USAID | StopPalu

ANNUAL REPORT OF ENTOMOLOGICAL MONITORING ACTIVITIES YEAR 5

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ANNUAL REPORT OF ENTOMOLOGICAL MONITORING ACTIVITIES YEAR 5

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Abbreviations

CDC	Centers for Disease Control and Prevention
CREC	Centre de Recherche Entomologique de Cotonou
CSP	circumsporozoite protein
ELISA	enzyme-linked immunosorbent assay
FY	fiscal year
GFATM	Global Fund to Fight AIDS, Tuberculosis and Malaria
GOG	Government of Guinea
HLC	human landing collection
IRS	indoor residual spraying
kdr	knock down 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
PCR	polymerase chain reaction
PMI	President's Malaria Initiative
PSNLIV	National Strategic Plan for Integrated Vector Management 2018–2022
RR	Homozygous for the kdr mutation
RS	Heterozygous for the kdr mutation
SS	Homozygote mutation for the kdr mutation
UGANC	Gamal Abdel Nasser University of Conakry
USAID	United States Agency for International Development
WHO	World Health Organization
WHOPES	WHO Pesticide Evaluation Scheme

Executive Summary

The President's Malaria Initiative (PMI) Program Component (*StopPalu*) activities are implemented to reduce the malaria burden in Guinea. These activities include building in-country capacity to perform entomological surveys. During November 1, 2016–October 31, 2017 (the period covered by this annual report), *StopPalu* supported the National Malaria Control Program (NMCP) to perform field activities and to establish an insectary and a laboratory to conduct entomological survey activities. *StopPalu* recruited two entomologists, procured necessary equipment, and hired an international consultant. The project trained NMCP staff to operate the insectary and the laboratory and to perform field activities. The aim of *StopPalu*'s entomological focus is to support the NMCP in developing a National Vector Control Strategy to control vector abundance, identify areas affected by insecticide resistance, and implement the most appropriate interventions.

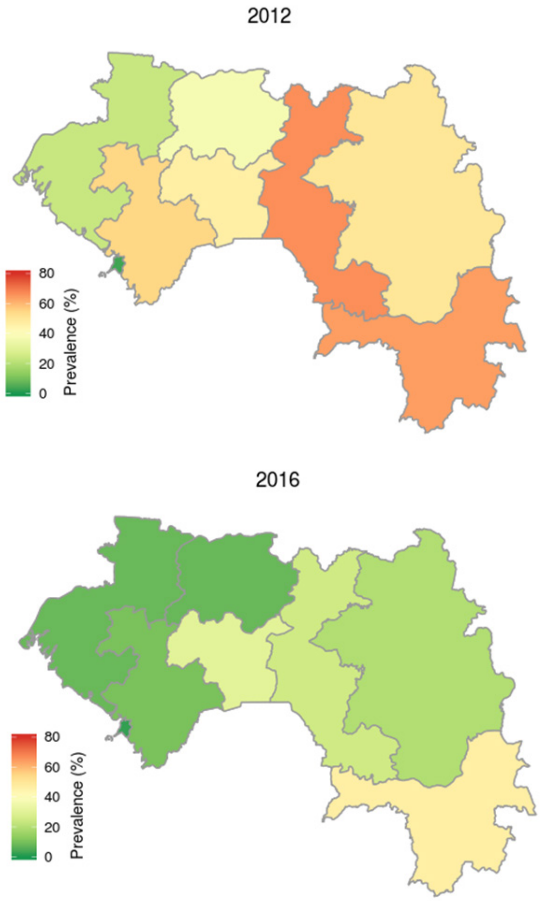
During the first year of supporting in-country entomological activities, *StopPalu* accomplished the following:

- Built an insectary and associated laboratory, which are fully operative
- Built an enclosure housing rabbits to support mosquito production
- Bred Kisumu strain mosquitoes for multiple generations
- Identified sentinel sites in Boké, Kankan, Kissidougou, Labé, Dabola, and Faranah where mosquito collection was performed to facilitate national entomological surveillance
- Developed a National Vector Control Strategy covering the period 2018–2022, which also includes an insecticide management plan to address the spread of insecticide-resistant vectors

1 Background

In the last five years, Guinea has made tremendous progress in its fight against malaria, leading to a reduction in malaria prevalence in children under 5 years of age, annual malaria incidence, and in-patient deaths (***Figure 1***).[1,2,3] Progress was such that in 2016, the Government of Guinea (GOG) received an Excellence Award from the African Leaders Malaria Alliance for its fight against malaria.[4] Much of this progress is due to the GOG's leadership and commitment to scale up key malaria interventions, 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: Malaria prevalence in children under 5 years of age



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.[5] These challenges invariably impact programmatic intervention coverage and effectiveness. Consequently, malaria remains the most burdensome communicable disease in Guinea: the entire 12.1 million population is at risk, with approximately 1 million cases reported in 2016, accounting for 31% 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) and, to a very limited extent, indoor residual spraying (IRS) with propoxur, deltamethrin, and pirimiphos-methyl.

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1 Institut National de la Statistique and MEASURE DHS (2012) *Enquête Démographique et de Santé et à Indicateurs Multiples (EDS-MICS 2012)*. Conakry, Guinée, pp. 1–528.

2 Institut National de la Statistique (2016) *Enquête par Grappes à Indicateurs Multiples avec Volet Renforcé sur le Paludisme (MICS-Palu, 2016)*. Conakry, Guinée.

3 Ministry of Health (MOH) (2017) *Plan Stratégique National de Lutte Contre le Paludisme 2018–2022*. Conakry, Guinée, pp. 1–69.

4 African Heads of State Celebrate Progress Against Malaria - PMI

5 MOH (2017) *Revue des Performances du Plan Stratégique de Lutte contre le Paludisme 2013–2017*. Conakry, Guinée, pp. 1–75.

The resistance status of malaria vectors in Guinea was recently evaluated in six sites (Boké, Labé, Maferinyah, Faranah, Kankan, and Kissidougou).[6] Resistance to DDT, permethrin, alpha-cypermethrin, and lambda-cyhalothrin was detected in all *An. gambiae s.l.* populations tested; populations were susceptible to deltamethrin and bendiocarb in two out of three sites sampled. The kdr (knock down resistance) West mutation was widespread, and the 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 reported for deltamethrin and malathion (unspecified doses).[7]

2 *StopPalu* Results Framework

PMI's main operational malaria platform from 2013 onward has been the RTI International-led USAID Guinea *StopPalu* project. *StopPalu*'s goal is to assist the GOG to achieve the PMI target of reducing malaria-related mortality by 50%, compared with pre-initiative levels, by (1) improving malaria prevention in support of the National Malaria Strategy; (2) improving diagnostic testing and malaria treatment capacity; and (3) enhancing the NMCP's technical capacity to plan, design, manage, and coordinate a comprehensive malaria control program.

The overarching aims of *StopPalu*-supported entomological activities 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:

Objective 1: Build in-country entomological capacity by

- Establishing an entomology laboratory and insectary in Conakry
- Establishing an *An. gambiae* (Kisumu strain) colony
- Training NMCP and *StopPalu* staff in CSP-ELISA (circumsporozoite protein / enzyme-linked immunosorbent assay)
- Training student interns
- Supporting Vector Control Technical Working Group meetings
- Developing national vector control strategies and policies

Objective 2: Conduct entomological surveillance by

- Performing mosquito collection in selected sentinel sites
- Assessing vector distribution and abundance
- Assessing vector biting behavior

Objective 3: Determine insecticide resistance of malaria vectors by

- Monitoring phenotypic resistance
- Monitoring genotypic resistance

[6] Keita K *et al.* (2017) Species identification and resistance status of *Anopheles gambiae s.l.* (Diptera: Culicidae) mosquitoes in Guinea. *J Med Entomol. 54*: 677–681.

[7] AngloGold Ashanti (2007), unpublished.

3 Achievements

3.1 Objective 1: Build in-country entomological capacity

Establishment of an entomology laboratory and insectary in Conakry

In November 2016, *StopPalu* recruited Professor Martin Akogbeto, Director of the Centre de Recherche Entomologique de Cotonou (CREC) in Benin, as a consultant to support the establishment of a laboratory and insectary at Gamal Abdel Nasser University of Conakry (UGANC). During the two weeks of engagement, Prof. Akogbeto carried out the following:

- Supported the operational set-up of the UGANC entomological laboratory and insectary, including installation of equipment procured by *StopPalu*;
- Developed an inventory list of all the equipment and materials needed for the continued maintenance and operational functioning of the laboratory and insectary (see **Annex**);
- Supported recruitment of two *StopPalu* entomological technicians;
- Conducted a field visit to Maferinyah to collect mosquitoes;
- Conducted entomological training for NMCP and *StopPalu* staff to ensure continuous functioning of the laboratory and insectary.

As result of these activities, the insectary and the laboratory are now fully functional for performing entomological surveys. A detailed description of the insectary and laboratory follows.

Laboratory

Physical space to establish a laboratory and insectary was made available at UGANC in 2016, the rehabilitation of which was supported by *StopPalu* with PMI support (***Figure 2***). The laboratory is composed of two large air-conditioned, ventilated, and equipped rooms with shelves and large tables. The available equipment and materials are being used to carry out entomological surveillance and insecticide resistance testing. The first room serves as an entomology laboratory: it is equipped with two binocular microscopes, one remote temperature measuring device, two kits for measuring physico-chemical parameters of water (Accumet™ portable laboratory), and one giant suction pump supplied with batteries and chargers for sampling adult mosquitoes. The second room is reserved for molecular biology and biochemistry testing. Equipment and materials allow for the following analyses: CSP-ELISA to determine anopheles infection rates, blood meal ELISA to identify mosquito host preference, polymerase chain reaction (PCR) testing to perform species identification of anopheline mosquitoes, and PCR and biochemical tests to identify insecticide resistance mechanisms (kdr allele frequencies and insecticide detoxification enzyme activity).

Figure 2: UGANC entomological laboratory and insectary

Insectary

The insectary consists of a large room divided into two compartments (compartments A and B). Compartment A is assigned to be the adult breeding room. It includes two large shelves holding approximately 20 adult holding cages. Compartment B of the insectary is assigned to breeding mosquito larvae. This room is air-conditioned to maintain a suitable temperature for the development of mosquitoes (25°–28°C). The first breeding activities started with rearing *An. gambiae s.s.* Kisumu strain of mosquitoes brought from Cotonou (Benin). An animal enclosure is annexed to the insectary room; the enclosure hosts two pairs of rabbits used to feed adult mosquitoes.

The entomological equipment and lab materials are summarized in Annex tables.

Since the official opening of the laboratory and insectary, several activities have been undertaken, including (1) breeding the *An. gambiae s.s.* Kisumu susceptible strain; (2) training staff on performing ELISA testing; (3) holding meetings, organized by staff members, with the NMCP Vector Control Unit; and (4) training student interns.

Establishment of *an. gambiae s.s.* (Kisumu strain) colony

An. gambiae s.s. are being reared as per standard procedures[8] (***Figure 3***):

- Female mosquitoes are allowed to take a blood meal from a rabbit immobilized on a device designed for this purpose and placed at the top of the cage in which the mosquitoes are housed. Mosquitoes are fed almost seven days after emergence.
- Mosquitoes are transferred in an oviposition tub after the second feeding; egg laying happens after 24 or 48 hours.
- Eggs are transferred into labeled water tanks filled with 500 ml of spring water to allow hatching.
- Hatched larvae are transferred to tanks and categorized according to their stage of development; tetramin fish food is fed to mosquito larvae.
- When mosquitoes reach the nymphal stage, they are transferred to the rearing cages for emergence.
- Adults are kept in holding cages until use and provided with water and sugar solution ad libitum.

Figure 3: Breeding An. gambiae s.s. at the UGANC insectary

During the reporting period, the laboratory produced 14 mosquito generations.

Training of NMCP and *StopPalu* staff on CSP-ELISA

From July 14 to 26, 2017, five staff members (two from the NMCP, two from *StopPalu*, and one from the National Institute of Public Health) received training on how to perform the CSP-

[8] Das S, Garver L, and Dimopoulos G (2007) Protocol for mosquito rearing (*A. gambiae*). *J Vis Exp*: 221.

ELISA to determine mosquito positivity for *Plasmodium falciparum* (sporozoite index). The training was performed at the laboratory in Conakry by CREC's Head of the Laboratory of Molecular Biology Mr. Aboubakar Sidick. During this training, the following topics were covered:

- Preparation of buffers and solutions for the testing
- Reconstitution of lyophilized monoclonal antibodies
- Cutting the heads and thoraxes of mosquitoes
- Manipulation and preparation of ELISA plates
- Reading and interpreting ELISA results

A total of 685 *An. gambiae* gathered from human landing collections (HLCs) and pyrethrum spray collection methods were tested using CSP-ELISA (see below).

Training on malaria entomology

From October 23 to December 3, 2017, four technicians—three regional staff (Kissidougou, Boké, and Labé) and one from *StopPalu*—attended a six-week training on basic techniques in malaria entomology at CREC. The training focused on the following:

- General behavior and bioecology of malaria vectors
- Realization of mosquito breeding at the insectarium
- Vector susceptibility tests for different insecticides
- Determination of physiological age through ovarian dissection
- Performing tunnel tests
- Evaluation of the effectiveness of mosquito nets and other impregnated equipment
- Identification of the different *Anopheles* species (larvae and adults)
- Knowledge of different sampling methods

Training of student interns

UGANC students interested in a career in entomology took a test to determine their eligibility for training. Ten candidates took the test, which was designed to determine their knowledge of computer science, level of study, availability, knowledge of insect vectors, and predisposition to work outside Conakry for periods ranging from two to four weeks.

Six candidates were selected for an internship (***Figure 4***), and these interns were trained in all techniques for breeding mosquitoes and maintaining an insectary. Interns supported the maintenance of the insectary and various entomological surveillance efforts.

Figure 4: Student interns at the insectary

Support of Vector Control Technical Working Group meetings

In fiscal year (FY) 2017, *StopPalu* supported the NMCP Vector Control Unit to organize eight monthly meetings. During these meetings, vector control activities for past months/quarters were reviewed and discussed, and activities for upcoming months/quarters were planned.

Development of national vector control strategies and policies

Development of the integrated National Vector Control Strategy (2018–2022)

In March 2017, *StopPalu* recruited Prof. Martin Akogbeto as a consultant to support the development of the National Vector Control Strategy. During his visit, he implemented the following tasks:

- Conducted an analysis of the situation based on the available information on vector control in Guinea;
- Consulted the NMCP Vector Control Unit, the technical working group, and other stakeholders involved in malaria vector control;
- Developed the strategic framework, interventions, and strategies to be implemented;
- Incorporated comments and recommendations from various stakeholders, finalized the new National Strategic Plan for Integrated Vector Management (PSNLIV) 2018–2022, and submitted the final version of the plan to the NMCP and *StopPalu* (see ***Box 1***).

Box 1. PSNLIV 2018–2022

Objectives outlined in the PSNLIV 2018–2022 are:

- Ensure universal access to prevention measures against mosquito bites (LLINs, IRS of households with insecticide, larviciding, environmental management).
- Ensure availability of LLINs.
- Strengthen communication for changing people's behavior with a view to increased use of mosquito bite prevention measures.
- Strengthen the coordination and management capacities of the NMCP vector control service at all levels.
- Strengthen partnerships in the country to mobilize substantial funding through the budget of the State, private sector, and other partners.

Five implementation strategies ("axes") will be carried out to achieve the PSNLIV objectives. Each axis includes strategic approaches and implementation modalities.

- Strategic axis 1: Strengthening legislation on integrated vector control
- Strategic axis 2: Strengthening vector control
- Strategic axis 3: Strengthening vector surveillance
- Strategic axis 4: Strengthening monitoring and evaluation
- Strategic axis 5: Strengthening national capacities in human, material, and financial resources as well as research promotion

Because of the celebration of the World Malaria Day, the validation workshop was not held in April 2017 as planned. The draft of the strategic plan was reviewed during the two-day workshop organized on May 4–5, 2017, in Dubréka. The comments from the workshop were sent to Prof. Akogbeto, who incorporated them into the final document. The final version of the PSNLIV 2018–2022 is available at the NMCP.

Development of the National Insecticide Resistance Management Plan

In July 2017, S*topPalu* supported the NMCP to develop a National Insecticide Resistance Management Plan. The purpose of this plan is to propose vector control strategies based on

the rational use of insecticides in order to limit the expansion of vector insecticide resistance (see ***Box 2***).

Box 2. National Insecticide Resistance Management Plan

Strategic Framework of the Plan

1. Strategic approaches

Capacity building in the area of resistance management:

For a sound implementation of resistance management, the plan provides for reinforcement of human resources (training a socio-anthropologist for the central level and two technicians on basic techniques in medical entomology by region). To avoid the problem of recruitment after training, candidates could be identified among the staff working at the NMCP and in the health facilities.

Resistance monitoring:

Monitoring of resistance and the mechanisms involved is already being done, but the plan proposes that the monitoring be strengthened, taking into account the ecological facies according to the following plan:

- Update existing data, taking into account all of Guinea's regions and various ecological zones (urban areas, rural areas, agricultural zones [rice-growing perimeters, vegetable gardens, cotton zones], areas with high use of insecticides in public health)
- Update the distribution insecticide resistance map
- Monitor the evolution of resistance and resistance mechanisms
- Record resistance data in the NMCP database
- Manage resistance

The theory on the management of insecticide resistance in vectors is known: rotation or mixture of insecticides with different modes of action, use of insecticides in mosaics, and a combination of interventions.[9] Among the measures to be considered by the NMCP are the following:

- The criteria and method for selecting an insecticide before use will be made by a competent structure, and it will be ensured that the product selected is already approved by the WHO Pesticide Evaluation Scheme (WHOPES)10 and has proved its effectiveness on target mosquitoes. The choice will be validated by the Central Coordination Unit for Integrated Vector Control.
- The Vector Control Technical Working Group will be responsible for vector control.
- Any control intervention in the field of mosquito bite prevention will be submitted to the Central Coordination Unit and will receive approval. The creation, composition, and functioning of the Coordination Unit are provided for in the PSNLIV 2018–2022.

[9] World Health Organization (WHO) (2012) *Global plan for insecticide resistance management in malaria vectors*. https://apps.who.int/iris/bitstream/handle/10665/44846/9789241564472_eng.pdf?sequence=1

[10] Now WHO pre-qualification process.

2. Vector control interventions selected

Interventions are chosen with full consideration of judicious insecticide use. The management of resistance involves reducing the amount of insecticide used in a given area and the need to avoid exposing mosquitoes to the same products over several years. For this reason, the plan establishes an integrated vector management strategy by combining commonly used chemical methods with non-chemical methods.

i. *Resistance management based on the use of non-chemical methods:*

In large cities, only non-chemical methods of mosquito control will be used to strengthen protection provided by LLIN use, the rationale being to use a minimum of insecticide in densely populated urban areas in order to minimize the environmental impact of insecticide use. The PSNLIV 2018–2022 therefore proposes the management of water sources to prevent the formation of mosquito breeding sites in collaboration with the authorities and local communities.

ii. *Anti-larval control based on bacteria, stopping after two or three years of implementation:*

In rural areas where mosquito breeding sites are small and easily identifiable, the plan also considers non-chemical methods. The method recommended for rural areas is larval control with bacteria (*Bacillus thuringiensis israelensis* [Bti], *Lysinibacillus sphaericus*[11]), which the PSNLIV 2018–2022 proposes be carried out in collaboration with communities. Since this method cannot be applied systematically in all eligible areas, the plan provides for stopping the intervention every two or three years to avoid a possible emergence of mosquito resistance to the bacteria used and allow the extension from intervention to other areas. The shutdown period will be alternated with a massive distribution of LLINs to the whole population, followed by awareness raising for better use of these nets.

iii. *Chemical methods:*

Promotion of LLINs: Currently, the use of mosquito nets is the main means of prevention against malaria in Guinea; LLINs are WHO-approved, with most being pyrethroid based.

IRS with insecticide rotation and withdrawal periods: The plan calls for IRS in targeted areas (areas with a short peak in malaria transmission, malaria pre-elimination areas, refugee camps, and during "malaria epidemic" periods). Two non-pyrethroid products with different modes of action will be used in rotation. The two products currently being used are bendiocarb and pirimiphos-methyl, and both are—to date—effective against malaria vectors. Both products will be used in rotation for three consecutive years each.

iv. *Combination of interventions:*

If necessary, intervention combinations (e.g., LLINs and IRS) will be considered in zones of strong malaria recrudescence or epidemics. Due to the strong resistance of vectors to pyrethroids, IRS will be performed with non-pyrethroid products.

[11] *L. sphaericus* was reclassified; it was previously known as *Bacillus sphaericus*. (https://ijs.microbiologyresearch.org/content/journal/ijsem/10.1099/ijs.0.63867-0)

3. Monitoring and evaluation framework

Entomological surveillance, the monitoring of resistance and resistance mechanisms, the implementation of control interventions, and their monitoring and evaluation are the direct responsibility of the NMCP, in collaboration with the research institutions involved in the field of entomology and the Coordination Unit.

3.2 Objective 2: Conduct entomological surveillance

Perform Mosquito collection in selected sentinel sites

Entomological surveillance activities were performed to investigate distribution, abundance, seasonality, behavior, infection rate, and insecticide resistance status of malaria vectors in six selected districts: Boké, Labé, Kankan, Kissidougou, Dabola, and Faranah—districts were selected to represent the four main areas of differing malaria endemicity in Guinea. In each district, three villages with the highest malaria prevalence were selected as sentinel sites (see ***Table 1***).

During the reporting period (November 2016–October 2017), *StopPalu* and the NMCP team conducted quarterly visits to Boké, Kankan, Kissidougou, and Labé sentinel sites; Dabola and Faranah sentinel sites were visited one time each because the objective of these visits was to assess the possibility of conducting IRS.

Table 1: Sentinel sites for entomology surveillance activities in FY 2017

Designation	Regions	Districts	Sentinel sites
Priority sites	Basse Guinée	Boké	Kaboye, Dioumaya, Guilere
	Moyenne Guinée	Labé	Thialy, Banty, Tountouroun
	Haute Guinée	Kankan	Dalabani, Balandou, Makonon
	Guinée Forestière	Kissidougou	Gbangbadou, Kérédou, Tongbèkoro
Secondary sites	Haute Guinée	Dabola	Bissikrima, Saourou, Sognéssa
	Haute Guinée	Faranah	Balayani, Foulaya, Tindo

During the visits to the 18 sentinel sites, three mosquito collection methods were used: (1) HLCs; (2) Centers for Disease Control and Prevention (CDC) light traps; and (3) pyrethrum spray catches (***Table 2***). These methods were carried out to collect information on vector species composition, mapping abundance, physiological status, and host-seeking behavior.

Table 2: Summary of the three quarterly visits to the six sites in FY 2017

Site	Location	Timing of visits								
		Visit #1 (Jan–Mar)			Visit #2 (Apr–Jul)			Visit#3 (Jul–Nov)		
		HLC	LT	PYRT	HLC	LT	PYRT	HLC	LT	PYRT
Priority sites	Boké	6	6	30	6	6	30	6	6	30
	Labé	6	6	30	6	6	30	6	6	30
	Kankan	6	6	30	6	6	30	6	6	30
	Kissidougou	6	6	30	6	6	30	6	6	30
Subtotal (priority)		**24**	**24**	**120**	**24**	**24**	**120**	**24**	**24**	**120**
Secondary sites	Faranah	0	0	0	0	0	0	6	6	30
	Dabola	0	0	0	6	6	30	0	0	0
Subtotal (secondary)		**0**	**0**	**0**	**6**	**6**	**30**	**6**	**6**	**30**
Total		**24**	**24**	**120**	**30**	**30**	**150**	**30**	**30**	**150**

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

Assess vector distribution and abundance

Human landing collections

HLCs are a standard for human-vector contact determination and specifically assess mosquito biting behavior. HLCs were carried out inside and outside houses in each of the 18 selected villages (see photos below). Two collectors were situated inside and outside a house in each site from 1800 to 0700 hrs; a first pair of collectors worked from 1800 to 0100 hrs and a second pair of collectors worked from 0100 to 0700 hrs. Collectors used individual tubes to capture mosquitoes that were trying to bite on exposed legs; tubes were packaged in different plastic bags for each collection hour to determine biting time throughout the night. This activity was conducted in two houses per village and for two nights per visit. Collected mosquitoes were returned to the laboratory for identification to species according to standard morphological keys.

HLC outside a home

HLC inside a home

The results of HLCs are shown in ***Table 3***. Of 8,654 mosquitoes collected across sites, 6,462 (75%) were anopheline mosquitoes—6,250 *An. gambiae s.l.* and 212 *An. funestus*. Among collected anopheline mosquitoes, 3,360 (52.0%) (range across sites: 32.6%–76.9%) were caught during indoor collections. Almost half of all indoor and outdoor HLC-collected *An. gambiae s.l.* (48.5% and 49.5%, respectively) were caught in Kissidougou. While in 10 sites more *An. gambiae s.l.* were caught indoors, in 5 sites more were collected outdoors than indoors; in 3 sites, comparative numbers of *An. gambiae s.l.* were caught indoors and outdoors.

Table 3: Abundance and distribution of mosquitoes collected indoors and outdoors using HLC

Districts	Sentinel sites	HLC results FY 2017							
		An. gambiae s.l.		*An. funestus*		*Culex*		*Aedes* / others	
		Int	Ext	Int	Ext	Int	Ext	Int	Ext
Boké	Kaboye	20	6	0	0	170	159	2	0
	Guillèrè	10	8	0	0	304	85	14	8
	Dioumaya	16	10	0	0	178	260	5	3
	Total	**46**	**24**	**0**	**0**	**652**	**504**	**21**	**11**
Kankan	Dalabani	129	153	1	1	57	38	0	0
	Makono	395	346	1	3	142	147	0	0
	Balandou	156	120	1	0	164	159	0	0
	Total	**680**	**619**	**3**	**4**	**363**	**344**	**0**	**0**
Kissidougou	Gbangbadou	289	207	0	0	12	11	0	0
	Kérédou	444	406	12	15	21	9	0	0
	Tongbèkoro	852	864	2	1	7	13	0	0
	Total	**1,585**	**1,477**	**14**	**16**	**40**	**33**	**0**	**0**
Labé	Banty	18	23	1	0	33	23	0	0
	Tountouroun	29	23	0	0	24	33	0	0
	Thialy	15	31	0	0	28	15	0	0
	Total	**62**	**77**	**1**	**0**	**85**	**71**	**0**	**0**
Dabola	Saourou	140	139	4	3	1	1	0	0
	Sognéssa	222	165	1	0	2	4	2	0
	Bissikrima	132	129	6	9	5	3	0	0
	Total	**494**	**433**	**11**	**12**	**8**	**8**	**2**	**0**
Faranah	Balayani	238	185	4	22	10	3	8	12
	Foulaya	116	129	31	47	2	0	5	9
	Tindo	48	37	27	20	0	0	0	1
	Total	**402**	**351**	**62**	**89**	**12**	**3**	**13**	**22**
Total (interior / exterior)		**3,269**	**2,981**	**91**	**121**	**1,160**	**963**	**36**	**33**
% Indoor		**52%**	**48%**	**43%**	**57%**	**55%**	**45%**	**52%**	**48%**
Overall species totals		**6,250**		**212**		**2,123**		**69**	

CDC light traps

CDC light traps were used to collect indoor and outdoor host-seeking mosquitoes. In each village, two houses were randomly 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.

The results of CDC light trap collections are shown in ***Table 4***. Of 1,967 mosquitoes collected across sites, 633 (32%) were anopheline mosquitoes—554 *An. gambiae s.l.* and 79 *An. funestus*. Among collected anopheline mosquitoes, 416 (65.7%) (range across sites: 36.8%–100%) were caught indoors. Of all *An. gambiae s.l.* collected with CDC light traps indoors and outdoors, 24.3% and 14.1%, respectively, were caught in Kissidougou.

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 indoors and outdoors using CDC light traps

Districts	Sentinel sites	CDC light trap results FY 2017							
		An. gambiae s.l.		*An. funestus*		*Culex*		*Aedes* / others	
		Int	Ext	Int	Ext	Int	Ext	Int	Ext
Boké	Kaboye	7	2	0	0	52	78	0	2
	Guillèrè	2	2	0	0	86	263	0	0
	Dioumaya	3	2	0	0	66	147	0	4
	Total	**12**	**6**	**0**	**0**	**204**	**488**	**0**	**6**
Kankan	Dalabani	8	4	3	1	28	16	0	1
	Makono	36	5	5	4	68	111	0	3
	Balandou	56	3	3	0	65	85	0	0
	Total	**100**	**12**	**11**	**5**	**161**	**212**	**0**	**4**
Kissidougou	Gbangbadou	66	31	0	1	21	18	0	0
	Kérédou	37	28	3	11	14	53	0	0
	Tongbèkoro	51	30	2	5	46	48	0	0
	Total	**154**	**89**	**5**	**17**	**81**	**119**	**0**	**0**
Labé	Banty	0	0	3	0	1	0	0	0
	Tountouroun	1	2	9	5	0	0	1	2
	Thialy	6	1	2	0	0	1	6	1
	Total	**7**	**3**	**14**	**5**	**1**	**1**	**7**	**3**
Dabola	Saourou	19	24	0	0	0	0	0	0
	Sognéssa	35	22	2	1	0	0	0	0
	Bissikrima	5	9	2	3	1	6	0	0
	Total	**59**	**55**	**4**	**4**	**1**	**6**	**0**	**0**
Faranah	Balayani	21	10	1	2	0	3	3	6
	Foulaya	11	8	5	1	6	2	3	4
	Tindo	7	0	5	0	6	7	0	0
	Total	**39**	**18**	**11**	**3**	**12**	**12**	**6**	**10**

Districts	Sentinel sites	CDC light trap results FY 2017							
		An. gambiae s.l.		*An. funestus*		*Culex*		*Aedes* / others	
		Int	Ext	Int	Ext	Int	Ext	Int	Ext
Totals (interior/exterior)		371	183	45	34	460	838	13	23
Percentage indoor		67%	33%	57%	43%	35%	65%	36%	64%
Overall species totals		554		79		1,298		36	

Pyrethrum spray catches

This method of capture consists of collecting adult mosquitoes inside the houses to determine species present indoors and their physiological state. Captures were made in the 18 selected villages; in each village, 10 houses/huts were randomly chosen 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 spray collections in Kankan

The results of pyrethrum spray catches are shown in ***Table 5***. Of 434 female anopheline mosquitoes collected across sites, 420 (96.8%) were *An. gambiae s.l.* and 14 (3.2%) *An. funestus*. Of female *An. gambiae s.l.,* 274 (65.2%) were fed, 60 (14.3%) were unfed, 50 (11.9%) were semi-gravid, and 36 (8.6%) were gravid; more than half (55.5%) of the *An. gambiae s.l.* collected by pyrethrum spray catches were collected in Kankan.

Larvae collection

As part of the mosquito surveillance efforts, a larvae 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 (May–July), with sites selected through searching, either during visits to or when driving to and from sentinel sites. Larvae and pupae 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. Larvae were fed with ground fish food and transferred to standard insect rearing cages after pupation; they were provided with 5%-10% glucose solution ad libitum before being tested.



Collection of larvae in the village of Dabadou (Kankan)

Table 5: Abundance and distribution of mosquitoes collected indoors and outdoors using pyrethrum spray catches

Districts	Sentinel sites	Pyrethrum spray catch results FY 2017										
		An. gambiae s.l.				*An. funestus*				*Information*		
		Unfed	Fed	SG	G	Unfed	Fed	SG	G	Beds	LLINs	Sleepers
Boké	Kaboye	0	1	0	0	0	0	0	0	30	22	46
	Guillèrè	0	1	0	0	0	0	0	0	30	28	34
	Dioumaya	0	2	0	0	0	0	0	0	30	25	31
	Total	**0**	**4**	**0**	**0**	**0**	**0**	**0**	**0**	**90**	**75**	**111**
Kankan	Dalabani	13	56	0	0	0	0	0	0	32	27	80
	Makono	9	78	0	0	0	0	0	0	34	34	75
	Balandou	14	81	0	0	0	0	0	0	40	40	91
	Total	**36**	**215**	**0**	**0**	**0**	**0**	**0**	**0**	**106**	**101**	**246**
Kissidougou	Gbangbadou	1	15	0	1	0	0	0	0	31	25	82
	Kérédou	2	9	6	1	0	0	0	0	31	28	73
	Tongbèkoro	2	2	19	17	0	0	0	0	31	31	83
	Total	**5**	**26**	**25**	**19**	**0**	**0**	**0**	**0**	**93**	**84**	**238**
Labé	Banty	0	1	0	0	0	0	0	0	30	19	61
	Tountouroun	0	2	0	0	0	0	0	0	41	34	80
	Thialy	0	0	0	0	0	0	0	0	36	28	50
	Total	**0**	**3**	**0**	**0**	**0**	**0**	**0**	**0**	**107**	**81**	**191**
Dabola	Saourou	Not Done										
	Sognéssa											
	Bissikrima											
	Total											
Faranah	Balayani	1	18	0	0	1	2	0	0	11	11	25
	Foulaya	11	8	0	0	5	1	0	0	10	9	14
	Tindo	7	0	0	0	5	0	0	0	10	7	29
	Total	**19**	**26**	**0**	**0**	**11**	**3**	**0**	**0**	**31**	**27**	**68**
Totals		**60**	**274**	**50**	**36**	**11**	**3**	**0**	**0**	**427**	**368**	**854**

Assessment of vector biting behavior

An analysis of the results of the vectors' biting behavior using HLC, recorded during hour-long intervals, shows biting behavior inside and outside houses in all six districts from 2000 until 0600 hrs. Whereas peak indoor biting was observed between 0100 and 0200 hrs, peak outdoor biting was observed between 0200 and 0300 hrs (**Figure 5**); note that peak biting times indoors and outdoors varied substantially between districts (***Table 6; Figure 6***).

Table 6: *An. gambiae s.l.* biting behavior as determined using HLCs

Time intervals	Boké		Labé		Kankan		Kissidougou		Dabola		Faranah		Total	
	Int	Ext	Int	Ext	Int	Ext	Int	Ext	Int	Ext	Int	Ext	Int	Ext
1800–1900	0	0	0	0	0	0	5	2	1	0	0	0	6	2
1900–2000	0	0	3	1	6	5	5	7	7	6	3	2	24	21
2000–2100	0	1	2	2	9	12	25	20	15	13	26	4	77	52
2100–2200	2	1	5	5	36	31	45	50	21	20	31	25	140	132
2200–2300	0	0	10	9	68	59	86	75	45	42	43	38	252	223
2300–0000	2	3	10	7	84	66	118	145	49	44	48	46	311	311
0000–0100	10	5	10	8	115	83	179	170	55	51	50	48	419	365
0100–0200	7	5	8	10	130	98	190	191	69	68	55	51	459	423
0200–0300	11	8	3	16	95	72	212	213	73	72	57	55	451	436
0300–0400	10	1	1	9	61	67	231	210	78	75	61	58	442	420
0400–0500	3	0	6	7	40	72	233	225	50	28	12	10	344	342
0500–0600	1	0	4	2	31	48	192	146	21	9	10	9	259	214
0600–0700	0	0	0	1	5	6	64	23	10	5	6	5	85	40
Total	**46**	**24**	**62**	**77**	**680**	**619**	**1,585**	**1,477**	**494**	**433**	**402**	**351**	**3,269**	**2,981**

Figure 5: Biting behavior *An. gambiae s.l.* (as determined by HLC)

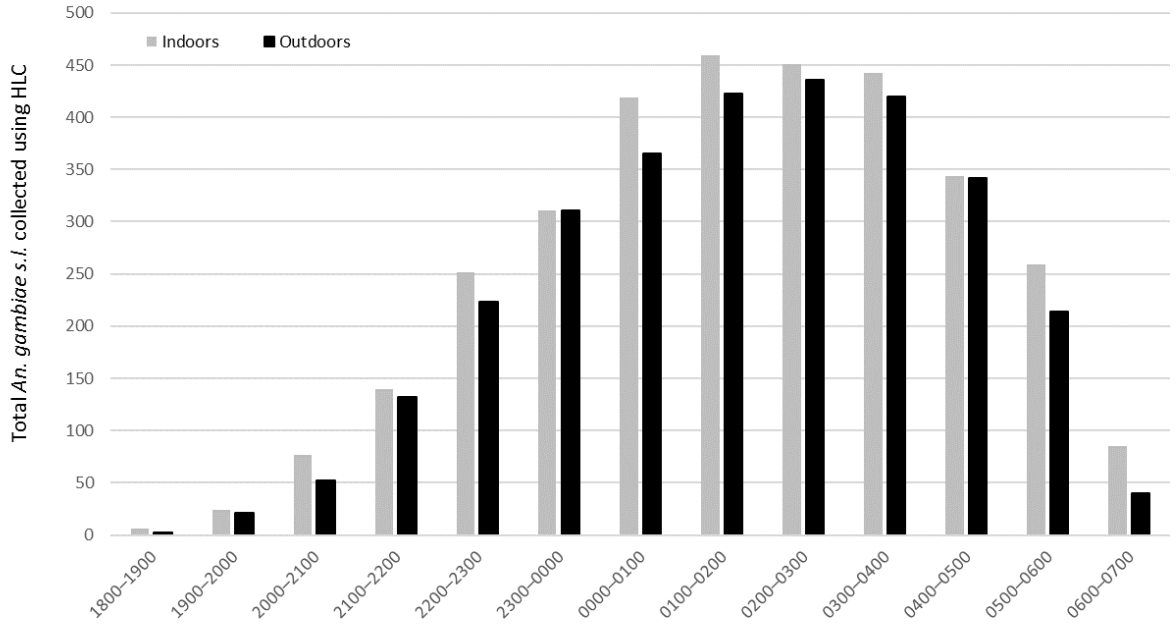
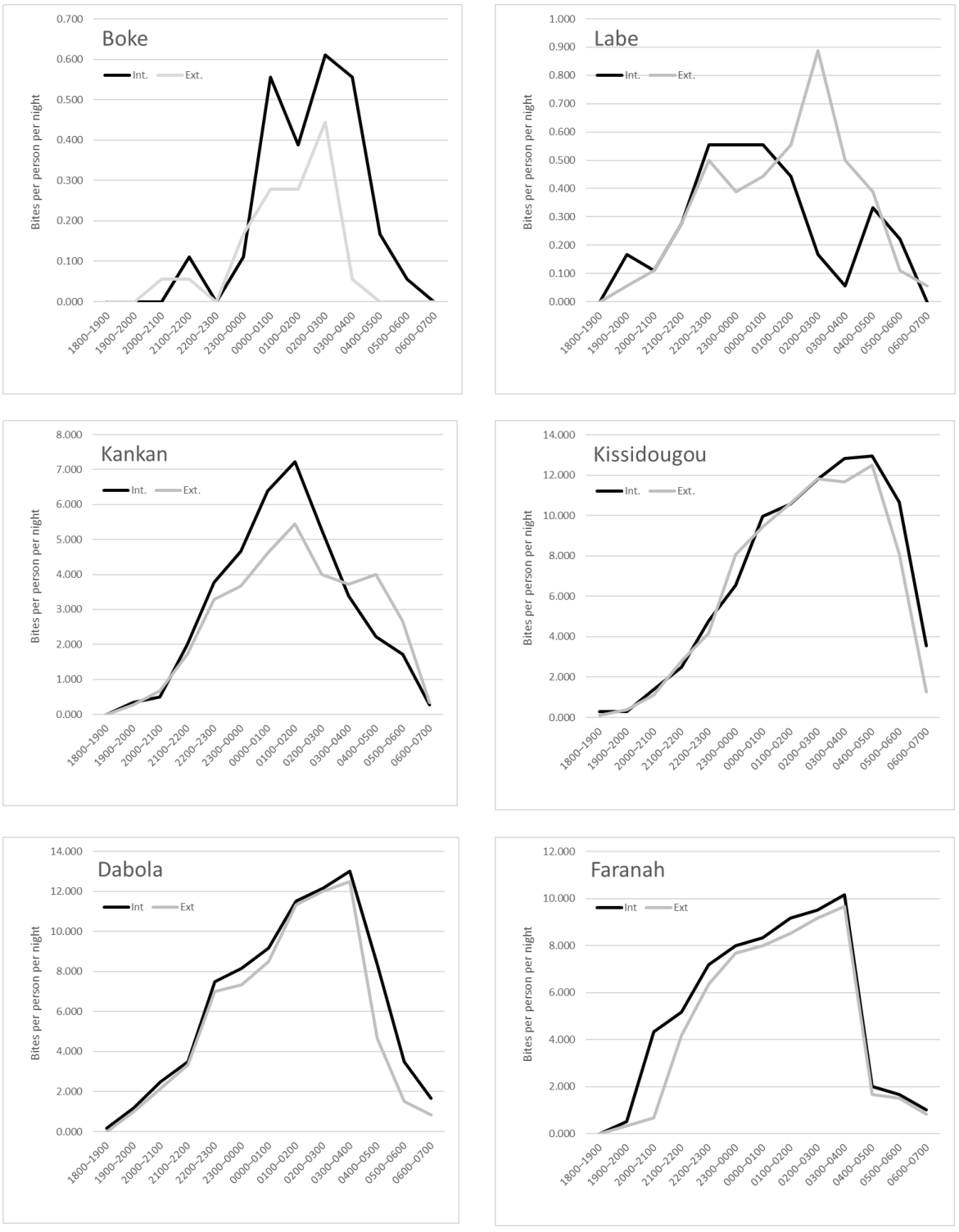


Figure 6: Biting behavior *An. gambiae s.l.* (across districts)



Species identification and sporozoite index

All the Anopheles mosquitoes collected by the different sampling methods (HLCs, CDC light traps, and pyrethrum spray catches) in the 18 villages were identified morphologically by genus and species using standardized keys. *Anopheles* were placed individually in tubes containing alcohol and stored at 95˚C. Each tube was numbered and coded. The heads and thoraxes were analyzed in specialized laboratories using the CSP-ELISA method for

circumsporozoite antigen identification or at the insectary and associated laboratory of the University of Conakry.

Legs and wings were used in PCR tests to identify species of the *An. gambiae* complex, and the abdomens will be used for blood meal type analysis by ELISA.

Anopheles identification, coding, and conditioning at the Kankan site

PCR for species identification

For PCR, we sent 1,000 mosquitoes to Cotonou and 680 to the US CDC. The results for various *An. gambiae s.l.* mosquitoes from locations in Guinea analyzed using PCR, kdr, and Ace-1 are presented below:

The molecular identification of the species of the *An. gambiae s.l.* in Guinea was conducted by PCR *SINE200* according to the protocol of Santolamazza et al. (2008).[12] This PCR makes it possible to determine both the species and the molecular forms within *An. gambiae s.l.*

In total, 997 female *An. gambiae s.l.* from locations in Guinea were analyzed using *SINE* PCR. The results of the molecular tests carried out are presented in ***Table 7***. Three species were found in the locations: *An. gambiae s.s., An. coluzzii*, and *An. arabiensis*. In all locations, *An. gambiae* was the most common species (range between districts: 71%–100%), followed by *An. coluzzii* (range between districts: 0%–29%); *An. arabiensis* was comparatively rare.

Table 7: Distribution of results of *An. gambiae s.l.* species in Guinea (2017)

Locations	Number tested	*An. gambiae s.s.*	*An. coluzzii*	*An. arabiensis*
Kankan	266	71%	29%	0%
Kissidougou	264	96%	2%	1%
Labé	9	100%	0%	0%
Dabola	191	96%	0%	4%
Faranah	267	94%	5%	1%
Total	**997**	**89%**	**10%**	**1%**

ELISA to determine sporozoite index

During the reporting period, 685 *An. gambiae s.l*. were tested with CSP-ELISA, 8 of which were positive for a mean sporozoite index of 1.16% (range across districts: 0.0%–2.6%) (***Table 8***).

[12] Santolamazza F *et al.* (2008). Insertion polymorphisms of *SINE200* retrotransposons within speciation islands of *Anopheles gambiae* molecular forms. *Malaria J 7*: 163.

Table 8: *An. gambiae s.l.* sporozoite index

Sites	Species	Capture method		Number tested	Number positive	Positive (%)
		HLC	Pyrethrum spray catch			
Boké	*An. gambiae s.l.*	22	1	23	0	0
Kankan	*An. gambiae s.l.*	137	18	155	1	0.6
Kissidougou	*An. gambiae s.l.*	142	13	155	4	2.6
Labé	*An. gambiae s.l.*	52	2	54	0	0
Dabola	*An. gambiae s.l.*	145	05	150	0	0
Faranah	*An. gambiae s.l.*	148	00	148	3	2.0
Total		**646**	**39**	**685**	**8**	**1.16**

3.3 Objective 3: Determine insecticide resistance of malaria vectors

Monitoring Phenotypic resistance

Resistance tests were carried out on nulliparous female mosquitoes derived from larvae and pupae collected at the district sites as per standard WHO protocol, i.e., 1-hour exposure of mosquitoes to insecticide-treated filter papers in WHO test tubes and monitoring 24-hour mortality following exposure.[13] Six insecticides were tested: deltamethrin 0.05%, alphacypermethrin 0.25%, permethrin 075%, pirimiphos-methyl 0.25%, propoxur 0.1%, and bendiocarb 0.1%. The surviving mosquitoes were preserved, and molecular analysis was performed in a reference laboratory to identify the mechanism of resistance.

With the exception of Kankan, which was only sampled once, all districts were sampled twice to determine insecticide susceptibility of local anopheline mosquito populations. Tests indicated resistance or suspected resistance to permethrin and alphacypermethrin in all four sampled sites; no resistance to pirimiphos-methyl or bendiocarb was observed.

WHO test tube bioassay showed that sampled vector populations are resistant to permethrin and alphacypermethrin, but not to deltamethrin; no resistance to pirimiphos-methyl and bendiocarb was observed, though only a limited number of populations (sites) were sampled (***Table 9***).

Table 9: Summary of insecticide susceptibility testing of vector mosquitoes

Insecticide	Insecticide strength	Boké	Labé	Kankan	Kissidougou	Dabola	Faranah
Deltamethrin	0.05%	100	100	100	95	ND	ND
Permethrin	0.75%	70	96	75	67	ND	ND
Alphacypermethrin	0.05%	80	95	94	88	ND	ND
Pirimiphos-methyl	0.25%	ND	ND	ND	ND	100	100
Bendiocarb	0.1%	ND	ND	ND	ND	ND	98

ND = Not done.

[13] WHO (2016) *Test procedures for insecticide resistance monitoring in malaria vector mosquitoes*. WHO, Geneva, Switzerland.

Genotypic resistance

Detection of kdr West and Ace-1R mutations in An. gambiae s.l.

Determination of the kdr L1014F (kdr West) and Ace-1 mutations was carried out following the protocol of Martinez-Torres et al. (1998)[14] and Weill et al. (2004),[15] respectively.

Of 997 mosquitoes analyzed for kdr PCR in all locations, 728 were resistant homozygotes, 190 heterozygotes, and 79 susceptible homozygotes. The allelic frequency of the kdr mutation in *An. gambiae s.s.* ranged from 78% in Labé to 96% in Kissidougou (***Table 10***).

Of 997 mosquitoes analyzed for Ace-1 PCR in all locations, 0 were resistant homozygotes, 68 heterozygotes, and 929 susceptible homozygotes. The allelic frequency of the Ace-1 mutation in *An. gambiae s.s.* ranged from 0.5% in Kankan to 22.2% in Labé (***Table 10***).

Table 10: Summary of the detection of kdr West and Ace-1R mutations in *An. gambiae s.s.*

Location	Mosquito species	*n*	*kdr West*				*Ace-1*			
			RR	RS	SS	Frequency (%)	RR	RS	SS	Frequency (%)
Kankan	*An. gambiae*	188	150	28	10	94.7	0	1	187	0.5
	An. coluzzii	78	61	13	4	94.9	0	3	75	3.8
Kissidougou	*An. gambiae*	255	209	35	11	95.7	0	20	235	7.8
	An. arabiensis	2	1	0	1	50.0	0	0	2	0.0
	An. coluzzii	7	4	2	1	85.7	0	2	5	28.6
Labé	*An. gambiae*	9	6	1	2	77.8	0	2	7	22.2
Dabola	*An. gambiae*	183	125	40	18	90.2	0	19	164	10.4
	An. arabiensis	8	3	4	1	87.5	0	2	6	25.0
Faranah	*An. gambiae*	250	159	62	29	88.4	0	17	233	6.8
	An. arabiensis	3	2	0	1	66.7	0	0	3	0.0
	An. coluzzii	14	8	5	1	92.9	0	2	12	14.3
Total	*An. gambiae*	**885**	**649**	**166**	**70**		**0**	**59**	**826**	
	An. arabiensis	**13**	**6**	**4**	**3**		**0**	**2**	**11**	
	An. coluzzii	**99**	**73**	**20**	**6**		**0**	**7**	**92**	

[14] Martinez-Torres D *et al.* (1998) Molecular characterization of pyrethroid knockdown resistance (kdr) in the major malaria vector *Anopheles gambiae s.s. Insect Mol Biol 7*: 179–84.

[15] Weill M *et al.* (2004) The unique mutation in Ace-1 giving high insecticide resistance is easily detectable in mosquito vectors. *Insect Mol Biol 13*: 1–7.

Annex

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

Table A-1: Entomological equipment and laboratory materials

Items	Quantity	Observations
Generator	1	Good condition
Inverters for 4000 et 5000 W	2	Good condition
Spectrophotometer, calibration from 450 to 630 nanometers	1	Good condition
Desktop computer with accessories	1	Good condition
Stabilizer and two wall thermometers	1	Good condition
Refrigerators (for the cold chain)	2	Good condition
Waste bins and plastic trash bags	8	Good condition
One-liter coffee maker	1	Good condition
Cat food	1	Good condition
Pair of rabbits and food	2	Good condition
Sanitary equipment kit	1	Good condition
Large bucket (60 liters)	2	Good condition
Small bowls	16	Good condition
Table	1	Good condition
Stools	6	Good condition
Rolling chairs	1	Good condition
Lab coats	4	Good condition

The reagents are summarized in ***Table A-2***.

Table A-2: Laboratory reagents

Items	Quantity	Observation
Citric acid	1	Good condition
Casein from bovine	1	Good condition
Sodium Chloride ACS	1	Good condition
Albumin from bovine serum BSA	1	Good condition
Ortotolidine	1	Good condition
Tween 20	1	Good condition
N.N Dimethyl formamide / 250ml	1	Good condition
Igepal CA - 630 / 50ml	1	Good condition
Thimerosal 10g	1	Good condition
Phenol Red Sodium Salt 5g	1	Good condition
Hydrogen peroxide 100ml	1	Good condition
Sulfuric acid 500ml	1	Good condition
Glycerol (Aliquot)	1	Good condition
Disodium Phosphate (Na2HPO4) Aliquot	1	Good condition
Potassium chloride (KCl)	1	Good condition
Potassium Phosphate KH2PO4	1	Good condition
PBS 8 tablets / Aliquot	1	Good condition
Sodium hydroxide (NaOH) / Aliquot	1	Good condition

Items	Quantity	Observation
Sterile water	1	Good condition
Anticorps de capture (pf)	1	Good condition
Anticorps conjugues (pf)	1	Good condition
Antigens (PF0 Témoins Positifs)	1	Good condition
Plaque Nunc (ELISA)	1	Good condition