USAID | *StopPalu+*

ANNUAL REPORT OF ENTOMOLOGICAL SURVEILLANCE ACTIVITIES (NOVEMBER 2018–OCTOBER 2019)

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Abbreviations

Ace-I	Acetylcholinesterase
CDC	U.S. Centers for Disease Control and Prevention
CSP	Circumsporozoite protein
CREC	Centre de Recherches Entomologiques de Cotonou
DDT	Dichloro-diphenyl-trichloroethane
EIR	Entomological inoculation rate
ELISA	Enzyme-linked immunosorbent assay
GFATM	Global Fund to Fight AIDS, Tuberculosis and Malaria
HLC	Human landing collection
ITN	Insecticide-treated bednet
kdr	Knockdown resistance (mutation)
LLIN	Long-lasting insecticide-treated net
MICS Palu	Multiple Indicator Cluster Survey (on Malaria)
MOH	Ministry of Health
NMCP	National Malaria Control Program
PBO	Piperonyl butoxide
PCR	Polymerase chain reaction
PMI	U.S. President's Malaria Initiative
PSC	Pyrethrum spray catch
UGANC	Gamal Abdel Nasser University of Conakry
USAID	United States Agency for International Development
WHO	World Health Organization

Summary

The President's Malaria Initiative (PMI) Program Component (*StopPalu+*) activities are implemented to reduce the malaria burden in Guinea.[1] These activities include building in-country capacity to carry out entomological surveys. During November 2018 to October 2019 (the period covered by this annual report), *StopPalu+* supported the National Malaria Control Program's (NMCP's) field activities and supported the operation of the insectary and laboratory for conducting entomological investigations. The focus of *StopPalu+*'s entomological activities is to continue to support the NMCP in developing a national vector control strategy to (1) control vector populations across the country, (2) identify areas affected by insecticide resistance and characterize resistance patterns, and (3) implement evidence-based interventions for impact.

During the reporting period, *StopPalu+* accomplished the following:

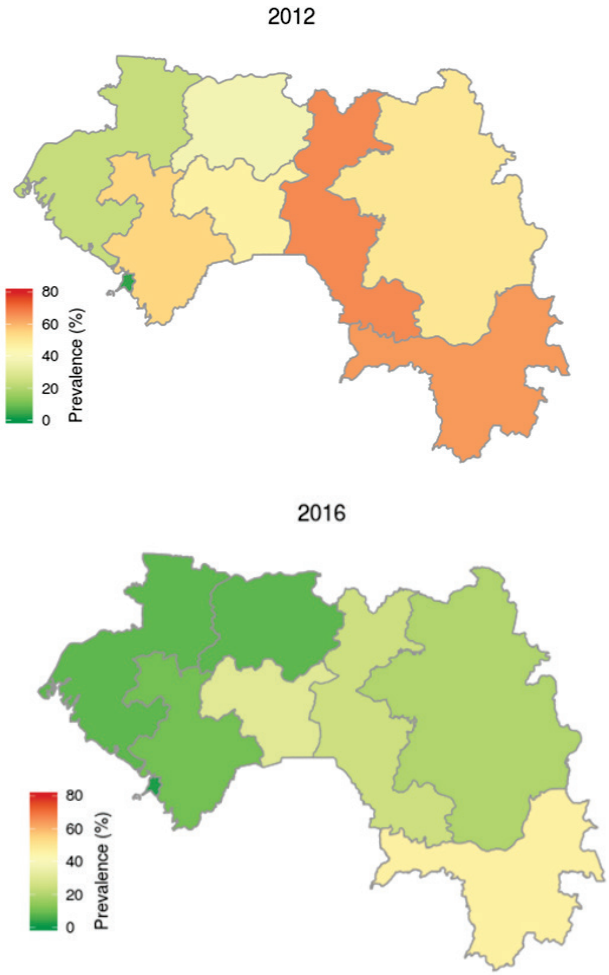
- Continued to support the breeding of Kisumu strain mosquitoes for multiple generations
- Conducted a monthly study of vector seasonality in Boké and Faranah
- Determined susceptibility of *Anopheles gambiae* s.l. to 3 insecticides (alphacypermethrin, deltamethrin, permethrin) recommended by World Health Organization (WHO) for use on long-lasting insecticide-treated nets (LLINs) in 6 sites: Boké, Labé, Kankan, Dabola, Faranah, and Kissidougou
- Determined susceptibility of *An. gambiae* s.l. to 3 insecticides (alphacypermethrin, deltamethrin, permethrin) after prior exposure to piperonyl butoxide (PBO)
- Conducted intensity tests to alphacypermethrin, deltamethrin, and permethrin in 6 sites
- Equipped and maintained the Insectarium and the lab at the Gama Abdel Nasser University in Conakry (UGANC)
- Strengthened capacities of students and trainees (UGANC, NMCP)
- Supported the NMCP Prevention Technical Working Group meetings

[1] *StopPalu+* is the five-year follow-on project to the original *StopPalu* project. *StopPalu+* started in December 2017 and will run through December 2022.

1 Context

Over the past five years, Guinea has made enormous progress in malaria control, leading to a reduction in malaria prevalence in children under five years of age (***Figure 1***), annual malaria incidence, and in-patient deaths Progress was such that in 2016, the Government of Guinea received an Award of Excellence from the Alliance of African Leaders Against Malaria for the country's efforts against malaria.[2] Much of this progress is due to the Guinean Government's leadership and commitment to scale up key interventions against malaria, backed by substantial external financial support, specifically the Global Fund to Fight AIDS, Tuberculosis, and Malaria (GFATM) and the U.S. President's Malaria Initiative (PMI).

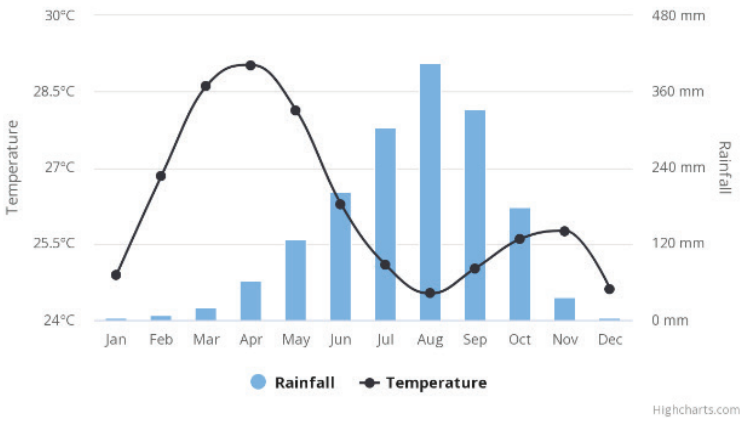
Figure 1: Malaria prevalence in children under 5 years



Guinea's malaria efforts face several challenges, however, including major transmission heterogeneity between regions and districts (*préfectures*); a constrained malaria commodity procurement supply chain; uneven technical and program planning, implementation, and management capacity at national, regional, and district levels; and mounting insecticide resistance.[3] These challenges invariably impact programmatic intervention coverage and effectiveness. Consequently, malaria remains the most burdensome communicable disease in Guinea: the entire population of 12.1 million people is at risk, with approximately 1 million cases reported in 2016, accounting for 31% of outpatient visits.[3]

[2] https://www.pmi.gov/african-heads-of-state-celebrate-progress-against-malaria/
[3] MOH. (2017). *Performance Review of the Strategic Plan for Malaria Control 2013–2017*. Conakry, Guinea, p. 1-75.

Figure 2: Average monthly temperature and rainfall in Guinea for 1991–2016[4]



The main malaria vector in Guinea is *Anopheles gambiae sensu lato* (s.l.), specifically *An. gambiae sensu stricto* (*s.s.*), *An. coluzzii*, *An. arabiensis*, and *An. melas*. The *An. funestus* complex is also present, but studies published to date indicate that *An. funestus* is less prevalent than the *An. gambiae* complex. Vector control interventions implemented in Guinea include the mass distribution of LLINs supported by GFATM and PMI and, to a very limited extent, indoor residual spraying with propoxur, deltamethrin, and pirimiphos-methyl, supported by Guinean mining companies. The resistance status of malaria vectors in Guinea has been investigated in several sites (Boké, Labé, Maferinyah, Faranah, Kankan, Fandie, Madinagbe, Maferinyah, Moribayah, Senguelen, and Yindi).[5,6,7] Resistance to DDT (dichloro-diphenyl-trichloroethane), permethrin, alpha-cypermethrin, and lambda-cyhalothrin was detected in all *An. gambiae* s.l. populations tested; part of the sample site showed susceptibility to deltamethrin and bendiocarb. Keita et al. (2017)[5] found that the *kdr* (knockdown resistance) *West* mutation was widespread, and frequency was 60% or more in all sites sampled; the *Ace-1* mutation was present in low levels. There is limited information on the insecticide resistance status in *An. funestus*, with complete susceptibility to deltamethrin and malathion (unspecified dose), reported.[8]

Furthermore, seasonal factors, such as temperature and rainfall, impact vector quantities and the resulting malaria transmission opportunities. As ***Figure 2*** shows, over a period of 25 years, the rainy season in Guinea covers approximately 5 months (with a concentration of rainfall occurring in July, August, and September), and the dry season is, on average, 7 months long. During the dry season, mosquito breeding sites vanish, and mosquito populations drop; however, when the rains return, there is a surge in mosquito population growth, matching the start of the malaria transmission season.[9]

[4] World Bank Group. The Climate Change Knowledge Portal. Accessed July 27, 2020 (https://climateknowledgeportal.worldbank.org/)
[5] Keita, K. et al. (2017). Species identification and resistance status of *Anopheles gambiae* s.l. (Diptera: Culicidae) mosquitoes in Guinea. *Journal of Medical Entomology, 54*, 677–681.
[6] Collins E, Vaselli NM, Sylla M, et al. The relationship between insecticide resistance, mosquito age and malaria prevalence in Anopheles gambiae s.l. from Guinea. Sci Rep. 2019;9(1):8846. Published 2019 5
[7] Stica C, Jeffries CL, Irish SR, et al. Characterizing the molecular and metabolic mechanisms of insecticide resistance in Anopheles gambiae in Faranah, Guinea. Malar J. 2019;18(1):244. y
[8] AngloGold Ashanti. (2007). Unpublished.
[9] Magombedze, G., Ferguson, N., and Ghani, A. (2018). A trade-off between dry season survival longevity and wet season high net reproduction can explain the persistence of *Anopheles* mosquitoes. *Parasites & Vectors, 11*(576).

2 *StopPalu+* Results Framework

PMI's main operational platform for malaria efforts from 2013 onward has been the USAID Guinea *StopPalu* project (2013–2017) and the current follow-on project *StopPalu+* (December 2017–December 2022), both led by RTI International. *StopPalu+*'s goal is to assist the Government of Guinea to achieve the PMI target of reducing malaria-related morbidity and mortality by 75% compared with 2016 levels. This will be achieved through (1) increasing the use of LLINs by the population; (2) increasing the use of intermittent preventive treatment of malaria in pregnancy during antenatal care visits; (3) increasing prompt care-seeking and treatment; (4) increasing the number of full doses of seasonal malaria chemoprevention that are delivered promptly; (5) increasing community involvement in and support for malaria prevention, care, and treatment activities; and (6) strengthening the technical capacity of the National Malaria Control Program (NMCP) to manage, implement, and monitor prevention, care, and treatment activities.

The overarching aims of entomological activities supported by *StopPalu+* are to strengthen the country's entomological capacity and generate malaria vector data that will help the NMCP and stakeholders to develop and implement Guinea's vector control strategy.

Entomological activities supported by *StopPalu+* have three main objectives during this second year:

Objective 1: Strengthen national entomological capacity by:

- Supporting the operation of an entomology laboratory and insectary at the Gamal Abdel Nasser University of Conakry (UGANC)
- Maintaining a colony of *An. gambiae* s.s. (Kisumu strain)
- Training UGANC students and the NMCP interns in maintenance techniques for the insectary
- Supporting the Vector Control Technical Working Group meetings

Objective 2: Conduct entomological surveillance by:

- Collecting mosquitoes in selected sentinel sites
- Assessing vector distribution and abundance
- Assessing vector seasonality
- Assessing vector biting behavior
- Performing species identification and sporozoite indexing

Objective 3: Determine insecticide resistance status of malaria vectors by:

- Carrying out susceptibility testing in sentinel sites
- Carrying out intensity tests for deltamethrin, permethrin, and alpha-cypermethrin at different doses
- Collecting semi-gravid mosquitoes for chromosomal analysis to understand the genetic basis of insecticide resistance

3 Achievements

3.1 Objective 1: Strengthen the national entomological capacity

Since the official opening of the entomology laboratory and insectary at UGANC, *StopPalu+* has conducted several activities, including (1) supporting the operation of the laboratory and insectary, (2) maintaining a susceptible *An. gambiae* s.s. population (Kisumu strain); (3) holding meetings, organized by the staff, with the NMCP Vector Control Unit; and (4) training student interns.

3.1.1 Supporting the functioning of the entomology laboratory and insectary at UGANC

The laboratory and insectary were established on the UGANC grounds in November 2016 with support from Professor [Redacted] (Entomology Research Center of Cotonou, Benin); rearing of colony mosquitoes in the insectary is ongoing, while mosquito identification by microscopy is currently the main activity occurring the laboratory.

Laboratory

During the reporting period, the project conducted the following activities in the laboratory:

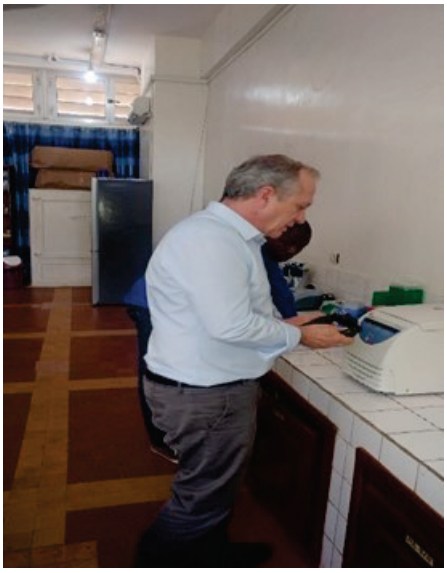
Identifying, coding, and packaging *Anopheles*

Whole *Anopheles* collected by various collection methods (human bait, light trap, and pyrethrum spray catch) in the sentinel sites were morphologically identified by sex, species, and physiological state. *Anopheles* were placed individually in tubes containing silica gel, and each tube was numbered and codified.

The legs and wings of collected mosquitoes are being saved for polymerase chain reaction (PCR) testing to identify sibling species and molecular forms of *An. gambiae* complex *(Anopheles gambiae s.s. vs. An. coluzzi)*.

Procurement of equipment for PCR tests

During the reporting period, the project procured materials and equipment for PCR tests. The lists of equipment, materials, and consumables are summarized in the table in Annex 1.

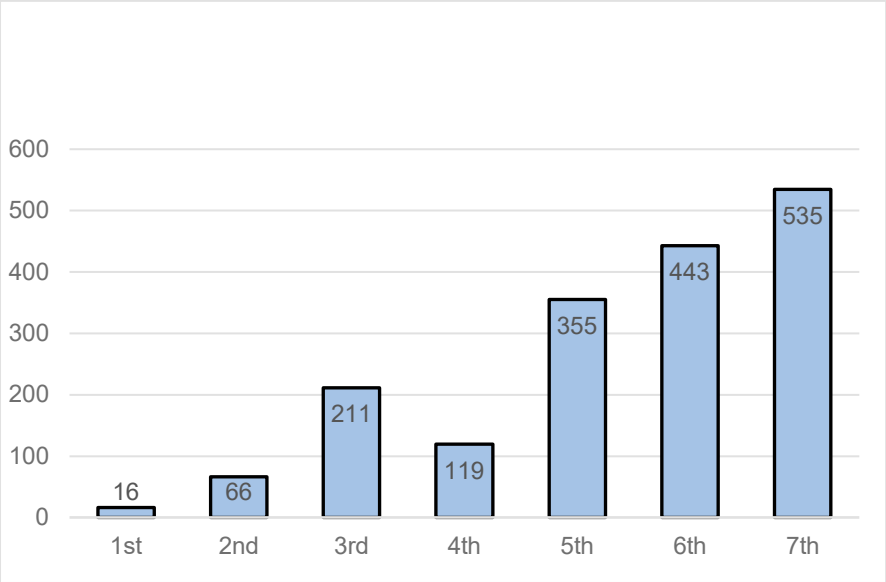


UGANC entomology laboratory and insectary

Insectary

On May 30, 2019, the insectary team received *An. gambiae* Kisumu strain mosquito eggs from Switzerland. The project entomology team maintained this colony. As of the end of October 2019, 7 generations of mosquitoes were produced with a total of 1,445 mosquitoes (see ***Figure 3***). Each generation had led to an increase in the number of mosquito progeny.

Figure 3: Number of mosquitoes produced per generation



3.1.2 Training student interns

As part of the collaboration between the *StopPalu+* project and the University, the team continued to support students as part of their initiation to basic entomology. During the reporting period, the project entomologist team, assisted by the NMCP Vector Control Unit team, continued to train 6 students and 5 interns from the NMCP at the insectarium.

Trainees at the insectary

3.1.3 Supporting the Vector Control Technical Working Group meetings

During the reporting period, *StopPalu+* supported the NMCP's Vector Control Unit in organizing monthly meetings. During these meetings, vector control activities for the month/quarter were reviewed and discussed, and activities for the months/quarters ahead were planned. It is important to mention that for this period, the main point discussed was the organization of the national LLIN mass distribution campaign. The participants also reviewed the agenda for the entomological monitoring visits, the number of sites to be visited, and the timing of the visits.

3.2 Objective 2: Conduct entomological surveillance

Entomological surveillance activities were conducted in six districts: Boké, Dabola, Faranah, Labé, Kankan, and Kissidougou (see Annex 2). These districts were selected based on the Health Management Information System (HMIS) data and represented the four main malaria endemicity zones in Guinea. In each district, three villages with the highest malaria prevalence were selected as sentinel sites (***Table 1***).

Insecticide resistance testing in malaria vectors was conducted in Boké, Dabola, Faranah, Labé, Kankan, and Kissidougou during the rainy season. To investigate the seasonality of

malaria vector distribution, abundance, behavior, and infection rate, monthly surveys were done in Boké and Faranah. *StopPalu+* and the NMCP team conducted these surveys together.

Table 1: Sentinel sites for entomology surveillance activities

Activity	Natural regions	Districts	Villages studies	Period of visits (2019)
Insecticide resistance testing	Lower Guinea	Boké	Kaboye, Guilere, Djumaya	16 Oct–4 Sep
	Middle Guinea	Labé	Banty, Thialy, Tountouroun	8–28 Jun
	Upper Guinea	Dabola	Bissikrima, Saourou, Sognessa	11–31Jul
		Kankan	Dalabani, Balandou, Makonon	8–28 Jun
		Faranah	Balayani, Foulaya, Tindo	11–31Jul
	Forest Guinea	Kissidougou	Gbangbadou, Kérédou, Tongbèkoro	16 Oct–4 Sep
Monthly entomological monitoring (seasonality)	Lower Guinea	Boké	Kaboye, Dioumaya, Guilere	16 Oct–4 Sep
	Upper Guinea	Faranah	Balayani, Foulaya, Tindo	11–31Jul

During the visits to the 18 villages, three mosquito collection methods were used: (1) human landing collection catch (HLC), (2) Centers for Disease Control and Prevention (CDC) light traps, and (3) pyrethrum spray catches (PSC) (***Table 2***). These methods were used to gather information on the vector species composition, abundance, physiological status, and host-seeking behavior.

Table 2: Summary of the frequency of collection and the number of homes sampled in both seasonal sites during the 2019 activity year

		Number of collections per month (houses sampled)															
		Feb		**Mar**		**Apr**		**May**		**Jun**		**Jul**		**Aug**		**Sep**	
		HLC/LT	PSC	HLC/LT	PSC	HLC/LT	PSC	HLC/LT	PSC	HLC/LT	PSC	HLC/LT	PSC	HLC/LT	PSC	HLC/LT	PSC
	Date	Feb 12 to 26		Mar 11 to 25		April 11 to 25		May 11 to 25		Jun 15 to 29		Jul 12 to 26		Aug 15 to 29		ND	ND
Districts	**Village**																
Boké	Kaboye	2 (1)	2 (5)	2 (1)	2 (5)	2 (1)	2 (5)	2 (1)	2 (5)	2 (1)	2 (5)	2 (1)	2 (5)	2 (1)	2 (5)	2 (1)	2 (5)
	Dioumaya	2 (1)	2 (5)	2 (1)	2 (5)	2 (1)	2 (5)	2 (1)	2 (5)	2 (1)	2 (5)	2 (1)	2 (5)	2 (1)	2 (5)	2 (1)	2 (5)
	Guilere	2 (1)	2 (5)	2 (1)	2 (5)	2 (1)	2 (5)	2 (1)	2 (5)	2 (1)	2 (5)	2 (1)	2 (5)	2 (1)	2 (5)	2 (1)	2 (5)
	Date	Feb 12 to 26		Mar 11 to 25		April 11 to 25		May 11 to 25		Jun 15 to 29		Jul 12 to 26		Aug 15 to 29		Sep 12 to 26	
	Village																
Faranah	Balayani	2 (1)	2 (5)	2 (1)	2 (5)	2 (1)	2 (5)	2 (1)	2 (5)	2 (1)	2 (5)	2 (1)	2 (5)	2 (1)	2 (5)	2 (1)	2 (5)
	Foulaya	2 (1)	2 (5)	2 (1)	2 (5)	2 (1)	2 (5)	2 (1)	2 (5)	2 (1)	2 (5)	2 (1)	2 (5)	2 (1)	2 (5)	2 (1)	2 (5)
	Tindo	2 (1)	2 (5)	2 (1)	2 (5)	2 (1)	2 (5)	2 (1)	2 (5)	2 (1)	2 (5)	2 (1)	2 (5)	2 (1)	2 (5)	2 (1)	2 (5)
Total houses sampled		12	60	12	60	12	60	12	60	12	60	12	60	12	60	12	60

ND=Not done; HLC=human landing collection; LT=CDC light trap; PSC=pyrethrum spray collection; Alternating collections were made in two houses over two nights using HLCs and CDC light traps, while five different houses per village per day (over two days) per month were sampled using PSCs (see Annex 3 for sampling approach).

3.2.1 Assessing vector distribution and abundance

Human landing collection (HLC)

HLCs are a standard method for determining human-vector contact and for specifically assessing mosquito biting behavior. HLCs were conducted inside and outside of one house per night in each of the 6 selected villages. Four collectors were situated inside and outside of a house in each village from 1800 to 0700 hrs; the first pair of collectors worked from 1800 to 0100 hrs and a second pair of collectors worked from 0100 to 0700 hrs. All collectors were offered malaria chemoprophylaxis before mosquito collections. Collectors used individual tubes to capture mosquitoes that were trying to bite their exposed legs; the tubes were packaged in different plastic bags for each collection hour to determine the timing of aggressive behavior throughout the night. This activity was carried out in two houses (one different house on each consecutive night) per village for two nights per visit from February to August 2019 in Boké and from February to September 2019 in Faranah (see Annex 3). (Please note that Boké was not assessed in September because the local entomologist was not available.) The collected mosquitoes were returned to the laboratory for species identification by standard morphological keys.

HLC inside a house

HLC outside a house

In total, 2,675 mosquitoes were collected in the two sites. Of these 2,675 mosquitoes, 2,340 (87%) were *An. gambiae s.l*. There were no other *Anopheles* identified. Among the *Anopheles* mosquitoes collected, 56% (1,299 of 2,340) were caught outdoors; the range in outdoor collection across sites varied between 50% and 59%. *Anopheles* caught indoors were 44% (1,041 of 2,340); the range in indoor collection across sites varied between 41% and 46%.

In Boké, 306 mosquitoes were collected, and in Faranah 2,369 mosquitoes were collected. In Boké, 23% (69 of 306) of mosquitoes caught were *Anopheles* (57% of *Anopheles* were collected indoors, 43% of *Anopheles* were collected outdoors). In Faranah, 96% (2,274 of 2,369) of mosquitoes caught were *Anopheles* (44% of *Anopheles* were collected indoors and 56% of *Anopheles* were collected outdoors).

Of the two sites, the Faranah villages collected the most *Anopheles*—96.2% of the total *Anopheles* collected indoors across the two sites (1,002 of 1,041) and 97% of the total *Anopheles* collected outdoors (1,254 of 1,299) were in Faranah villages.

The overall mean of mosquitoes collected through HLC is presented in ***Table 3***.

Table 3: Mean abundance and distribution of mosquitoes collected using HLC over 14 collection nights (2 nights every 7 months) for Boké and 16 collection nights for Faranah (2 nights every 8 months) in one house per village

Prefecture	Sentinel Sites	HLC: Average number of mosquitoes per house per collection*					
		An. gambiae s.l.		*Culex spp.*		*Ae. aegypti*	
		Int	Ext	Int	Ext	Int	Ext
Boké	Kaboye	0.93	0.71	2.28	2.86	0	0
	Guilere	0.85	0.93	2.50	3.50	0	0
	Dioumaya	1.00	0.50	2.78	3.00	0	0
	Boké mean	**0.93**	**0.71**	**2.52**	**3.11**	**0**	**0**
Faranah	Balayani	22.94	29.38	0.06	0	0.25	0.81
	Foulaya	25.87	32.20	0.06	0.19	0.31	1.00
	Tindo	13.81	17.75	1.12	0.87	0.25	1.19
	Faranah mean	**20.87**	**26.43**	**0.41**	**0.35**	**0.20**	**1.00**
Overall indoor and outdoor mean		**65.40**	**81.47**	**8.81**	**10.42**	**0.81**	**3.00**
Percentage		**45%**	**55%**	**46%**	**54%**	**21%**	**79%**
Overall mean per species		**13.00**		**1.52**		**0.32**	

Int = interior; Ext = exterior

*Given that there have been 7 months of activity in Boké and 8 months in Faranah and the team did 2 nights of captures per month per village, we divided the results of Boké by 14 and by 16 in Faranah to calculate the means.

CDC light traps

CDC light traps were used to collect indoor and outdoor host-seeking mosquitoes. In each village, two houses were systematically selected and sampled for one night, with one trap located indoors (near sleeping spaces, if possible) and a second trap located outdoors from 1800 to 0700 hrs (where feasible these traps were hung under the eaves of the house).

The results of CDC light trap collection are presented in ***Table 4***. Of 1,248 mosquitoes collected in the two sites, 47% (590 of 1248) were *An. gambiae* s.l. There were no other *Anopheles* identified. Among the collected *Anopheles* mosquitoes, 56% (318 of 590) were caught outdoors. Of all the *An. gambiae* collected using CDC light traps indoors and outdoors, 86% and 93%, respectively, were caught in Faranah.

A CDC light trap outside a house

A CDC light trap inside a house

Table 4: Mean abundance and distribution of mosquitoes collected using CDC light traps over 14 collection nights (2 nights every 7 months) for Boké and 16 collection nights for Faranah (2 nights every 8 months) in one house per village

		CDC light traps: Number of mosquitoes per light trap per collection					
		***An. gambiae* s.l.**		***Culex spp.***		***Ae. aegypti***	
Prefecture	**Sentinel Sites**	**Int**	**Ext**	**Int**	**Ext**	**Int**	**Ext**
Boké	**Kaboye**	0.78	0.43	4.00	4.43	0	0.14
	Guilere	0.86	0.57	2.14	4.43	0	0.36
	Dioumaya	1.00	0.57	2.21	4.00	0	0.14
	Boké mean	**0.88**	**0.52**	**2.78**	**4.28**	**0**	**0.21**
Faranah	**Balayani**	5.31	6.94	3.00	3.37	0.50	0.94
	Foulaya	6.31	7.44	3.44	2.37	0.44	0.81
	Tindo	3.06	4.12	3.37	2.37	0.50	0.87
	Faranah mean	**4.89**	**6.16**	**3.27**	**2.70**	**0.47**	**0.87**
Overall indoor and outdoor mean		**17.32**	**20.07**	**18.16**	**20.97**	**1.44**	**3.26**
Percentage		**46%**	**54%**	**46%**	**54%**	**31%**	**69%**
Overall species mean		**3.27**		**3.24**		**0.41**	

Int = interior; Ext = exterior

*Given that there have been 7 months of activity in Boké and 8 months in Faranah and the team did 2 nights of captures per month per village, we divided the results of Boké by 14 and by 16 in Faranah to calculate the means.

Pyrethrum spray catches

This method of capture consists of collecting adult mosquitoes inside the houses to determine species present indoors and their physiological state. Collections were conducted in the 6 selected villages; in each village, 10 houses/huts were systematically selected for pyrethrum spray catches. In each house, the floor was covered with white sheets to collect knocked-down mosquitoes, with collection teams receiving permission from all households before spraying. The collections took place from 0700 to 1000 hrs. An operator sprayed the indoor area with a pyrethroid insecticide after taking all necessary precautions, including removing all food and other sensitive materials. Houses were sprayed from the outside inward through the openings to prevent mosquitoes from escaping. After spraying, all windows and doors were kept closed for 15 minutes. After 15 minutes, the sheets were taken out of the house to collect mosquitoes that had fallen on the sheets. Mosquitoes were brought to the laboratory, where their species were identified and their physiological status (unfed, fed, gravid, and semi-gravid) was determined.

Pyrethrum collection in Faranah

Of the 388 mosquitoes collected, all were female *An. gambiae* s.l. No other mosquitoes were collected. Of the 388 female *An. gambiae* s.l., 295 (76%) were fed, 93 (24%) were unfed, and none were semi-gravid or gravid. The overall mean of mosquitoes collected with the pyrethrum spray catch is presented in ***Table 5***.

Table 5: Mean abundance and distribution of mosquitoes collected using indoor pyrethrum spray catch over 7 months of collection in Boké and 8 months of collection in Faranah using in 10 houses per village

		Pyrethrum spray catch results: Number of *An. gambiae* s.l. per house-collection*				
Prefecture		**Unfed**	**Fed**	**Semi-gravid**	**Gravid**	**Overall site mean**
Boké	**Kaboye**	0	0.01	0	0	0.01
	Guilere	0	0.03	0	0	0.03
	Dioumaya	0.01	0.03	0	0	0.04
	Boké overall	**0.01**	**0.07**	**0**	**0**	**0.08**
Faranah	**Balayani**	0.34	1.67	0	0	2.01
	Foulaya	0.44	1.04	0	0	1.48
	Tindo	0.37	0.91	0	0	1.28
	Faranah overall	1.15	**3.62**	**0**	**0**	**4.77**
Overall stage mean		**1.16**	**3.69**	**0**	**0**	**0.91**

* **Given that there have been 7 months of activity in Boké and 8 months in Faranah, and the team visited 10 houses per village per month, we divided the results by 70 for Boké and by 80 for Faranah to calculate the means.**

Larvae collection

As part of mosquito surveillance efforts, a larval collection was performed in each sentinel village. The goal of this collection was to gather information on mosquito breeding sites and collect mosquitoes for insecticide susceptibility tests. Collections were performed during the rainy season (June– August 2019), with sites selected during searches at the sentinel sites, either during visits or when driving to and from the sentinel sites. Pupae and larvae were collected from different water bodies using pans and dippers, and they were then raised in the laboratories located in the Regional and Prefectural Health Directorates and health centers. The larvae were fed with grounded fish food and transferred to standard insect rearing cages after pupation; they received a 5%–10% glucose solution ad libitum before being tested.

Larvae collection

3.2.1 Assessing vector seasonality

The Boké and Faranah sites were sampled to understand vector seasonality. Human biting rates (HBR) results are presented in ***Tables 6, 7, and 8***; total number of *An. gambiae* s.l. mosquitoes collected are present in ***Annex 4, 5, and 6***.

Overall, the month of peak human biting rate and mosquito trap rate of *An. gambiae* s.l. was in July as measured by the HLCs and the CDC light trap, respectively (***Tables 6 and 7***). However, Faranah contributed mostly to this trend. In Boké specifically, the peak biting rate and mosquito trap rate tended to be in August. Generally, monthly trends for indoor and outdoor collections were similar. In Faranah, there were slightly more mosquitoes collected outdoors than indoors using CDC and HLCs, while in Boké the numbers were more equal.

For the indoor resting density, June to September had the higest abundance overall in the two sites, with Faranah contributing mostly to the trend (***Table 8***).

Table 6: Seasonal human biting rate (bites per person per night) of *An. gambiae* s.l. collected in Boké and Faranah villages as determined using HLCs

Prefecture	Village	Location	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Overall annual biting rate
Boké	**Dioumaya**	Indoor	0.00	1.50	0.50	0.00	0.00	2.00	3.00	ND	1.00
		Outdoor	0.50	0.50	0.00	0.50	0.00	0.50	1.50	ND	0.50
		Combined	**0.25**	**1.00**	**0.25**	**0.25**	**0.00**	**1.25**	**2.25**	ND	**0.75**
	Guilere	Indoor	0.00	0.50	1.50	0.50	0.00	1.50	2.00	ND	0.86
		Outdoor	0.50	2.00	0.50	0.00	0.50	1.00	2.00	ND	0.93
		Combined	**0.25**	**1.25**	**1.00**	**0.25**	**0.25**	**1.25**	**2.00**	ND	**0.89**
	Kaboye	Indoor	1.00	0.50	0.50	0.00	0.00	1.50	3.00	ND	0.93
		Outdoor	0.00	1.00	0.00	0.00	0.00	2.50	1.50	ND	0.71
		Combined	**0.50**	**0.75**	**0.25**	**0.00**	**0.00**	**2.00**	**2.25**	ND	**0.82**
		Boké overall	**0.33**	**1.00**	**0.50**	**0.17**	**0.08**	**1.50**	**2.17**	ND	**0.82**
Faranah	**Balayani**	Indoor	4.00	16.50	19.50	21.00	31.00	45.00	26.50	20.00	22.94
		Outdoor	6.00	14.00	27.50	20.50	43.00	64.50	34.50	25.00	29.38
		Combined	**5.00**	**15.25**	**23.50**	**20.75**	**37.00**	**54.75**	**30.50**	**22.50**	**26.16**
	Foulaya	Indoor	5.50	22.50	23.50	21.00	39.50	43.50	28.00	23.50	25.88
		Outdoor	6.50	23.50	34.50	21.50	45.50	62.00	36.00	28.00	32.19
		Combined	**6.00**	**23.00**	**29.00**	**21.25**	**42.50**	**52.75**	**32.00**	**25.75**	**29.03**
	Tindo	Indoor	0.50	13.00	10.00	11.50	17.50	27.00	19.00	12.00	13.81
		Outdoor	0.50	12.50	16.50	13.50	25.00	37.50	24.50	12.00	17.75
		Combined	**0.50**	**12.75**	**13.25**	**12.50**	**21.25**	**32.25**	**21.75**	**12.00**	**15.78**
		Faranah overall	**3.83**	**17.00**	**21.92**	**18.17**	**33.58**	**46.58**	**28.08**	**20.08**	**23.66**
Overall monthly biting rate			**2.08**	**9.00**	**11.21**	**9.17**	**16.83**	**24.04**	**15.13**	**20.08**	**13.00**
ND = Not done											

Table 7: Seasonal trap nights (mosquitoes per trap per night) of *An. gambiae s.*l. collected in Boké and Faranah as determined using CDC light traps

Prefecture	Village	Location	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Overall annual trap nights
Boké	**Dioumaya**	Int	0.50	1.00	0.50	0.00	0.50	1.50	3.00	ND	1.00
		Ext	0.00	0.00	0.00	0.00	0.00	0.50	3.50	ND	0.57
		Combined	**0.25**	**0.50**	**0.25**	**0.00**	**0.25**	**1.00**	**3.25**	ND	**0.79**
	Guilere	Int	0.50	0.00	1.50	1.00	0.00	1.00	2.00	ND	0.86
		Ext	0.00	0.50	0.50	0.00	0.00	1.00	2.00	ND	0.57
		Combined	**0.25**	**0.25**	**1.00**	**0.50**	**0.00**	**1.00**	**2.00**	ND	**0.71**
	Kaboye	Int	0.50	0.50	0.50	0.00	0.00	1.00	3.00	ND	0.79
		Ext	0.00	0.50	0.00	0.00	0.00	1.50	1.00	ND	0.43
		Combined	**0.25**	**0.50**	**0.25**	**0.00**	**0.00**	**1.25**	**2.00**	ND	**0.61**
		Boké overall	**0.25**	**0.42**	**0.50**	**0.17**	**0.08**	**1.08**	**2.42**	ND	**0.70**
Faranah	**Balayani**	Int	0.50	4.50	8.00	5.00	7.00	8.00	4.50	5.00	5.31
		Ext	0.50	3.00	12.00	6.00	8.00	13.50	6.00	6.50	6.94
		Combined	**0.50**	**3.75**	**10.00**	**5.50**	**7.50**	**10.75**	**5.25**	**5.75**	**6.13**
	Foulaya	Int	0.50	5.00	8.50	6.00	6.50	11.00	8.00	5.00	6.31
		Ext	0.50	3.00	13.50	5.00	9.50	15.00	5.50	7.50	7.44
		Combined	**0.50**	**4.00**	**11.00**	**5.50**	**8.00**	**13.00**	**6.75**	**6.25**	**6.88**
	Tindo	Int	0.00	3.50	3.50	3.00	4.00	6.00	2.00	2.50	3.06
		Ext	0.00	3.00	7.00	3.00	5.50	8.50	3.00	3.00	4.13
		Combined	**0.00**	**3.25**	**5.25**	**3.00**	**4.75**	**7.25**	**2.50**	**2.75**	**3.59**
		Faranah overall	**0.33**	**3.67**	**8.75**	**4.67**	**6.75**	**10.33**	**4.83**	**4.92**	**5.53**
Overall monthly trap nights			**0.29**	**2.04**	**4.63**	**2.42**	**3.42**	**5.71**	**3.63**	**4.92**	**3.28**

ND = Not Done

Table 8: Seasonal indoor resting density (mosquitoes per house per night) of *An. gambiae* s.l. collected in Boké and Faranah using pyrethrum spray catches

Prefecture	Village	February	March	April	May	June	July	August	September	Overall annual indoor resting density
Boké	Dioumaya	0.00	0.00	0.00	0.00	0.00	0.10	0.20	ND	0.04
	Guilere	0.00	0.00	0.10	0.00	0.00	0.00	0.10	ND	0.03
	Kaboye	0.00	0.00	0.00	0.00	0.00	0.10	0.00	ND	0.01
	Combined	**0.00**	**0.00**	**0.03**	**0.00**	**0.00**	**0.07**	**0.10**	ND	**0.03**
Faranah	Balayani	0.20	1.20	1.50	2.00	3.30	3.00	2.30	2.60	2.01
	Foulaya	0.30	1.50	1.40	0.70	2.00	2.50	1.60	1.80	1.48
	Tindo	0.10	0.70	0.90	1.60	2.30	2.00	3.20	1.80	1.58
	Combined	**0.20**	**1.13**	**1.27**	**1.43**	**2.53**	**2.50**	**2.37**	**2.07**	**1.69**
Overall monthly indoor resting density		**0.10**	**0.57**	**0.65**	**0.72**	**1.27**	**1.28**	**1.23**	**2.07**	**0.91**
ND = Not done										

3.2.2 Assessing vector biting behavior

Analysis of the results of the vectors' biting behavior using HLC, recorded during hour-long intervals, shows biting behavior inside and outside houses in the sites between 1900 and 0600 hrs. Whereas peak indoor and outdoor biting were observed between 0000 and 0200 (***Figure 4***). Note, peak biting times indoors and outdoors varied substantially between sites (***Figures 5 and 6***).

Figure 4: Biting behavior of *An. gambiae* s.l. across the six sites in Boké and Faranah (as determined by HLC)

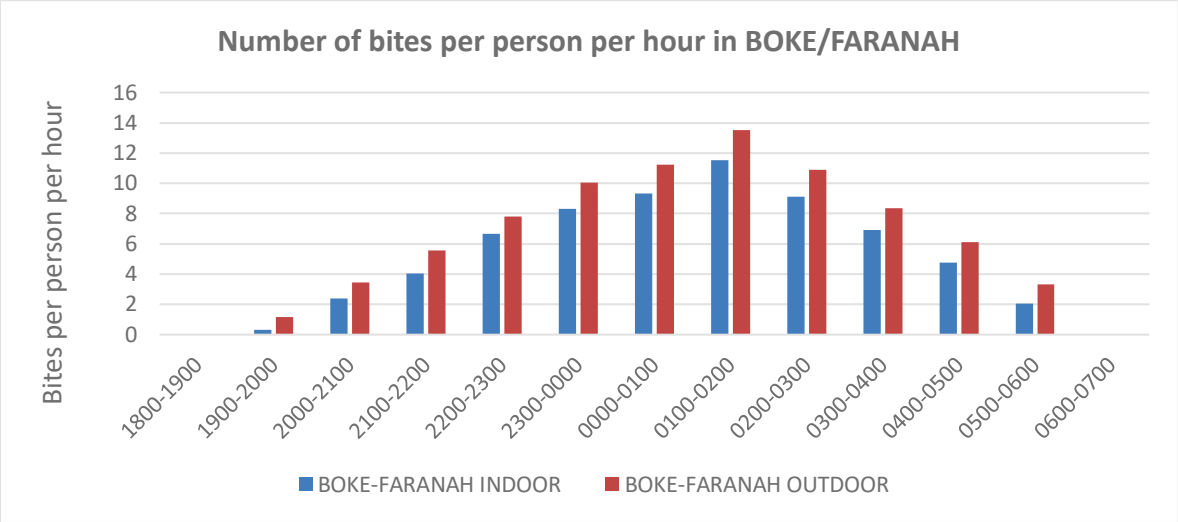
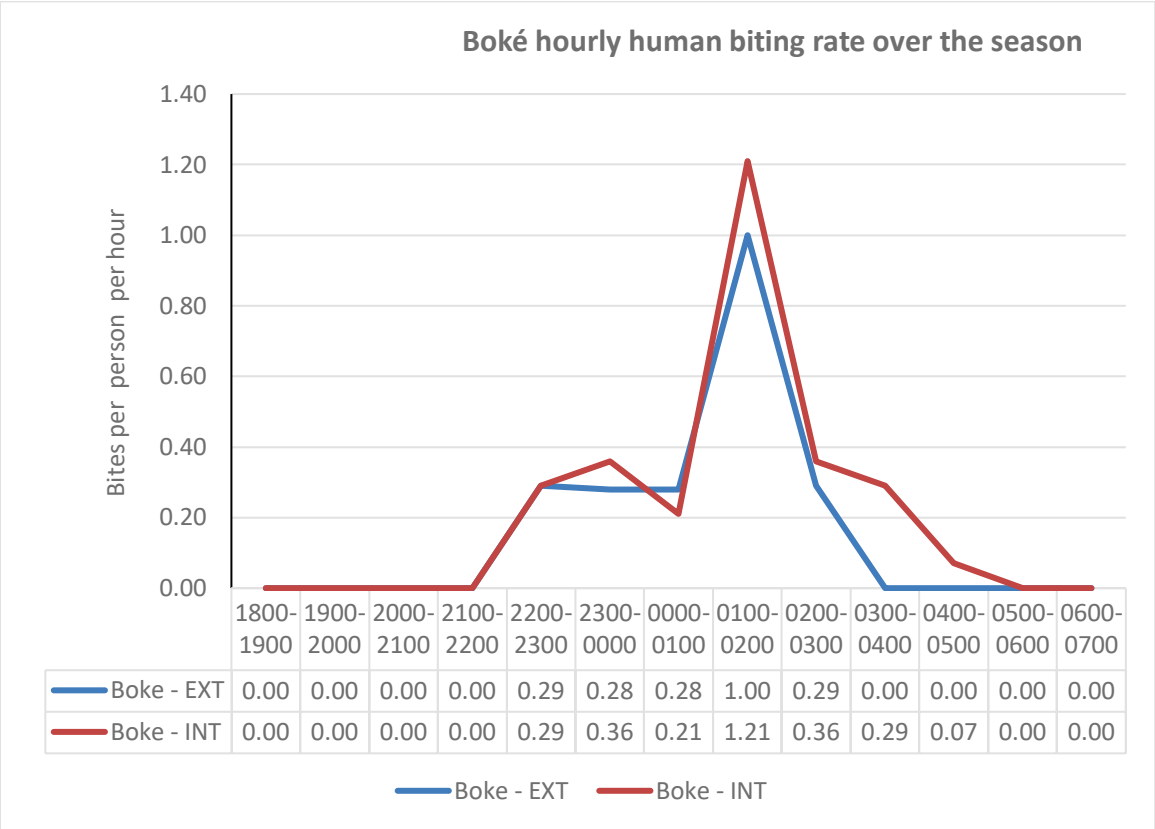
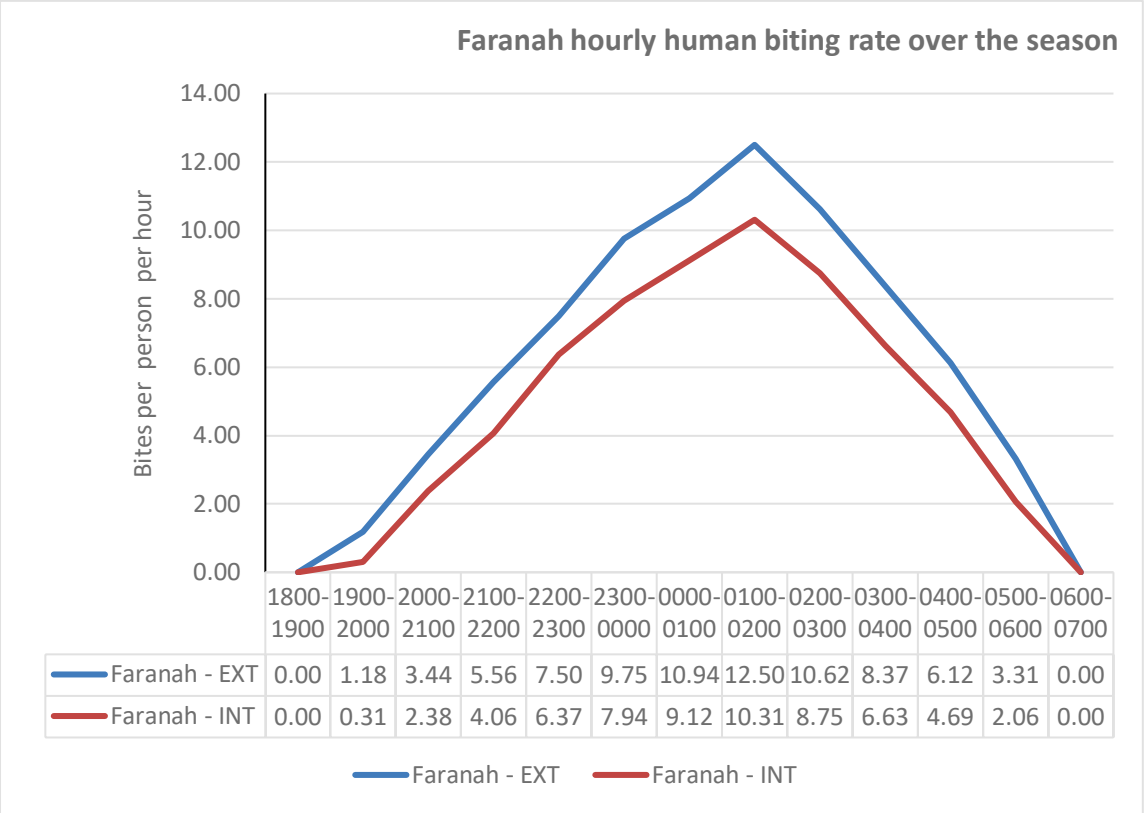


Figure 5: Biting behavior of *An. gambiae* s.l. in Boké



	1800-1900	1900-2000	2000-2100	2100-2200	2200-2300	2300-0000	0000-0100	0100-0200	0200-0300	0300-0400	0400-0500	0500-0600	0600-0700
Boke - EXT	0.00	0.00	0.00	0.00	0.29	0.28	0.28	1.00	0.29	0.00	0.00	0.00	0.00
Boke - INT	0.00	0.00	0.00	0.00	0.29	0.36	0.21	1.21	0.36	0.29	0.07	0.00	0.00

Figure 6: Biting behavior of *An. gambiae* s.l. in Faranah



3.2.3 Species identification and sporozoite index

All *Anopheles* mosquitoes collected by the different sampling methods (HLCs, CDC light traps, and pyrethrum spray catch) in the 24 villages were morphologically identified by genus and species using the standard keys. *Anopheles* were placed individually in tubes containing silica gel, numbered, coded, and preserved in refrigerators at UGANC. For the test, we selected 200 *Anopheles gambiae* s.l. from Faranah and 58 *Anopheles gambiae* s.l. from Boké.

Of the 258 *Anopheles* mosquitoes tested from both sites using *Pf*-CSP ELISA to determine the infection rate, 2 positives were obtained from Faranah only. The sporozoite index in Faranah was 1%. The positive mosquitoes were collected in Balayani in March and Foulaya in May[10] (***Table 9***). The estimated annual entomological inoculation rate (EIR) (based on the product of the sporozoite rate and the human biting rate in Table 6) in Faranah and overall was 86.4 and 38.0 infectious bites per person per year, respectively.

Table 9: Sporozoite index and EIR of *An. gambiae* s.l. in Boké and Faranah

Sites	Number tested	Number of positive	Percentage of positive (%)	EIR/ night	EIR/ year
Boké	58	0	0	0	0
Faranah	200	2	1.0	0.237	86.4
Overall	**258**	**2**	**0.8**	**0.104**	**38.0**

[10] Sample reference code for the positive samples are Fa19BaCa148 for the March specimen and Fa19FoCa655 for the May specimen.

3.2.4 Monitoring phenotypic resistance

Resistance tests were carried out on nulliparous female mosquitoes derived from larvae and pupae collected at the six sentinel sites (Boké, Labe, Kankan Dabola, Faranah, and Kissidougou) as per standard World Health Organization (WHO) protocol, (i.e., 1-hour exposure of mosquitoes to insecticide-treated filter papers in WHO test tubes and monitored for mortality 24 hours after exposure[11]). Three insecticides were tested: 0.05% deltamethrin, 0.05% alpha-cypermethrin, and 0.75% permethrin to determine the susceptibility of malaria vectors. For each test, 100 mosquitoes were used. The susceptibility and intensity tests were performed once at all sites during the months of June, July, August, September, and October 2019. Tests on pyrethroids included: the diagnostic dose (1×), 5 times the diagnostic dose, and 10 times the diagnostic dose. To better understand the contribution of oxidases to pyrethroid resistance in mosquitoes from Guinea, we used the following synergist: piperonyl butoxide (PBO) 4%.

The three pyrethroids and synergists were selected for susceptibility testing because they are insecticides used on LLINs available in the market[12]. This will help guide decision-making in LLINs purchase for routine and mass distributions. Wherever the mosquito mortality in the diagnostic dose (1×) was 100%, it was not necessary to perform insecticide resistance tests at 5× or 10× the diagnostic dose. Similarly, whenever the 5× dose killed 100% of mosquitoes, it was not necessary to conduct tests using the 10× dose. All efforts were made to test each insecticide on the same day

The WHO test tube bioassay showed that sampled vector populations are resistant to permethrin and alpha-cypermethrin, but susceptible to deltamethrin (***Table 10)***.

Table 10: Summary of insecticide susceptibility testing of mosquito vectors (% mortality)

Insecticides	Dose	Boké	Dabola	Faranah	Kankan	Kissidougou	Labé
Deltamethrin	1×	100	98	99	98	100	100
Permethrin	1×	80	63	65	70	75	66
Alpha-cypermethrin	1×	78	93	68	78	80	75

Twenty-five mosquitoes per site were used in the control of which no mortality was observed.

Susceptibility (98 – 100% mortality); Possibility of resistance (90 – 97%); Resistance (<90%)

Table 11: Summary of insecticide susceptibility testing of mosquito vectors with PBO 4% (% mortality)

Insecticides	Dose	Boké	Dabola	Faranah	Kankan	Kissidougou	Labé
Deltamethrin*	1×+PBO	100	100	100	100	100	100
Permethrin	1×+PBO	95	88	72	90	93	84
Alpha-cypermethrin	1×+PBO	95	95	84	96	98	94

Twenty-five mosquitoes per site were used in the control of which only 4 mosquitoes in the Boké control group died.

*Though this test was done, it was not necessary to do so since mosquitoes were already shown to be susceptible to deltamethrin in standard assays

Susceptibility (98 – 100% mortality); Possibility of resistance (90 – 97%); Resistance (<90%)

[11] WHO (2016) *Test procedures for monitoring insecticide resistance in malaria mosquitoes.* WHO, Geneva, Switzerland.
[12] Presently, Guinea is not using PBO-ITNs, but may do so in the future if data suggests it.

For permethrin, the mean and median increase in mortality after pre-exposure to PBO was 17.2% and 18.0%, respectively; the range of increased mosquito mortality was between 7% in Faranah and 20% in Kankan; For alpha-cypermethrin, the mean and median increase in mortality after pre-exposure to PBO was 15.0% and 17.5 %, respectively; the range of increased mosquito mortality was between 2% in Dabola and 19% in Labé. PBO was only able to restore alpha-cypermethrin to WHO susceptibility levels in Kissidougou where mosquito mortality rate went from 80% to 98% (***Table 10*** and ***Table 11***).

Intensity tests in CDC bottles

To measure the intensity of mosquito resistance to insecticides that are advocated for use in LLINs, intensity tests were conducted with deltamethrin, permethrin, and alpha-cypermethrin. For this test, 250 ml bottles are coated with the insecticides, dried, and then mosquitoes are exposed for 30 minutes (diagnosis time) to determine susceptibility. Various doses are tested: While the diagnostic dose is used to define the susceptibility/resistance, tests at more concentrated doses (5×, 10×) determine the intensity of resistance.

The results showed that the sample vectors are susceptible to permethrin and alpha-cypermethrin at 5× and 10× doses. ***Table 12*** shows a summary of test results to date.

Table 12: Summary of insecticide intensity tests with CDC bottle bioassay (% mortality)

Insecticides	Dose	Boké	Dabola	Faranah	Kankan	Kissidougou	Labé
Permethrin	5×	100	100	100	100	100	100
	10×	ND	ND	ND	ND	ND	ND
Alpha-cypemethrin	5×	100	98	100	98	100	100
	10×	ND	100	ND	100	ND	ND

Twenty-five mosquitoes per site were used in the control of which no mortality was observed. ND = Not done

Susceptibility (98 – 100% mortality); Possibility of resistance (90 – 97%); Resistance (<90%)

Conclusion

For the 2019 calendar year, entomological study activities were carried out in the six prefectures where we noticed resistance with permethrin 0.75%, alpha-cypermethrin 0.05% but sensitivity to deltamethrin 0.05%.

An. gambiae s.l. were found to be susceptible to deltamethrin based on WHO criteria. This result suggests that standard deltamethrin LLINs are still effective in Guinea. While there were two instances where PBO did not increase mosquito mortality above 10%, PBO pre-exposure did show an overall increase in mosquito mortality to pyrethroid in the tested study sites, with one instance where PBO increased mosquito mortality to WHO susceptibility levels in Kissidougou. This suggests that PBO LLINs with permethrin and alpha-cypermethrin may have a programmatic impact. However, the intensity test showed that mosquitoes were still susceptible at 5× and 10× the diagnostic dose. This suggests that standard permethrin or alpha-cypermethrin LLINs may still be operationally effective in Guinea, but enhanced monitoring may be needed.

The seasonal human biting rate was monitored for 7 months in Boké and 8 months in Faranah, where we observed more biting outdoors rather than indoors. We also observed the peak of biting activity to be between 1 am to 2 am. Faranah had a higher biting rate than Boké. Based on these results, it may be useful to conduct some human behavior studies to understand the risk of mosquito exposure at night. It may also be useful to understand why Faranah has higher human biting rates than Boké. In addition, given that Faranah had a sporozoite index of 1.0, it may be useful to determine the relationship between mosquito abundance and transmission.

The Kisumu strain from Switzerland is in its 7th generation. However, although there is a steady increase in the colony population, the overall numbers continue to be too low to perform any bioassay. Rearing will continue with the goal of increasing the mosquito numbers.

The laboratory analysis in this report took place at CREC. However, new equipment and PCR reagents have been received in Guinea. The next step is to operationalize laboratory activities to allow in-country analysis of mosquitoes. *StopPalu+* will organize a training in which CREC will send its staff to Guinea to provide in-country training on entomological molecular techniques.

In the collaboration between the project and the University, the team continued to help the students in their initiation to fundamental entomology.

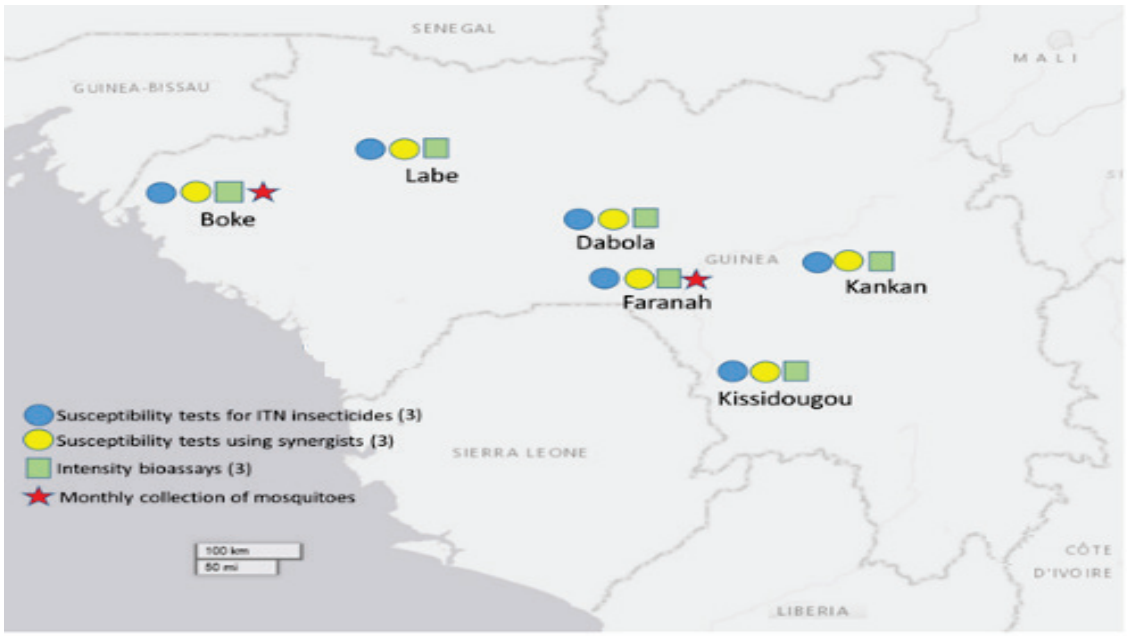
Annex 1. Laboratory Equipment, Materials, and Reagents

Entomological laboratory equipment and materials are summarized in ***Table A-1***.

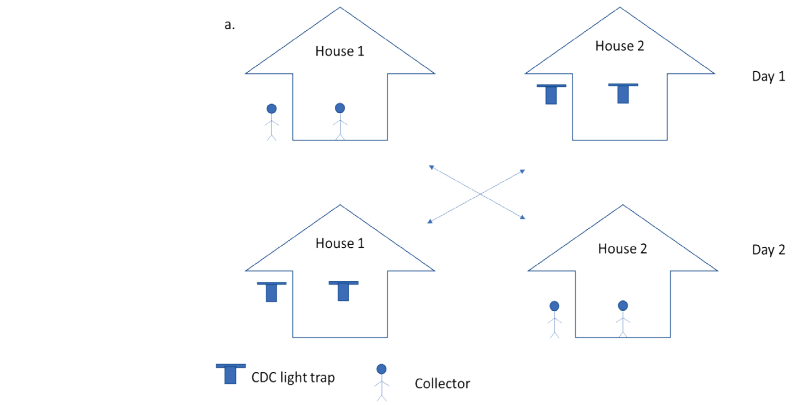
Table A-1: PCR equipment and materials

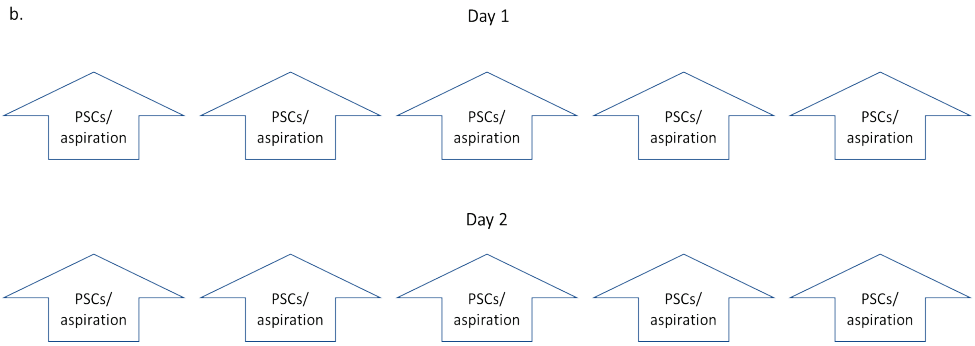
N° D	REFERENCES	DESIGNATION	QUANTITY
1	GG 4010X	*Freezer LIEBHERR GG 4010*	1
2	UVC/T-M-AR	*PCR hood UVC-T-M-AR with UV and anti-bacterial air recirculation system*	1
3	GB902 A-HCX	*Ice machine GB902, granular ice, with reservoir*	1
4	161126001	*Vilber lourmat E-B-X CX5TS Edge imaging system*	1
5	321000	*APELEX Midigel 2 Complete electrophoresis cuvette*	1
6	80433.1	*Ethylenediamine tetraacetic acid-250g Disodium salt*	1
	HP46.1	*Ethidium bromide-15ml*	1
	3170.1	*TRIS-100g*	1
	6943.4	*Boric acid 500g buffer for Molecular Biology*	1
	A512.3	*Bromophenol blue-25g Sodium salt for electrophoresis*	1
	777531	*Agarose E 1000g bottle*	1
	2018T	*Indoor thermometer / outdoor probe*	1
	046072	*Magnetic bar with swivel plate*	10
7	095099	*Hawo HD260MS8 manual pulse sealers*	1
8	280120121	*Trans illuminator TFS-20M Edge UV PAD 20M*	1
9	ALJ 250 4A	*Kerm analytical balance ALJ 250-4A*	1

Annex 2. Map of insecticide resistance and entomological monitoring sites in Guinea



Annex 3. Schematic diagram of mosquito sampling approach in the seasonal monitoring sites in Guinea





a. Alternating house sampling using human landing catches (HLC) and CDC light trap (LTs) over two nights of collection

b. Collection of 5 houses using pyrethrum spray catches (PSCs) or a mechanical aspirator over two days

Annex 4. Total number of *An. gambiae* s.l. mosquitoes collected in Boké and Faranah villages as determined using HLCs

Prefecture	Sentinel site	February		March		April		May		June		July		August		September	
		Int	Ext	Int	Ext	int	Ext	Int	Ext	Int	Ext	Int	Ext	Int	Ext	Int	Ext
Boké	Kaboye	2	0	1	2	1	0	0	0	0	0	3	5	6	3	-	-
	Guilere	0	1	1	4	3	1	1	0	0	1	3	2	4	4	-	-
	Dioumaya	0	1	3	1	1	0	0	1	0	0	4	1	6	3	-	-
	Total	**2**	**2**	**5**	**7**	**5**	**1**	**1**	**1**	**0**	**1**	**10**	**8**	**16**	**10**	**-**	**-**
Faranah	Balayani	8	12	33	28	39	55	42	41	62	86	90	129	53	69	40	50
	Foulaya	11	13	45	47	47	69	42	43	79	91	87	124	56	72	47	56
	Tindo	1	1	26	25	20	33	23	27	35	50	54	75	38	49	24	24
	Total	**20**	**26**	**104**	**100**	**106**	**157**	**107**	**111**	**176**	**227**	**231**	**328**	**147**	**190**	**111**	**130**
Total		**22**	**28**	**109**	**107**	**111**	**158**	**108**	**112**	**176**	**228**	**241**	**336**	**163**	**200**	**111**	**130**
Percentage		**44**	**56**	**50**	**50**	**41**	**59**	**49**	**51**	**44**	**56**	**42**	**58**	**45**	**55**	**46**	**54**

Annex 5. Total number of *An. gambiae* s.l. mosquitoes collected in Boké and Faranah as determined using CDC light traps

Prefecture	Sentinel site	February		March		April		May		June		July		August		September	
		Int	Ext	Int	Ext	int	Ext	Int	Ext	Int	Ext	Int	Ext	Int	Ext	Int	Ext
Boké	Kaboye	1	0	1	1	1	0	0	0	0	0	2	3	6	2	-	-
	Guilere	1	0	0	1	3	1	2	0	0	0	2	2	4	4	-	-
	Dioumaya	1	0	2	0	1	0	0	0	1	0	3	1	6	7	-	-
	Total	**3**	**0**	**3**	**2**	**5**	**1**	**2**	**0**	**1**	**0**	**7**	**6**	**16**	**13**	**-**	**-**
Faranah	Balayani	1	1	9	6	16	24	10	12	14	16	16	27	9	12	10	13
	Foulaya	1	1	10	6	17	27	12	10	13	19	22	30	16	11	10	15
	Tindo	0	0	7	6	7	14	6	6	8	11	12	17	4	6	5	6
	Total	**2**	**2**	**26**	**18**	**40**	**65**	**28**	**28**	**35**	**46**	**50**	**74**	**29**	**29**	**25**	**34**
Total		**5**	**2**	**29**	**20**	**45**	**66**	**30**	**28**	**36**	**46**	**57**	**80**	**45**	**42**	**25**	**34**
Percentage		**71**	**29**	**59**	**41**	**41**	**59**	**52**	**48**	**44**	**56**	**42**	**58**	**52**	**48**	**42**	**58**

Annex 6. Total number of *An. gambiae* s.l. mosquitoes collected in Boké and Faranah using pyrethrum spray catches

Prefecture	Sentinel site	February				March				April				May				June				July				August				September			
		U	F	S	G	U	F	S	G	U	F	S	G	U	F	S	G	U	F	S	G	U	F	S	G	U	F	S	G	U	F	S	G
Boké	Kaboye	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	-	-	-	-
	Guilere	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	-	-	-	-
	Dioumaya	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	2	0	0	-	-	-	-
	Total	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**2**	**0**	**0**	**0**	**2**	**0**	**0**	**-**	**-**	**-**	**-**
Faranah	Balayani	0	2	0	0	10	2	0	0	0	15	0	0	1	19	0	0	1	32	0	0	5	25	0	0	10	13	0	0	0	26	0	0
	Foulaya	2	1	0	0	8	7	0	0	8	6	0	0	1	6	0	0	2	18	0	0	5	20	0	0	6	10	0	0	3	15	0	0
	Tindo	1	0	0	0	7	0	0	0	3	6	0	0	0	16	0	0	5	18	0	0	6	14	0	0	5	9	9	9	9	9	0	0
	Total	**3**	**3**	**0**	**0**	**25**	**9**	**0**	**0**	**11**	**27**	**0**	**0**	**2**	**41**	**0**	**0**	**8**	**68**	**0**	**0**	**16**	**59**	**0**	**0**	**21**	**32**	**9**	**9**	**12**	**50**	**0**	**0**
U = unfed; F = fed; S = semi-gravid, g = gravid																																	