USAID | *StopPalu+*

ANNUAL REPORT OF ENTOMOLOGICAL SURVEILLANCE ACTIVITIES (JANUARY–OCTOBER 2020)

September 2020

This document was produced for review by the US Agency for International Development (USAID). It was made possible by the support of the American people. It was prepared by RTI International for the USAID | StopPalu+ Project.

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Cooperative Agreement No. 72067518CA000015
Contractual period: December 2017–December 2022

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Abbreviations

AI	Active ingredient
CDC	United States Centers for Disease Control and Prevention
COVID-19	Coronavirus disease 2019
CREC	*Centre de Recherche Entomologique de Cotonou* (Entomological Research Center of Cotonou)
CSP	Circumsporozoite protein
ELISA	Enzyme-linked immunosorbent assay
GFATM	Global Fund to Fight AIDS, Tuberculosis and Malaria
HBR	Human biting rate
HLC	Human landing collection
kdr	Knockdown resistance (mutation)
LSHTM	London School of Hygiene and Tropical Medicine
LLIN	Long-lasting insecticide-treated net
MTR	Mosquito trap rate
NMCP	National Malaria Control Program
PBO	Piperonyl-butoxide
PCR	Polymerase chain reaction
PMI	United States President's Malaria Initiative
PSC	Pyrethrum spray catches
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 surveillance. During January to October 2020 (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. *StopPalu+*'s entomological activities focus on continuing 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 laboratory and the insectary
- Conducted a monthly study of vector seasonality in Boké and Faranah
- Procured equipment and materials for polymerase chain reaction (PCR) tests
- Developed the fiscal year (FY) 2020 entomology work plan

As part of implementing activities related to vector control under the NMCP's Prevention Unit Operational Action Plan, an investigative study on the sensitivity of malaria vectors to insecticides was conducted in Faranah, September 24–October 14, 2020.

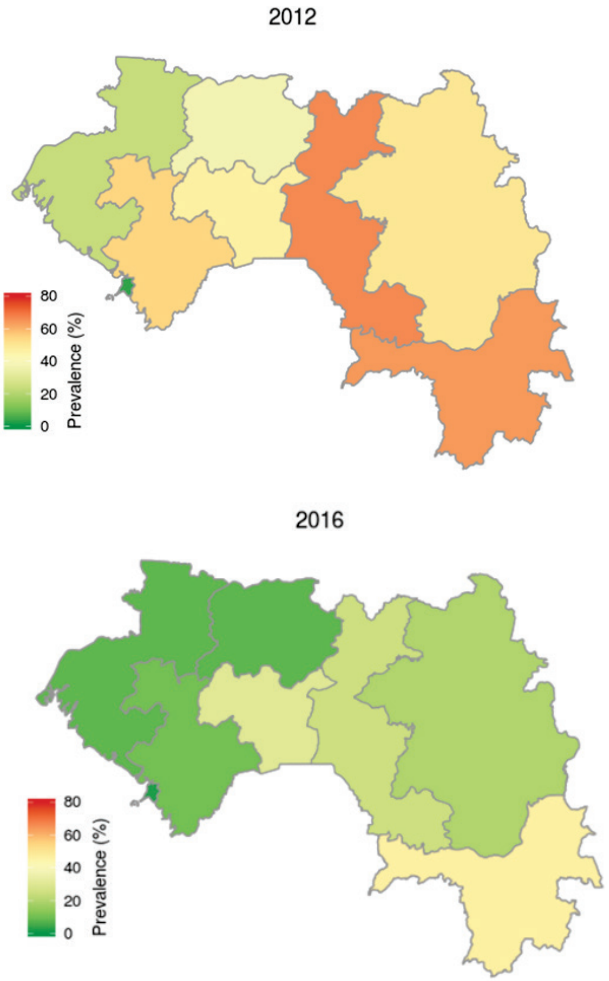
The NMCP organized this study in collaboration with the *StopPalu+* project, with technical and financial support from PMI.

[1] *StopPalu+* is the 5-year follow-on project to the original *StopPalu* project. *StopPalu+* started in December 2017 and will run through December 2022.

1 General context

Over the past five years, Guinea has made enormous progress in malaria control, leading to a reduction in malaria prevalence in children under 5 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 US President's Malaria Initiative (PMI).

Figure 1: The prevalence of malaria 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 1,777,726 cases reported in 2019, accounting for 37% of outpatient visits.[3]

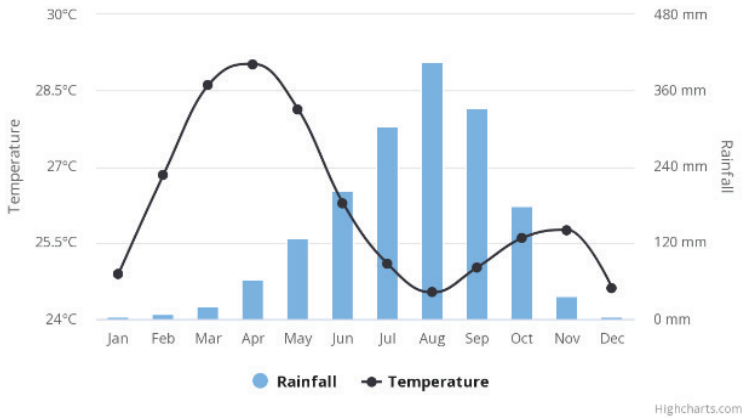
The main malaria vector in Guinea is *Anopheles gambiae sensu lato* (s.l.), specifically *An. gambiae sensu stricto* (s.s.), *An. coluzzii*, *An. arabiensis*, and *An. melas*. The *An. funestus* complex is also present, but studies published to date indicate that *An. funestus* is less prevalent than the *An. gambiae* complex. Vector control interventions implemented in Guinea include the mass distribution of long-lasting insecticide-treated mosquito nets (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).[4] Resistance to DDT (dichloro-diphenyl-trichloroethane), permethrin, alpha-cypermethrin, and lambda-cyhalothrin was detected in all *An. gambiae* s.l.

[2] African Heads of State Celebrate Progress Against Malaria - PMI
[3] Malaria monthly reports in DHIS2
[4] 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.

populations tested; part of the sample site showed susceptibility to deltamethrin and bendiocarb. Keita et al. (2017)[4] 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.[5]

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. In countries neighboring Guinea (Mali specifically), it has been shown that 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.[7]

Figure 2: Average monthly temperature and rainfall in Guinea for 1991–2016 [6]



The Faranah Region is located in east-central Guinea and is surrounded by the other Guinean regions of Kankan (to the east), Mamou (to the west), Nzérékoré (to the southeast), and Labé (to the northwest). The Faranah Region also shares national borders with the countries of Sierra Leone to the southwest and Mali to the north. Average temperatures in Faranah range from 22°C in January to 29°C to April. The hottest period occurs between February and May. Average rainfall ranges from 0.2 mm in December to 293 mm in August. The months of peak rainfall occur from May to October.

The prevalence of malaria in Faranah region is 25%.[8]

[5] AngloGold Ashanti. (2007). Unpublished.
[6] World Bank Group. The Climate Change Knowledge Portal. Accessed July 27, 2020 (https://climateknowledge-portal.worldbank.org/)
[7] 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).
[8] MICS Palu (2016).

2 Context specific for the Faranah study

In Faranah, the dominant economic activity is agriculture. Rice is one of the main crops cultivated on many of the plains, with pesticides used heavily in an uncontrolled manner.

In 2018, a research team from the London School of Hygiene and Tropical Medicine (LSHTM) conducted a study in Faranah sub-prefecture on the insecticide sensitivity of malaria vectors in this locality.[9] Using the U.S. Centers for Disease Control and Prevention (CDC) bottle bioassay with adult mosquitoes, this study revealed that the *An. gambiae* s.l. population in Faranah were resistant to deltamethrin in the sites visited. Deltamethrin mortality rates ranged between 73% in Faranah city center to 95% in Foulaya village at the diagnostic dose. However, at 2×, 5×, and 10× of the diagnostic dose of deltamethrin, mortality was consistently 100%.

These results raised questions about vector resistance to deltamethrin, given that previous investigations at the same sites and in the same vectors by the NMCP and *StopPalu+* had not detected deltamethrin resistance at the diagnostic dose.[10,11] These previous studies by the NMCP and *StopPalu+* used World Health Organization (WHO) insecticide resistance tests.

The Faranah Region was selected by the NMCP to receive piperonyl-butoxide (PBO) LLINs in the 2022 mass LLIN campaign. The decision to distribute PBO LLINs in Faranah was based on (1) the high prevalence of malaria in the region, (2) the results of the LSHTM study, and (3) the scale-up of malarious sub-Saharan African countries switching to next-generation LLINs (PBO LLINs and dual active ingredient (AI) LLINs [chlorfenapyr-alpha-cypermethrin and deltamethrin-pyriproxyfen]) to mitigate the threat of widespread pyrethroid resistance and sustain malaria reduction gains.

Because of questions raised by the 2018 research, another study was conducted to try to repeat the results reported in 2018. A joint team from the NMCP and *StopPalu+* went to Faranah to measure the same parameters to draw conclusions, using a protocol developed, adapted, and validated by a group of major stakeholders (NMCP, PMI, *StopPalu+*).

[9] Stica C, Jeffries CL, Irish SR, Barry Y, Camara D, Yansane I, Kristan M, Walker T, Messenger LA. Characterizing the molecular and metabolic mechanisms of insecticide resistance in Anopheles gambiae in Faranah, Guinea. Malar J. 2019 Jul 17;18(1):244. doi: 10.1186/s12936-019-2875-y. PMID: 31315630; PMCID: PMC6637595.

[10] K. Keita, D. Camara, Y. Barry, R. Ossè, L. Wang, M. Sylla, D. Miller, L. Leite, P. Schopp, G. G. Lawrence, M. Akogbéto, E. M. Dotson, T. Guilavogui, M. Keita, S. R. Irish, Species Identification and Resistance Status of Anopheles gambiae s.l. (Diptera: Culicidae) Mosquitoes in Guinea, Journal of Medical Entomology, Volume 54, Issue 3, May 2017, Pages 677–681, https://doi.org/10.1093/jme/tjw228

[11] PMI/StopPalu+ Annual report of entomological monitoring (November 2017–October 2018) (https://www.pmi.gov/)

3 *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 in a timely manner; (5) increasing community involvement in and support for malaria prevention, care, and treatment activities; and (6) strengthening the technical capacity of the 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.

However, the implementation of this plan was impacted by the COVID-19 pandemic. In fact, following the outbreak of this pandemic, WHO and PMI drew up guidelines that prohibited the collection of adult mosquitoes. The pandemic has also delayed the supply of insecticides necessary for insecticide resistance testing. During the reporting period, *StopPalu+* carried out the following activities:

- Continued to support the breeding of the Kisumu strain of mosquitoes for multiple generations
- Conducted a monthly study of vector seasonality in Boké and Faranah
- Equipped and maintained the insectary and laboratory at the Gama Abdel Nasser University of Conakry (UGANC)
- Strengthened capacities of students and trainees (UGANC, NMCP)
- Supported the NMCP Prevention Technical Working Group meetings

4 Achievements

4.1 Objective 1: Strengthened 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.

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

To ensure the continuity and sustainability of the entomology activities in the laboratory and insectary at UGANC, several activities have been achieved.

Laboratory

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 A.

Insectary

On May 30, 2019, the insectary team received *An. gambiae* s.s. Kisumu strain mosquito eggs from Switzerland. The project entomology team has been maintaining this colony. A total of 17 mosquito generations have been produced from these eggs, with a total of 1,709 mosquitoes. On average 190 mosquitoes have been produced per generation with a minimum 173 mosquitoes in each generation, a maximum 302 mosquitoes in each generation, and a median of 855 mosquitoes overall.

Training student interns

As part of the collaboration between the project and UGANC, the team continued to support students as part of their initiation to basic entomology. During the reporting period, the project entomology team, assisted by the NMCP Vector Control Unit team, continued to train the six students and five interns from the NMCP at the insectary. The training topics included mosquito rearing, mosquito collection, laboratory analysis, insecticide resistance etc. The trainings are conducted through theorical sessions and practical activities in the field.

4.1.2 Supporting the Vector Control Technical Working Group meetings

During the reporting period, *StopPalu+* supported the NMCP's Vector Control Unit in organizing four monthly meetings. Two of these meetings were held via video conferencing because of COVID-19 restrictions that require social distancing. During these meetings, vector control activities for the month/quarter were reviewed and discussed, and activities for the months/quarters ahead were planned.

4.2 Objective 2: Conducted routine entomological surveillance

During the reporting period, entomological surveillance activities were conducted in the two seasonality monitoring sites of Boké and Faranah only for the months of February and March. In April, PMI recommended to stop collection of adult mosquitoes during the COVID-19 pandemic. In each prefecture, three villages with the highest malaria prevalence were selected as sentinel sites (***Table 1***).

Table 1: Sentinel sites for entomology surveillance activities

Designation	Region	Districts	Sites
Seasonality study sites	Basse Guinée	Boké	Kaboye, Dioumaya, Guilere
	Haute Guinée	Faranah	Balayani, Foulaya, Tindo

During the visits to the six villages in Boké and Faranah, three mosquito collection methods were used in each village over a 2-day period: (1) human landing collection (HLC), (2) CDC light traps, and (3) pyrethrum spray catches (***Table 2***). These methods were used to gather information on the vector species composition, abundance (for mapping), physiological status, and host-seeking behavior.

Table 2: Summary of the frequency of collections and number of houses sampled in both seasonal sites†

Prefectures	Villages	Number of collections per month (houses sampled)					
		February			March		
		HLC	LT	PYR	HLC	LT	PYR
Boké	Kaboye	2(1)	2(1)	2(5)	2(1)	2(1)	2(5)
	Guilere	2(1)	2(1)	2(5)	2(1)	2(1)	2(5)
	Dioumaya	2(1)	2(1)	2(5)	2(1)	2(1)	2(5)
Faranah	Balayani	2(1)	2(1)	2(5)	2(1)	2(1)	2(5)
	Foulaya	2(1)	2(1)	2(5)	2(1)	2(1)	2(5)
	Tindo	2(1)	2(1)	2(5)	2(1)	2(1)	2(5)
Total number of houses sampled		**12**	**12**	**60**	**12**	**12**	**60**

† One house per village was sampled over two nights using HLCs and light traps, while 10 houses pre village were sampled using pyrethrum spray catches over one day, for a total of 10 different houses per month.

HLC = human landing collection; LT = CDC light trap; PYR = pyrethrum spray collection.

4.2.1 Assessing vector distribution and abundance

Human landing collection (HLC)

HLCs are a standard for determining human–vector contact and for specifically assessing mosquito biting behavior. HLCs were conducted inside and outside of one house in each of the six selected villages. In each village, a total of four collectors were recruited to conduct HLCs. These four collectors worked in two shifts—two collectors from 1800 to 0100 hours (shift 1 – evening to early night) and the other two collectors from 0100 to 0700 hours (shift 2 – late night to early morning). At any given hour during the collection period, one collector was situated inside the house and one collector was situated outside the house. The second night, collectors rotated their stations inside or outside, to reduce collector bias. All collectors were offered malaria chemoprophylaxis prior to 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 biting behavior throughout the night. This activity was carried out in one house on the first night and different house on the second night per village for the months of February and March 2020. The collected mosquitoes were returned to the laboratory for species identification by standard morphological keys.

HLC inside a house

HLC outside a house

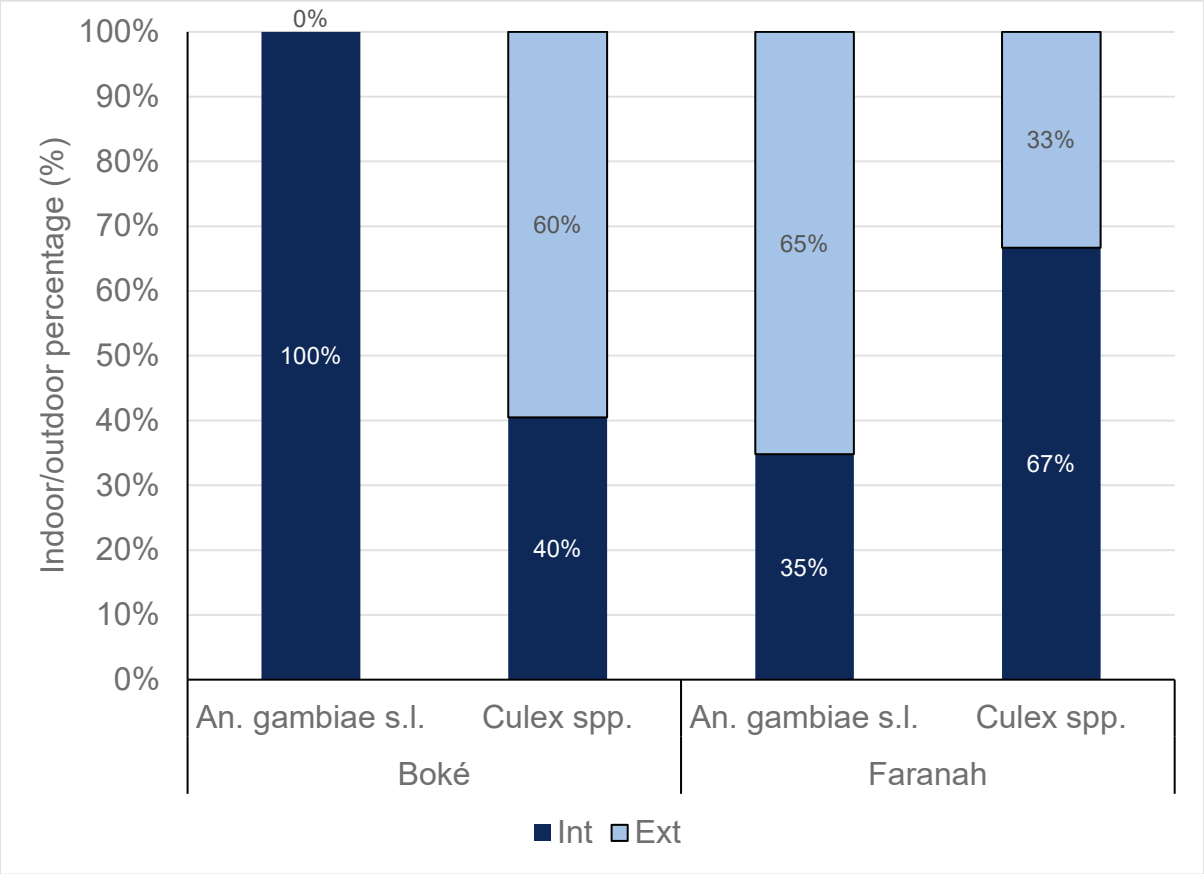
A total of 210 of mosquitoes were collected at the two sites. Of 210 mosquitoes collected, 165 (79%) were *An. gambiae* s.l. There were no other *Anopheles* identified. Among the *Anopheles* mosquitoes collected, 62% (103 of 165) were caught outdoors; the range across sites varied between 0% and 100%. *Anopheles* caught indoors were 38% (62 of 165).

In Boké, 49 mosquitoes were collected, and in Faranah, 161 mosquitoes were collected. In Boké, 14% (7 of 49) of mosquitoes caught were *Anopheles* (100% of *Anopheles* were collected indoors). In Faranah, 98% (157 of 161) of mosquitoes caught were *Anopheles* (35% of *Anopheles* were collected indoors and 65% of *Anopheles* were collected outdoors). The overall numbers of mosquitoes collected through the HLC method are presented in ***Table 3***.

Table 3: Abundance and distribution of mosquitoes collected using HLC over 4 nights (2 nights every 2 months) of collection in one house per village

-	-	HLC (number of mosquitoes per house per collection)			
-	-	***An. gambiae* s.l.**		***Culex* spp.**	
Prefecture	**Sentinel site**	**Int**	**Ext**	**Int**	**Ext**
Boké	Dioumaya	1.00	0.00	1.00	1.50
	Guilere	0.25	0.00	1.75	3.25
	Kaboye	0.50	0.00	1.50	1.50
	Boké overall	**0.58**	**0.00**	**1.42**	**2.08**
Faranah	Balayani	6.00	11.00	0.00	0.25
	Foulaya	5.00	10.00	0.00	0.00
	Tindo	2.75	4.75	0.50	0.00
	Faranah overall	**4.58**	**8.58**	**0.17**	**0.08**
Overall Indoor/Outdoor		**2.58**	**4.29**	**0.79**	**1.08**

Figure 3: Percentage of mosquitoes found indoors and outdoors by species in Boké and Faranah using HLCs

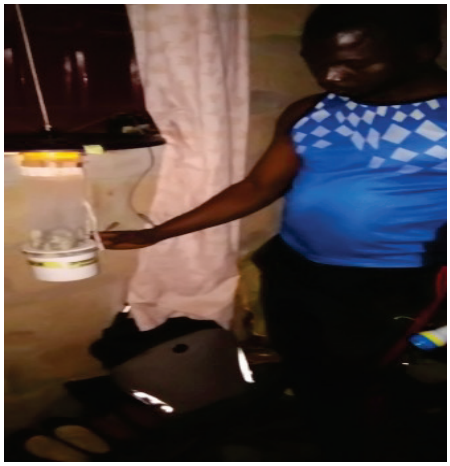


CDC light traps

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 each (i.e., house #1 sampled for one night and house #2 sampled the next consecutive night). Within the house one trap was placed indoors (near sleeping spaces, if possible) traps were place approximately 0.5 to 1.5 meters depending on the height of the sleeping surface. A second trap was placed outdoors; where feasible, outdoor traps were hung under the eaves of the house or within 2 to 3 meters of the houses. Outdoor traps were hung at a height of approximately 1.5 to 2.0 meters from the ground. Indoor and outdoor CDC light traps were hung from1800 to 0700 hours.

The results of CDC light trap collection are presented in ***Table 4***. Of 117 mosquitoes collected in the two sites, 43% (50 of 117) were *An. gambiae* s.l. There were no other *Anopheles* identified. Among the collected *Anopheles* mosquitoes, 52% (26 of 50) were caught outdoors and 48% (24 of 50) were caught indoors. Of all the *An. gambiae* collected using CDC light traps indoors and outdoors, 79% and 100%, respectively, were caught in Faranah.

A CDC light trap placed outside to capture mosquitoes

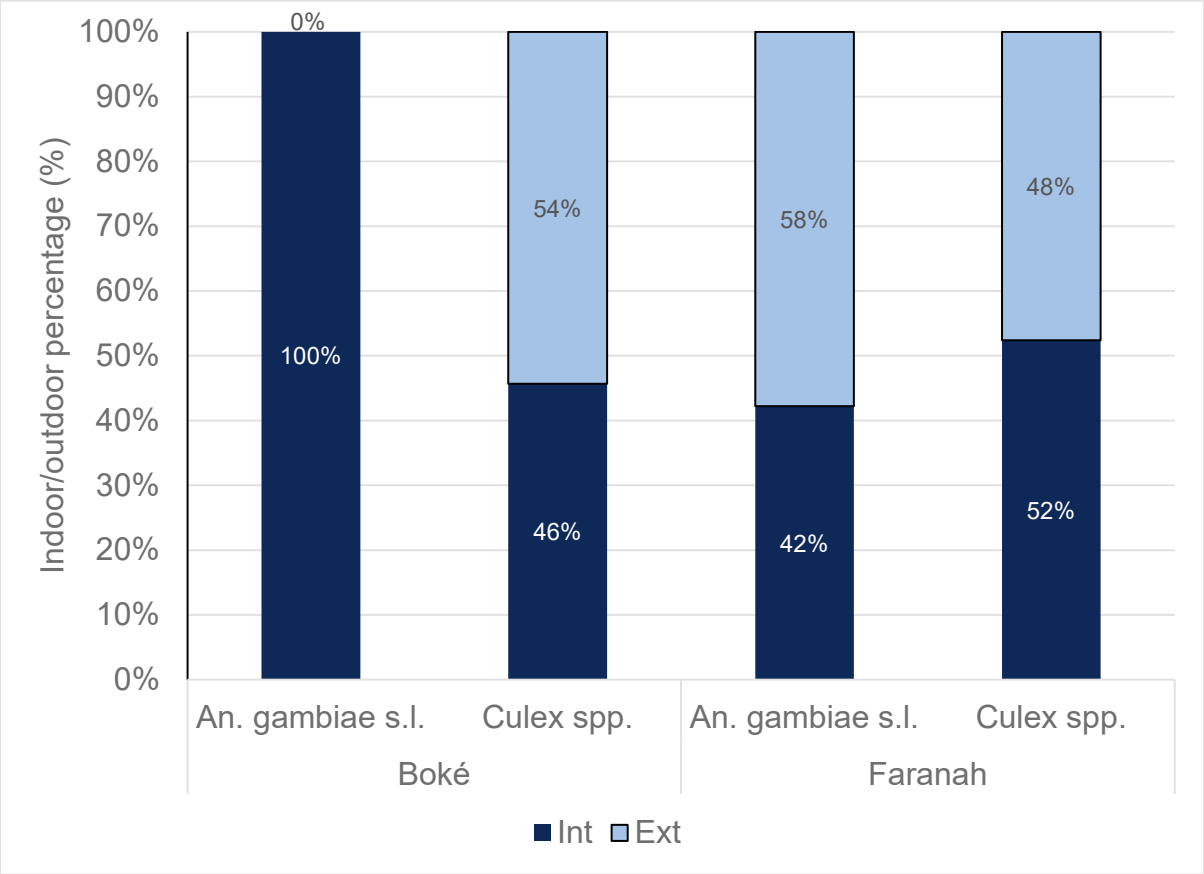


A CDC light trap placed inside to capture mosquitoes

Table 4: Abundance and distribution of mosquitoes collected using CDC light traps over 4 nights (2 nights every 2 months) of collection in two houses per village

-	-	Light trap (number of mosquitoes per collection)			
-	-	***An. gambiae* s.l.**		***Culex* spp.**	
Prefecture	**Sentinel sites**	**Int**	**Ext**	**Int**	**Ext**
Boké	Dioumaya	0.75	0.00	1.50	2.25
	Guilere	0.25	0.00	1.50	1.75
	Kaboye	0.25	0.00	2.25	2.25
	Boké overall	**0.42**	**0.00**	**1.75**	**2.08**
Faranah	Balayani	1.75	3.00	1.00	0.50
	Foulaya	2.25	2.75	1.00	0.25
	Tindo	0.75	0.75	0.75	1.75
	Faranah overall	**1.58**	**2.17**	**0.92**	**0.83**
Overall Indoor/Outdoor		**1.00**	**1.08**	**1.33**	**1.46**

Figure 4: Percentage of mosquitoes found indoors and outdoors by species in Boké and Faranah using CDC light traps



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 six 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 hours. 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.

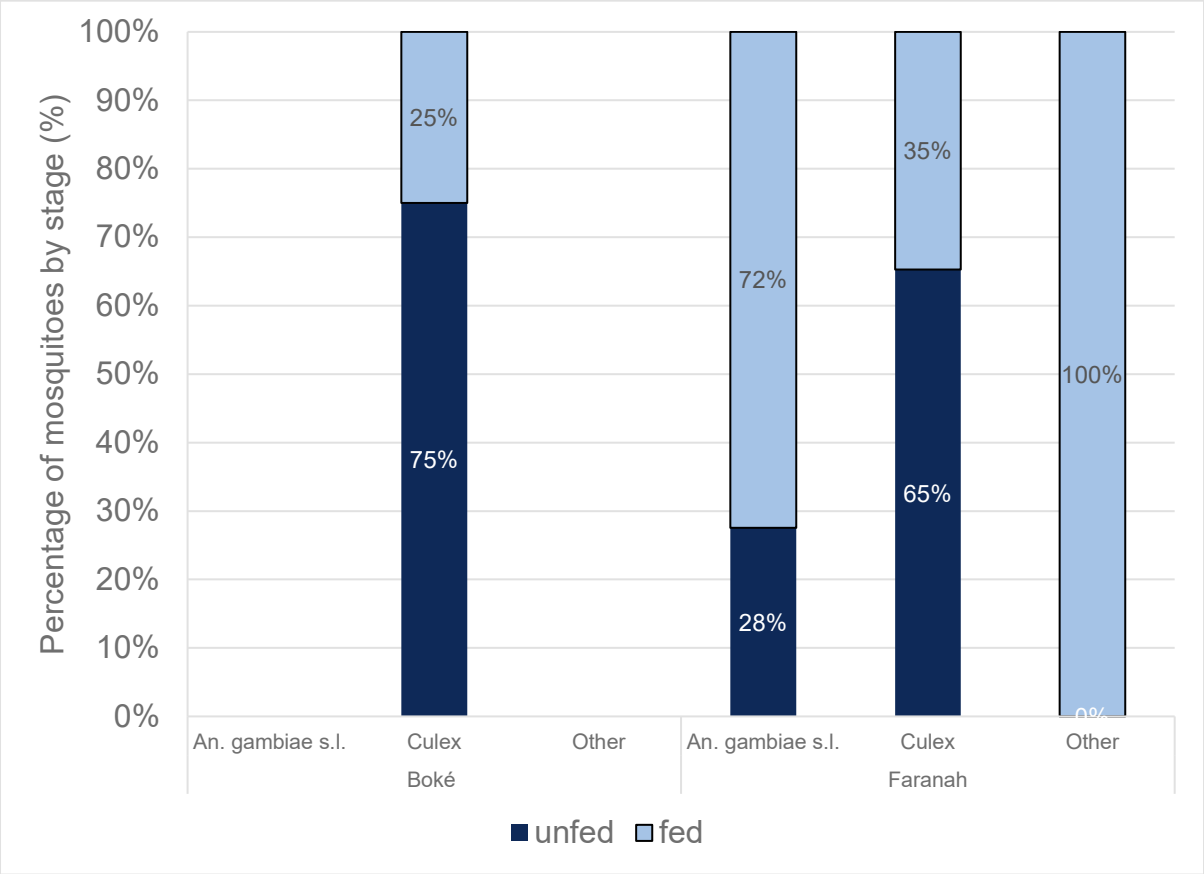
Pyrethrum collection in Faranah

Of the 127 mosquitoes collected, 69 (46%) were female *An. gambiae* s.l. Of the 69 female *An. gambiae* s.l., 50 (72%) were fed, 19 (28%) were unfed, and none were semi-gravid or gravid. The overall mean of mosquitoes collected with the pyrethrum spray catch are presented in ***Table 5***.

Table 5: Abundance and distribution of mosquitoes over 2 months of collection using indoor pyrethrum spray catches in 10 houses per village

-	-	PSCs: Number of mosquitoes per house-collection*								
-	-	***An. gambiae* s.l.**			***Culex* spp.**			**Other**		
Prefecture	**Sentinel site**	**Unfed**	**Fed**	**All**	**Unfed**	**Fed**	**All**	**Unfed**	**Fed**	**All**
Boké	Dioumaya	0.00	0.00	0.00	0.20	0.00	0.20	0.00	0.00	0.00
	Guilere	0.00	0.00	0.00	0.10	0.10	0.20	0.00	0.00	0.00
	Kaboye	0.00	0.00	0.00	0.30	0.10	0.40	0.00	0.00	0.00
	Boké overall	**0.00**	**0.00**	**0.00**	**0.20**	**0.07**	**0.27**	**0.00**	**0.00**	**0.00**
Faranah	Balayani	1.10	2.50	3.60	1.60	0.10	1.70	0.00	0.00	0.00
	Foulaya	0.80	1.50	2.30	1.00	0.60	1.60	0.00	0.10	0.10
	Tindo	0.00	1.00	1.00	0.60	1.00	1.60	0.00	0.00	0.00
	Faranah overall	**0.63**	**1.67**	**2.30**	**1.07**	**0.57**	**1.63**	**0.00**	**0.03**	**0.03**
Overall		**0.32**	**0.83**	**1.15**	**0.63**	**0.32**	**0.95**	**0.00**	**0.02**	**0.02**

Figure 5: Percentage of mosquitoes by stage and species in Boké and Faranah using pyrethrum spray catches



4.2.2 Assessing vector seasonality

The Boké and Faranah sites were sampled to understand vector seasonality. However, due to COVID monitoring only occurred in February and March. The results of the monitoring are presented in ***Tables 6, 7,*** and ***8*** and **Figure 6, 7,** and **8**. Only *An. gambiae* s.l. were captured.

Table 6: Seasonal human biting rate (HBR) of *An. gambiae* s.l. collected in Boké and Faranah villages as determined using the HLC method

-	-	February		March		Overall HBR by site
Prefecture	**Sentinel site**	**Int**	**Ext**	**Int**	**Ext**	
Boké	Dioumaya	2.00	0.00	0.00	0.00	0.50
	Guilere	0.50	0.00	0.00	0.00	0.13
	Kaboye	1.00	0.00	0.00	0.00	0.25
	Boké overall	**1.17**	**0.00**	**0.00**	**0.00**	**0.29**
Faranah	Balayani	2.00	6.00	10.00	16.00	8.50
	Foulaya	2.00	6.50	8.00	13.50	7.50
	Tindo	1.00	2.50	4.50	7.00	3.75
	Faranah overall	**1.67**	**5.00**	**7.50**	**12.17**	**6.58**
Overall monthly HBR		**1.42**	**2.50**	**3.75**	**6.08**	**3.44**

Figure 6: Seasonal percentage of mosquitoes found indoors and outdoors by species in Boké and Faranah using HLCs

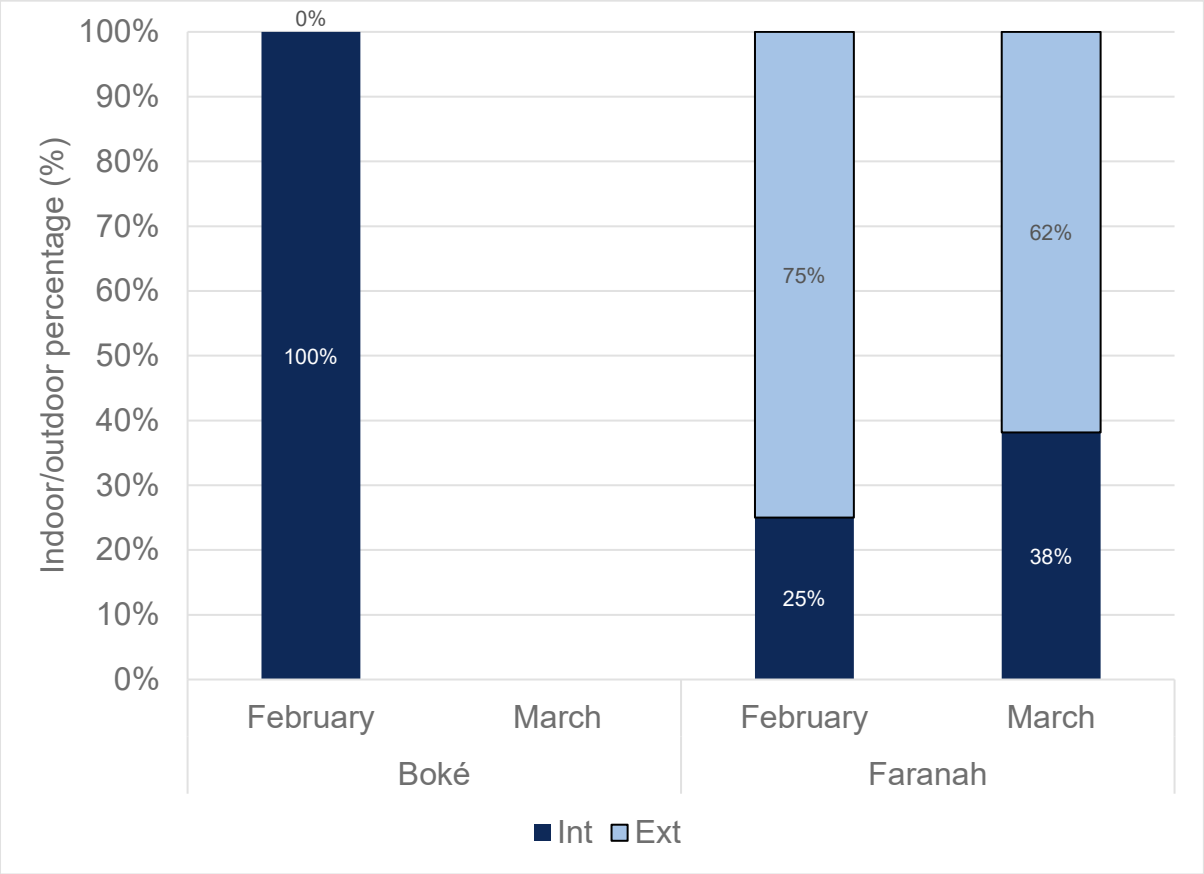


Table 7: Seasonal human biting rate of *An. gambiae* s.l. collected in Boké and Faranah as determined using CDC light traps

		February		March		Overall mosquito per trap by site
Prefecture	**Sentinel site**	**Int**	**Ext**	**Int**	**Ext**	
Boké	Dioumaya	1.50	0.00	0.00	0.00	0.38
	Guilere	0.50	0.00	0.00	0.00	0.13
	Kaboye	0.50	0.00	0.00	0.00	0.13
	Boké overall	**0.83**	**0.00**	**0.00**	**0.00**	**0.21**
Faranah	Balayani	0.50	2.00	3.00	4.00	2.38
	Foulaya	1.00	1.00	3.50	4.50	2.50
	Tindo	0.00	0.50	1.50	1.00	0.75
	Faranah overall	**0.50**	**1.17**	**2.67**	**3.17**	**1.88**
Overall monthly mosquito per trap		**0.67**	**0.58**	**1.33**	**1.58**	**1.04**

Figure 7: Seasonal percentage of mosquitoes found indoors and outdoors by species in Boké and Faranah using CDC light traps

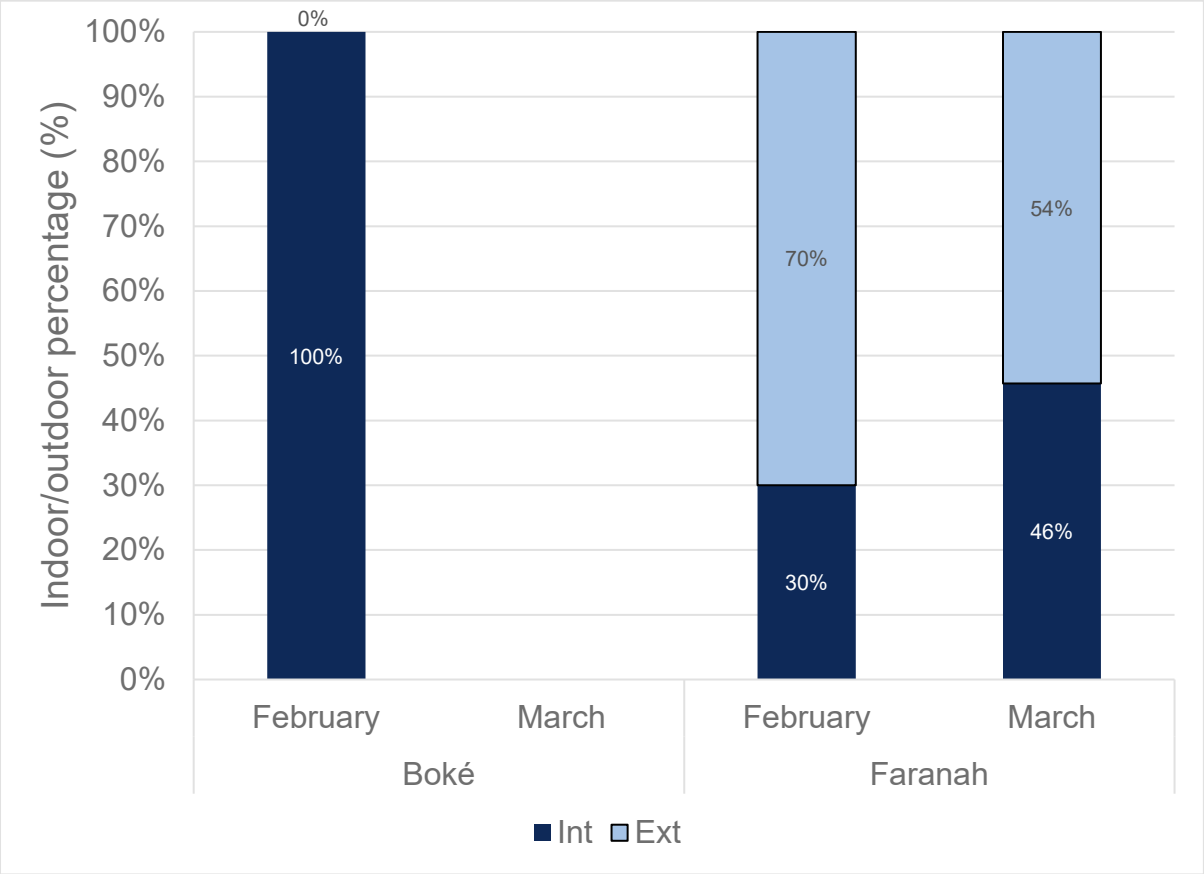
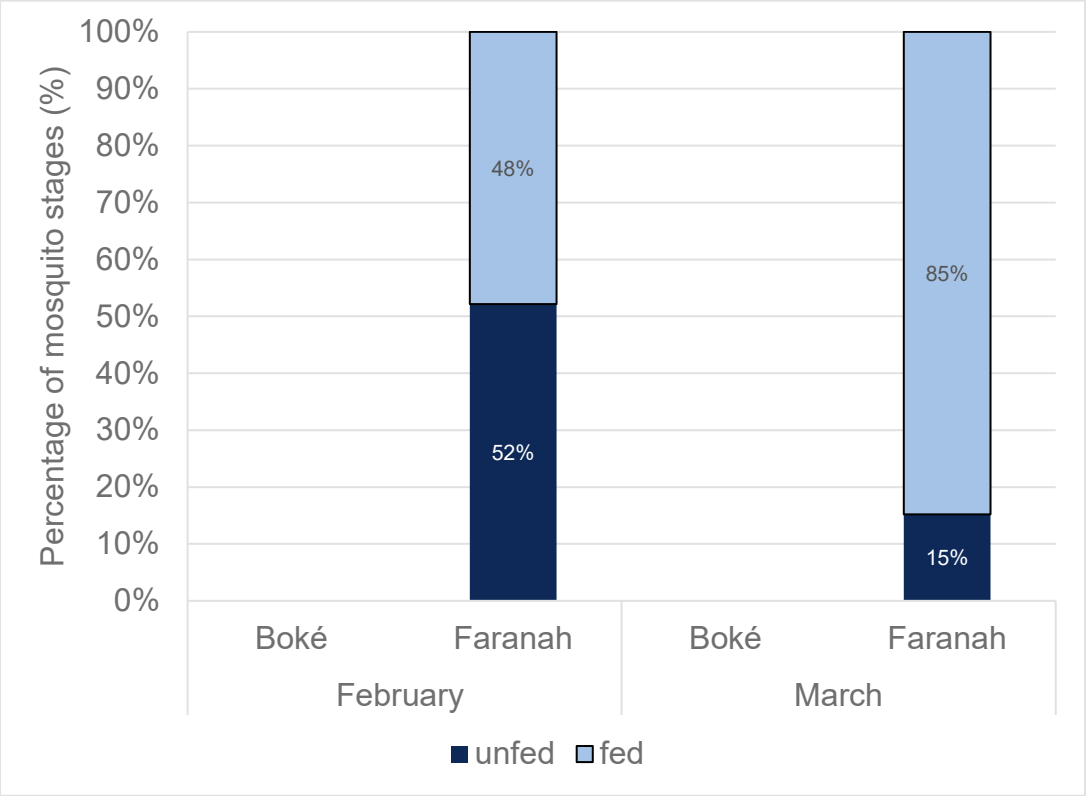


Table 8: Seasonal indoor resting density of *An. gambiae* s.l. collected in Boké and Faranah as determined using indoor pyrethrum spray catches

Prefecture	Sentinel site	February	March	Overall indoor resting density by site
Boké	Dioumaya	0.00	0.00	0.00
	Guilere	0.00	0.00	0.00
	Kaboye	0.00	0.00	0.00
	Boké overall	**0.00**	**0.00**	**0.00**
Faranah	Balayani	1.60	2.00	1.80
	Foulaya	0.50	1.80	1.15
	Tindo	0.20	0.80	0.50
	Faranah overall	**0.77**	**1.53**	**1.15**
Overall monthly indoor resting density		**0.38**	**0.77**	**0.58**

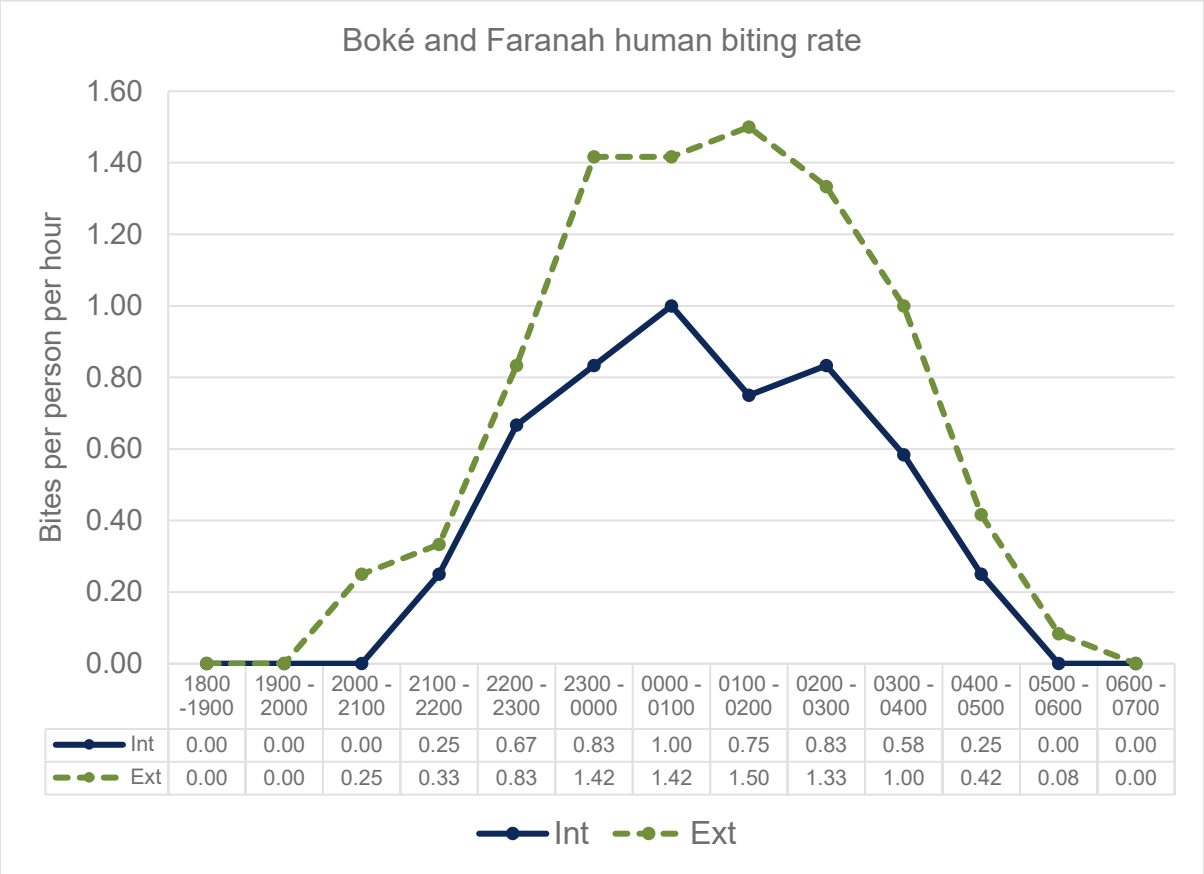
Figure 8: Seasonal percentage of *An. gambiae* s.l. by stage and species in Boké and Faranah using pyrethrum spray catches



4.2.3 Assessing vector biting behavior

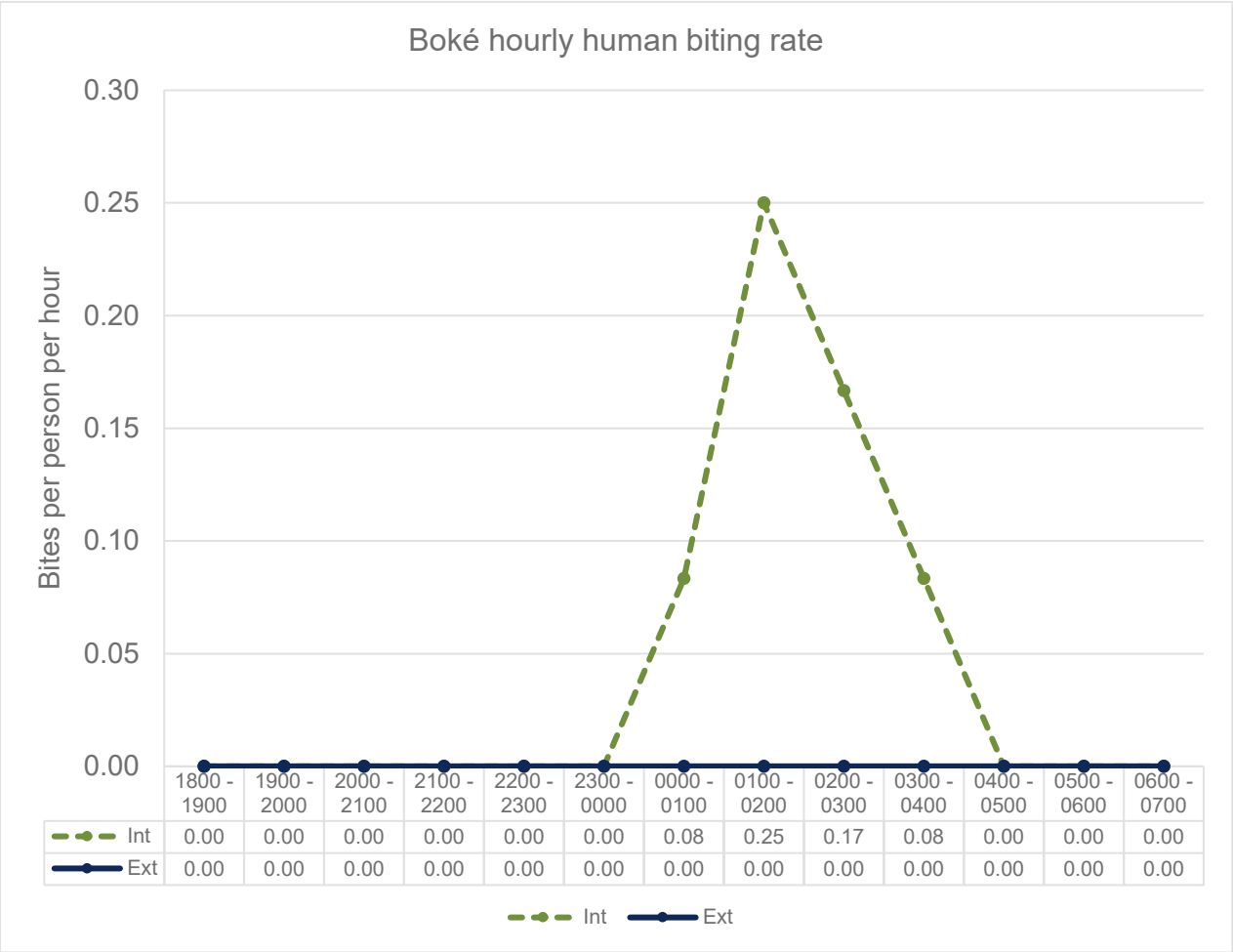
Analysis of the results of the vectors' biting behavior using HLC, recorded during hour-long intervals, shows biting behavior inside and outside houses in the sites between 1900 and 0700 hrs. The peak indoor biting was observed between 0000 hours and 0100, while the peak outdoor biting was observed between 0100 and 0200 hours (***Figure 9***). Also, among the six sites in the two prefectures, Boké had lower biting rates than Faranah (***Figures 10*** and ***11***).

Figure 9: Interior and exterior biting behavior of *An. gambiae* s.l. across the six sites in Boké and Faranah (as determined by HLC)



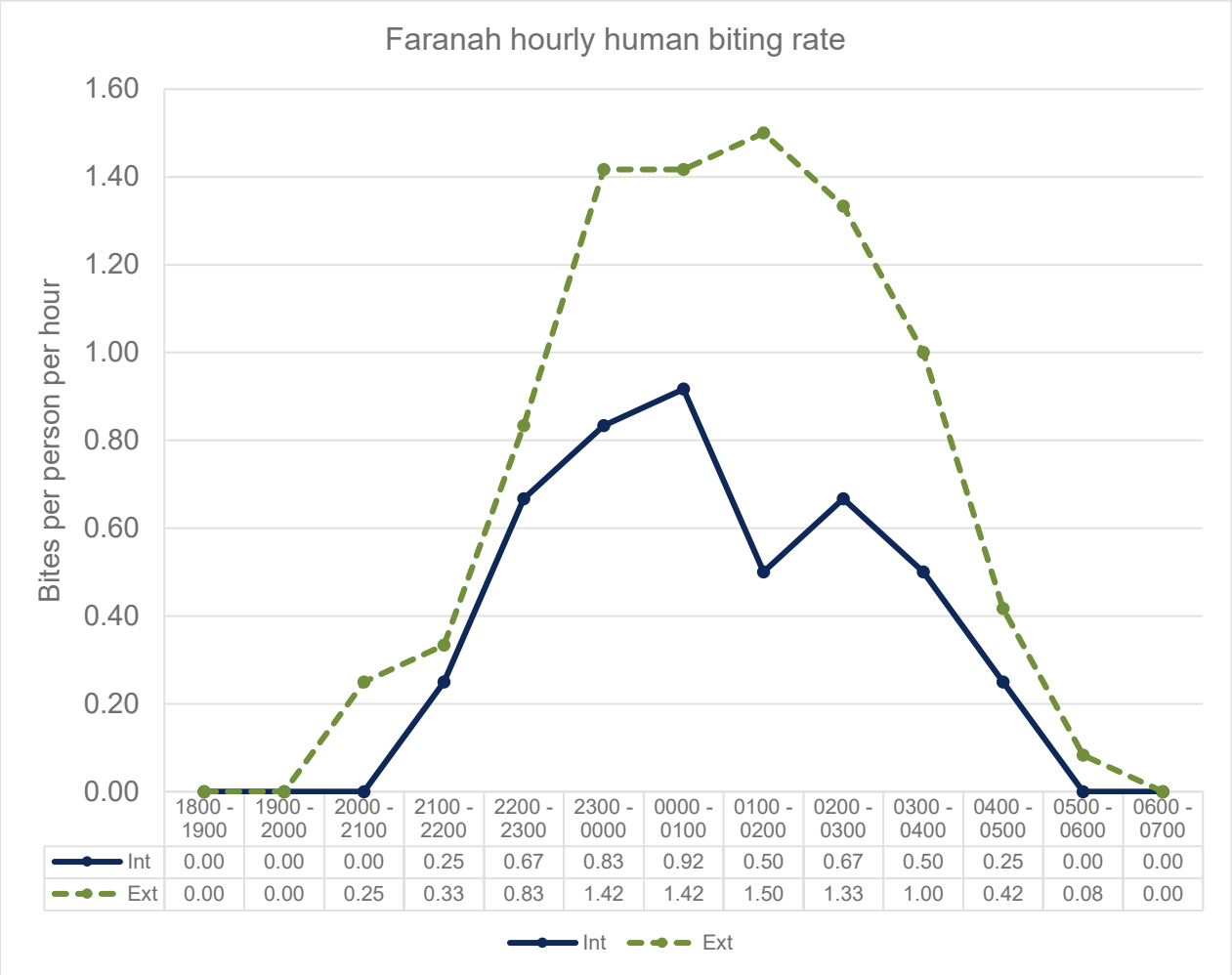
	1800 -1900	1900 - 2000	2000 - 2100	2100 - 2200	2200 - 2300	2300 - 0000	0000 - 0100	0100 - 0200	0200 - 0300	0300 - 0400	0400 - 0500	0500 - 0600	0600 - 0700
Int	0.00	0.00	0.00	0.25	0.67	0.83	1.00	0.75	0.83	0.58	0.25	0.00	0.00
Ext	0.00	0.00	0.25	0.33	0.83	1.42	1.42	1.50	1.33	1.00	0.42	0.08	0.00

Figure 10: Interior and exterior biting behavior of *An. gambiae* s.l. (HLC, 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
Int	0.00	0.00	0.00	0.00	0.00	0.00	0.08	0.25	0.17	0.08	0.00	0.00	0.00
Ext	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Figure 11: Interior and exterior biting behavior of *An. gambiae* s.l. (HLC, Faranah)



	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
Int	0.00	0.00	0.00	0.25	0.67	0.83	0.92	0.50	0.67	0.50	0.25	0.00	0.00
Ext	0.00	0.00	0.25	0.33	0.83	1.42	1.42	1.50	1.33	1.00	0.42	0.08	0.00

4.3 Objective 3: Conducted an entomological investigation of malaria vectors in Faranah

4.3.1 General objectives

- Collect adult mosquitoes; identify the time periods when peak mosquito biting behavior occurs in the Faranah sentinel site
- Assess the susceptibility of malaria vectors to insecticides at the Faranah sentinel site

4.3.2 Specific objectives

During this work plan period, the project targeted the following activities:

- Collect malaria larvae in Faranah using standard dipping techniques for insecticide resistance testing
- Collect adult mosquitoes using three methods—human landing collection (HLC), light traps, and pyrethrum spray catch—in the Faranah sentinel site
- Determine the genera and identify the *Anopheles* fauna at the Faranah sentinel sites
- Collect data on the use of LLINs in the communities of the Faranah sentinel sites

- Determine the peak vector biting time from 6 p.m. to 7 a.m. and the preferred biting location (indoors or outdoors) at the Faranah sentinel sites
- Determine the sensitivity of *An. gambiae* s.l. to three insecticides (alpha-cypermethrin, deltamethrin, permethrin) recommended by WHO for use on insecticide-treated nets in the Faranah region
- Determine the sensitivity of *An. gambiae* s.l. to three insecticides (alpha-cypermethrin, deltamethrin, permethrin) after pre-exposure to PBO
- Perform intensity tests for alpha-cypermethrin, deltamethrin, and permethrin
- Compare the results of insecticide resistance tests between adult and larval samples
- Compare WHO test results with CDC test results
- Perform laboratory analysis of samples to determine mosquito species, *Plasmodium* spp. infection status, and molecular resistance markers

4.4 Methodology

The period of work was from September 24 to October 14, 2020.

4.4.1 Summary

- Entomological monitoring and insecticide resistance tests were carried out in the field laboratories of the four sentinel sites in Faranah prefecture.
- The WHO Insecticide Susceptibility Test and the CDC Insecticide Resistance Test were used for testing.
  - The WHO test was carried out in Balayani and Tindo.
  - The CDC test was performed in Foulaya and in city center
- Larval mosquitoes and adult mosquitoes were used in the tests.
- The results of insecticide resistance tests were reported individually for each of the four localities, even if the quota of 100 mosquitoes per test was not reached.
- Considering the workload, an extra day was necessary to finalize the work in Foulaya. During this final day, the mosquito count reached 100.

4.4.2 Sentinel sites and collections

Sentinel sites

This activity was carried out in three villages and the city center of Faranah prefecture (***Table 9***).

Table 9: Sentinel sites for entomological surveillance in 2020

Natural region	Prefecture	Sub-prefecture	Villages Sentinel sites	Longitude	Latitude
Upper Guinea	Faranah	Urban commune of Faranah	Balayani	10.1325	- 10.7443
Upper Guinea	Faranah	Urban commune of Faranah	Foulaya	10.144633	- 10.749717
Upper Guinea	Faranah	Tindo	Tindo	9.9612230	- 10.7016560
Upper Guinea	Faranah	City center	City center	10.057518	- 10.732869

Collections of larval mosquitoes

The larval collections in the four localities were carried out for resistance tests.

- The larval sampling procedure described in the WHO "Malaria entomology and vector control training module" [12,13] was used for the larval collections.
- Larval samples were taken from sites selected through active research and / or known to have been productive in previous years.
- The collectors carried out larval collections in each village to obtain an adequate number of mosquitoes for testing.
- The larval sites were visited daily until enough mosquitoes for testing were acquired.
- The larval sites were identified, geo-referenced, and characterized.
- The GPS coordinates were recorded by the teams by taking the latitude and longitude of all the larval sites sampled for the resistance tests.
- Standard dipping techniques were used to collect samples of mosquito larvae and pupae.
- To identify the larval sites, collectors used information from village residents about the locations of standing water and a sampling transect (i.e., a road, river, or other convenient centerline).
  - Collectors recorded the name and type (i.e., road, river, other) of the transect used for sampling in each of the locations.
  - The sampling interval along the transect was chosen appropriately depending on the size and shape of the village (i.e., 20, 50, or 100 meters). However, once the sampling interval was selected, the collector recorded the approximate sampling interval along the transect.
  - Typical larval sites sampled were puddles, swamps, rivers, rice plains, and ditches.
- The larvae and pupae collected from the larval sites in each village were reared as adults to perform the susceptibility tests.
- Larvae and pupae were collected using larval dippers, buckets, and larval pans.
- The larval collections were brought back an improvised insectary room in the Faranah Market Health Center, where the live larvae were fed with fish food (TetraMin® Tropical Fish Food Flakes).
- The pupae were removed daily, placed in plastic cups, and transferred to mosquito cages.
- Emerging adult mosquitoes were given a 10% glucose solution before bioassay.

Collection of adult mosquitoes

Adult collections in selected localities were performed for resistance tests.

- The HLC method, described below, was used to collect live mosquitoes for insecticide resistance testing.
- After the consent from the household owner, field agents (collectors) were placed in the home. (Ministry of Health COVID-19 guidelines were followed to protect household residents and collectors.)
- Mosquitoes attempting to land and feed on the field agents' exposed feet were collected from 6 p.m. to 7 a.m.
- Live mosquitoes were stored in tubes, returned to the Faranah Insectarium, and given a 10% sugar solution prior to insecticide resistance testing.

[12] https://www.who.int/malaria/publications/atoz/9789241505819/en/
[13] https://www.paho.org/hq/dmdocuments/2012/2012-cha-manuel-entomologie-paludisme.pdf

4.4.3 Susceptibility tests, including synergist bioassays and biological resistance intensity tests

Insecticide resistance tests

The three pyrethroids and a synergist were chosen for the susceptibility tests because these are the insecticides that are used on LLINs. This will help guide decision making for purchasing insecticide-treated nets for routine and mass distributions. Three comparisons will be made with data from bioassays that will guide the choice of insecticide for LLINs:

1. Are mosquitoes susceptible to pyrethroids used on standard LLINs?
2. Does pre-exposure to PBO increase the activity of the insecticide, resulting in increased vector mortality?
3. Is the resistance, where it exists, intense (for example, are mosquitoes able to survive 5 or 10 times the diagnostic dose)?

The best way to answer these questions was to have the team collect a large number of larvae, which allowed them to perform multiple tests during the same time period. The proposed insecticides and test concentrations used, and the targeted number of mosquitoes to be tested are shown in ***Tables 10*** and ***11***.

Collecting larvae in breeding sites

Maintaining larvae at the Circostance Laboratory

The larvae of Anopheles at different stages

Table 10: Planned tests and target number of larval mosquitoes to be tested to measure the susceptibility of *An. gambiae* s.l. in three villages in the prefecture of Faranah, Guinea

Villages	Test	Deltamethrin				Permethrin				Alpha-cypermethrin				Total
		1×	5×	10×	1× + PBO	1×	5×	10×	1× + PBO	1×	5×	10×	1× + PBO	
Balayani	WHO	100	100	100	100	100	100	100	100	100	100	100	100	**1200**
Foulaya	CDC	100	100	100	100	100	100	100	100	100	100	100	100	**1200**
Tindo	WHO	100	100	100	100	100	100	100	100	100	100	100	100	**1200**
City center	CDC	100	100	100	100	100	100	100	100	100	100	100	100	**1200**
Total		**400**	**400**	**400**	**400**	**400**	**400**	**400**	**400**	**400**	**400**	**400**	**400**	**4800**
Control trials with papers impregnated only with silicone oil was also carried out using approximately 100 to 200 mosquitoes (40 to 50 mosquitoes at each concentration) per insecticide in each village; in total, about 4800 are targeted by this pyrethroid test.														

Table 11: Planned tests and target number of adult mosquitoes to be tested to measure the susceptibility of *An. gambiae* s.l. in three villages in Faranah prefecture, Guinea

Villages	Test	Deltamethrin				Permethrin				Alpha-cypermethrin				Total
		1×	5×	10×	1× + PBO	1×	5×	10×	1× + PBO	1×	5×	10×	1× + PBO	
Balayani	WHO	100	100	100	100	100	100	100	100	100	100	100	100	**1,200**
Foulaya	CDC	100	100	100	100	100	100	100	100	100	100	100	100	**1,200**
Tindo	WHO	100	100	100	100	100	100	100	100	100	100	100	100	**1,200**
City center	CDC	100	100	100	100	100	100	100	100	100	100	100	100	**1200**
Total		**400**	**400**	**400**	**400**	**400**	**400**	**400**	**400**	**400**	**400**	**400**	**400**	**4800**
Control trials with papers impregnated only with silicone oil was carried out using approximately 100 to 200 mosquitoes (40 to 50 mosquitoes at each concentration) per insecticide in each village; in total, about 4,800 are targeted by this pyrethroid test.														

Diagnostic and intensity test

Sensitivity tests were carried out by exposing susceptible unfed *An. gambiae* s.l. to insecticide-impregnated papers aligned with the WHO standard,[14] and exposing them to insecticide-coated bottles for the CDC insecticide resistance test.[15] (See ***Table 12***.)

- The larval mosquitoes and pupae sampled and used in the test were reared to adulthood and used when 2–5 days old.
- Adult mosquitoes collected from HLC were used in the test and were given 10% sugar until they could be tested.

The following insecticides were tested in September–October 2020.

Table 12: Insecticides and associated concentrations (per WHO and CDC tests)

Insecticide	WHO	CDC
Deltamethrin	0.05%	12.5 µg / bottle
Permethrin	0.75%	21.5 µg / bottle
Alphacypermethrin	0.05%	12.5 µg / bottle

- Pyrethroids testing included the diagnostic dose (1×) and, if necessary, 5 times the diagnostic dose (5×) and 10 times the diagnostic dose (10×).
- For the WHO test, a control consisting of a control paper soaked with silicon oil only was also included.
- For the CDC test, a control consisting of a 250 ml bottle coated with 95%–98% acetone only was also included.

Bioassays with synergist, PBO

In these synergistic bioassays, mosquitoes were pre-exposed to PBO before exposure to pyrethroid insecticides. WHO and CDC bioassays were used [4, 5].

All efforts were made to use 30–100 mosquitoes per test (5–25 mosquitoes for control) for each insecticide on the same day.

If a ≥ 98% mortality rate was achieved at the diagnostic dose (1×), it was not necessary to perform the tests at 5× or 10× the diagnostic dose or the PBO test. Likewise, if the 5× dose achieved a ≥ 98% mortality rate, the 10× dose did not need to be performed.

Analysis of insecticide resistance at the surveillance site laboratories

At the end of each exposure period, the team separated the mosquitoes according to status—surviving (resistant), knocked down, or dead (susceptible)—and these separated test mosquitoes as well as the control mosquitoes were stored in silica gel and deposited at the University of Conakry laboratory for downstream molecular analysis. Mosquitoes from the WHO test and CDC test were packed and labeled to determine the *An. gambiae* s.l. species collected and the frequency of target site mutations: *kdr-west* and *kdr-east* for pyrethroids and *ace-1R*.

[14] WHO. (2018). *Test procedure for insecticide resistance monitoring in malaria vector mosquitoes - 2nd ed.* (https://www.who.int/en/) and (https://inreskit.usm.my/mosquito/mosquito-adults-dt)
[15] CDC Bottle Bioassay (https://www.cdc.gov/parasites/education_training/lab/bottlebioassay.html)

4.4.4 Vector composition and behavior tests

Three entomological sampling methods to collect adult mosquitoes were used at sentinel sites throughout the work plan period to obtain information on species composition and feeding behavior (e.g., indoor and outdoor biting behavior, peak biting time, etc.). The team collected mosquitoes in the prefecture of Faranah in three villages—Balayani, Foulaya, and Tindo—and the city center. During the September–October visit, in each locality, 2 capture sessions using HLCs, 2 capture sessions using CDC light traps, and 10 sessions using indoor pyrethrum spray catches (5 houses per 2 collection visits) were performed. Because there were two collection nights for the HLCs and light trap methods (at one house per village each), and two collection days for the indoor spray captures (at 5 houses per village), the total number of visits provided 8 capture sessions using HLC, 8 capture sessions using CDC light traps, and 40 sessions using indoor pyrethrum spray catches (***Table 13***).

Table 13: Collection methods and number of houses visited in Faranah

Site	Method	Number of houses
Faranah	HLC [a]	8
	CDC light trap [a]	8
	Indoor pyrethrum spray catches [b]	40
[a] Two collection nights × 4 villages × 1 house; [b] Two collection days × 4 villages × 5 houses		

Collection of host-seeking mosquitoes

Faranah was the prefecture selected for entomological seasonal monitoring. The NMCP team and the village chiefs chose the houses according to certain selection criteria. Households identified with the highest number of malaria cases were selected, while ensuring a minimum of 60 to 100 meters between houses. The same houses were used on each visit. The team collected host-seeking adult female mosquitoes through two methods—HLC and CDC light traps.

Human landing collections (HLC)

HLC is a standard method for determining human-vector contact and for specifically assessing mosquito behavior. HLCs were conducted for two nights in each of the four locations during each pass. The HLC session took place from 6 p.m. to 7 a.m. In each village, a total of four collectors were recruited to serve as the live human bait. These collectors worked in shifts from 6 p.m. to midnight (evening to night) and from 1 to 7 a.m. (night to early morning); More precisely, a first pair of collectors (1 indoor and 1 outdoor) sampled mosquitoes from 6 p.m. to midnight and a second pair of collectors (1 indoor and 1 outside) sampled mosquitoes from 1 a.m. to 7 a.m. Having two shifts of mosquito collection was to reduce collector fatigue and sleep while working. Every hour, the collectors rotated for indoors to outdoor and vice versa to reduce the collection bias related to variation in collectors' attractiveness to mosquito and sampling ability of the collectors. The number of sleeping spaces and LLINs in the house were recorded. The people involved in collecting and supervising the work received chemoprophylaxis to protect them from malaria.

Community volunteer collecting mosquitoes

The HLC method has been used to assess mosquito biting time and feeding behavior (indoors and / or outdoors), and to monitor species composition and sporozoite rates. Mosquito collection containers were pre-labeled with the date, time, location (indoor / outdoor), house code and collection site (village). At each respective hour, the mosquitoes were put into the collection containers. At the end of each collection hour, the name of the collector was placed on the container. Live mosquitoes captured using HLC were also used in the WHO and CDC insecticide resistance test.

CDC light traps

CDC light trap

Light traps are an effective method of sampling for malaria vectors, especially indoors. The light trap collections were conducted for two nights in each of the four locations during each visit. The traps were placed between 5:00 p.m. and 6:00 p.m. and were recovered between 7:00 a.m. and 8:00 a.m. the next morning. The exterior light trip was suspended under the eaves of the house and at least 3 meters from the ground when possible. Inside the light trap was at the foot of the bed and 3 meters from the ground.

Indoor pyrethrum spray catches

Collecting mosquitoes that have fallen after exposure to pyrethrum

The indoor pyrethrum spray catch method was used to collect resting mosquitoes inside homes. This method involves collecting adult mosquitoes inside houses to determine the species present inside, their physiological status, and vector density. In each house, the team requested permission from the residents to spray the house. Once the residents consented, the team took all necessary precautions, including removing all food and other sensitive materials before spraying. The team covered the floor with white sheets to collect the fallen mosquitoes. The collections took place in the morning from 7:00 a.m. to 10:00 a.m. A member of the team sprayed the inside of each house with an insecticide aerosol, while another team sprayed outside of the house toward the interior through openings (i.e., eaves or other structural gaps) to prevent mosquitoes from escaping. A local insecticide with the brand name Fatala—active ingredients tetramethrin 0.33% and cypermethrin 0.05%, available in 600 ml bottles—was used. After spraying, all windows and doors were kept closed for 15 minutes. After 15 minutes, the sheets were taken out of the house to pick up the fallen mosquitoes. Mosquitoes were placed in labeled storage tubes containing the following information: date, time, house code, and site (village) of collection.

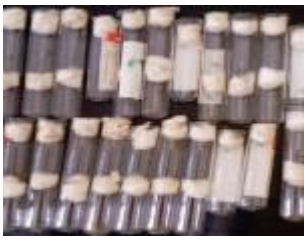
The number of sleeping spaces and the brand of LLINs in the house were recorded.

Mosquito identification and downstream analysis

The morphological identification of the mosquitoes was made in the field laboratories using an *Anopheles* morphological key (Gillis & et Meillon 1968; Diagne et al.,1994). In addition to the morphological analysis, an analysis of the species and molecular form of *An. gambiae* s.l. will be performed in a reference laboratory.

An. gambiae s.l.

Mosquito conditioning session



Labeled tubes

4.5 Results

4.5.1 Mapping and scouting for mosquito larvae

This activity was conduted during the rainy season, when rainfall was abundant in the health district of Faranah. The team carried out sampling transects by foot in the four localities to identify and characterize potential larval development sites.

In general, the breeding sites that were located were reservoirs or puddles of water created by vehicles rolling along the roads. The samples of larvae suspected to be *Anopheles* were taken and reared in an improvised insectary room located at the Marché Health Center to identify the species. A total of 51 larval development sites were surveyed in the four localities, and 18 larval development sites were positive for *Anopheles* larvae.

- In Balayani out of 15 larval sites, 6 (40%) were positive.
- In Foulaya, of the 7 larval sites evaluated, none was positive.
- In Tindo, out of the 7 larval sites sampled, 3 (43%) were positive.
- In the Faranah city center, out of the 22 breeding sites sampled, 9 (43%) were positive.

Overall, the positivity rate of *Anopheles* in larval sites was 35% (18 out of 51 breeding sites). Only the Foulaya site did not record any larval sites that were positive for *Anopheles* larvae; its 3 positive breeding sites only contained *Culex* larvae. Given that Balayani and Foulaya are close to each other (approximately 1 kilometer) and share the same plain (bank of the Niger river) and given the lack of *Anopheles* larvae in the Foulaya breeding sites, we believe that most of the adult mosquitoes in Foulaya come from Balayani, which is located in the same cluster. (See the Annex B table showing latitude/longitude coordinate information for the larval breeding sites.)

4.5.2 Mosquito density, biting and resting behavior

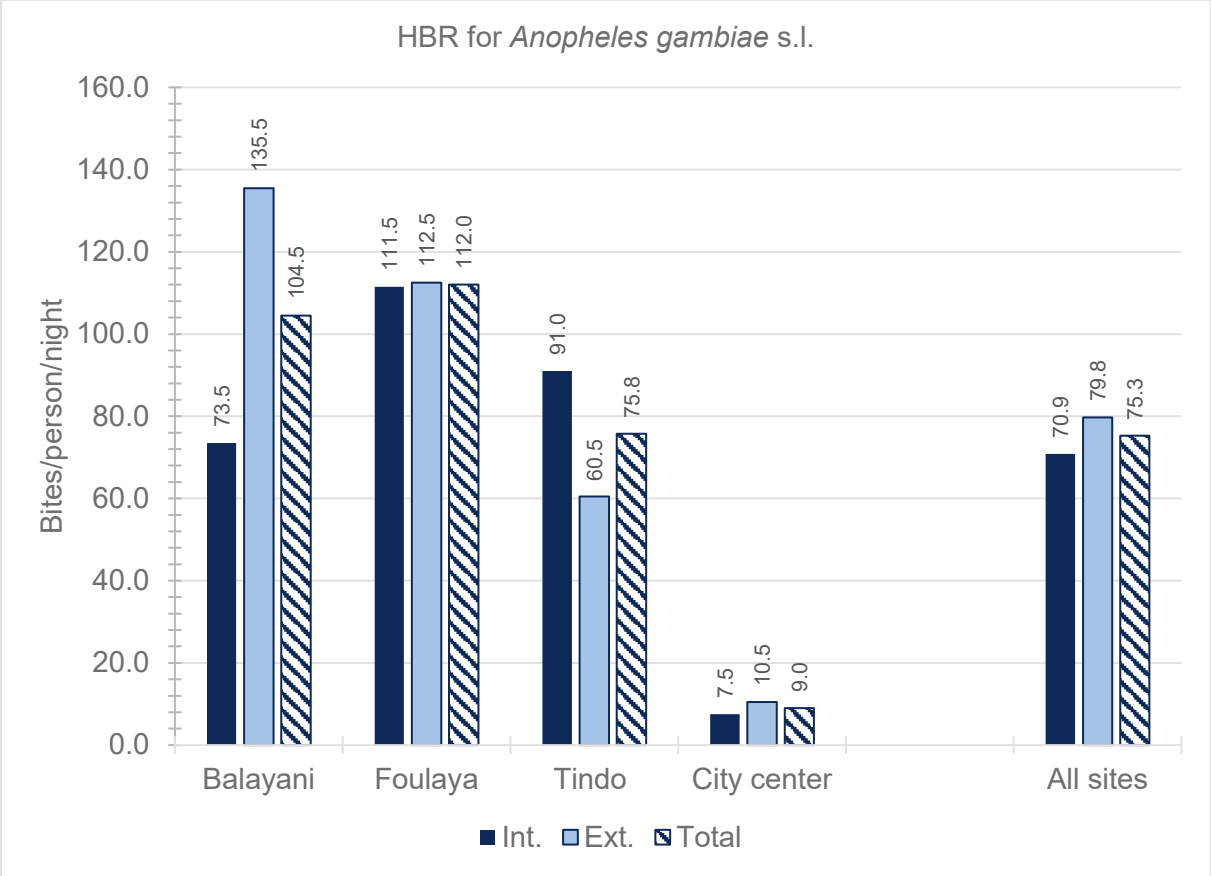
Human landing catches (HLC) of mosquitoes to determine the human biting rate

In Balayani, a total of 421 mosquitoes were collected using the HLC method, including 418 *An. gambiae* s.l., 1 *Culex* spp. and 2 *Aedes* spp. In total, 147 out of 418 (35.17%) *An. gambiae* s.l. were captured indoors, and 271 out of 418 (64.83%) were captured outdoors. In Foulaya, a total of 468 mosquitoes were collected using HLC, including 448 *An. gambiae* s.l., 17 *Culex* spp. and 3 *Aedes* spp. In total, 223 out of 448 (49.78%) *An. gambiae* s.l. were captured indoors, and 225 out of 448 (50.22%) were caputred outdoors. In Tindo, a total of 324 mosquitoes were collected using HLC, including 303 *An. gambiae* s.l. 20 *Culex* spp. and 1 *Aedes* spp. In total, 182 out of 303 (60.07%) *An. gambiae* s.l. were captured indoors and 121 out of 303 (39.93%) captured outdoors. In the Faranah city center, a total of 296 mosquitoes

collected using HLC, including 36 *An. gambiae* s.l. and 260 *Culex*. In total, 15 out of 36 (41.67%) *An. gambiae* s.l. were captured indoors and 21 of out 36 (58.33%) were captured outdoors.

The human biting rates (HBRs) indoors and outdoors of *An. gambiae* s.l. in the 4 sites and overall are presented in ***Figure 12***. When comparing the 4 sites, Faranah city center had the lowest HBR indoors, outdoor and total. HBR totals were highest in Balyani (104.5 bites/person/night) and Foulaya (112.0 bites/person/night). However, Balayani had the highest outdoor HBR (135.5 bites/person/night), which was greater than indoor HBR (73.5 bites/person/night), and had a higer outdoor HBR than all the other sites. For Foulaya, biting rates were similar for indoors (111.5 bites/person/night) and outdoors (112.5 bites/person/night). For Tindo, biting rates were higher indoors (91.0 bites/person/night) than outdoors (60.5 bites/person/night).

Figure 12: HBR of *Anopheles gambiae* s.l.in Faranah by HLC

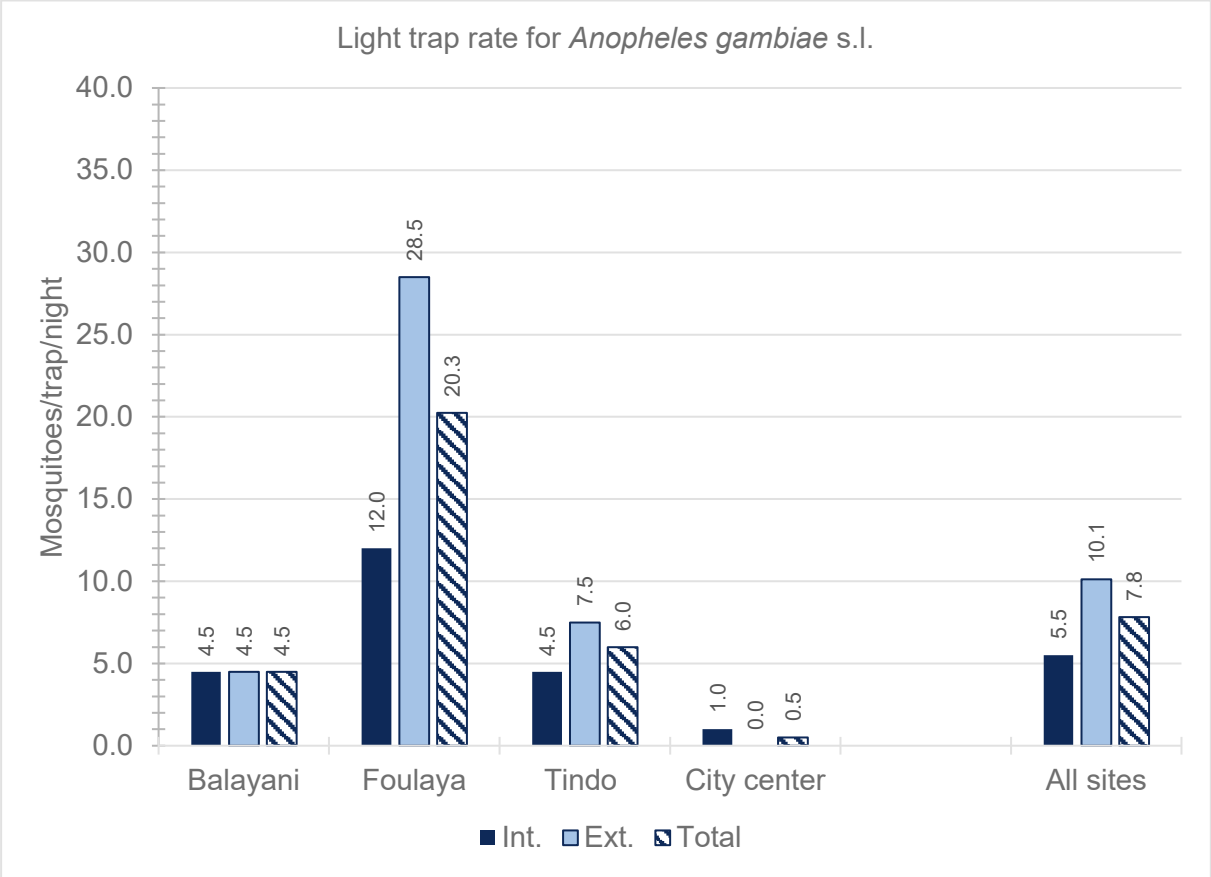


Light trapping of mosquitoes to determine mosquito trap rates (MTR)

Using light traps in Balayani, a total of 30 mosquitoes were collected, including 18 *Anopheles* (9 inside, 9 outside), 11 *Culex*, and 1 *Aedes*. In Foulaya, out of a total of 100 mosquitoes collected using light traps, 81 were *Anopheles*; 24 of those (29.63%) were captured indoors, and 57 of those (70.37%) were captured outdoors. During this capture, 19 *Culex* were identified. In Tindo out of a total of 47 mosquitoes collected using light traps, 24 were *Anopheles*, with 9 of those (37.50%) captured indoors and 15 (62.50%) captured outdoors, which shows exophagy in *Anopheles* in this locality. Also using light traps, 20 *Culex* and 3 *Aedes* were identified. In Faranah city center, using the light traps, a total of 82 mosquitoes were collected, including 2 *Anopheles*, 79 *Culex*, and 1 *Aedes*.

The MTR indoors and outdoors of *An. gambiae* s.l. in the 4 sites and overall are presented in ***Figure 13***. While the magnitude of the MTR was lower than the HBR, the trends were similar: Foulaya had the highest MTR (20.3 mosquitoes/trap/night) and Faranah city center had the lowest MTR (0.05 mosquitoes/trap/night). Outdoor biting was on average higher across sites (10.1 mosqutioes/trap/night).

Figure 13: Mosquito light trap rate of *Anopheles gambiae* s.l. in Faranah using the CDC light trap

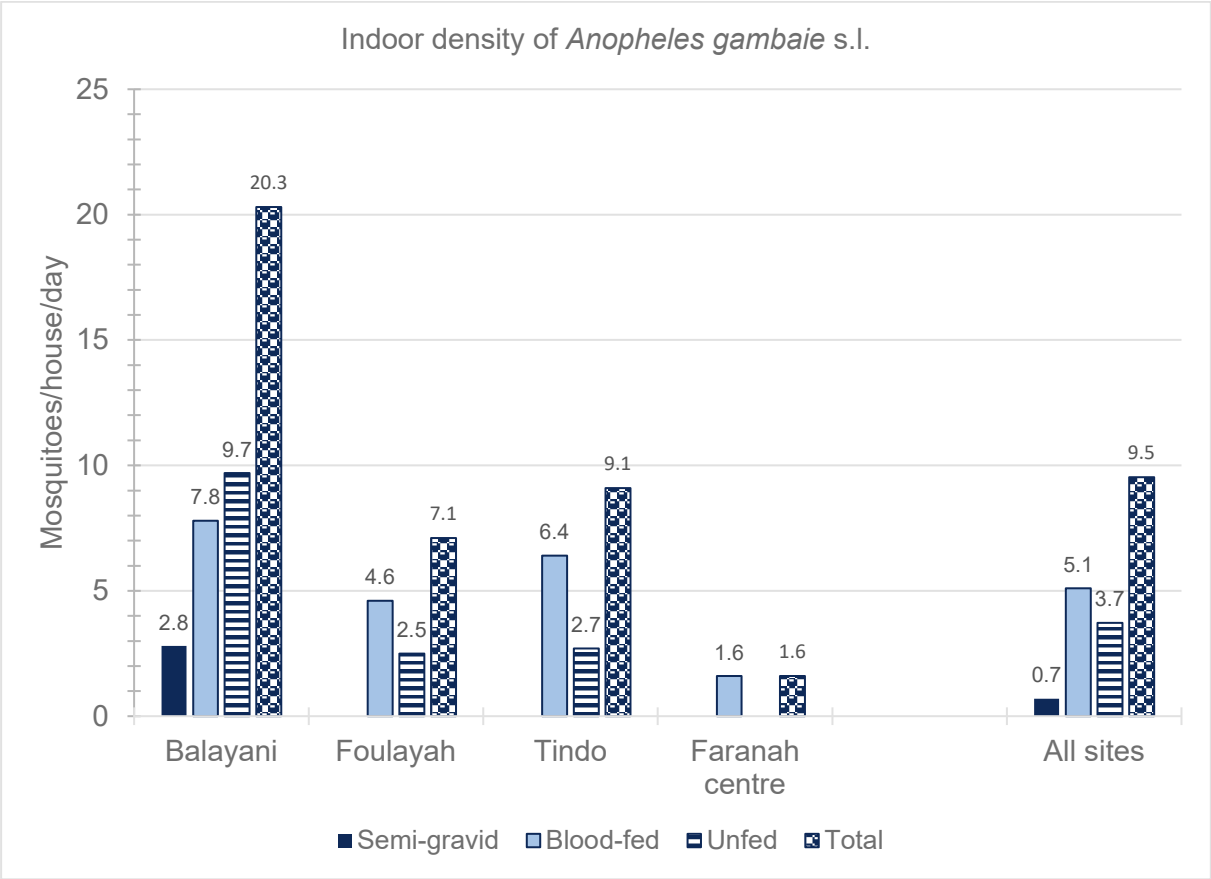


Pyrethrum spray catches (PSC) of mosquitoes to determine indoor resting density of *An. gambiae* s.l.

In Balayani, a total of 204 mosquitoes were collected using PSC, including *203 An. gambiae* s.l., 1 *Culex* fed. In total, 203 out of 204 (99.51%) *An. gambiae* s.l. were captured inside. Of the 203 *An. gambiae* s.l. 28 were semi-gravid, 78 blood-fed, and 97 were unfed. In Foulaya, a total of 72 mosquitoes were collected using PSC, including 71 *An. gambiae* s.l., 1 *Culex* unfed. In total, 71 out of 72 (98.61%) *An. gambiae* s.l. were captured inside. Of the 71 *An. gambiae* s.l., 46 were blood-fed and 25 unfed. In Tindo, a total of 94 mosquitoes were collected using PSC, 91 of which were *An. gambiae* s.l and 3 *Culex* (2 blood-fed and 1 unfed). In total, 91 out of 94 (96.80%) *An. gambiae* s.l. were captured inside. Of the 91 *Anopheles*, 46 were blood-fed and 25 unfed. In the city center of Faranah, a total of 128 mosquitoes were collected using PSC, including 16 *An. gambiae* sl. and 112 *Culex* (52 blood- fed and 60 unfed. All the 16 *Anopheles* were blood-fed.

In ***Figure 14***, the indoor resting density of *An. gambiae* s.l. is shown for semi-gravid, blood fed, unfed mosquitoes and overall.

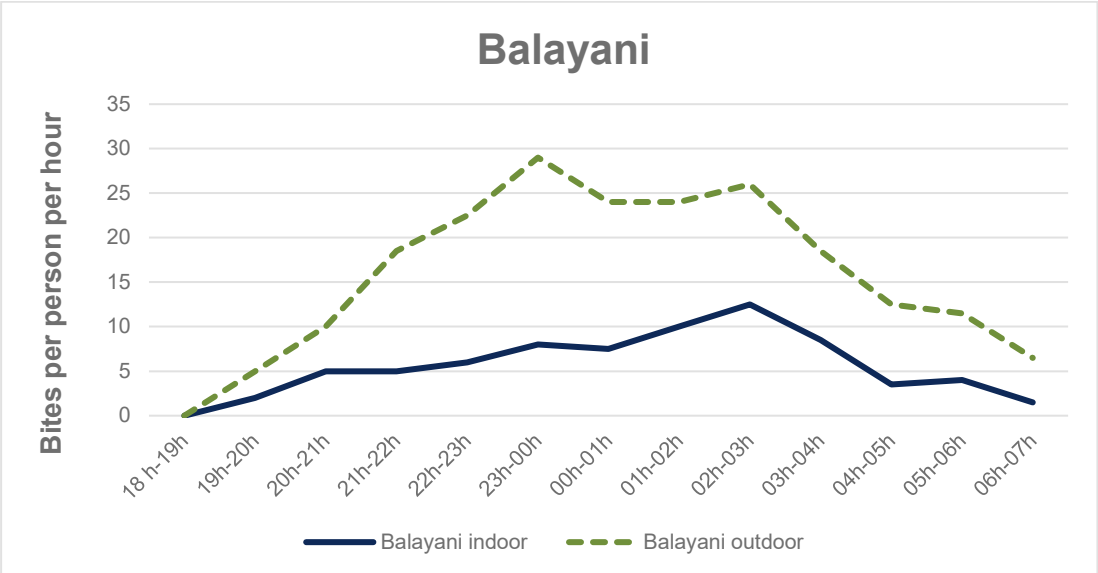
Figure 14: Indoor resting density of *Anopheles gambiae* s.l. in Faranah by PSC



Hourly biting behavior of the *Anopheles gambiae* s.l.

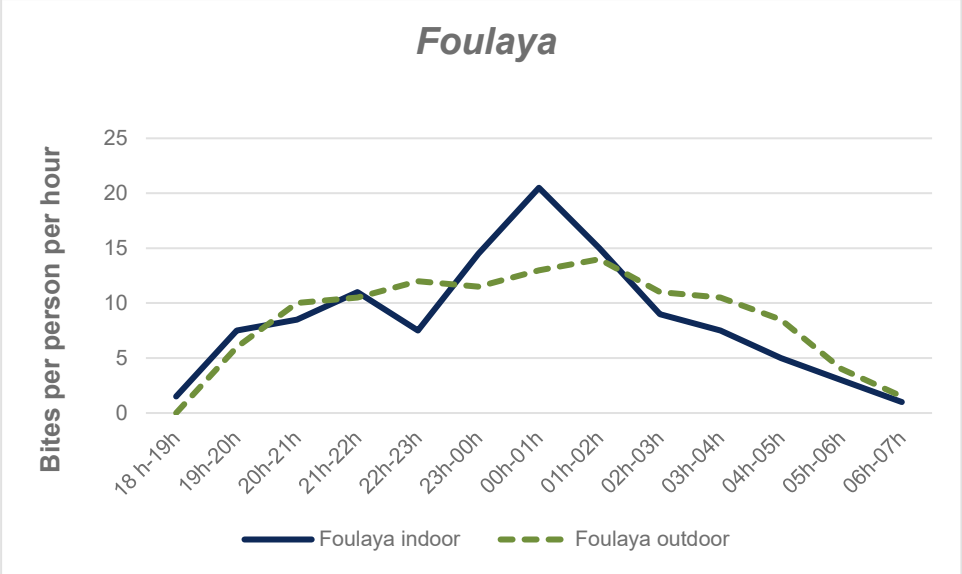
The biting behavior of the mosquitoes was observed from 6 p.m. to 7 a.m. The following figures present trends for biting behavior by time, for both interior and exterior.

Figure 15: Interior and exterior biting behavior of mosquitoes in Balayani (measured during HLC)



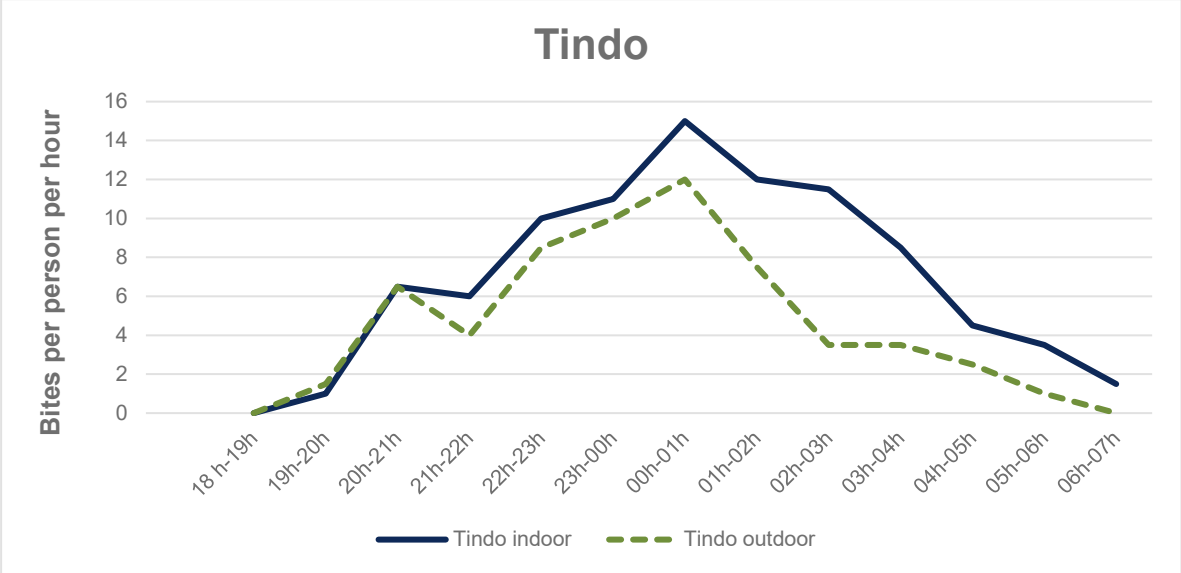
In Balayani, the peak biting time was observed between 11 p.m. to midnight for the outdoors, while indoors it was observed between 2 a.m. and 3 a.m. (***Figure 15***).

Figure 16: Interior and exterior biting behavior of mosquitoes in Foulaya (measured during HLC)



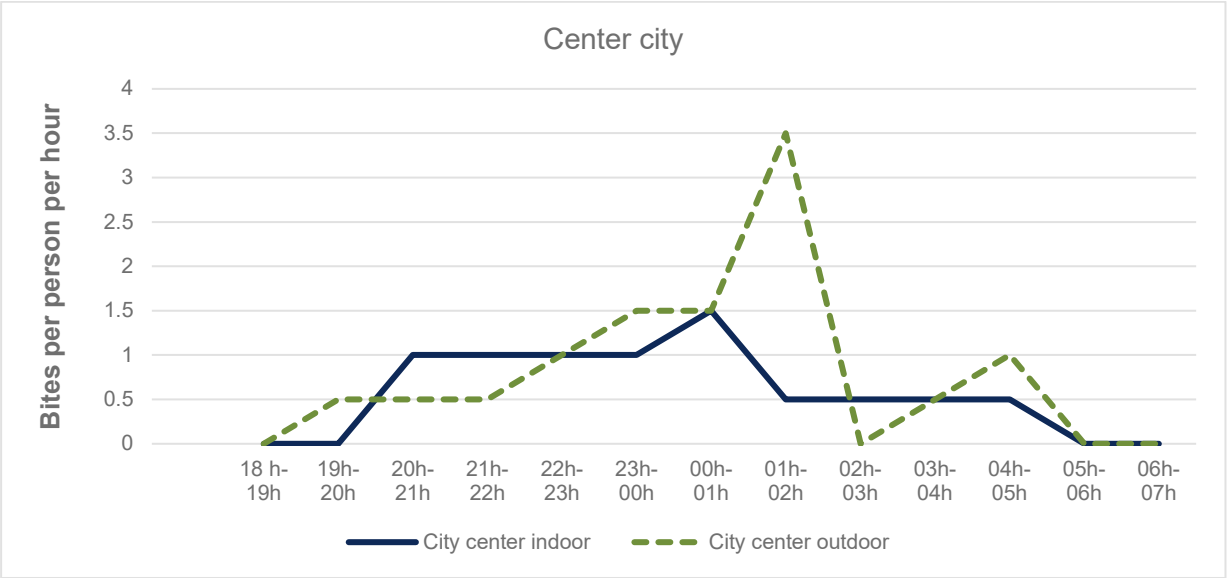
In Foulaya, the peak biting time was observed between midnight and 1 a.m. for indoors, while outdoors it was observed between 1 a.m. and 2 a.m. (***Figure 16***).

Figure 17: Interior and exterior biting behavior of mosquitoes in Tindo (measured during HLC)



In Tindo, peak biting time was observed between midnight and 1 a.m. for both indoors and outdoors (***Figure 17***).

Figure 18: Interior and exterior biting behavior of mosquitoes in the Faranah city center (measured during HLC)



In Faranah city center, the peak was observed between midnight and 1 a.m. for indoors and between 1 a.m. and 2 p.m. outdoors (***Figure 18***).

4.5.3 Insecticide Resistance tests

Table 14 provides the results of the insecticide resistance testing at the diagnostic dose. At the end of the tests carried out on the mosquitoes raised from the harvested larvae, the team noted suspected resistance of *An gambiae* s.l. to deltamethrin 0.05% (1×), which varied between 95% and 97%. In Foulaya, suspected resitance was also detected for alpha-cypermethrin (95%). Confirmed resistance was observed in all sites for permethrin, and in Balayani, Tindo, and the city center for alpha-cypermethrin (mortality less 90%).

Table 14: Mortality rate of *An. gambiae* s.l. when tested with deltamethrin, permethrin, and alpha-cypermethrin at the diagnostic dose (1×)

Insecticide	Dose	Balayani	Foulaya	Tindo	City center
		% (n)	% (n)	% (n)	% (n)
Deltamethrin	1×	95 (100)	97 (100)	96 (100)	96 (60)
Permethrin	1×	75 (100)	85 (100)	77 (100)	65 (60)
Alphacypermethrin	1×	69 (100)	95 (60)	77 (100)	68 (60)

Control mosquitoes were less than <5% in mortality, therefore no Abbott correction was done.
Green: >98% mortality
Yellow; 90 to 97% mortality
Red: <90%

Table 15 provides the results of the insecticide resistance testing after pre-exposure to PBO. The team observed sensitivity to deltamethrin 1× + PBO as follows: Balayani (98%), Foulaya (99%), Tindo (98%), and the city center (100%). Resistance to permethrin 1× + PBO varied between 75% and 88%, and to alpha-cypermethrin 1× + PBO between 70% and 85% in the four localities. There was an increase in pyrethroid-induced mortality after PBO pre-exposure in all sites except in Foulaya, where mortality dropped to less the 90% mortality, which is classified as confirmed resistance by the WHO classification.

Table 15: Mortality of *An. gambiae* s.l. when tested with deltamethrin, permethrin, and alpha-cypermethrin at the diagnostic dose after pre-exposure to PBO

Insecticide	Dose	Balayani % (n)	Foulaya % (n)	Tindo % (n)	City center % (n)
Deltamethrin	1× + PBO	98 (100)	99 (100)	98 (100)	100 (30
Permethrin	1× + PBO	88 (100)	85 (100)	88 (100)	75 (30)
Alphacypermethrin	1× + PBO	71 (100)	85 (60)	84 (100)	70 (30)
Control mosquitoes were less than <5% in mortality, therefore no Abbott correction was done. Green: >98% mortality Yellow; 90 to 97% mortality Red: <90%					

Tables 16 and ***17*** provide the results of the intensity insecticide resistance. In Balayani, the team observed sensitivity to permethrin 5× (98%) and suspected resistance to alpha-cypermethrin 5× (96%). In Foulaya, there was suspected resistance to permethrin 5× (92%) and alpha-cypermethrin 5× (95%). In the city center, the team observed resistance to permethrin 5x (89%) and alpha-cypermethrin 5× (77%). At 10× the diagnostic dose, the mortality at all sites were ≥98%

Table 16: Intensity test for *Anopheles* at the 5× dose in the four localities

	Dose	Balayani % (n)	Foulaya % (n)	Tindo % (n)	City center % (n)
Permethrin	5×	98 (100)	92 (30)	97 (100)	89 (30)
Alphacypermethrin	5×	96 (100)	95 (30)	94 (100)	77 (30)
Control mosquitoes were less than <5% in mortality, therefore no Abbott correction was done. Green: >98% mortality Yellow; 90 to 97% mortality Red: <90%					

Table 17: Intensity test for *Anopheles* at the 10× dose in the four localities

Insecticide	Dose	Balayani % (n)	Foulaya % (n)	Tindo % (n)	City center % (n)
Permethrin	10×		98 (30)	100 (100)	98 (30)
Alphacypermethrin	10×	100 (100)	100 (30)	100 (100)	98 (30)
Control mosquitoes were less than <5% in mortality, therefore no Abbott correction was done. Green: >98% mortality Yellow; 90 to 97% mortality Red: <90%					

4.6 Conclusion of Faranah study

- During this investigation, carried out in September–October 2020 (which corresponds to the end of the rainy season), 51 breeding sites were identified, of which 18 were positive for *Anopheles* larvae.
- A total of 1,830 mosquitoes were collected, including 1,407 *Anopheles* (77.88%) by two methods (HLC, light trap). This number indicates that the population is still at high risk of exposure to bites from the mosquitoes (*Anopheles*) that transmit malaria.
- Overall, there was relatively high number of *An. gambiae* s.l. outside (HBR: 79.8 outdoors versus 70.9 indoors). This may suggest exophagy in this locality, but additional monitoring would be useful to characterize the pattern.
- In Faranah city center, the team found a higher number of *Culex* spp than *An. gambiae* s.l. However, this may be expected as *An. gambiae* s.l. tend to be more rurally distributed than in urban areas.
- Mosquito biting started at 7:00 p.m. and continued until 7:00 a.m. in general. Peak biting times occurred between 11 p.m. and 2 a.m. in the localities visited.
- The collection of mosquitoes using indoor pyrethrum spray catches in the four localities yielded a total of 498 mosquitoes, including 307 *Anopheles* (61.64%).
- In addition, the study showed a total of 120 people slept under 37 LLINs, which gave an average coverage of 3.5 people per LLIN in all localities. This rate, close to the universal coverage (two people per LLIN) results from the recent 2019 LLIN mass distribution campaign. However, efforts still need to be made to improve this coverage.
- During this study, 32 tests of vector sensitivity to insecticides were carried out in the sentinel sites according to the WHO and CDC protocols. The following conclusions were drawn:
    - Suspected resistance to deltamethrin 1× in the four localities
    - Sensitivity to deltamethrin with PBO proven in the four localities
    - Persistent resistance to permethrin and alpha-cypermethrin at the 5× dose in the city center
    - Suspected resistance to permethrin and alpha-cypermethrin at the 5× dose in Balayani, Foulaya, and Tindo
    - Sensitivity to permethrin 10× and alpha-cypermethrin 10× in the four localities
- The results of study suggest the resistance to deltamethrin is present in the *An. gambiae* s.l. population in Faranah, but based on intensity results, resistance levels are still low (mortality at the diagnostic dose (1×) <90% but the mortality at 5× the diagnostic dose ≥98%). Resistance levels in *An. gambiae* s.l. for permethrin and alpha-cypermethrin are moderate (mortality at the diagnostic dose (1×) and 5× the diagnostic dose <90%, but mortality at 10× the diagnostic dose ≥98%).
- Restoration of deltamethrin to WHO levels of susceptibility classification (mortality ≥98%) with PBO was observed. However, PBO only increased mortality from 0% to 13% for permethrin and alpha-cypermethrin.
- In Faranah, the dominant economic activity is agriculture. Rice is one of the main crops cultivated on many of the plains, with pesticides used heavily in an uncontrolled manner, which could be a factor causing mosquitoes to become resistant to certain insecticidal doses (deltamethrin at 0.05%) whereas previous studies showed them as being sensitive.
- These results were similar to the LSHTM study and confirm the results collected in 2018.

4.7 Next steps

The mosquitoes used in the tests were coded by insecticide and sent to the laboratory at UGANC for analysis of the resistance mechanism.

Mosquitoes captured by HLC, light traps, and pyrethrum were coded for analysis (ELISA-CSP, EC1, etc.).

These samples will be sent to CDC or the Entomological Research Center of Cotonou (CREC) for additional analysis.

Report of the field results to the Deputy Coordinator / NMCP

4.8 Recommendations

The resistance to pyrethroids observed in this study revives the debate on indoor residual spraying with a view to the pre-elimination of malaria in Guinea. At this level, in the zones with high transmission rates, it is advisable to foresee the coupling of indoor residual spraying with the use of LLINs, since the populations often attend late vigils or outdoor meetings, which expose them to the risk of mosquito bites. Alternatively, new types of LLINs (i.e., PBO LLINs or dual AI LLINs) could also be considered to address resistance.

Technical working sessions with the managers of the Prevention Unit

5 Challenges

During the reporting period, the project encountered challenges.

1. COVID-19 disruptions

   (a) *StopPalu+* activities were impacted by the COVID-19 pandemic. *StopPalu+* had to stop seasonal entomological monitoring activities, as recommended by PMI and WHO guidance for the COVID-19 context.

   (b) Insecticide resistance could not be performed because orders placed to WHO for insecticide-treated papers were significantly delayed.

2. FY 2021 funding delays

   (a) Another challenge was the delay in obtaining FY 2021 funds, resulting in USAID requesting that RTI plan closeout and postpone all activities until funds are available.

To mitigate these challenges:

1. RTI will monitor closely any changes in PMI and WHO guidance regarding entomological activities.

2. *StopPalu+* will continue to follow up with WHO on the procurement of the insecticide-treated papers.

3. RTI and the *StopPalu+* project will resume all entomological monitoring activities as soon as the FY 2021 funds are obligated.

Issue	Mitigation
WHO test kits were delayed due to COVID-19. Therefore resistance testing will not be done until August.	We contacted the WHO supplier to try to expedite the shippment of the inseticides. We are also worked with PMI to accqurie insecticides for testing.
CREC training on labroatory analysis was been delayed due travel restrictions and coordination issues.	We were in dialogue with CREC to determine alternate days where the training could occur.
Samples were not able to be tested in Guinea.	We looked for options to outsource laboratory analysis.

6 Continued Activities

- Continue to support the operation of the entomology laboratory and insectary at UGANC
- Conduct entomological surveillance in selected sentinel sites
- Determine insecticide resistance of malaria vectors in sentinel sites
- Perform the enzyme immunoassay (ELISA) circumsporozoite protein (CSP) and PCR tests
- Support NMCP Vector Technical Working Group meetings

It is important to mention that the implementation of these activities will depend on the evolution of the COVID-19 pandemic.

Annex A. Laboratory Equipment, Materials, and Reagents

Table A-1: PCR equipment and materials available at the laboratory

	Materials, reagents, and small equipment for PCR ordered	Quantity
1	Thermocycleur en temps réel Life Technologie quant Studio 3 avec ordinateur Life Technology quant Studio 3 real-time thermal cycler with computer	1
2	Séquence Détection Primer – 10,000 pmol Plasmodium falciparum 205 bp Sequence Détection Primer – 10,000 pmol Plasmodium falciparum 205 bp	2
3	Séquence Détection Primer – 10,000 pmol Plasmodium malaria 144 bp Sequence Détection Primer – 10,000 pmol Plasmodium malaria 144 bp	2
4	Séquence Détection Primer – 10,000 pmol Plasmodium oval 800 bp Sequence Detection Primer – 10,000 pmol Plasmodium oval 800 bp	2
5	Séquence Détection Primer – 10,000 pmol Plasmodium vivax 800 bp Sequence Detection Primer – 10,000 pmol Plasmodium vivax 800 bp	2
6	Distillateur (Eau stérile) Distiller (sterile water)	1
7	Incubateur bactériologique Incubator bacteriological	1
8	Piston Pellet a usage unige en PP, pour microtubes 1,5 ml Single-use pellet piston PP, for microtubes 1,5 ml	1
9	Microtube ClearLine 1,5 ml à capuchon Snap Microtube ClearLine 1,5 ml hooded Snap	35
10	ABI MicroAmp Optical 96-Well Reaction Plate Thermo Scientific lot de 10 plaques ABI MicroAmp Optical 96-Well Reaction Plate Thermo Scientific lot 10 plates	10
11	Film Adhesif Optique MicroAmp Thermo Scientific pour Appareil PCR en temps réel QS3 Plaques de 100 films Film Adhesif Optique MicroAmp Thermo Scientific for engine PCR real-time QS3 Plates to 100 films	1
12	Pointes à filtre Multiguard MµIti 1 -20 µL Head filter Multiguard MµIti 1 -20 µL	3
13	Pointes à filtre Multiguard MµIti 1 -200 µL Head filter Multiguard MµIti 1 -200 µL	
14	Pointes Multiguard 1000 µL sur support à Couvercle Coiffant Multi-sur rack de 100 pointes Multiguard 1000 µL tips on multi-styling cover support on rack of 100 tips	3
15	Pointes types Gilson pour micropipettes, en vrac 0,5 -10 µL lot de 1000 Gilson type tips for micropipettes, bulk 0.5 -10 µL pack of 1000	3
16	Pointes types Gilson pour micropipettes, en vrac 2 -200 µL lot de 1000 Gilson type tips for micropipettes, bulk 2 -200 µL pack of 1000	3
17	Pointes types Gilson pour micropipettes, en vrac 100 -1000 µL lot de 1000 Gilson type tips for micropipettes, bulk 100 -1000 µL pack of 1000	3
18	Micropipette monocanal Thermo Labsystems Finnpipette F2 Plaque De volume 0,2 – 2 µL Thermo Labsystems Finnpipette F2 single-channel micropipette Plate Volume 0.2 - 2 µL	3
19	Micropipette monocanal Thermo Labsystems Finnpipette F2 Plaque De volume 2 – 20 µL Thermo Labsystems Finnpipette F2 single-channel micropipette Plate Volume 2 - 20 µL	3
20	Micropipette monocanal Thermo Labsystems Finnpipette F2 Plaque De volume 20 – 200 µL Thermo Labsystems Finnpipette F2 single-channel micropipette Plate Volume 20 - 200 µL	3
21	Micropipette monocanal Thermo Labsystems Finnpipette F2 Plaque De volume 100 – 1000 µL Thermo Labsystems Finnpipette F2 single-channel micropipette Plate Volume 100 - 1000 µL	3

Table A-2: List of equipment and materials existing in the laboratory

Item	Quantity	Status
Générateur Generator	1	Good Condition
Onduleur pour 4000 et 5000 Watt Inverter for 4000 and 5000 Watt	2	Good Condition
Spectrophotomètre, calibration de 450 à 660 nanomètres Ou Biotek Spectrophotometer, calibration from 450 to 660 nanometers Or Biotek	1	Good Condition
Ordinateur de bureau avec des accessoires Desktop computer with accessories	1	Good Condition
Stabilisateur et 2 thermomètres muraux Stabilizer and 2 wall thermometers	1	Good Condition
Réfrigérateur pour chaine de froid Refrigerator for cold chain	4	Good Condition
Etagères Shelves	5	Good Condition
Louches pour la prospection larvaire Mosquito dippers for larval prospecting	12	Good Condition
Bain Marie Water bath	1	Good Condition
Grand seau (60 litres) Large bucket (60 liters)	2	Good Condition
Petits pots Small jars	16	Good Condition
Tables Tables	4	Good Condition
Selles Stools	10	Good Condition
Chaises roulantes Rolling chairs	3	Good Condition
Manteaux de laboratoire Lab coats	14	Good Condition
Loupe binoculaire Binocular magnifier/dissecting microscope	3	Good Condition
Microscope Light Microscope	1	Good Condition
Centrifugeuse électrique Electric centrifuge	1	Good Condition
Pipette électrique Electric pipette	1	Good Condition
Accumet XL 200 avec accessoires Accumet XL 200 with accessories (pH meter)	1	Good Condition
Ficher scientifique isotherme Ficher scientifique isotherme	2	Good Condition
Hotte pour surface chauffante Extractor hood	1	Good Condition
Appareil millipore Millipore device	1	Good Condition

Item	Quantity	Status
Beacher 1000 ml (Ficher brand 1000 ml)	12	Good Condition
Beacher 600 ml	15	Good Condition
Ficher 300 ml	15	Good Condition
Pyrex 100 ml	12	Good Condition
Flacon 250 ml	9	Good Condition
Beacher 100 ml	74	Good Condition
Baecher 300 ml	44	Good Condition
Accumet portable Portable accumet (pH meter)	2	Good Condition
Kit d'étude entomologique Entomological study kit	3	Good Condition
Blouse de protection Protective gown	8	Good Condition
Aspirateur mécanique Mechanical aspirator	3	Good Condition
Cones D10, D200, D300	1	Good Condition
Porte tube Tube holder	1	Good Condition
Lambeau Flap	1	Good Condition
Chargeur de batterie Battery charger	11	Good Condition
Congélateur LIEBHERR GG 4010 (frigo -20 degré) LIEBHERR GG 4010 freezer (fridge -20 degree)	1	Good Condition
Hotte PCR UVC-T-M-AR avec UV et système de recirculation d'air anti bactérien UVC-T-M-AR PCR hood with UV and anti bacterial air recirculation system	1	Good Condition
Machine à glace GB902, glace grains, avec resserve GB902 ice machine, grain ice, with refill	1	Good Condition
Système d'imagerie Vilber lourmat E-B-X CX5TS Edge Vilber lourmat E-B-X CX5TS Edge Imaging System	1	Good Condition
Cuve d'électrophorèse APELEX Midigel 2 Complete APELEX Midigel 2 Complete electrophoresis tank	1	Good Condition
Thermomètre intérieur /sonde extérieur Indoor thermometer / outdoor probe	1	Good Condition
Baro magnétique à baque pivot Magnetic baro with swivel base	10	Good Condition
Soudeuse a impulsion manuelle Hawo HD260MS8 Hawo HD260MS8 manual impulse welder	1	Good Condition
Trans illuminateur TFS-20M Edge UV PAD 20M Trans illuminator TFS-20M Edge UV PAD 20M	1	Good Condition
Balance d'analyse Kerm ALJ 250-4A Kerm ALJ 250-4A analytical balance	1	Good Condition

Annex B. Mapping of Larval Breeding Sites

Villages	Laval sites	Types	Points GPS		Positive sites	Negative sites	Sub-family
			Latitude	Longitude			
Balayani	1	Road puddle	10°7.9140	10°44.6780	+		Anophelinae
	2	Road puddle	10°7.9000	10°44.6760	+		Anophelinae
	3	Road puddle	10°7.9240	10°44.6740	+		Anophelinae + Culicinae
	4	Road puddle	10°7.9380	10°44.6760		-	
	5	Road puddle	10°7.9520	10°44.6760		-	
	6	Road puddle	10°7.9800	10°44.6770		-	
	7	Road puddle	10°7.8890	10°44.6740		-	
	8	Road puddle	10°7.8800	10°44.6720		-	
	9	Road puddle	10°7.8730	10°44.6720		-	
	10	Bas-fond Lowland ground pools	10°7.8640	10°44.6700		-	
	11	Bas-fond Lowland ground pools	10°7.8910	10°44.6470		-	
	12	Road puddle	10°7.597795	10°44.409321	+		Anophelinae
	13	Road puddle	10°7.595478	10°44.407921	+		Anophelinae
	14	Road puddle	10°7.563762	10°44.407475	+		Anophelinae
	15	Bas-fond	10°7.8790	10°44.7480		-	
Foulaya	1	Road puddle	10°83.788729	10°44.5984851		-	
	2	Road puddle	10°83.64797	10°44.581712	+		Culicinae
	3	Road puddle	10°83.66702	10°44.585048	+		Culicinae
	4	Road puddle	10°83.69288	10°44.58717	+		Culicinae
	5	Road puddle	10°83.6702	10°44.58119		-	
	6	Road puddle	10°84.67705	10°44.58770		-	
	7	Road puddle	10°85.28448	10°44.592594		-	
Tindo	1	Bas-fond	10°2.31664	10°44.140361	+		Anophelinae + Culicinae
	2	Road puddle	9°5752.4413	10°4146.4394	+		Anophelinae + Culicinae
	3	Road puddle	9°5752.0105	10°4146.7701	+		Anophelinae + Culicinae
	4	Road puddle	9°5751.1326	10°4145.7651		-	

Villages	Laval sites	Types	Points GPS		Positive sites	Negative sites	Sub-family
			Latitude	Longitude			
	5	Road puddle	9°57'50.4686"	10°41'45.4329"	+		Culicinae
	6	Road puddle	9°56'40.7833"	10°41'57.1851"		-	
	7	Road puddle	9°56'41.4874"	10°41'57.958"		-	
Centre ville	1	Road puddle	10°22'6.0268"	10°44'46.8056"	+		Anophelinae + Culicinae
	2	Road puddle	10°22'4.1991"	10°44'47.7825"	+		Anophelinae + Culicinae
	3	Road puddle	10°22'8.9125"	10°44'48.6261"	+		Anophelinae + Culicinae
	4	Road puddle	10°2'43.1100"	10°44'18.6600"		-	
	5	Road puddle	10°2'32.1122"	10°44'14.6052"		-	
	6	Road puddle	10°3'31.8597"	10°44'15.5200"		-	
	7	Road puddle	10°2'29.7107"	10°44'43.1911"	+		Culicinae
	8	Road puddle	10°2'34.4735"	10°44'47.1270"	+		Anophelinae + Culicinae
	9	Road puddle	10°2'35.6198"	10°44'48.8204"	+		Anophelinae
	10	Road puddle	10°2'23.6220"	10°44'56.1933"	+		Anophelinae + Culicinae
	11	Road puddle	10°2'50.6583"	10°45'41.6917"		-	
	12	Road puddle	10°2'21.5092"	10°45'41.6917"	+		Anophelinae
	13	Road puddle	10°2'21.4858"	10°45'41.7117"		-	
	14	Road puddle	10°2'19.8315"	10°45'41.9183"		-	
	15	Road puddle	10°2'19.8315"	10°45'41.9183"		-	
	16	Road puddle	10°2'15.9548"	10°45'55.1549"		-	
	17	Road puddle	10°1'13.5142"	10°44'16.4448"	+		Culicinae
	18	Road puddle	10°0'53.1736"	10°44'24.6822"	+		Anophelinae + Culicinae
	19	Rice field	10°1'35.1347"	10°44'12.6900"	+		Anophelinae
	20	Road puddle	10°2'21.2885"	10°44'13.1040"		-	
	21	Road puddle	10°2'48.0165"	10°43'50.7327"		-	
	22	Road puddle	10°3'11.92923"	10°45'5.89295"		-	

Annex C. Details of Houses Visited, Numbers of LLINs, and State of Mosquitoes

Villages	N° House	Resident sleepers	Number of LLINs	Anophelinae				Total	Culicinae				Total	Total mosquitoes				Total
				Gravid	Semi-gravid	Fed	Unfed		Gravid	Semi-gravid	Fed	Unfed		Gravid	Semi-gravid	Fed	Unfed	
Balayani	1	2	3	0	8	22	18	48	0	0	1	0	1	0	8	23	18	49
	2	6	0	0	20	33	13	66	0	0	0	0	0	0	20	33	13	66
	3	2	1	0	0	0	1	1	0	0	0	0	0	0	0	0	1	1
	4	3	1	0	0	0	1	1	0	0	0	0	0	0	0	0	0	1
	5	8	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	6	6	1	0	0	0	38	38	0	0	0	0	0	0	0	0	38	38
	7	2	1	0	0	5	4	9	0	0	0	0	0	0	0	5	4	9
	8	4	3	0	0	3	1	4	0	0	0	0	0	0	0	3	1	4
	9	2	1	0	0	13	17	30	0	0	0	0	0	0	0	13	17	30
	10	1	1	0	0	2	4	6	0	0	0	0	0	0	0	2	4	6
		36	14	0	28	78	97	203	0	0	1	0	1	0	28	79	96	204

Villages	N° House	Resident sleepers	Number of LLINs	Anophelinae				Total	Culicinae				Total	Total mosquitoes				Total
				Gravid	Semi-gravid	Fed	Unfed		Gravid	Semi-gravid	Fed	Unfed		Gravid	Semi-gravid	Fed	Unfed	
Foulaya	1	5	2	0	0	0	6	6	0	0	0	0	0	0	0	0	6	6
	2	1	1	0	0	0	6	6	0	0	0	0	0	0	0	0	6	6
	3	4	1	0	0	0	0	0	0	0	0	1	1	0	0	0	1	1
	4	3	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	5	6	1	0	0	9	3	12	0	0	0	0	0	0	0	9	3	12
	6	4	1	0	0	3	0	3	0	0	0	0	0	0	0	3	0	3
	7	3	1	0	0	19	5	24	0	0	0	0	0	0	0	19	5	24
	8	7	0	0	0	8	2	10	0	0	0	0	0	0	0	8	2	10
	9	6	1	0	0	7	3	10	0	0	0	0	0	0	0	7	3	10
	10	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		40	9	0	0	46	25	71	0	0	0	1	1	0	0	46	26	72

Villages	N° House	Resident sleepers	Number of LLINs	Anophelinae				Total	Culicinae				Total	Total mosquitoes				Total
				Gravid	Semi-gravid	Fed	Unfed		Gravid	Semi-gravid	Fed	Unfed		Gravid	Semi-gravid	Fed	Unfed	
Tindo	1	2	0	0	0	12	5	17	0	0	2	0	2	0	0	14	5	19
	2	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	3	1	1	0	0	0	0	0	0	0	0	1	0	0	0	0	1	1
	4	1	1	0	0	0	11	11	0	0	0	0	0	0	0	0	11	11
	5	2	0	0	0	0	1	1	0	0	0	0	0	0	0	0	1	1
	6	2	0	0	0	34	5	39	0	0	0	0	0	0	0	34	5	39
	7	2	1	0	0	4	0	4	0	0	0	0	0	0	0	4	0	4
	8	4	1	0	0	9	0	9	0	0	0	0	0	0	0	9	0	9
	9	1	1	0	0	4	0	4	0	0	0	0	0	0	0	4	0	4
	10	2	0	0	0	1	5	6	0	0	0	0	0	0	0	1	5	6
		18	6	0	0	64	27	91	0	0	2	1	2	0	0	66	28	94

Villages	N° House	Resident sleepers	Number of LLINs	Anophelinae				Total	Culicinae				Total	Total mosquitoes				Total
				Gravid	Semi-gravid	Fed	Unfed		Gravid	Semi-gravid	Fed	Unfed		Gravid	Semi-gravid	Fed	Unfed	
Faranah centre ville	1	3	1	0	0	0	0	0	0	0	5	0	5	0	0	5	0	5
	2	4	1	0	0	0	0	0	0	0	19	30	39	0	0	19	30	49
	3	3	1	0	0	1	0	1	0	0	1	0	1	0	0	2	0	2
	4	2	1	0	0	0	0	0	0	0	2	0	2	0	0	2	0	2
	5	2	0	0	0	0	0	0	0	0	7	2	9	0	0	7	2	9
	6	5	1	0	0	9	0	9	0	0	3	6	9	0	0	12	6	18
	7	1	0	0	0	2	0	2	0	0	5	19	24	0	0	7	19	26
	8	1	1	0	0	0	0	0	0	0	4	0	4	0	0	4	0	4
	9	1	1	0	0	0	0	0	0	0	6	3	9	0	0	6	3	9
	10	4	1	0	0	4	0	4	0	0	0	0	0	0	0	4	0	4
01		26	8	0	0	16	0	16	0	0	52	60	102	0	0	68	60	128

Annex D: HBR (bites/person/night) of Mosquitoes Collected in Faranah

-	*An. gambiae* s.l.				Culicinae spp.				*Aedes* spp.		
Site	**Int.**	**Ext.**	**Total**		**Int.**	**Ext.**	**Total**		**Int.**	**Ext.**	**Total**
Balayani	73.5	135.5	104.5		0.5	0.0	0.3		0.0	1.0	0.5
Foulaya	111.5	112.5	112.0		6.0	2.5	4.3		0.0	1.5	0.8
Tindo	91.0	60.5	75.8		2.0	8.0	5.0		0.5	0.0	0.3
City center	7.5	10.5	9.0		52.0	78.0	65.0		0.0	0.0	0.0
All sites	**70.9**	**79.8**	**75.3**		**15.1**	**22.1**	**18.6**		**0.1**	**0.6**	**0.4**

Annex E: Mosquito Trap Rates (mosquitoes/trap/night) of Mosquitoes Collected in Faranah

-	*An. gambiae* s.l.				Culicinae spp.				*Aedes* spp.		
Site	**Int.**	**Ext.**	**Total**		**Int.**	**Ext.**	**Total**		**Int.**	**Ext.**	**Total**
Balayani	4.5	4.5	4.5		1.0	3.5	2.3		0.0	0.5	0.3
Foulaya	12.0	28.5	20.3		1.0	8.5	4.8		0.0	0.0	0.0
Tindo	4.5	7.5	6.0		1.5	8.5	5.0		1.0	0.5	0.8
City center	1.0	0.0	0.5		13.0	26.5	19.8		0.0	0.5	0.3
All sites	**5.5**	**10.1**	**7.8**		**4.1**	**11.8**	**7.9**		**0.3**	**0.4**	**0.3**