High Vector Diversity and Malaria Transmission Dynamics in Five Sentinel Sites in Cameroon

Etienne FONDJO
U.S. President's Malaria Initiative (PMI) VectorLink Project, Abt Associates
Jean-Claude. TOTO
Central African Organization for Endemic Disease Control
Magellan TCHOUAKI
Centre for Research in Infectious Diseases
E. EKOKO WOLFANG
Central African Organization for Endemic Disease Control
Salomon PATCHOKE
The Biotechnology Center
Benjamin MENZE
Centre for Research in Infectious Diseases
Boris NJEAMBOSAY
The Biotechnology Center
Francis ZEUKENG
The Biotechnology Center
Raymond TABUE
National Malaria Control Programme
Elyse MANDENG
Central African Organization for Endemic Disease Control
Emmanuel Elanga NDILLE
Centre for Research in Infectious Diseases
Edmond KOPYA
Central African Organization for Endemic Disease Control
Jerome Achille BINYANG
Centre for Research in Infectious Diseases
Cyrille NDO
Centre for Research in Infectious Diseases
Billy TENE-FOSSOG
Centre for Research in Infectious Diseases
Armel TEDJOU
Centre for Research in Infectious Diseases
Emmanuel NCHOUTPOUEN
Central African Organization for Endemic Disease Control
Frederic TCHOUINE
U.S. President's Malaria Initiative (PMI) VectorLink Project, Abt Associates
Dorothy ACHU
National Malaria Control Programme
Kelley AMBROSE

U.S. President's Malaria Initiative (PMI) VectorLink Project, Abt Associates
Judith HEDJE
PMI U.S. Agency for International Development (USAID)
Celestin KOUAMBENG
PMI U.S. Agency for International Development (USAID)
Jenny CARLSON
PMI, USAID
Sarah ZOHDY
PMI, U.S. Centers for Disease Control and Prevention (CDC)
Joseph Chabi (joseph_chabi@pmivectorlink.com)
U.S. President's Malaria Initiative (PMI) VectorLink Project, Abt Associates

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Research Article

Keywords:

Posted Date: July 22nd, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1871595/v1

Additional Declarations: No competing interests reported.

Version of Record: A version of this preprint was published at Malaria Journal on April 13th, 2023. See the published version at https://doi.org/10.1186/s12936-023-04552-z.

Abstract

Background

Malaria remains one of the main causes of morbidity and mortality in Cameroon. To inform vector control intervention decision making, malaria vector surveillance was conducted monthly from October 2018 to September 2020 in five selected sentinel sites (Gounougou and Simatou in the North, Bonabéri, Mangoum and Nyabessang in the South).

Methods

Human landing catches (HLCs), U.S. Centers for Disease Control and Prevention (CDC) light traps, and pyrethrum spray catches (PSCs) were used to assess vector density and species composition, human biting rate (HBR), endophagic index, indoor resting density (IRD), parity, sporozoite infection rates, entomological inoculation rate (EIR), and *Anopheles* vectorial capacity.

Results

A total of 139,322 *Anopheles* mosquitoes from 21 species were collected across all sites and methods. Out of the 21 species, eleven were implicated in malaria transmission including *An. gambiae* s.l.*, An. funestus* s.l., *An. nili*, *An. moucheti*, *An. paludis*, *An. demeilloni*, *An. pharoensis*, *An. ziemanni*, *An. multicinctus*, *An. rufipes,* and *An. marshallii*. *Anopheles gambiae* s.l. remains the major malaria vector (71% of the total *Anopheles*) collected across all sites, though *An. moucheti* and *An. paludis* had highest sporozoite rates in Nyabessang. The mean indoor HBR of *Anopheles* ranged from 11 bites/person/night (b/p/n) in Bonabéri to 104 b/p/n in Simatou, while outdoors, it varied from 24.2 b/p/n in Mangoum to 98.7 b/p/n in Simatou. *Anopheles gambiae* s.l. was actively biting until at least 8:00 a.m., and similarly for *An. moucheti* in Nyabessang. The mean *Anopheles* IRD was 17.1 females/room, and the parity rate was 68.9%. The monthly mean EIRs recorded was 46.1 infective bites/person/month (ib/p/m) in Gounougou, 91.2 ib/p/m in Simatou, 38.2 ib/p/m in Mangoum, 28.4 ib/p/m in Nyabessang, and 19.9 ib/p/m in Bonabéri. *Anopheles gambiae* s.l. was confirmed as the main malaria vector with the highest vectorial capacity in all sites based on sporozoite rate, except in Nyabessang.

Conclusion

These findings highlight the high malaria transmission occurring in Cameroon and will support the National Malaria Control Program to design evidence-based malaria vector control strategies, effective and integrated vector control interventions deployment to reduce malaria transmission and burden in Cameroon, where several *Anopheles* species could potentially maintain year-round transmission.

Introduction

Malaria remains a leading public health concern in Cameroon, accounting for 29.1% of health facility consultations and 17.2% of deaths in 2020 [1, 2]. Children under five years of age and pregnant women are disproportionately vulnerable. In 2020, hospital morbidity due to malaria was 40.1% among children under five years and 22.5% for pregnant women [1, 2]. In the last two decades, efforts and progress have been made worldwide to enable control of the disease by implementing several vector control measures in addition to therapeutic care. The use of insecticide treated nets (ITNs) has contributed to the drastic reduction of the disease burden [3, 4]. Nonetheless, sub-Saharan Africa is still at risk and provides most malaria cases and deaths worldwide. According to the 2021 World Malaria Report, there were globally an estimated 241 million malaria cases recorded in 2020, showing an increasing number of cases compared to 227 million cases in 2019, with the majority of the increased cases reported from countries in the WHO African Region [3, 4].

In Cameroon, the National Malaria Control Programme (NMCP) and the country partners have objectively applied a malaria response plan by implementing: i) free distribution of ITNs through mass campaigns and during antenatal consultations for pregnant women, ii) seasonal malaria chemoprevention for children aged 3 to 59 months, specifically in the North and Far

North regions, and iii) free treatment of uncomplicated and severe malaria for children under five and subsidized case management of malaria for the general population. However, the effectiveness of these control measures is being threatened by factors such as the resistance of vectors to the insecticides used in different ITNs [5, 6], the change in vector behaviors, human population behavior and movement throughout the night, and/or the resistance of the *Plasmodium falciparum* parasite to antimalarial drugs [7, 8]. Furthermore, the adaptation of the sporozoite infection to several malaria vectors has emerged as a new challenge for vector control and malaria treatments because the existing interventions target the behavior of specific species with more opportunities to transmit malaria parasites. Cameroon is at particular risk given it hosts several species of *Anopheles* mosquitoes that have been found to carry malaria parasites [8]. The complex vector-parasite ecology in Cameroon requires that efforts to control malaria include all vector species instead of targeting a single malaria vector. Continuous evaluation of vector bionomics in a changing landscape is required to improve the vector control strategy. While several entomological studies have been conducted in the country to describe malaria transmission parameters, these are often conducted within a short time frame or in a limited number of sites [8–11]. Based on this context, and to provide recent and extensive entomological data to the NMCP, the U.S. President's Malaria Initiative (PMI) VectorLink project conducted vector surveillance from 2018–2020 in five sentinel sites representing four ecological zones in the country. Vector bionomics and malaria transmission parameters were assessed to support the country's vector control strategy, including the selection and deployment of appropriate evidence-based vector control tools.

Methods

Study Sites

Two sites (Gounougou and Simatou) were selected in the northern part of the country and three (Mangoum, Nyabessang, and Bonabéri) in the southern part. Gounougou (13.55°E; 9.07°N) is a rice cultivation area located in the dry savannah zone of the North. It has a rainy season of about six months (May to October). Simatou (15°E; 10.34°N), situated in the Sahelian zone in the Far North, is also a rice cultivation area, with a short rainy season occurring from July to October.

The three southern sites included Mangoum (10,58°E; 5,47°N) in the wet savanna zone in the West region, Nyabessang (10.39°E; 2.4°N), a rural area located in the forest of the South region, surrounded by many rivers and dams with a high rainfall, and Bonabéri (9.65°E; 4.08°N), an urban area located in the coastal zone in the Littoral region (Fig. 1).

Vector Bionomics Monitoring

The study was conducted in the five sentinel sites from October 2018 to September 2020. Monthly entomological data collections were carried out in Gounougou and Simatou from October 2018 through September 2020 (except for November 2019 through March 2020 when collections were conducted every other month at both sites). Thus, a total of 19 collection-months were completed in these two sites over the survey period. In the southern sites, collections were done every other month in Mangoum and Nyabessang from October 2018 through February 2020 and from December 2018 through February 2020 in Bonabéri. Thereafter, the collections were conducted monthly from June to September 2020 at all three sites. A total of 13 months of field collections were completed in Mangoum and Nyabessang and 12 months in Bonabéri. No collections were conducted between April and May 2020 due to the COVID-19 pandemic while collections were adjusted to every other month per activity programming including expansion of insecticide resistance monitoring sites across the country.

Adult mosquitoes were collected in each of the sites using three collection methods including human landing catches (HLCs) which target human host seeking biting vectors, and U.S. Centers for Disease Control and Prevention (CDC) light traps which target a diversity of host seeking species, and pyrethrum spray catches (PSCs) which target indoor resting vectors. Each method enabled the estimation of different parameters which characterized the vector behavior and malaria transmission within the local populations.

HLCs were conducted in three randomly selected houses per site that were maintained throughout the collection period. Adult mosquito collections were done from 6:00 pm to 8:00 am for two consecutive nights per collection month. Four collectors were assigned to each house (two collectors indoors and two collectors outdoors). The collectors were monitored for malaria symptoms before and after collection and any malaria cases would be given treatment. For efficiency and accuracy, two teams of 12 collectors each worked in shift (from 6:00 p.m. to midnight and from midnight to 8:00 am) with a daily rotation. Additionally, the collectors rotated positions every hour throughout the night to account for variation in attractiveness among collectors. The collectors used hemolysis tubes to catch mosquitoes landing on their lower exposed limbs. Mosquitoes were collected hourly and put in separate bags. After each night of collection, mosquitoes were identified morphologically using taxonomic identification keys [12–14]. For each collection month and site, a subsample of randomly selected vectors underwent ovary dissection for parity rate determination [15].

CDC light traps were set indoors and outdoors from 6:00 p.m. to 6:00 a.m. in four houses for two consecutive nights per collection month. The indoor traps were baited and suspended nearby a bed with a mosquito net where household owners slept. Outdoor traps were hung on a tree with no bait. All traps were suspended 1.5 meters above the ground.

PSCs were carried out during two consecutive days per month in 20 houses between 6:00 a.m. and 8:00 a.m. A room in which inhabitants spent the night was selected in each house. A white sheet was placed in the room covering the floor and bed. A pyrethroid insecticide spray containing piperonyl butoxide (PBO) synergist was used to spray the room and to collect all indoor resting mosquitoes. When the house had opened eaves, those eaves were sprayed first from outside before spraying indoors to prevent the mosquitoes from escaping. After about 10 minutes post-spraying, the sheets were gently brought outdoors and the mosquitoes on the sheets were collected using forceps and preserved in petri dishes for morphological identification. The abdominal status of the collected vectors was determined, and the number and percentage of blood-fed mosquitoes were recorded during morphological identification [12].

Parity Assessment

To determine the parity rate of *Anopheles* species collected, approximately 20% of unfed, female *Anopheles* collected using HLCs were randomly selected each month for ovary dissection, following the methods described by Detinova 1962, by observing the degree of coiling by the ovarian tracheoles [15]. All *Anopheles* and the carcasses of the dissected *Anopheles* were individually stored in labeled Eppendorf tubes containing silica gel for further molecular analysis.

Molecular Characterization

A random subsample of about 100 field preserved mosquitoes morphologically identified as *An. gambiae* s.l. and *An. funestus* s.l. were selected per month, per collection method, and per site, and used for molecular species identification using polymerase chain reaction (PCR) methods to discriminate between sibling *Anopheles* species using wings and legs. Additionally, about 400 mosquitoes were randomly selected to detect sporozoite infections using the head and thorax using indirect enzyme-linked immunosorbent assay (ELISA), and 100 per month to determine blood meal sources using the abdomen of PSC-collected mosquitoes using direct ELISA.

Genomic DNA Extraction and Species Identification

Whole genomic DNA (gDNA) was extracted from each mosquito sample following the LIVAK method [16] and stored at -20ºC. A NanoDrop™ spectrophotometer (Thermo-Scientific, Wilmington, USA) was used to determine the concentration and purity of the extracted DNA.

Members of *An. gambiae* s.l. complex (*An. gambiae* s.s., *An. coluzzii*, and *An. arabiensis)* were discriminated using the Short Interspersed Nuclear Element (SINE) PCR protocol of Santolamazza *et al.* [17]. In the coastal sites such as Bonabéri and Nyabessang, where other species such as *An. melas* are present, the PCR-RFLP protocol described by Fanello *et al*. [18] was used to discriminate the *An. gambiae* s.l. complex species. *Anopheles funestus* group subspecies were characterized using a cocktail PCR according to Koekemoer *et al*., [19] with addition of the *An. rivulorum-like* primers. Genomic DNA from 100

randomly selected mosquitoes were processed per month and per site. PCR products were run via electrophoresis through a 1.5% agarose gel with Midori Green® (Gene flow, UK) and visualized under ultraviolet light.

Circumsporozoite Infection Detection

Determination of sporozoite infection rates and blood meal analysis of adult *Anopheles* mosquitoes collected using HLCs and PSCs were conducted using circumsporozoite ELISA (csELISA) following the method described by Burkot *et al.* [20] and modified by Wirtz *et al.* [21] 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 0.5mg/ml. Similarly, all reagents including phenol red, 1X Phosphate Buffered Saline (PBS), Blocking Buffer (BB), Grinding Buffer, and 1X PBS-Tween wash solution were prepared before starting the manipulation and according to the manufacturer guidance (MR4-890 kit). Diluted *P. falciparum* sporozoite recombinant 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 (OD) at 405 nm on an ELISA plate reader (Biotek Elx800, Swindon, UK). Positive samples were determined by OD readings two-fold greater than the negative controls and were tested a second time for validation.

Identification of Blood Meal Source

The source of the blood meal contained in the abdomen of resting mosquitoes collected by PSCs was determined using a direct ELISA technique described by Beier *et al*. [22]. This technique allows the identification of human, cow, pig, chicken, goat, horse, and dog blood. Peroxidase conjugated antibodies and animal heterologous serum were obtained from Sigma (St. Louis, U.S.). After manipulation, absorbance at 414 nm was determined using an ELISA plate reader. Samples were considered positive if absorbance values exceeded the mean plus three times the standard deviation of four negative control represented by unfed mosquitoes.

Vectorial Capacity of *Anopheles* Species

The vectorial capacity represents the ability of a population of vectors to transmit *Plasmodium* spp. In terms of the potential number of secondary inoculations originating per day from an infective person. The vectorial capacity is dependent upon a series of biological characteristics such as population density, blood meal preference, and the probability of the vector to survive per day. We used the MacDonald formula to estimate the vectorial capacity of each of the *Anopheles* species found with *P. falciparum* parasite across all the sentinel sites, as described below:

$$\mathbf{VC} = \frac{\left(ma^2\right)p^n}{-ln(p)}$$

Where m = the ratio of mosquitoes feeding on human, a = the HBR of the vector, the parasite's extrinsic incubation period (EIP, n days) which we considered as 12 days, and p = the mosquito's survival through one day calculated using the parity rate.

Data Management and Statistical Analysis

All entomological data was regularly entered in Epi-Info Version 3.5.4 by a database manager to facilitate analysis. The proportion of each identified mosquito species was calculated as a percentage of each species out of the total *Anopheles* collected. Infection rate, measured as the proportion of mosquitoes found with circumsporozoite antigen by ELISA, was calculated by dividing the number of positive mosquitoes by the total number of mosquitoes tested. Mean parity rate was determined by dividing the number of parous females by the total number dissected.

The EIR was calculated as the product of the HBR and circumsporozoite antigen rate as determined by ELISA. The human blood index (HBI) was calculated as the proportion of mosquitoes found to contain human IgG by ELISA out of the total mosquitoes tested.

Results

Anopheles Mosquito Species Composition

Overall, 139,322 *Anopheles* mosquitoes representing 18 distinct species were collected in the five sentinel sites using the three methods (HLC, CDC LT, and PSC). A total of 83,540 *Anopheles* mosquitoes (60.0%) were collected using HLC, 29,846 (21.4%) were collected with CDC LT and 25,936 (18.6%) were collected by PSC. *Anopheles gambiae* s.l. (98,867; 71.0%) was the predominant species and was collected in all sites using the three methods. *Anopheles moucheti* and *An. nili* were only found at Nyabessang (Table 1).

Table 1
Species Composition of Anopheles **Mosquitoes Collected*Across Five Sites in Cameroon**.

Species	Gounougou	Simatou	Mangoum	Nyabessang	Bonabéri	Total	Frequency (%)
An. gambiae s.l.	29,514	56,404	6,432	1,463	5,054	98,867	70.96
An. funestus s.l.	2,623	423	18	0	0	3,064	2.20
An. ziemanni	673	9,429	113	198	1	10,414	7.47
An. paludis	0	1	2	4,285	0	4,288	3.08
An. moucheti	0	0	0	3,546	0	3,546	2.55
An. rufipes	339	1,126	0	0	0	1,465	1.05
An. marshallii	0	0	0	180	0	180	0.13
An. pharoensis	341	11,850	0	0	0	12,191	8.75
An. christyi	2	0	0	0	0	2	0.00
An. multicinctus	226	0	0	0	0	226	0.16
An. nili	0	0	0	658	0	658	0.47
An. coustani	1	3	0	0	0	4	0.00
An. welcomei	0	8	0	0	0	8	0.01
An. tenebrosus	9	0	0	0	0	9	0.01
An. smithii	5	0	0	0	0	5	0.00
An. demeilloni	0	4,380	0	0	0	4,380	3.14
An. cinerus	3	0	0	0	0	3	0.00
An. hancocki	0	12	0	0	0	12	0.01
Total	**33,736**	**83,636**	**6,565**	**10,330**	**5,055**	**139,322**	**100.0**

*Mosquito collection methods include: HLC: human landing catches; CDC LT: Centers for Disease Control and Prevention light traps; PSC: pyrethrum spray catches

A total of 5,598 *An. gambiae* s.l. mosquitoes from all five sites were DNA extracted for species identification. Of these, 283 (5.1%) did not amplified, while 5,315 *An. gambiae* s.l. (1,513 from Simatou, 1,538 from Gounougou, 962 from Mangoum, 575 from Nyabessang, and 727 from Bonabéri) and 596 *An. funestus* s.l. (217 from Simatou, 368 from Gounougou, 9 from Mangoum, and 2 from Nyabessang) were successfully tested by PCR for molecular identification of the sub-species of each complex (Table 2). Three species from the *An. gambiae* s.l. complex were identified in Simatou and Gounougou including *An. gambiae* s.s. (1.1% in Simatou, 2.4% in Gounougou), *An. coluzzii* (90.0% in Simatou, 84.2% in Gounougou), and *An.*

arabiensis (8.7% in Simatou and 13.4% in Gounougou). Hybrids of *An. gambiae/An. coluzzii* (0.2%) were also recorded in Simatou.

In the three southern sites, two species of the *An. gambiae* s.l. complex were recorded, including *An. gambiae* s.s. (98.9% in Mangoum, 93.5% in Nyabessang, and 1.4% in Bonabéri) and *An. coluzzii* (0.6% in Mangoum, 5.2% in Nyabessang, and 98.6% in Bonabéri). A small proportion of hybrids of both species was also recorded in Mangoum (0.4%) and Nyabessang (1.2%).

For *An. funestus* s.l., two species of the group were found in Simatou and Gounougou: *An. funestus* s.s. (59.4% and 89.7%, respectively) and *An. leesoni* (40.5% and 10.3%, respectively). Prior to molecular identification, a second morphological identification was conducted by the laboratory team to ensure that the tested samples were *An. funestus* s.l. to avoid misidentification of the *An. leesoni* that could occur [23].

Table 2
Species Composition of *Anopheles gambiae* Complex and *An. funestus* Group Collected Across Five Sites in Cameroon

Sites	*An. gambiae* s.l.				Total *An. gambiae* s.l.	*An. funestus* s.l.		Total *An. funestus* s.l.
	An. gambiae s.s. (%)	An. coluzzii (%)	An. arabiensis (%)	An. coluzzii/ An. gambiae (%)		An. funestus s.s. (%)	An. leesoni (%)	
Simatou	17 (1.1%)	1,362 (90.0%)	131 (8.7%)	3 (0.2%)	**1,513**	129 (59.4%)	88 (40.6%)	**217**
Gounougou	37 (2.4%)	1,295 (84.2%)	206 (13.4%)	0 (0.0%)	**1,538**	330 (89.7%)	38 (10.3%)	**368**
Mangoum	951 (98.9%)	6 (0.6%)	1 (0.1%)	4 (0.4%)	**962**	9 (100.0%)	0	**9**
Nyabessang	538 (93.6%)	30 (5.2%)	0 (0.0%)	7 (1.2%)	**575**	0 (0.0%)	2 (100.0%)	**2**
Bonabéri	10 (1.4%)	717 (98.6%)	0 (0.0%)	0 (0.0%)	**727**	0 (0.0%)	0 (0.0%)	**0**
Total	**1,553 (29.2%)**	**3,410 (64.2%)**	**338 (6.4%)**	**14 (0.3%)**	**5,315**	**468 (78.5%)**	**128 (21.5%)**	**596**

Estimation of Malaria Transmission Parameters

Malaria Vectors and Human Biting Rates

The HLC method was the most productive collection method in all sites. Of the 83,540 *Anopheles* mosquitoes collected, *An. gambiae* s.l. represented the main vector species in all sites, except in Nyabessang where *An. moucheti* and *An. paludis* were predominant. The average human biting rate of *Anopheles* mosquitoes collected using HLC varied across the sites: 101.3 bites/person/night (b/p/n) in Simatou, 39.2 b/p/n in Gounougou, 15.9 b/p/n in Mangoum, 30.5 b/p/n in Nyabessang and 17.5 b/p/n in Bonabéri. Of these, *An. gambiae* s.l. recorded an HBR of 73.9 b/p/n in Simatou, 35.6 b/p/n in Gounougou, 15.6 b/p/n in Mangoum, 3.5 b/p/n in Nyabessang, and 17.5 b/p/n in Bonabéri. *An. gambiae* s.l. yielded a similar biting pattern both indoors and outdoors throughout the night in all sites with the highest biting recorded between 11:00 pm and 5:00 am. Simatou and Gounougou recorded the highest hourly peak biting with 8.1 bites per person per hour (b/p/h) and 4.5 b/p/h, respectively.

Trends in monthly HBR of *An. gambiae* s.l. over time differed in the southern and the northern sites, which could be related to the eco-geographical location of the sites. In Gounougou, the lowest HBRs were recorded between October 2018 and January

2019 and in May and June 2019. Two peaks were observed in February (69.4 b/p/n) and August 2019 (106.0 b/p/n). In Simatou, the lowest HBRs were recorded from October 2018 to February 2019 and in May 2019. However, two relatively high peaks were observed in July 2019 (281.2 b/p/n) and July 2020 (327.2 b/p/n) (Fig. 2). The average monthly HBR of *An. gambiae* s.l. was 15.6 b/p/n in Mangoum with the lowest HBR recorded in August 2020 (3.2 b/p/n) and the highest peak in April 2019 (42.9 b/p/n). In Nyabessang, the highest and only peak of 14.0 b/p/n was recorded in December 2018. From February to August 2019, recorded biting was much lower at between 2.0 b/p/n and 4.8 b/p/n. Furthermore, the peak biting of the predominant *An. paludis* (34.5 b/p/n) and *An. moucheti* (34.3 b/p/n) in Nyabessang was recorded in February 2019 and 19.3 b/p/n for *An. moucheti* in June 2020, showing a replacement of vector population with *An. gambiae* s.l. which recorded its lowest density during the same period. In Bonabéri, *An. gambiae* s.l. biting peaked three times in the study period —first in February 2019 (32.7 b/p/n), then in August 2019 (39.0 b/p/n), and finally in July 2020 (35.0 b/p/n). The lowest HBR was observed in December 2019 with 1.7 b/p/n (Fig. 3).

In addition to the main malaria vector *An. gambiae* s.l., the mean HBRs of all other potential vectors were also estimated per site. In the three sites where *An. funestus* s.l. were collected, an average HBR of 0.4 b/p/n was recorded in Simatou, 2.2 b/p/n in Gounougou, and 0.1 b/p/n in Mangoum. The mean HBR of *An. ziemanni* was 2.3 b/p/n in Simatou, 0.3 b/p/n in Gounougou, and 0.4 b/p/n in Mangoum. *Anopheles paludis* and *An. moucheti* were mostly found in Nyabessang with mean HBRs of 13.2 b/p/n and 10.5 b/p/n respectively. *Anopheles nili* and *An. marshallii* recorded a mean HBR of 2.0 b/p/n and 0.6 b/p/n, respectively. *Anopheles pharoensis* and *An. demeilloni* were only collected in the northern sites with 18.6 b/p/n and 0.5 b/p/n for *An. pharoensis* in Simatou and Gounougou, while *An. demeilloni* showed 6.0 b/p/n in Simatou. Other potential vectors recorded included *An. coustani* and *An. welcomei* in Simatou, and *An. tenebrosus*, *An. smithii*, and *An. christyi* in Gounougou (Table 3 & Supp. data 1).

Table 3
Mean Human Biting Rate of *Anopheles* Mosquitoes Collected using Human Landing Catches from Oct 2018 to Sept 2020 Across the Five Sites in Cameroon

Simatou			**Gounougou**		**Mangoum**		**Nyabessang**		**Bonabéri**	
Species	Total collected	Mean HBR (bpn)	Total collected	Mean HBR (bpn)	Total collected	Mean HBR (bpn)	Total collected	Mean HBR (bpn)	Total collected	Mean HBR (bpn)
An. gambiae s.l.	33,703	**73.91**	16,231	**35.59**	4,859	**15.57**	1,108	3.55	5,031	**17.47**
An. funestus s.l.	186	0.41	1,007	2.21	18	0.06	0	nd	0	nd
An. ziemanni	1,041	2.28	144	0.32	110	0.35	196	0.63	1	0.00
An. paludis	1	0.00	0	0	2	0.01	4,121	**13.21**	0	nd
An. rufipes	40	0.09	13	0.03	0	nd	0	nd	0	nd
An. pharoensis	8,482	**18.60**	248	0.54	0	nd	0	nd	0	nd
An. coustani	3	0.01	1	0.00	0	nd	0	nd	0	nd
An. welcomei	8	0.02	0	nd	0	nd	0	nd	0	nd
An. demeilloni	2,736	**6.00**	0	nd	0	nd	0	nd	0	nd
An. multicinctus	0	nd	151	0.33	0	nd	0	nd	0	nd
An. tenebrosus	0	nd	9	0.02	0	nd	0	nd	0	nd
An. smithii	0	nd	5	0.01	0	nd	0	nd	0	nd
An. christyi	0	nd	1	0.00	0	nd	0	nd	0	nd
An. moucheti	0	nd	0	nd	0	nd	3,271	**10.48**	0	nd
An. marshallii	0	nd	0	nd	0	nd	175	0.56	0	nd
An. nili	0	nd	0	nd	0	nd	638	2.04	0	nd
Total	**46,200**	**101.32**	**17,810**	**39.06**	**4,989**	**15.99**	**9,509**	**30.48**	**5,032**	**17.47**

nd = not determined because the vector was not collected at those specific sites. Bold human biting rates (HBRs) represented the vectors with high bites/person/night (bpn) per specific site.

Entomological Inoculation Rate

A total of 9,778 *Anopheles* mosquitoes were tested by ELISA of which 393 were found with circumsporozoite antigen of *Plasmodium*, for a total average infection rate of 4.0%. Eleven *Anopheles* species were found carrying *Plasmodium* parasites including *An. nili, An. moucheti, An. demeilloni, An. pharoensis, An. ziemanni, An. multicinctus, An. marshallii, An. rufipes* and *An. paludis.* The infection rates recorded across sites were as follows: Gounougou (3.6%), Simatou (3%), Mangoum (7.9%),

Nyabessang (3.1%), and Bonabéri (3.8%). *Anopheles gambiae* s.l. from all five sites and *An. ziemanni* from the four sites where it was collected tested positive for *Plasmodium* circumsporozoite antigen (Table 4).

The entomological inoculation rate (EIR) varied from 19.9 infected bites/person/month (ib/p/m) in Bonabéri to 91.2 ib/p/m in Simatou. Among all species, *An. gambiae* s.l. contributed most to malaria transmission in all sites. At least two *Anopheles* species were involved in malaria transmission in four of the sites (Table 4). Simatou and Nyabessang recorded the largest number of malaria vectors with six *Anopheles* species involved in the transmission of the parasite. Gounougou recorded four vectors, while in Bonabéri, the only malaria vector found was *An. gambiae* s.l. The monthly indoor and outdoor HBRs recorded throughout the collection period, coupled with the EIRs showed that the higher transmission period did not always coincide with the higher biting period in the southern part of the country. EIRs were high when densities were relatively low in June 2019 in Mangoum and Bonabéri and in August 2019 in Nyabessang and all three southern sites. In contrast, EIRs in the northern sites of Simatou and Gounougou peaked when densities were high in July and August 2019, respectively. Furthermore, indoor and outdoor biting rates were relatively similar in all sites and across all *Anopheles* collected. The endophagic rate of *An. gambiae* s.l. was 50% in Simatou, 47% in Gounougou, 53% in Mangoum, and 49% in Nyabessang, indicating that the vectors bite equally indoors and outdoors. Only *An. gambiae* s.l. from Bonabéri were found to bite more outdoors than indoors with an endophagic rate of 30%. The same trends were observed with *An. funestus* s.l. in Simatou (53%) and Gounougou (57%), with all other *Anopheles* collected in specific sites yielding a mean endophagic rate of about 50%.

Table 4
Entomological Inoculation Rate of *Anopheles* Mosquitoes Collected Across the Five Sites in Cameroon from October 2018 to September 2020

Sentinel Site	Species	HBR	Infection Rate	EIR (infected bites/person/night)	Monthly EIR (infected bites/person/month)
Gounougou	*An. gambiae* s.l.	35.6	0.04	1.424	42.72
	An. funestus s.l.	2.2	0.03	0.066	1.98
	An. ziemanni	0.3	0.01	0.003	0.09
	An. multicinctus	0.33	0.09	0.0297	0.891
Total EIR		**38.4**	**0.04**	**1.536**	**46.08**
Simatou	*An. gambiae* s.l.	73.91	0.05	3.696	110.87
	An. funestus s.l.	0.41	0.01	0.004	0.12
	An. ziemanni	2.28	0.004	0.009	0.27
	An. demeilloni	6.0	0.03	0.180	5.40
	An. rufipes	0.09	0.03	0.003	0.08
	An. pharoensis	18.6	0.01	0.186	5.58
Total EIR		**101.3**	**0.03**	**3.039**	**91.17**
Mangoum	*An. gambiae* s.l.	15.6	0.08	1.248	37.44
	An. ziemanni	0.37	0.03	0.0111	0.333
Total EIR		**15.92**	**0.08**	**1.274**	**38.21**
Nyabessang	*An. gambiae* s.l.	3.55	0.05	0.1775	5.325
	An. moucheti	10.48	0.02	0.2096	6.288
	An. nili	2.04	0.017	0.0347	1.0404
	An. ziemanni	0.63	0.05	0.0315	0.945
	An. paludis	13.21	0.02	0.2642	7.926
	An. marshallii	0.56	0.11	0.0616	1.848
Total EIR		30.5	0.031	0.9455	**28.37**
Bonabéri	*An. gambiae* s.l.	17.47	0.038	0.6639	19.92
Total EIR		**17.47**	**0.038**	0.6639	19.92

EIR = entomological inoculation rate; HBR = human biting rate.

Parity Rate

Across all five sites, ovaries of 11,051 *Anopheles* species were dissected during the collection period. The average parity rate across all sites was 68.9% and ranged from 57.1% (Nyabessang) to 76.4% (Gounougou) (Table 5). The parity rate of *An. gambiae* s.l. in Gounougou (75.1%) was significantly higher than that of the four other sites (Chi-Square = 201.3, ddl = 3, $p < 10^{-6}$). However, all *Anopheles* species dissected showed high parity rates across all sites (Supp. data 2).

Table 5
Parity Rate of the Anopheles **Mosquitos Across All Five Sites in Cameroon**

Sentinel site	Species	Total dissected	#Parous	% Parous
Gounougou	*An. gambiae* s.l.	2,244	1,685	75.1
	An. funestus s.l.	291	245	84.2
	An. ziemanni	50	46	92
	An. pharoensis	37	27	73
	An. multicinctus	49	38	77.5
Total		2,679	2,048	76.5
Simatou	*An. gambiae* s.l.	2,360	1,585	67.2
	An. funestus s.l.	89	63	70.8
	An. ziemanni	278	108	38.9
	An. rufipes	15	11	73.3
	An. pharoensis	1,469	1,090	74.2
	An. welcomei	13	10	76.9
	An. demeilloni	393	281	71.5
Total		4,619	3,150	68.2
Mangoum	*An. gambiae* s.l.	650	436	67.1
	An. ziemanni	13	9	69.2
	Total	665	445	66.9
Nyabessang	*An. gambiae* s.l.	369	228	61.8
	An. ziemanni	59	38	64.4
	An. paludis	527	270	51.2
	An. moucheti	664	381	57.4
	An. marshallii	27	15	55.6
	An. nili	137	86	62.8
Total		1,785	1,019	57.1
Bonabéri	*An. gambiae* s.l.	1,301	944	72.6
Total		1,303	945	72.6

Malaria Vector Resting Behavior

Ten *Anopheles* species were collected resting indoors using PSCs representing 18.6% (25,936) of the total *Anopheles* mosquitoes collected at all sites and during the collection period. Similar to HLCs, Simatou and Gounougou recorded the larger number and largest diversity of *Anopheles* species collected through PSCs. Seven of the 10 species collected were found in both sites and included *An. gambiae* s.l., *An. funestus* s.l., *An. ziemanni*, *An. rufipes*, *An. pharoensis*, *An. hancocki*, and *An. demeilloni.* Two *An. multicinctus* were also collected in Gounougou while *An. moucheti* and *An. nili* were found in Nyabessang. In Mangoum and Bonabéri, *An. gambiae* s.l. was the only species collected.

Indoor Resting Density Across Sites

The average density per room of *Anopheles* mosquitoes resting indoors (IRD) was 17.1 females/room (f/r) (25,929 total females/1,520 rooms visited). Table 6 describes the IRD per site. The highest mean IRD was recorded in Simatou (39.6 f/r) in the north, while the lowest was observed in Bonabéri (0.04 f/r) in the south and varied by month and season. The mean IRD of *An. gambiae* s.l. was 34.7 f/r in Simatou and 23.4 f/r in Gounougou. The highest was observed in July 2020 (135.2 f/r) in Simatou and in July 2019 (77.5 f/r) in Gounougou. In the southern sites, the mean IRDs were low compared to the northern sites with the mean of 1.8 f/r in Mangoum, 0.04 f/r in Bonabéri and 0.6 f/r in Nyabessang. Seasonal variation was also observed in the southern sites where the highest IRD was recorded in October 2018 (4.3 f/r) in Mangoum, in July 2020 (0.2 f/r) in Bonabéri, and in September 2020 (1.2 f/r) in Nyabessang.

Table 6
Mean Indoor Resting Density of *Anopheles* Mosquitos Collected by Pyrethrum Spray Catches across the Five Sites from October 2018 to September 2020

	Simatou		**Gounougou**		**Mangoum**		**Nyabessang**		**Bonabéri**	
Species	Total collected	Mean IRD (f/r)	Total collected	Mean IRD (f/r)	Total collected	Mean IRD (f/r)	Total collected	Mean IRD (f/r)	Total collected	Mean IRD (f/r)
An. gambiae s.l.	13,190	34.7	8,905	23.4	467	1.8	140	0.6	9	0.04
An. funestus s.l.	112	0.3	1,127	3.0	0	nd	10	0.04	0	nd
An. rufipes	695	1.8	213	0.6	0	nd	0	0	0	nd
An. ziemanni	13	0.03	5	0.01	0	nd	5	0.02	0	nd
An. pharoensis	82	0.2	0	nd	0	nd	0	0	0	nd
An. multicinctus	0	nd	2	0.01	0	nd	0	0	0	nd
An. pharoensis	0	nd	3	0.01	0	nd	0	0	0	nd
An. hancocki	11	0.03	0	nd	0	nd	0	0	0	nd
An. demeilloni	940	2.5	0	nd	0	nd	0	0	0	nd
Total	15,043	39.6	10,255	27	467	1.8	155	0.6	9	0.04

IRD = indoor resting density; nd = not determined because the vector was not collected at those specific sites.

Host Preference of *Anopheles* Species Across Sites

Nine *Anopheles* species collected from the five sites were screened for blood meal sources to detect if the bloodmeal taken was from either human, cow, sheep, chicken, pig, or horse. A total of 2,994 blood-fed *Anopheles* mosquitoes were analyzed using ELISA, including 2,144 *An. gambiae* s.l., 225 *An. funestus* s.l., 252 *An. rufipes*, 83 *An. demeilloni*, 31 *An. pharoensis*, 246 *An. ziemanni*, 2 *An. moucheti*, 1 *An. nili*, and 10 *An. hancocki.* Only 1,151 of the blood-fed mosquitoes analyzed were found to have fed on humans, giving a HBI of 38.4%. The overall HBI varied from 34.3% in Gounougou to 82.3% in Mangoum. The HBIs of *An. gambiae* s.l. in Mangoum (82.3%) and Nyabessang (64.2%) located in the south were significantly higher than the one recorded in the two northern sites (CHI. Square = 14.18 144.5, ddl = 3, $p < 10^{-6}$). Furthermore, the HBI of *An. funestus* s.l. was 44.4% among the samples collected in Simatou and Gounougou. Out of the 252 *An. rufipes* tested in Simatou and Gounougou, only 12 (4.8%) were found with human blood meal while *An. pharoensis* tested in Simatou showed a HBI of 40% (12/30).

Vectorial Capacity

The vectorial capacity, described as the ability to serve as a vector, was determined for seven species (*An. gambiae* s.l., *An. funestus* s.l., *An. ziemanni*, *An. rufipes*, *An. pharoensis*, *An. demeilloni* and *An. welcomei*) in Simatou, six in Gounougou (*An. gambiae* s.l., *An. funestus* s.l., *An. ziemanni*, *An. rufipes*, *An. pharoensis*, and *An. multicinctus)*, five in Nyabessang (*An. gambiae* s.l., *An. ziemanni*, *An. marshallii, An. moucheti* and *An. nili)* and only *An. gambiae* s.l. in Mangoum and Bonabéri. *Anopheles gambiae* s.l. showed the highest vectorial capacity in all sites, except in Nyabessang where *An. moucheti* represented the main potential malaria vector (26.1 infectious bites/person/day (infectious b/p/d) versus 1.9 infectious b/p/d). Simatou (2365.2 infectious b/p/d) and Gounougou (676.9 infectious b/p/d) yielded higher capacity of *An. gambiae* s.l. to transmit malaria compared to the other *Anopheles* species reported with sporozoite infections. In contrast, *An. gambiae* s.l. was the main vector collected in Mangoum (152.8 infectious b/p/d) and Bonabéri (216.0 infectious b/p/d) (Table 7). *Anopheles funestus* s.l. was the second contributor of persistent malaria in Gounougou, while *An. pharoensis* and *An. demeilloni* represented the two secondary vectors in Simatou (Supp. data 2).

Table 7
Vectorial Capacity of *Anopheles* Species Collected Across the Five Sites in Cameroon

	Infectious bites per person per day (infectious b/p/d)				
Species	Simatou	Gounougou	Nyabessang	Bonabéri	Mangoum
An. gambiae s.l.	2365.2	676.9	1.9	216.0	152.8
An. ziemanni	0.02	0.003	0.0	nd	nd
An. funestus s.l.	0.2	11.8	nd	nd	nd
An. pharoensis	151.0	0.0	nd	nd	nd
An. rufipes	0.001	0.01	nd	nd	nd
An. welcomei	0.0	nd	nd	nd	nd
An. demeilloni	30.9	nd	nd	nd	nd
An. multicinctus	nd	0.0	nd	nd	nd
An. moucheti	nd	nd	26.1	nd	nd
An. marshallii	nd	nd	0.0	nd	nd
An. nili	nd	nd	0.0	nd	nd

nd = not determined because either the vector was not collected at those specific sites, or the mosquitoes collected were not ovary dissected.

Discussion

Entomological vector surveillance is key to describing vector populations and behavior and thereby informing the development of appropriate vector control strategies and tailored deployment of tools. This study, conducted in different ecological and geographical areas of Cameroon, indicated a high diversity and density of *Anopheles* species in the country. Of the 18 *Anopheles* species collected, 10 were found to be positive for *P. falciparum* sporozoites. Other recent studies conducted in Cameroon have revealed a high diversity of malaria vectors distributed across different geographical locations within the country [8–11]. However, *An. gambiae* s.l. was the dominant vector and was found in all sites. *Anopheles moucheti* and *An. nili* were observed only in Nyabessang, which is surrounded by large rivers and dams offering suitable breeding sites for the development of larvae of these two species. *Anopheles arabiensis* was found in the two sites in the northern part of the country where the climate is drier than in the southern regions, which corresponds to reports from other sub-Saharan African countries where *An. arabiensis* was also found in drier areas [24, 25]. Furthermore, *An. gambiae* s.s. was predominant in Mangoum and Nyabessang while *An. coluzzii* represented the main species of the complex in the other three sites. It is known that *An. gambiae* s.s. prefers larval habits with lots of sun exposure, while *An. coluzzii* was mostly found in the man-made areas such as rice fields and more humid areas [26, 27]. The findings of this study corroborate with previously reported data [8, 10, 11], as Simatou and Gounougou are rice cultivation areas and Bonabéri is in the southern humid area. Similar results have also been reported from previous studies conducted in similar eco-geographical areas in the country [28], though this study assessed vector bionomics over two consecutive years. Reviewing the trends over this period of time can help decision makers to assess and determine not only which would be the most effective tools, but also what timing of deployment will optimize their impact. For example, the eco-geographical location of Nyabessang, surrounded by many rivers and dams with a high rainfall, favored the development of *An. paludis* and *An. moucheti* over leading *An. gambiae* s.l. at a specific period of the year. This high density of both species observed during the same period of the year could be a good indicator for integrated strategies to control all species. On the other hand, Mangoum recorded the lowest species diversity with predominantly *An. gambiae* s.l. and few *An. ziemanni* recorded throughout both years of collections. This could be due to the location of the site and farming activities including corn and tomato gardening. Mangoum is a humid and sunny area located within a forest savannah, which is favorable for *An. gambiae* s.l. breeding sites, implying targeted *An. gambiae* s.l. effective vector control tools such as any appropriate combination of ITNs.

The HBR of the *Anopheles* species varied seasonally at each site and peaked with either increasing rainfall and/or rice cultivation. Rainfall and rice paddies are known factors contributing to an increase in biting and consequently an increase in the malaria incidences in all endemic countries [29, 30]. The peak biting in both Gounougou and Simatou was observed during the rainy season, which coincides with rice cultivation. This trend was observed over both survey years and highlights the need for malaria control strategy implementation, particularly during the peak transmission which seems to recur from year to year. The NMCP has already anticipated some actions by conducting seasonal malaria chemoprevention (SMC) in the northern regions [31] and providing free malaria treatment of children under five across the country. In addition, ITNs are distributed through mass campaigns and routine channels. However, it may be necessary to consider additional vector control measures such as complementary larval source management (LSM) or indoor residual spraying (IRS) An impact evaluation may be useful to determine if LSM is appropriate in areas where rice cultivation is conducted, such as in Simatou, and Gounougou to help control the diversity of *Anopheles* that were found in these areas. In contrast to the north, several biting peaks were recorded at the southern sites, where two rainy seasons are observed yearly, except during the short dry season from November to January. Interestingly, all the vectors showed outdoor biting patterns throughout both years and at all sites, even though several animal shelters were found in the northern sites, which could drive the mosquitoes outdoors [32, 33]. Furthermore, the endophagic rates recorded in Gounougou and Simatou were lower than those of the southern sites because of the presence of great number of cattle farms. However, HBRs were still high in Cameroon compared to some sub-Saharan African countries with similar geographical and climatic conditions [34–38].

The highest transmission was observed in Simatou where six *Anopheles* species were found infected and a higher HBR was recorded compared to the other sites. This could continue to worsen given recent reports on the potential transmission by

sub-species of *An. funestus* s.l. in addition to already known *An. gambiae* s.l. complex [39, 40]. Though the diversity of malaria vectors has been described in the country [8, 9, 41], no specific vector control measures targeting the various species have been developed to date. All control efforts are channeled towards the main vector *An. gambiae* s.l., with the expectation that they will also have effects on the other vectors. However, the vectorial capacity of other vectors that are currently considered as secondary vectors needs to be closely monitored. Furthermore, this diversity of *Anopheles* vectors constituted a cause of concerns considering that the current vector control interventions target mostly endophagic and endophilic *An. gambiae* s.l. only, which may have the potential to alter vector dynamics creating opportunities for niche partitioning and for other vectors to fill in the missing niche left by reduced populations of *An. gambiae* s.l. As observed in Nyabessang, *An. moucheti* and *An. paludis* yielded higher vectorial capacity and entomological inoculation rates compared to *An. gambiae* s.l. This may require deeper investigation into the ecology, transmission, and epidemiological impact of these vectors for targeted vector control interventions. Despite climate difference between the two northern sites, the pattern of the malaria transmission was similar, and all vectors recorded seem to be living long enough to enable them to transmit the disease, as the parity rates recorded at all sites were high for most of the vectors. This observation suggests that the current vector control tools implemented by the NMCP may have limited impact on the vectors. Cameroon has recommended universal coverage and mass distribution of pyrethroid-only ITNs since the last decade until the recent plan to introduce new types of nets during the 2022 mass ITN distribution campaigns. Even though some positive results were recorded on the decrease of morbidity due to malaria, more efforts are needed to reach elimination of the disease in the country. It is known that the use of ITNs was still low among the population [42], therefore calling for appropriate social and behavior communication programs that need to be undertaken by the NMCP.

Conclusion

Cameroon has a particularly diverse and high density of *Anopheles* species, with *An. gambiae* s.l., as the main malaria vector overall and other subspecies spread in different geographical regions of the country. Nyabessang showed the contrast in vector population, recording *An. moucheti*, *An. nili*, and *An. paludis*. Seasonal variations of HBRs and the indoor resting density of *An. gambiae* s.l. were observed in all sites. *Anopheles gambiae* s.l. was observed to bite more indoors in Mangoum and more outdoors in Gounougou, Bonabéri, and Nyabessang, with biting occurring until the early morning hours at all sites.

Eleven *Anopheles* species and subspecies were involved in malaria transmission and *An. gambiae* s.l. highly contributed at all sites, except in Nyabessang where *An. moucheti* and *An. paludis* were highly active in transmitting the disease. This study highlights the urgent need for integrated vector control interventions considering all potential vectors to reduce malaria transmission and burden in Cameroon. Furthermore, there is a necessity to investigate the human behavior overnight to understand the impact of outdoor transmission of the different vectors for appropriate vector control strategies. The data could also support the deployment of IRS in targeted sites with timing determined by the trends observed over the collection years.Targeted LSM could be an additional option to reduce the peak biting and transmission in northern areas, where rice cultivation increases the mosquito population density, but should be implemented in the context of an impact evaluation.

Declarations

Acknowledgements

We are grateful to all site mosquito collectors and administrative authorities for facilitating the field data collections.

Authors' contributions

EF and JC[#] (the corresponding author) drafted the manuscript. JT; MT; CN; EEW; SP; BM; RT; EM; EE; EC and EK supported, supervised field collections, and reviewed the manuscript. DA; KE; JH; CK, JC and SZ revised the manuscript for

improvement. All corresponding authors reviewed the draft, provided inputs, which were collated and incorporated by JC[#]. All authors read and approved the final manuscript.

Funding

This study was supported by the United States President's Malaria Initiative through the United States Agency for International Development (USAID) Abt Associates / VectorLink Project.

Disclaimer

The findings and conclusions expressed herein are those of the authors and do not necessarily represent the official position of USAID, PMI, nor the Centers for Disease Control and Prevention (CDC).

Availability of data and materials

All data generated or analyzed during this study are included in this published article and its supplementary information files.

Declarations

Ethics approval and consent to participate

Ethical IRB approval was determined to be unnecessary for this study in Cameroon as vector monitoring did not involve any human blood collection. This entomological monitoring work received ethical clearance under CDC protocol number 0900f3eb819cbc38. Administrative clearance was requested anytime that the entomological field collections were organized an official letter signed by the Director of the NMCP was sent to the Public Health Regional Delegate to inform about the survey.

Consent for publication

This manuscript was formally cleared through CDC and PMI approval system for external publications.

Competing interests

The authors declare that they have no competing interests.

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Figures

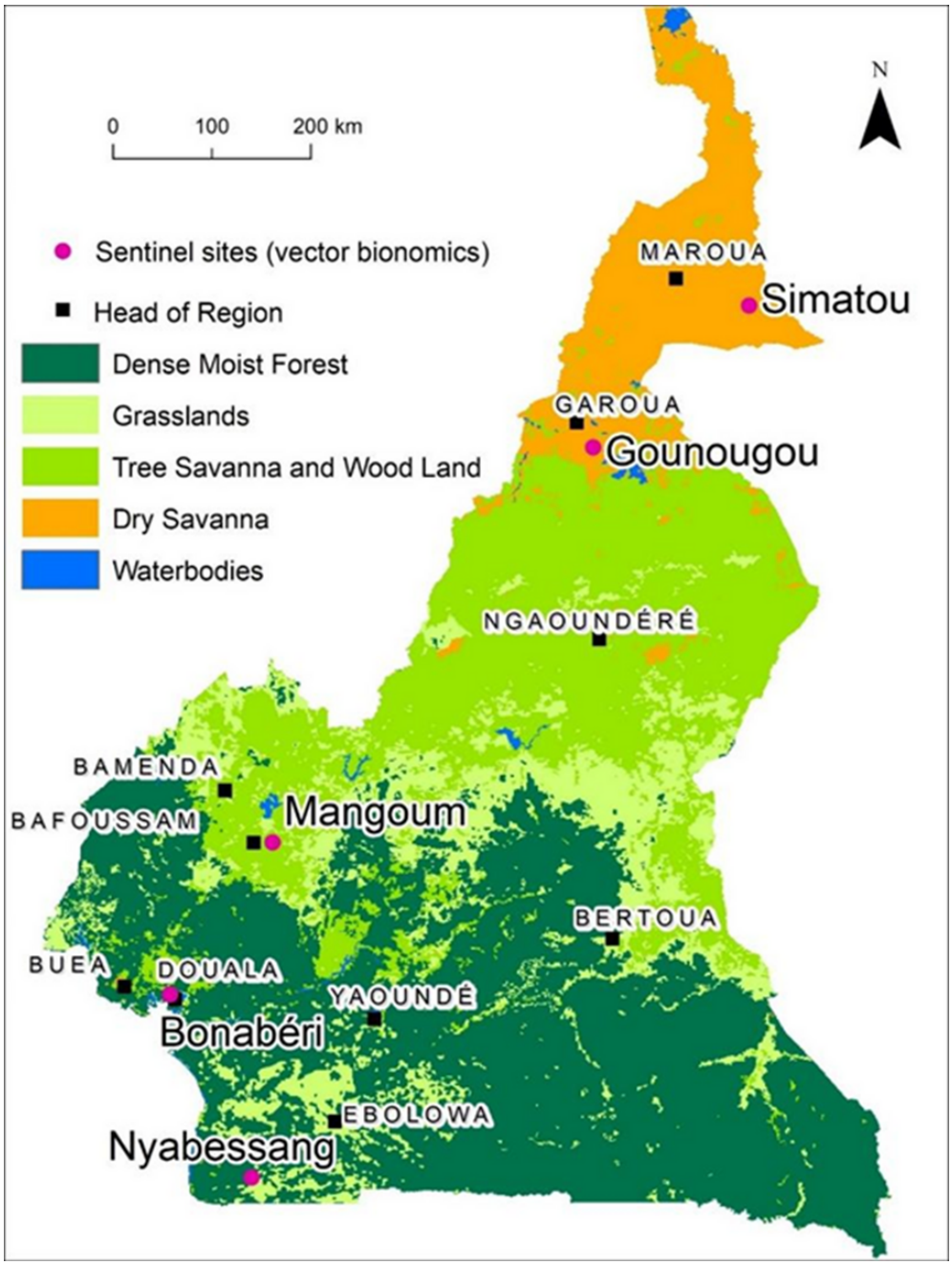

Figure 1

Map of Cameroon Showing the Location of the Five Vector Surveillance Sentinel Sites

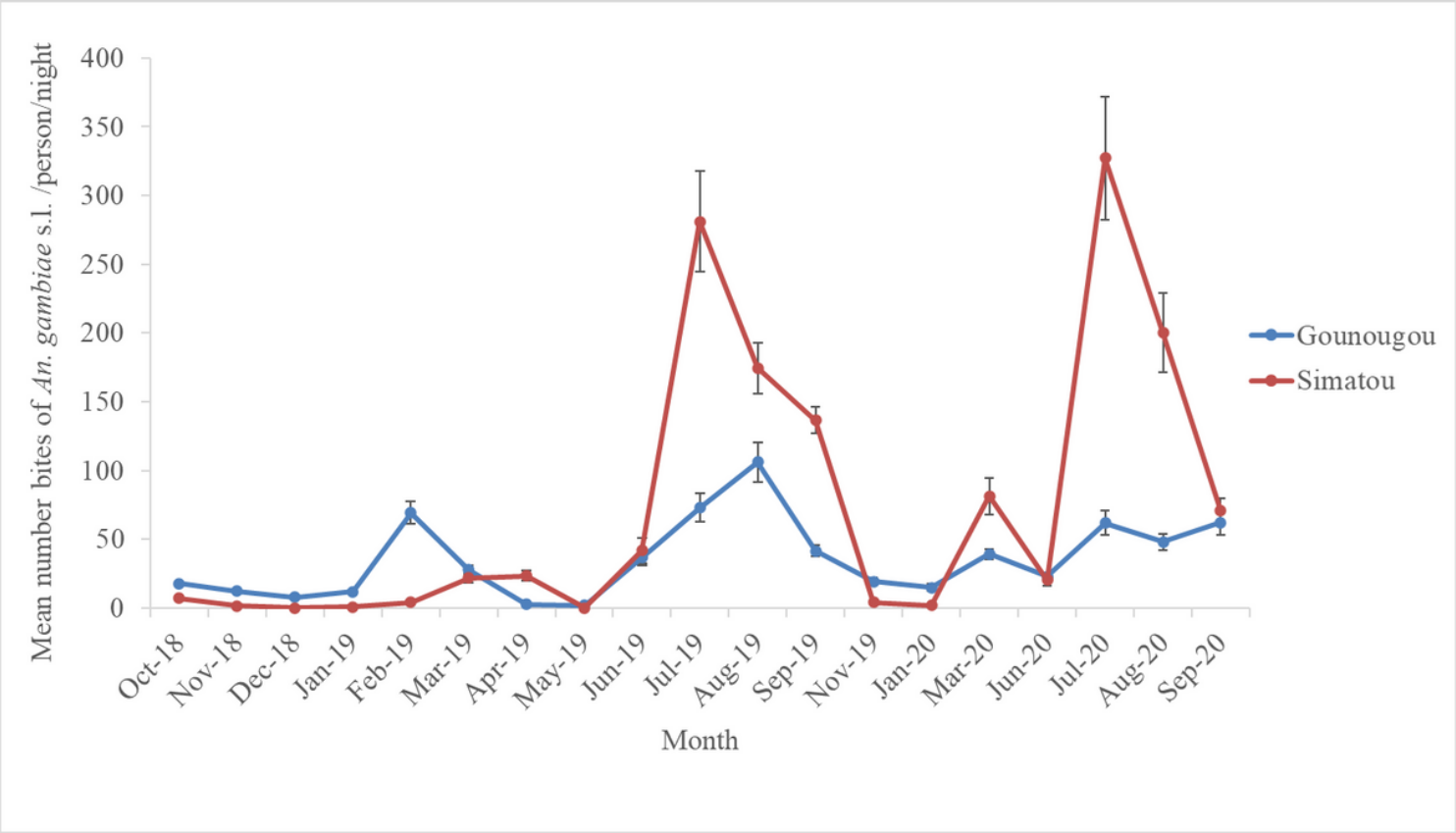

Figure 2

Mean Monthly Human Biting Rates of *Anopheles gambiae* s.l. in the Northern Sites of Simatou and Gounougou (Oct 2018-Sept 2020)

Error bars represent the standard errors

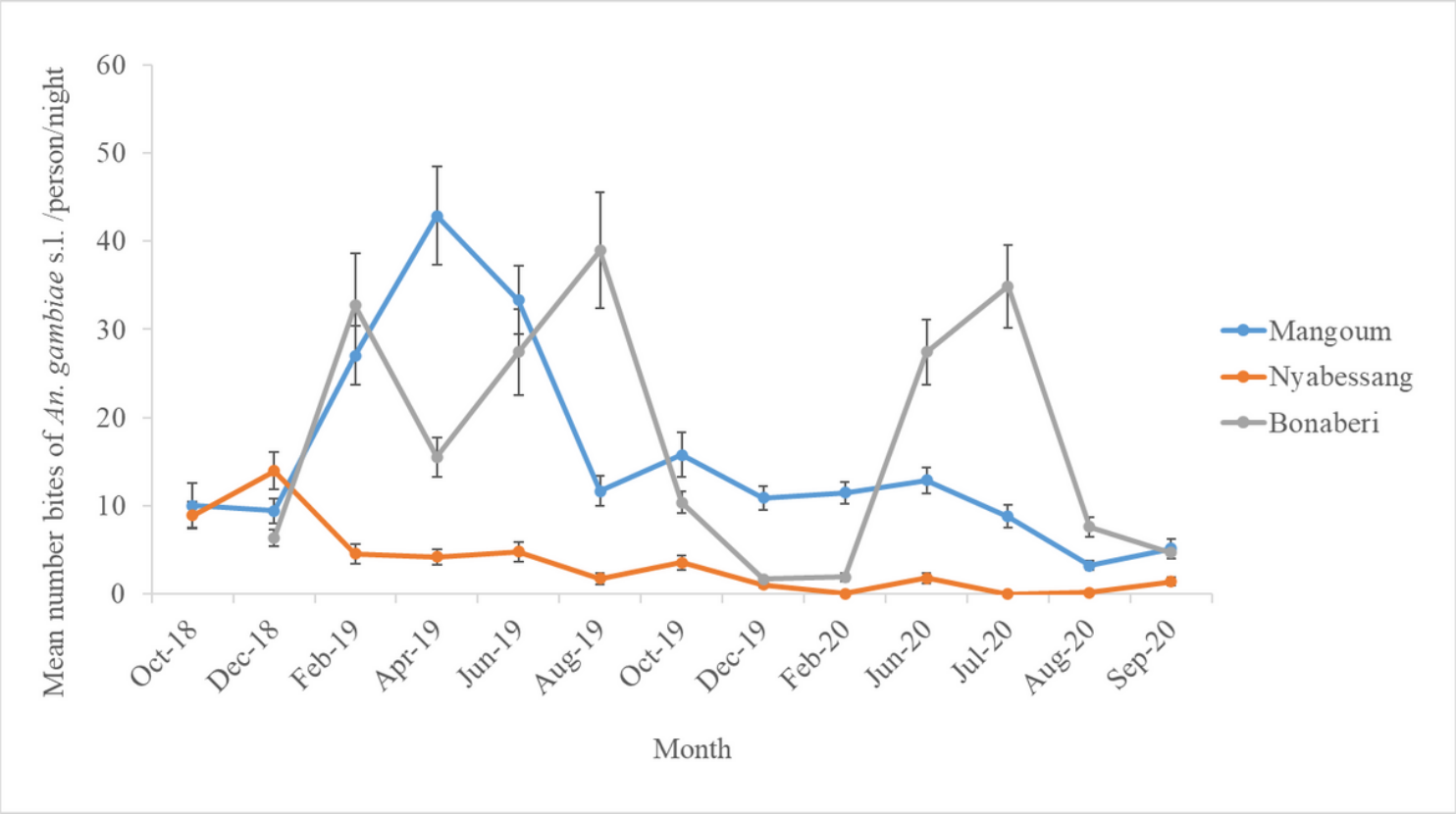

Figure 3

Mean Monthly Human Biting Rates of *Anopheles gambiae* s.l. in the Southern Sites of Mangoum, Nyabessang and Bonabéri (Oct 2018-Sept 2020)

Error bars represent the standard errors

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementarydata1.xlsx
- Supplementarydata2.xlsx