plan to investigate the insecticide and pesticide usage and frequency in the selected sites will help understand the different trends observed. Furthermore, clothianidin tested on paper using WHO tube test yielded susceptibility at all sites while four sites recorded resistance using bottle assays. Even though both protocols should not be compared due to the different ingredients used, the results observed may require further tests for confirmation.

Considering the vector densities, diversity and level of transmission, appropriate selection of vector control tools and additional vector control strategy such as indoor residual spraying could be considered in select location (e.g., Simatou, where indoor resting density is very high) to reduce the malaria transmission and burden in the country. These data could also be used by the NMCP to estimate the impact of ongoing vector control strategies, namely ITN distributions.

ANNEX A: SPECIES COMPOSITION OF *ANOPHELES* BY METHOD AND SITE

Table A1: *Anopheles* Species Collected by HLCs by Site (October 2020-September 2021)

Anopheles Species	Site					Total
	Gounougou	Simatou	Mangoum	Nyabessang	Bonabéri	
An. gambiae s.l.	4,737	5,174	5,062	243	2,117	**17,333**
An. pharoensis	71	1,489	0	0	0	**1,560**
An. paludis	7	0	0	2,696	0	**2,703**
An. moucheti	0	0	0	1,173	0	**1,173**
An. demeilloni	0	10	0	0	0	**10**
An. ziemanni	52	510	66	1	0	**629**
An. funestus s.l.	368	49	0	0	0	**417**
An. nili	0	0	0	136	0	**136**
An. marshallii	0	0	0	26	0	**26**
An. multicinctus	34	0	0	0	0	**34**
An. rufipes	1	28	0	0	0	**29**
An. squamosus	5	0	0	0	0	**5**
Total	**5,475**	**7,260**	**5,128**	**4,275**	**2,117**	**24,055**

Table A2: *Anopheles* Species Collected by CDC LTs by Site (October 2020-September 2021)

Anopheles Species	Site					Total
	Gounougou	Simatou	Mangoum	Nyabessang	Bonabéri	
An. gambiae s.l.	1,606	905	1,289	24	19	3,843
An. ziemanni	68	711	11	0	0	790
An. pharoensis	5	820	0	0	0	825
An. demeilloni	0	0	0	0	0	0
An. funestus s.l.	69	12	0	0	0	81
An. rufipes	13	65	0	0	0	78
An. moucheti	0	0	0	98	0	98
An. paludis	5	0	0	14	0	19
An. multicinctus	2	0	0	0	0	2
An. nili	0	0	0	3	0	3
Total	**1,768**	**2,513**	**1,300**	**139**	**19**	**5,739**

Table A3: *Anopheles* Species Collected by PSC by Site (October 2020-September 2021)

Anopheles Species	Site					Total
	Gounougou	Simatou	Mangoum	Nyabessang	Bonabéri	
An. gambiae s.l.	3,621	5,054	454	12	2	**9,143**
An. funestus s.l.	478	48	0	0	0	**526**
An. rufipes	84	131	0	0	0	**215**
An. pharoensis	0	4	0	0	0	**4**
An. ziemanni	1	1	0	0	0	**2**
An. moucheti	0	0	0	19	0	**19**
An. paludis	0	0	0	9	0	**9**
An. multicinctus	7	0	0	0	0	**7**
An. squamosus	5	0	0	0	0	**5**
Total	**4,196**	**5,238**	**454**	**40**	**2**	**9,930**

Table A4: *Anopheles* Species Collected by Prokopack by Site (July and September 2021)

Anopheles Species	Gounougou	Simatou	Mangoum	Nyabessang	Bonabéri	Total
An. gambiae s.l.	271	704	1	1	0	**977**
An. funestus s.l.	10	0	0	0	0	**10**
An. rufipes	2	0	0	0	0	**2**
An. pharoensis	0	7	0	0	0	**7**
An. ziemanni	0	8	0	0	0	**8**
An. moucheti	0	0	0	1	0	**1**
Total	**283**	**719**	**1**	**2**	**0**	**1,005**

Table A5: Summary of Samples Collected in the Five Sentinel Sites and Sent to CRID for Analysis (October 2020-September 2021)

Designation	Gounougou		Simatou		Mangoum		Nyabessang		Bonabéri		Total
	An. gambiae s.l.	*An. funestus* s.l.	*An. gambiae* s.l.	*An. funestus* s.l.	*An. gambiae* s.l.	*An. funestus* s.l.	*An. gambiae* s.l.	*An. funestus* s.l.	*An. gambiae* s.l.	*An. funestus* s.l.	
# of samples sent to CRID for analysis	1,283	121	1,713	48	1,151	0	200	0	828	0	**5,344**
# of samples molecularly identified	1,170	111	1,659	48	1,116	0	187	0	805	0	**5,096**
# of samples that did not amplify	113	10	54	0	35	0	13	0	23	0	**248**

Table A6: Species Composition of *An. gambiae* s.l. and *An. funestus* s.l. by Site (October 2020-September 2021)

Sites	*An. gambiae* s.l.				*An. funestus* s.l.		
	An. gambiae s.s.	*An. arabiensis*	*An. coluzzii*	Total	*An. funestus* s.s.	*An. leesoni*	Total
Gounougou	3	66	420	**489**	117	8	**125**
Simatou	0	68	798	**866**	70	0	**70**
Mangoum	509	0	0	**509**	22	0	**22**
Nyabessang	87	0	8	**95**	22	0	**22**
Bonabéri	2	0	467	**469**	22	0	**22**
Total	**601**	**134**	**1,693**	**2,428**	**253**	**8**	**261**

ANNEX B: HUMAN BITING RATE OF *ANOPHELES* MOSQUITO SPECIES BY SITE

Table B1: Human Biting Rate of *Anopheles* Mosquitoes from Gounougou Using HLCs (October 2020-September 2021)

Species	October 2020		November 2020		December 2020		January 2021		February 2021		March 2021	
	TC	HBR	TC	HBR	TC	HBR	TC	HBR	TC	HBR	TC	HBR
An. gambiae s.l.	554	23.08	283	11.79	94	3.92	864	36.00	1,105	46.04	371	15.46
An. funestus s.l.	85	3.54	45	1.88	5	0.21	39	1.63	81	3.38	35	1.46
An. ziemanni	2	0.08	10	0.42	3	0.13	3	0.13	0	0.00	2	0.08
An. multicinctus	0	0.00	0	0.00	0	0.00	2	0.08	3	0.13	5	0.21
An. pharoensis	5	0.21	3	0.13	2	0.08	3	0.13	19	0.79	16	0.67
An. rufipes	0	0.00	1	0.04	0	0.00	0	0.00	0	0.00	0	0.00
An. paludis	0	0.00	0	0.00	0	0.00	0	0.00	2	0.08	5	0.21

Species	April 2021		May 2021		July 2021		September 2021		Total	
	TC	HBR	TC	HBR	TC	HBR	TC	HBR	TC	HBR
An. gambiae s.l.	34	1.42	60	5.00	736	61.33	636	53.00	**4,737**	**23.22**
An. funestus s.l.	18	0.75	18	1.50	30	2.50	12	1.00	**368**	**1.80**
An. ziemanni	9	0.38	1	0.08	5	0.42	17	1.42	**52**	**0.25**
An. multicinctus	0	0.00	0	0.00	0	0.00	24	2.00	**34**	**0.17**
An. pharoensis	2	0.08	1	0.08	11	0.92	9	0.75	**71**	**0.35**
An. rufipes	0	0.00	0	0.00	0	0.00	0	0.00	**1**	**0.00**
An. paludis	0	0.00	0	0.00	0	0.00	0	0.00	**7**	**0.03**

TC=Total collected, HBR=Human Biting Rate

Table B2: Human Biting Rate of *Anopheles* Mosquitoes and Endophagic Index in Gounougou (October 2020-September 2021)

Species	October 2020				November 2020				December 2020			
	HBR in	HBR out	Total HBR	EI	HBR in	HBR out	Total HBR	EI	HBR in	HBR out	Total HBR	EI
An. gambiae s.l.	23.42	22.75	23.08	0.51	14.00	9.58	11.79	0.59	2.58	5.25	3.92	0.33
An. funestus s.l.	3.50	3.58	3.54	0.49	2.42	1.33	1.88	0.64	0.17	0.25	0.21	0.40
An. ziemanni	0.00	0.17	0.08	0.00	0.08	0.75	0.42	0.10	0.08	0.17	0.13	0.33
An. pharoensis	0.00	0.42	0.21	0.00	0.08	0.17	0.13	0.33	0.00	0.17	0.08	0.00
An. rufipes	0.00	0.00	0.00	0.00	0.00	0.08	0.04	0.00	0.00	0.00	0.00	0.00
An. paludis	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Species	January 2021				February 2021				March 2021				April 2021			
	HBR in	HBR out	Total HBR	EI	HBR in	HBR out	Total HBR	EI	HBR in	HBR out	Total HBR	EI	HBR in	HBR out	Total HBR	EI
An. gambiae s.l.	38.83	33.17	36.00	0.54	40.75	51.33	46.04	0.44	13.50	17.42	15.46	0.44	1.92	0.92	1.42	0.68
An. funestus s.l.	1.33	1.92	1.63	0.41	3.33	3.42	3.38	0.49	1.75	1.17	1.46	0.60	1.33	0.17	0.75	0.89
An. ziemanni	0.00	0.25	0.13	0.00	0.00	0.00	0.00	0.00	0.08	0.08	0.08	0.50	0.17	0.58	0.38	0.22
An. pharoensis	0.17	0.08	0.13	0.67	0.67	0.92	0.79	0.42	0.92	0.42	0.67	0.69	0.08	0.08	0.08	0.50
An. rufipes	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
An. paludis	0.00	0.00	0.00	0.00	0.00	0.17	0.08	0.00	0.17	0.25	0.21	0.00	0.00	0.00	0.00	0.00

Species	May 2021				July 2021				September 2021				Total			
	HBR in	HBR out	Total HBR	EI	HBR in	HBR out	Total HBR	EI	HBR in	HBR out	Total HBR	EI	HBR in	HBR out	Total HBR	EI
An. gambiae s.l.	5.83	4.17	5.00	0.58	59.83	62.83	61.33	0.49	49.67	56.33	53.00	0.47	22.67	23.77	23.22	0.49
An. funestus s.l.	1.67	1.33	1.50	0.56	3.33	1.67	2.50	0.67	1.00	1.00	1.00	0.50	1.98	1.63	1.80	0.55
An. ziemanni	0.17	0.00	0.08	1.00	0.00	0.83	0.42	0.00	0.33	2.50	1.42	0.12	0.08	0.43	0.25	0.15
An. pharoensis	0.00	0.17	0.08	0.00	0.83	1.00	0.92	0.45	0.33	1.17	0.75	0.22	0.29	0.40	0.35	0.42
An. rufipes	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00
An. paludis	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.05	0.03	0.29

HBR=Human Biting Rate, EI = Endophagic Index

Table B3: Human Biting Rate of *Anopheles* Mosquitoes from Simatou Using HLCs (October 2020-September 2021)

Species	October 2020		November 2020		December 2020		January 2021		February 2021	
	TC	HBR	TC	HBR	TC	HBR	TC	HBR	TC	HBR
An. gambiae s.l.	377	15.71	87	3.63	14	0.58	23	0.96	41	1.71
An. funestus s.l.	25	1.04	19	0.79	1	0.04	0	0.00	1	0.04
An. ziemanni	23	0.96	4	0.17	0	0.00	2	0.08	0	0.00
An. pharoensis	29	1.21	57	2.38	28	1.17	88	3.67	250	10.42
An. rufipes	12	0.50	4	0.17	1	0.04	1	0.04	2	0.08

Species	March 2021		April 2021		May 2021		July 2021		September 2021		Total	
	TC	HBR	TC	Total HBR	TC	HBR	TC	HBR	TC	HBR	TC	Total HBR
An. gambiae s.l.	90	3.75	519	21.63	389	32.42	2,896	241.33	738	61.50	**5,174**	**25.36**
An. funestus s.l.	0	0.00	3	0.13	0	0.00	0	0.00	0	0.00	**49**	**0.24**
An. ziemanni	0	0.00	1	0.04	103	8.58	0	0.00	377	31.42	**510**	**2.50**
An. pharoensis	44	1.83	448	18.67	298	24.83	40	3.33	207	17.25	**1,489**	**7.30**
An. rufipes	4	0.17	2	0.08	2	0.17	0	0.00	0	0.00	**28**	**0.14**

TC=Total collected, HBR=Human Biting Rate

Table B4: Human Biting Rate and Endophagic Index in Simatou Using HLCs (October 2020-September 2021)

Species	October 2020				November 2020				December 2020			
	HBR in	HBR out	Total HBR	EI	HBR in	HBR out	Total HBR	EI	HBR in	HBR out	Total HBR	EI
An. gambiae s.l.	15.00	16.42	15.71	0.48	4.50	2.75	3.63	0.62	0.75	0.42	0.58	0.64
An. funestus s.l.	1.17	0.92	1.04	0.56	1.17	0.42	0.79	0.74	0.00	0.08	0.04	0.00
An. ziemanni	1.08	0.83	0.96	0.57	0.25	0.08	0.17	0.75	0.00	0.00	0.00	0.00
An. pharoensis	1.08	1.33	1.21	0.45	2.75	2.00	2.38	0.58	1.58	0.75	1.17	0.68
An. rufipes	0.67	0.33	0.50	0.67	0.00	0.33	0.17	0.00	0.08	0.00	0.04	1.00
An. demeilloni	0.25	0.33	0.29	0.43	0.17	0.08	0.13	0.67	0.00	0.00	0.00	0.00

Species	January 2021				February 2021				March 2021			
	HBR in	HBR out	Total HBR	EI	HBR in	HBR out	Total HBR	EI	HBR in	HBR out	Total HBR	EI
An. gambiae s.l.	1.17	0.75	0.96	0.61	2.00	1.42	1.71	0.59	3.75	3.75	3.75	0.50
An. funestus s.l.	0.00	0.00	0.00	0.00	0.00	0.08	0.04	0.00	0.00	0.00	0.00	0.00
An. ziemanni	0.08	0.08	0.08	0.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
An. pharoensis	4.17	3.17	3.67	0.57	11.83	9.00	10.42	0.57	2.50	1.17	1.83	0.68
An. rufipes	0.08	0.00	0.04	1.00	0.17	0.00	0.08	1.00	0.08	0.25	0.17	0.24
An. demeilloni	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

HBR=Human Biting Rate, EI = Endophagic Index

Table B4 (continued)

Species	March 2021				April 2021				May 2021			
	HBR in	HBR out	Total HBR	EI	HBR in	HBR out	Total HBR	EI	HBR in	HBR out	Total HBR	EI
An. gambiae s.l.	3.75	3.75	3.75	0.50	20.17	23.08	21.63	0.47	32.83	32.00	32.42	0.51
An. funestus s.l.	0.00	0.00	0.00	0.00	0.17	0.08	0.13	0.67	0.00	0.00	0.00	0.00
An. ziemanni	0.00	0.00	0.00	0.00	0.08	0.00	0.04	1.00	9.17	8.00	8.58	0.53
An. pharoensis	2.50	1.17	1.83	0.68	18.17	19.17	18.67	0.49	26.83	22.83	24.83	0.54
An. rufipes	0.08	0.25	0.17	0.24	0.17	0.00	0.08	0.00	0.33	0.00	0.17	1.00
An. demeilloni	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Species	July 2021				September 2021				Total			
	HBR in	HBR out	Total HBR	EI	HBR in	HBR out	Total HBR	EI	HBR in	HBR out	Total HBR	EI
An. gambiae s.l.	250.67	232.00	241.33	0.52	65.17	57.83	61.50	0.53	**26.08**	**24.65**	**25.36**	**0.51**
An. funestus s.l.	0.00	0.00	0.00	0.00	0.00	0.00	0.00	-	**0.29**	**0.19**	**0.24**	**0.61**
An. ziemanni	0.00	0.00	0.00	0.00	27.67	35.17	31.42	0.44	**2.34**	**2.66**	**2.50**	**0.47**
An. pharoensis	3.33	3.33	3.33	0.50	16.00	18.50	17.25	0.46	**7.67**	**6.93**	**7.30**	**0.53**
An. rufipes	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**0.17**	**0.11**	**0.14**	**0.61**
An. demeilloni	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**0.05**	**0.05**	**0.05**	**0.00**

HBR=Human Biting Rate, EI = Endophagic Index

Table B5: Human Biting Rate of *Anopheles* Mosquitoes from Mangoum Using HLCs (October 2020-September 2021)

Species	October 2020		November 2020		December 2020		January 2021		February 2021		March 2021		April 2021	
	TC	Total HBR	TC	Total HBR	TC	Total HBR	TC	Total HBR	TC	Total HBR	TC	Total HBR	TC	Total HBR
An. gambiae s.l.	467	19.46	199	8.29	238	9.92	522	21.75	790	32.92	1,524	63.50	612	25.50
An. ziemanni	58	2.42	4	0.17	1	0.04	1	0.04	0	0.00	0	0.00	0	0.00

Species	May 2021		July 2021		September 2021		Total	
	TC	Total HBR	TC	Total HBR	TC	Total HBR	Total Collected	Total HBR
An. gambiae s.l.	508	42.33	27	2.25	175	14.58	**5,062**	**24.81**
An. ziemanni	0	0.00	2	0.17	0	0.00	**66**	**0.32**

TC=Total collected, HBR=Human Biting Rate

Table B6: Human Biting Rate of *Anopheles* Mosquitoes and Endophagic Index in Mangoum (October 2020-September 2021)

Species	October 2020				November 2020				December 2020				January 2021			
	HBR in	HBR out	Total HBR	EI	HBR in	HBR out	Total HBR	EI	HBR in	HBR out	Total HBR	EI	HBR in	HBR out	Total HBR	EI
An. gambiae s.l.	17.42	21.50	19.46	0.45	8.17	8.42	8.29	0.49	10.08	9.75	9.92	0.51	18.83	24.67	21.75	0.43
An. ziemanni	0.50	4.33	2.42	0.10	0.00	0.33	0.17	0.00	0.00	0.08	0.04	0.00	0.00	0.08	0.04	0.00

Species	February 2021				March 2021				April 2021				March 2021			
	HBR in	HBR out	Total HBR	EI	HBR in	HBR out	Total HBR	EI	HBR in	HBR out	Total HBR	EI	HBR in	HBR out	Total HBR	EI
An. gambiae s.l.	27.25	38.58	32.92	0.41	63.17	63.83	63.50	0.50	23.50	27.50	25.50	0.46	63.17	63.83	63.50	0.50
An. ziemanni	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	-

Species	April 2021				May 2021				July 2021				September 2021				Total			
	HBR in	HBR out	Total HBR	EI	HBR in	HBR out	Total HBR	EI	HBR in	HBR out	Total HBR	EI	HBR in	HBR out	Total HBR	EI	HBR in	HBR out	Total HBR	EI
An. gambiae s.l.	23.50	27.50	25.50	0.46	37.33	47.33	42.33	0.44	1.83	2.67	2.25	0.41	10.50	18.67	14.58	0.36	**22.74**	**26.89**	**24.81**	**0.46**
An. ziemanni	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.17	0.17	0.17	0.50	0.00	0.00	0.00	0.00	**0.07**	**0.58**	**0.32**	**0.11**

HBR=Human Biting Rate, EI = Endophagic Index

Table B7: Human Biting Rate of *Anopheles* Mosquitoes from Nyabessang Using HLCs (October 2020-September 2021)

Species	October 2020		November 2020		December 2020		January 2021		February 2021		March 2021		April 2021	
	TC	Total HBR	TC	Total HBR	TC	Total HBR	TC	Total HBR	TC	Total HBR	TC	Total HBR	TC	Total HBR
An. gambiae s.l.	107	4.46	22	0.92	4	0.17	9	0.38	30	1.25	39	1.63	13	0.54
An. moucheti	214	8.92	298	12.42	171	7.13	204	8.50	56	2.33	49	2.04	12	0.50
An. paludis	413	17.21	845	35.21	562	23.42	229	9.54	115	4.79	125	5.21	154	6.42
An. nili	47	1.96	17	0.71	4	0.17	1	0.04	3	0.13	43	1.79	5	0.21

Species	May 2021		July 2021		September 2021		Total	
	TC	Total HBR	TC	Total HBR	TC	Total HBR	TC	Total HBR
An. gambiae s.l.	10	0.83	2	0.17	7	0.58	**243**	**1.19**
An. moucheti	39	3.25	79	6.58	51	4.25	**1,173**	**5.75**
An. paludis	119	9.92	110	9.17	24	2.00	**2,696**	**13.22**
An. nili	1	0.08	3	0.25	12	1.00	**136**	**0.67**

TC=Total collected, HBR=Human Biting Rate

Table B8: Human Biting Rate of *Anopheles* Mosquitoes and Endophagic Index in Nyabessang (October 2020-September 2021)

Species	October 2020				November 2020				December 2020				January 2021			
	HBR in	HBR out	Total HBR	EI	HBR in	HBR out	Total HBR	EI	HBR in	HBR out	Total HBR	EI	HBR in	HBR out	Total HBR	EI
An. gambiae s.l.	5.75	3.17	4.46	0.64	1.00	0.83	0.92	0.55	0.17	0.17	0.17	0.50	0.17	0.58	0.38	0.22
An. moucheti	9.83	8.00	8.92	0.55	11.58	13.25	12.42	0.47	7.92	6.33	7.13	0.56	6.25	10.75	8.50	0.37
An. paludis	11.92	22.50	17.21	0.35	12.58	57.83	35.21	0.18	18.92	27.92	23.42	0.40	8.83	10.25	9.54	0.46
An. nili	1.83	2.08	1.96	0.47	0.75	0.67	0.71	0.53	0.25	0.08	0.17	0.75	0.00	0.08	0.04	0.00

Species	February 2021				March 2021				April 2021				May 2021			
	HBR in	HBR out	Total HBR	EI	HBR in	HBR out	Total HBR	EI	HBR in	HBR out	Total HBR	EI	HBR in	HBR out	Total HBR	EI
An. gambiae s.l.	1.33	1.17	1.25	0.53	1.42	1.83	1.63	0.44	0.83	0.25	0.54	0.77	1.00	0.67	0.83	0.60
An. moucheti	1.17	3.50	2.33	0.25	1.50	2.58	2.04	0.37	0.42	0.58	0.50	0.42	4.67	1.83	3.25	0.72
An. paludis	4.33	5.25	4.79	0.45	2.58	7.83	5.21	0.25	4.58	8.25	6.42	0.36	12.17	7.67	9.92	0.61
An. nili	0.08	0.17	0.13	0.33	1.75	1.83	1.79	0.49	0.25	0.17	0.21	0.60	0.00	0.17	0.08	0.00

Species	July 2021				September 2021				Total			
	HBR in	HBR out	Total HBR	EI	HBR in	HBR out	Total HBR	EI	HBR in	HBR out	Total HBR	EI
An. gambiae s.l.	0.33	0.00	0.17	1.00	0.67	0.50	0.58	0.57	**1.37**	**1.01**	**1.19**	**0.58**
An. moucheti	9.50	3.67	6.58	0.72	4.33	4.17	4.25	0.51	**5.64**	**5.86**	**5.75**	**0.49**
An. paludis	9.33	9.00	9.17	0.51	1.33	2.67	2.00	0.33	**8.84**	**17.59**	**13.22**	**0.33**
An. nili	0.33	0.17	0.25	0.67	0.83	1.17	1.00	0.42	**0.65**	**0.69**	**0.67**	**0.49**

HBR=Human Biting Rate, EI = Endophagic Index

Table B9: Human Biting Rate of *Anopheles* Mosquitoes from Bonabéri Using HLCs (October 2020-September 2021)

Species	October 2020		November 2020		December 2020		January 2021		February 2021	
	Total collected	Total HBR	Total collected	Total HBR	Total collected	Total HBR	Total collected	Total HBR	Total collected	Total HBR
An. gambiae s.l.	89	3.71	40	1.67	22	0.92	32	1.33	39	1.63

Species	March 2021		April 2021		May 2021		July 2021		September 2021		Total	
	Total collected	Total HBR	Total collected	Total HBR	Total collected	Total HBR	Total collected	Total HBR	Total collected	Total HBR	Total collected	Total HBR
An. gambiae s.l.	263	10.96	430	17.92	579	48.25	277	23.08	346	28.83	**2,117**	**10.38**

HBR=Human Biting Rate

Table B10: Human Biting Rate and Endophagic Index of *An. gambiae* s.l. in Bonabéri (October 2020-September 2021)

Month	Indoor	HBR in	Outdoor	HBR out	Total	Total HBR	EI
October 2020	31	2.58	58	4.83	**89**	**3.71**	**0.35**
November 2020	8	0.67	32	2.67	**40**	**1.67**	**0.20**
December 2020	4	0.33	18	1.50	**22**	**0.92**	**0.18**
January 2021	7	0.58	25	2.08	**32**	**1.33**	**0.22**
February 2021	11	0.92	28	2.33	**39**	**1.63**	**0.28**
March 2021	76	6.33	187	15.58	**263**	**10.96**	**0.29**
April 2021	121	10.08	309	25.75	**430**	**17.92**	**0.28**
May 2021	199	33.17	380	63.33	**579**	**48.25**	**0.34**
July 2021	104	17.33	173	28.83	**277**	**23.08**	**0.38**
September 2021	101	16.83	245	40.83	**346**	**28.83**	**0.29**
TOTAL	**662**	**6.49**	**1,455**	**14.26**	**2,117**	**10.38**	**0.31**

HBR=Human Biting Rate, EI = Endophagic Index

ANNEX C: HUMAN BLOOD INDEX, PARITY, AND INFECTION RATES BY SITE

Table C1: Human Blood Index of *Anopheles* Mosquitoes Across Sentinel Sites

Sites	Host	*An. gambiae* s.l.	*An. funestus* s.l.	*An. moucheti*	*An. ziemanni*	*An. rufipes*	Total	Total HBI
Gounougou	Human	105	35	0	0	1	**141**	63.3%
	Animal	69	12	0	0	6	**87**	
	Mix	66	12	0	0	0	**78**	
	Not identified	23	10	0	0	7	**40**	
	Total	**263**	**69**	**0**	**0**	**14**	**346**	
Simatou	Human	262	1	0	0	0	**263**	58.7%
	Animal	150	0	0	0	5	**155**	
	Mix	189	2	0	0	0	**191**	
	Not identified	160	2	0	0	3	**165**	
	Total	**761**	**5**	**0**	**0**	**8**	**774**	
Mangoum	Human	24	0	0	0	0	**24**	100.0%
	Animal	0	0	0	0	0	**0**	
	Mix	2	0	0	0	0	**2**	
	Not identified	0	0	0	0	0	**0**	
	Total	**26**	**0**	**0**	**0**	**0**	**26**	
Nyabessang	Human	3	0	1	0	0	**4**	80.0%
	Animal	0	0	0	0	0	**0**	
	Mix	0	0	0	0	0	**0**	
	Not identified	0	0	1	0	0	**1**	
	Total	**3**	**0**	**2**	**0**	**0**	**5**	
Bonabéri	Human	**1**	**0**	**0**	**0**	**0**	**1**	100.0%
	Animal	**0**	**0**	**0**	**0**	**0**	**0**	
	Mix	**0**	**0**	**0**	**0**	**0**	**0**	
	Not identified	**0**	**0**	**0**	**0**	**0**	**0**	
	Total	**1**	**0**	**0**	**0**	**0**	**1**	

Table C2: Parity Rate of *Anopheles* Mosquitoes Across Sentinel Sites

Sites	Species	Ovaries dissected	# Parous	Parity Rate (%)
Gounougou	*An. gambiae* s.l.	1,021	837	81.98
	An. funestus s.l.	97	86	88.66
Simatou	*An. gambiae* s.l.	1,435	1,138	79.30
	An. funestus s.l.	34	34	100.00
Mangoum	*An. gambiae* s.l.	404	235	58.17
	An. funestus s.l.	0	0	0.00
Nyabessang	*An. gambiae* s.l.	91	65	71.43
	An. funestus s.l.	0	0	0.00
Bonabéri	*An. gambiae* s.l.	1,031	712	69.06
	An. funestus s.l.	0	0	0.00
Total	***An. gambiae* s.l.**	3,982	2,987	75.01
	***An. funestus* s.l.**	131	120	91.60

Table C3: Infection Rate of *Anopheles* Mosquitoes by Site (October 2020-September 2021)

Species	Gounougou			Simatou			Mangoum			Nyabessang			Bonabéri			Total		
	# Tested	# Pos.	% Infection	# Tested	# Pos.	% Infection	# Tested	# Pos.	% Infection	# Tested	# Pos.	% Infection	# Tested	# Pos.	% Infection	# Tested	# Pos.	% Infection
An. gambiae s.l.	620	20	3.2	870	10	1.1	1,048	38	3.6	79	7	8	915	29	3.1	3,532	104	2.9
An. funestus s.l.	79	3	3.7	0	0	0	0	0	0	0	0	0	0	0	0	79	3	3.7
An. pharoensis	19	0	0	175	1	0.5	0	0	0	0	0	0	0	0	0	194	1	0.5
An. ziemanni	20	0	0	36	0	0	56	3	5.3	0	0	0	0	0	0	112	3	2.6
An. moucheti	0	0	0	0	0	0	0	0	0	160	2	1.2	0	0	0	160	2	1.2
An. nili	0	0	0	0	0	0	0	0	0	54	2	3.7	0	0	0	54	2	3.7
An. marshallii	0	0	0	0	0	0	0	0	0	21	0	0	0	0	0	21	0	0
An. paludis	0	0	0	0	0	0	0	0	0	321	3	0.9	0	0	0	321	3	0.9
TOTAL	**738**	**23**	**3.1**	**1,081**	**11**	**1.01**	**1,104**	**41**	**3.7**	**635**	**14**	**2.2**	**915**	**29**	**3.1**	**4,473**	**118**	**2.6**

ANNEX D: WHO SUSCEPTIBILITY TEST AND CDC BOTTLE RESULTS

Table D1: *An. gambiae* s.l. WHO Susceptibility Test Results Across Sites in 2021

	Bertoua		Djohong		Garoua		Gazawa		Mada	
Insecticide	Total exposed	% Mortality	Total exposed	% Mortality	Total exposed	% Mortality	Total exposed	% Mortality	Total exposed	% Mortality
Pirimiphos-methyl 1x	96	89.7	97	84.0	100	60.0	99	93.0	84	96.4
Permethrin 1x	99	31.5	93	0.0	100	13.0	84	0.0	86	35.0
Permethrin 5x	99	96.0	90	58.6	92	47.3	89	35.9	87	53.1
Permethrin 10x	102	98.0	91	83.4	98	70.2	98	84.7	98	82.6
PBO + Permethrin	98	85.1	88	19.6	100	25.0	87	5.7	88	56.9
Deltamethrin 1x	100	81.1	87	3.5	100	19.0	85	0.0	85	38
Deltamethrin 5x	99	90.9	90	46.1	100	30.0	95	24.2	94	38.3
Deltamethrin 10x	95	95.1	95	75.5	100	54.0	95	41.9	89	44.5
PBO + Deltamethrin	97	99.0	84	95.1	100	38.0	87	13.6	84	72.6
Alpha-cypermethrin 1x	99	35.4	83	1.3	100	7.0	89	0.0	97	11.5
Alpha-cypermethrin 5x	100	76.1	92	19.9	100	15.0	92	3.3	102	35.2
Alpha-cypermethrin 10x	100	75.2	92	40.1	97	18.6	95	13.7	91	39.2
PBO + Alpha-cypermethrin	100	84.0	91	58.9	100	43.0	87	18.6	86	41.9
Bendiocarb 1x	101	82.2	97	95.8	100	97.0	95	100.0	85	100.0

Insecticide	Mogode		Ndelele		Ngaoundere		Njombe*		Touboro	
	Total exposed	% Mortality	Total exposed	% Mortality	Total exposed	% Mortality	Total exposed	% Mortality	Total exposed	% Mortality
Pirimiphos-methyl 1x	95	85.2	105	92.3	94	5.4	105	92.3	85	72.9
Permethrin 1x	97	8.3	100	38.0	98	4.0	83	73.5	92	4.3
Permethrin 5x	96	54.2	102	75.5	94	51.2	NT	NT	88	80.7
Permethrin 10x	100	84.0	100	89.0	92	70.8	NT	NT	84	86.7
PBO + Permethrin	96	21.9	100	59.2	90	9.9	83	82	86	75.5
Deltamethrin 1x	89	43.8	101	17.7	95	12.6	82	66.8	93	1.2
Deltamethrin 5x	96	51.1	100	52.0	87	28.4	NT	NT	85	16.6
Deltamethrin 10x	98	64.3	100	69.0	90	43.4	NT	NT	80	82.6
PBO + Deltamethrin	90	97.8	100	84.0	92	64.1	83	82.0	93	78.4
Alpha-cypermethrin 1x	95	5.3	100	3.0	89	1.1	83	82.0	88	1.1
Alpha-cypermethrin 5x	94	33.1	104	46.7	93	33.1	NT	NT	90	17.7
Alpha-cypermethrin 10x	99	48.6	106	77.4	90	25.6	NT	NT	81	33.3
PBO + Alpha-cypermethrin	95	80.9	100	60.0	91	49.3	83	90.6	84	59.7
Bendiocarb 1x	96	99.0	100	93.0	94	95.7	83	82.0	85	98.8

*NT = Not tested

Table D2: *An. gambiae* s.l. WHO Susceptibility Test Results with Clothianidin 2% Across Sites in 2021

	Bertoua		Djohong		Garoua		Gazawa		Mada	
Times (hours)	Total exposed	% Mortality	Total exposed	% Mortality	Total exposed	% Mortality	Total exposed	% Mortality	Total exposed	% Mortality
J1 (24 hours)	100	97.0	85	82.2	99	97.0	95	78.0	87	100.0
J2	100	100.0	85	92.1	100	100.0	95	97.9	87	100.0
J3	100	100.0	85	96.6	100	100.0	95	100.0	87	100.0
J4	100	100.0	85	97.8	100	100.0	95	100.0	87	100.0
J5	100	100.0	85	97.8	100	100.0	95	100.0	87	100.0
J6	100	100.0	85	98.8	100	100.0	95	100.0	87	100.0
J7	100	100.0	85	100.0	100	100.0	95	100.0	87	100.0

	Mogode		Ndelele		Ngaoundere		Njombe		Touboro	
Times (hours)	Total exposed	% Mortality	Total exposed	% Mortality	Total exposed	% Mortality	Total exposed	% Mortality	Total exposed	% Mortality
J1 (24 hours)	100	58.0	100	58.0	100	66.0	81	67.8	82	96.3
J2	100	73.0	100	73.0	100	81.0	81	82.2	82	100.0
J3	100	100.0	100	100.0	100	92.0	81	98.9	82	100.0
J4	100	100.0	100	100.0	100	97.0	81	98.9	82	100.0
J5	100	100.0	100	100.0	100	98.0	81	98.9	82	100.0
J6	100	100.0	100	100.0	100	100.0	81	100.0	82	100.0
J7	100	100.0	100	100.0	100	100.0	81	100.0	82	100.0

Table D3: *An. gambiae* s.l. CDC Bottle Assay Test Results with Clothianidin (4 µg/bottle) Across Sites in 2021

	Bertoua		Djohong		Garoua		Gazawa		Mada	
Times (hours)	Total exposed	% Mortality	Total exposed	% Mortality	Total exposed	% Mortality	Total exposed	% Mortality	Total exposed	% Mortality
J1 (24 hours)	97	97.9	99	94.8	99	97.9	96	100.0	110	100.0

	Mogode		Ndelele		Ngaoundere		Njombe		Touboro	
Times (hours)	Total exposed	% Mortality	Total exposed	% Mortality	Total exposed	% Mortality	Total exposed	% Mortality	Total exposed	% Mortality
J1 (24 hours)	93	100.0	100	98.0	96	66.2	101	96.0	108	37.5

Table D4: *An. gambiae* s.l. CDC Bottle Assay Test Results with Chlorfenapyr (100 µg/bottle) Across Sites in 2021

	Bertoua		Djohong		Garoua		Gazawa		Mada	
Times (hours)	Total exposed	% Mortality	Total exposed	% Mortality	Total exposed	% Mortality	Total exposed	% Mortality	Total exposed	% Mortality
J1 (24 hours)	100	99.0	92	85.8	99	92.1	98	100.0	109	100.0
J2	100	100.0	92	97.9	99	99.0	98	100.0	109	100.0
J3	100	100.0	92	98.9	99	99.0	98	100.0	109	100.0

	Mogode		Ndelele		Ngaoundere		Njombe		Touboro	
Times (hours)	Total exposed	% Mortality	Total exposed	% Mortality	Total exposed	% Mortality	Total exposed	% Mortality	Total exposed	% Mortality
J1 (24 hours)	94	50.9	100	98.0	100	83.0	99	99.0	102	16.8
J2	94	76.3	100	98.0	100	93.0	99	99.0	102	27.6
J3	94	89.3	100	100.0	100	95.0	99	100.0	102	31.5

Table D5: *An. gambiae* s.l. CDC Bottle Assay Test Results with Chlorfenapyr (200 µg/bottle) Across Sites in 2021

	Bertoua		Djohong		Garoua		Gazawa		Mada	
Times (hours)	Total exposed	% Mortality	Total exposed	% Mortality	Total exposed	% Mortality	Total exposed	% Mortality	Total exposed	% Mortality
J1 (24 hours)	100	100.0	99	86.8	100	97.0	95	100.0	101	100.0
J2	100	100.0	99	97.9	100	100.0	95	100.0	101	100.0
J3	100	100.0	99	98.9	100	100.0	95	100.0	101	100.0

	Mogode		Ndelele		Ngaoundere		Njombe		Touboro	
Times (hours)	Total exposed	% Mortality	Total exposed	% Mortality	Total exposed	% Mortality	Total exposed	% Mortality	Total exposed	% Mortality
J1 (24 hours)	93	63.8	100	98.0	96	87.9	100	99.0	113	99.1
J2	93	92.7	100	100.0	96	95.9	100	99.0	113	99.1
J3	93	98.0	100	100.0	96	96.9	100	99.0	113	100.0

Table D6: Frequency of Target Site Resistance Allele Across Sites in 2021

Sentinel Site	*Kdr*-west			*Kdr*-east			N1575Y			*Ace*-1		
	RR	RS	SS	RR	RS	SS	RR	RS	SS	RR	RS	SS
Bertoua	47	13	0	0	2	58	1	3	56	16	0	25
Djohong	46	0	1	0	4	53	0	9	48	4	0	38
Garoua	10	35	12	0	0	57	4	10	43	2	2	36
Gazawa	16	38	3	0	0	57	2	14	41	0	0	43
Mada	37	6	14	0	0	57	0	15	42	0	0	43
Mogode	62	0	0	0	0	61	0	5	57	0	0	39
Ndelele	66	0	0	1	14	51	0	5	61	5	0	21
Ngaoundere	33	2	2	0	0	45	0	9	36	3	0	25
Njombe	44	4	0	0	1	47	0	0	48	2	0	44
Touboro	46	10	3	0	1	58	0	10	49	2	0	38
Total	**407**	**108**	**35**	**1**	**22**	**544**	**7**	**80**	**481**	**34**	**2**	**352**

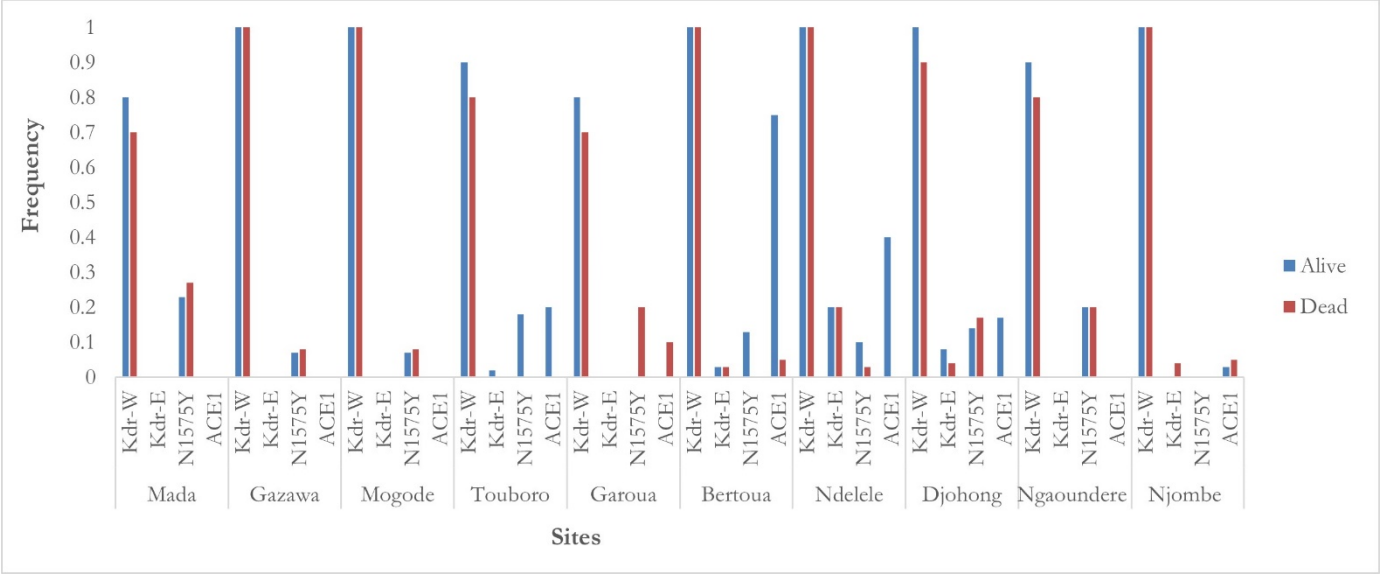
RR = Homozygous Resistant; RS = Heterozygous Resistant; SS = Homozygous Susceptible

Table D7: Frequency of Target Site Resistance Alleles in Dead and Alive *An. gambiae* s.l. Across Sites in 2021

Sentinel Sites	Status	*Kdr*-west			*Kdr*-east			N1575Y			*Ace*-1		
		RR	RS	SS	RR	RS	SS	RR	RS	SS	RR	RS	SS
Bertoua	Alive	24	6	0	0	1	29	1	3	26	15	0	5
	Dead	23	7	0	0	1	29	0	0	30	1	0	20
Djohong	Alive	25	0	0	0	3	31	0	5	29	4	0	19
	Dead	21	0	1	0	1	22	0	4	19	0	0	19
Garoua	Alive	6	20	9	0	0	35	2	5	28	1	2	24
	Dead	4	15	3	0	0	22	2	5	15	1	0	12
Gazawa	Alive	16	38	3	0	0	57	2	14	41	0	0	7
	Dead	0	0	0	0	0	0	0	0	0	0	0	36
Mada	Alive	17	0	4	0	0	21	0	5	16	0	0	3
	Dead	20	6	10	0	0	36	0	10	26	0	0	40
Mogode	Alive	38	0	0	0	0	37	0	3	35	0	0	22
	Dead	24	0	0	0	0	24	0	2	22	0	0	17
Ndelele	Alive	35	0	0	0	7	28	0	4	31	5	0	6
	Dead	31	0	0	1	7	23	0	1	30	0	0	15
Ngaoundere	Alive	25	1	0	0	0	30	0	6	24	3	0	11
	Dead	8	1	2	0	0	15	0	3	12	0	0	14
Njombe	Alive	24	2	0	0	0	26	0	0	26	1	0	27
	Dead	20	2	0	0	1	21	0	0	22	1	0	17
Touboro	Alive	42	9	2	0	1	52	0	10	43	2	0	8
	Dead	4	1	1	0	0	6	0	0	6	0	0	30
Total		**407**	**108**	**35**	**1**	**22**	**544**	**7**	**80**	**481**	**34**	**2**	**352**

RR = Homozygous Resistant; RS = Heterozygous Resistant; SS = Homozygous Susceptible

Figure 40: Frequency of Target Site Resistance Alleles in Dead and Alive *An. gambiae* s.l. Across Sites in 2021



ANNEX E: REFERENCES

Beier JC, Perkins PV, Wirtz RA, Koros J, Diggs D, Gargan TP, 2nd, et al. Bloodmeal identification by direct enzyme-linked immunosorbent assay (ELISA), tested on *Anopheles* (Diptera: Culicidae) in Kenya. J Med Entomol. 1988 Jan;25(1):9-16.

Burkot TR, Williams JL, Schneider I: Identification of *Plasmodium falciparum*-infected mosquitoes by a double antibody enzyme-linked immunosorbent assay. Am J Trop Med Hyg . 1984 Sep;33(5):783-8. doi: 10.4269/ajtmh.1984.33.783.

Coetzee, M. (2020): Key to the females of Afrotropical *Anopheles*, Malar J. 2020; 19:70.

Coetzee M, Craig M, le Sueur D 2000. Distribution of African malaria mosquitoes belonging to the *Anopheles gambiae* complex. Parasitol Today 16: 74-77.

Cohuet A, Simard F, Toto JC, Kengne P, Coetzee M, Fontenille D. Species identification within the *Anopheles funestus* group of malaria vectors in Cameroon and evidence for a new species. Am J Trop Med Hyg. 2003 Aug;69(2):200-5.

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

Fossog Tene B, Poupardin R, Costantini C, Awono-Ambene P, Wondji CS, Ranson H, et al. Resistance to DDT in an urban setting: common mechanisms implicated in both M and S forms of *Anopheles gambiae* in the city of Yaounde Cameroon. PLoS One. 2013;8(4): e61408.

Fouet, Caroline et al. 2020: Resistance of *Anopheles gambiae* to the new insecticide clothianidin associated with unrestricted use of agricultural neonicotinoids in Yaoundé, Cameroon. BioRxiv, 2020.

Jones CM, Liyanapathirana M, Agossa FR, Weetman D, Ranson H, Donnelly MJ, et al. Footprints of positive selection associated with a mutation (N1575Y) in the voltage-gated sodium channel of *Anopheles gambiae*. Proc Natl Acad Sci U S A. 2012 Apr 24;109(17):6614-9.

Koekemoer LL, Kamau L, Hunt RH, Coetzee M. A cocktail polymerase chain reaction assay to identify members of the *Anopheles funestus* (Diptera: Culicidae) group. Am J Trop Med Hyg. 2002 Jun;66(6):804-11.

Kwiatkowska RM, Platt N, Poupardin R, Irving H, Dabire RK, Mitchell S, et al. Dissecting the mechanisms responsible for the multiple insecticide resistance phenotype in *Anopheles gambiae* s.s., M form, from Vallee du Kou, Burkina Faso. Gene. 2013 Apr 25; 519(1):98-106.

Lindsay SW, Parson L, Thomas CJ 1998. Mapping the ranges and relative abundance of the two principal African malaria vectors, *Anopheles gambiae* sensu stricto and *An. arabiensis*, using climate data. Proc R Soc London B 265: 847-854.

Martinez-Torres D, Chandre F, Williamson MS, Darriet F, Berge JB, Devonshire AL, et al. Molecular characterization of pyrethroid knockdown resistance (kdr) in the major malaria vector *Anopheles gambiae* s.s. Insect Mol Biol. 1998;7:179 - 84.

Menze BD, Riveron JM, Ibrahim SS, Irving H, Antonio-Nkondjio C, Awono-Ambene PH, et al. Multiple insecticide resistance in the malaria vector *Anopheles funestus* from northern Cameroon is mediated by metabolic resistance alongside potential target site insensitivity mutations. PLoS One. 2016;11(10): e0163261.

Menze BD, Wondji MJ, Tchapga W, Tchoupo M, Riveron JM, Wondji CS. Bionomics and insecticides resistance profiling of malaria vectors at a selected site for experimental hut trials in Central Cameroon. Malaria Journal. 2018; In Press.

NMCP, 2020: Malaria Report.

NMCP, 2020: National Strategic Plan (2019-2023).

Ranson H, Jensen B, Vulule JM, Wang X, Hemingway J, Collins FH. Identification of a point mutation in the voltage-gated sodium channel gene of Kenyan *Anopheles gambiae* associated with resistance to DDT and pyrethroids. Insect Molec Biol. 2000;9(5):491 - 7.

Riveron JM, Yunta C, Ibrahim SS, Djouaka R, Irving H, Menze BD, et al. A single mutation in the GSTe2 gene allows tracking of metabolically based insecticide resistance in a major malaria vector. Genome Biol. 2014 Feb 25;15(2).

Santolamazza F, Mancini E, Simard F, Qi Y, Tu Z, della Torre A. Insertion polymorphisms of SINE200 retrotransposons within speciation islands of *Anopheles gambiae* molecular forms. Malar J. 2008;7:163.

Weill M, Malcolm C, Chandre F, Mogensen K, Berthomieu A, Marquine M, et al. The unique mutation in ace-1 giving high insecticide resistance is easily detectable in mosquito vectors. Insect Molecular Biology. 2004;13(1):1-7.

White GB 1974. *Anopheles gambiae* complex and disease transmission in Africa. Trans R Soc Trop Med Hyg 68: 278-301.

WHO (2016): A toolkit for integrated vector management in sub-Saharan Africa. ISBN 978 92 4 154965 3

WHO (2016): Test procedures for insecticide resistance monitoring in malaria vector mosquitoes – 2nd ed ISBN 978 92 4 151157 5.

WHO (2018): High burden to high impact: A targeted malaria response. CDS/GMP/2018.25.

Wirtz, RA, Zavala, F, Charoenvit, Y, Campbell, GH, Burkot, TR et al. (1987). Comparative testing of monoclonal antibodies against *Plasmodium falciparum* sporozoites for ELISA development. Bulletin of the World Health Organization, 65 (1), 39 - 45. https://apps.who.int/iris/handle/10665/264328.

Witzig C, Parry M, Morgan JC, Irving H, Steven A, Cuamba N, et al. Genetic mapping identifies a major locus spanning P450 clusters associated with pyrethroid resistance in kdr-free *Anopheles arabiensis* from Chad. Heredity (Edinb). 2013 Apr;110(4):389-97.