U.S. PRESIDENT'S MALARIA INITIATIVE

U.S. PRESIDENT'S MALARIA INITIATIVE

INTELLIGENT › INNOVATIVE › INTEGRATED

THE PMI VECTORLINK PROJECT ANGOLA ANNUAL ENTOMOLOGY REPORT

MAY 2019–APRIL 2020

Recommended Citation: The PMI VectorLink Project Angola, Annual Entomology Report. May 2019–April 2020. Rockville, MD. The PMI VectorLink Project, Abt Associates Inc.

Contract: AID-OAA-I-17-00008

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

Submitted to: United States Agency for International Development/PMI

Submitted on: June 4, 2020

Approved on: July 22, 2020

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

THE PMI VECTORLINK PROJECT ANGOLA ANNUAL ENTOMOLOGY REPORT

MAY 2019–APRIL 2020

CONTENTS

LIST OF TABLES

LIST OF FIGURES

ACRONYMS

AIRS	Africa Indoor Residual Spraying
Bti	*Bacillus thuringiensis israelensis*
CDC	(U.S.) Centers for Disease Control and Prevention
DHIS2	District Health Information Software 2
DPS	*Direcções Provincial de Saúde* (Provincial Health Directorate)
FETP	Field Epidemiology Training Program
INIS	*Instituto Nacional de Investigação em Saúde* (National Health Research Institute)
DNSP	*Direcção Nacional de Saúde Publica* (National Directorate of Public Health)
IRS	Indoor Residual Spraying
ITN	Insecticide-treated Net
LT	Light Trap
NMCP	National Malaria Control Program
PBO	Piperonyl butoxide
PMI	(U.S.) President's Malaria Initiative
SOP	Standard Operating Procedure
WHO	World Health Organization

EXECUTIVE SUMMARY

Malaria remains a serious public health problem in Angola despite long-term, albeit irregular, implementation of vector control strategies including mass distribution of insecticide-treated nets (ITNs) and focal indoor residual spraying (IRS). From 2012 to 2016, the U.S. President's Malaria Initiative (PMI) Africa Indoor Residual Spraying (AIRS) project conducted longitudinal entomological surveillance in Angola and collected data on key entomological indicators, such as vector species composition, seasonality, behavior, and insecticide resistance status and sporozoite infection rate, from three provinces (Cunene, Huambo, and Malanje), in collaboration with the *Direcções Provincial de Saúde* (DPS)/Provincial Health Directorates. However, since the end of the AIRS project, there has been very little and no systematic malaria entomological monitoring conducted in Angola. The need for up-to-date insecticide susceptibility data for malaria vectors is particularly urgent given Angola's reliance on the use of insecticides for malaria vector control through ITNs and IRS. A major task in Year 1 of the PMI VectorLink Angola project (May 2019 to April 2020) was to resurrect entomological surveillance in Angola by engaging with key partners at the national and local levels and recruiting and providing refresher training to entomological technicians who had not conducted entomology activities for several years. The ultimate goal was to start to generate entomological data that can aid vector control decision making by Angola's National Malaria Control Program (NMCP).

In Year 1, entomological surveillance was conducted in two sites—one in Huambo Province and the other in Lunda Sul Province. Originally, three sites were to receive entomological surveillance, but the sampling in Luanda Province was postponed due to the COVID-19 pandemic. Huambo Province was selected given the existence of an insectary and entomology technicians previously-trained through AIRS, and Lunda Sul Province was chosen due to the high number of malaria cases and the logistical ease of implementation. VectorLink conducted one month of entomological surveillance at each site during the peak mosquito season (January-March 2020) to determine species composition, vector behavior, and vector susceptibility to different insecticides. The team used U.S. Centers for Disease Control and Prevention (CDC) light traps (LTs) and Prokopack aspirators to trap mosquitoes and also conducted larval collections. The project hired a full-time Entomology Coordinator who facilitated preparations for data collection, including meeting with central, provincial, and district leadership, and obtaining ethical clearance from the *Direcção Nacional de Saúde Publica* (DNSP). With support from the VectorLink home office and an international consultant who stayed in Angola throughout the sampling season. In this first year, the project trained 15 people (eight members of the Provincial mosquito brigades, five technicians, a Field Epidemiology Training Program fellow, and the Entomology Coordinator) on adult and larval mosquito collection methods and morphological species identification.

The main malaria vector species collected in Huambo Province was *Anopheles funestus* s.l.; a smaller proportion of *An. gambiae* s.l. were also collected. Other secondary malaria vector and non-vector species collected included *An. coustani*, *An. squamosus*, and *An. rufipes*. The predominant malaria vector species in Lunda Sul Province was *An. gambiae* s.l., although there was local variation by village, with Tchaungo also having a substantial proportion of *An. funestus* s.l. In both Huambo and Lunda Sul provinces, the majority of *Anopheles* were collected using indoor CDC LTs, with early morning Prokopack aspiration catching very few *Anopheles*, suggesting that vector species exited houses very early (before sunrise) and did not rest indoors during the daytime.

World Health Organization (WHO) insecticide susceptibility tests showed that *An. gambiae* s.l. from Saurimo in Lunda Sul Province were resistant to permethrin and deltamethrin. While not a surprising result, the finding of pyrethroid resistance may mean that pyrethroid-based ITNs are not providing optimal control in Lunda Sul Province. Preliminary bioassays also indicated an increase in mortality with pre-exposure to piperonyl butoxide (PBO) followed by permethrin or deltamethrin. Further replicates were planned but had to be cancelled due to COVID-19 restrictions. Therefore, the synergist data should be considered as preliminary until more replicates can be conducted. All *Anopheles* species collected through indoor CDC LTs, Prokopack aspiration, and susceptibility tests have been stored individually in Eppendorf tubes and will be used later in 2020 for laboratory analyses at *Instituto Nacional de Investigação em Saúde* (INIS) in Luanda for species identification by polymerase chain reaction (PCR) and to determine malaria sporozoite infection rates.

During data collection, we had great support from NMCP, the DPS, other health authorities, the mosquito brigades, and community leaders. The team made strides in building basic local capacity which will need to be strengthened further in future years. Infrastructure was developed in Luanda at INIS, through the conversion of an existing structure into an insectary which will become fully established in Year 2.

1. Introduction

Malaria remains a serious public health problem in Angola despite sustained malaria control strategies. Malaria is endemic throughout the country and the entire population is at risk, but there is significant heterogeneity in transmission across the country. *Plasmodium falciparum* is the primary malaria parasite in Angola, responsible for an estimated 87% of malaria cases. The other three *Plasmodium* species known to cause human malaria—*P. vivax*, *P. malariae*, and *P. ovale*—are also present and cause about 7%, 3%, and 3% of reported malaria cases, respectively. There are five *Anopheles* species responsible for transmission in the country: *An. gambiae* s.s., *An. funestus*, *An. melas* (in coastal areas), *An. arabiensis*, and *An. pharoensis* (in southern, unstable mesoendemic areas). *An. rufipes* and *An. coustani* have been identified as secondary vectors in Huambo and Zaire Provinces (Malaria Operational Plan, 2018).

The Angola NMCP strategy includes three vector control methods—ITNs, IRS, and larviciding—and aims to cover 80% of the population at risk of malaria with at least one vector control and prevention measure. A survey in 2016 showed that 31% of households in Angola owned at least one ITN (2015–16 Demographic and Health Survey). By comparison, across all countries in sub-Saharan Africa in 2016, 80% of households owned at least one ITN[1]. PMI and the Global Fund have been key partners supporting procurement and distribution of pyrethroid ITNs. Angola took a major step in 2017 towards universal coverage of ITNs, conducting a three-phase nationwide mass campaign in 2017–2018, which resulted in the distribution of 6,693,503 ITNs to 2,379,943 registered household across 13 provinces. Following the campaign, a survey conducted in 2019 showed that 55.4% had access to a net in Kwanza Sul Province (use: access ratio 0.87), 51% in Cunene Province (0.69 use: access ratio) and 33.3% in Uige (0.77 use: access) (VectorWorks, 2019).

From 2012 to 2016, the PMI AIRS project conducted longitudinal entomological surveillance activities in Angola, collecting data on key entomological indicators—vector species composition, seasonality, behavior, and insecticide resistance status and sporozoite infection rate—from three provinces (Cunene, Huambo, and Malanje), in collaboration with the DPS. In 2014, PMI constructed and established an insectary in Huambo, the first insectary in Angola since the end of the civil war. PMI also supported the training of technicians on basic malaria entomology, including sampling of larval and adult mosquitoes, rearing of adults from field-collected larvae/pupae, morphological species identification, ovary dissection, cone bioassays, susceptibility testing using CDC bottle bioassay and WHO tube test methods, preservation of samples, and transport to a laboratory for molecular and biochemical assays. Insectary technicians also received on-the-job training and mentorship to continuously improve their skills. PMI also supported training of provincial and municipal health authorities from nine provinces representing three malaria transmission zones (Benguela, Cunene, Huambo, Huila, Luanda, Malanje, Namibe, Uige, and Zaire) where insecticide resistance was evaluated in 2015-2016. From 2016 to 2019, PMI did not support entomological monitoring activities in Angola.

In Year 1 of the PMI VectorLink Angola project (May 2019-April 2020), entomological surveillance was conducted in two sites—one in Huambo Province and the other in Lunda Sul Province. Entomological surveillance was meant to be conducted at three sites, but sampling collection and initiation of insectary activities in Luanda Province was postponed due to the COVID-19 pandemic. VectorLink conducted one month of entomological surveillance at each site during the peak mosquito season (January-March 2020) to determine species composition, vector behavior, and vector susceptibility to different insecticides. The project also hired a full-time Entomology Coordinator who facilitated preparations for data collection, including meeting with central, provincial, and district leadership, and obtaining ethical clearance from DNSP.

---

1 Koenker H, Arnold F, Ba F, Cisse M, Diouf L, Eckert E, et al. Assessing whether universal coverage with insecticide-treated nets has been achieved: is the right indicator being used? Malar J. 2018;17:355.

In Year 2, the project will continue to build entomological capacity at the country level and strengthen existing institutional structures.

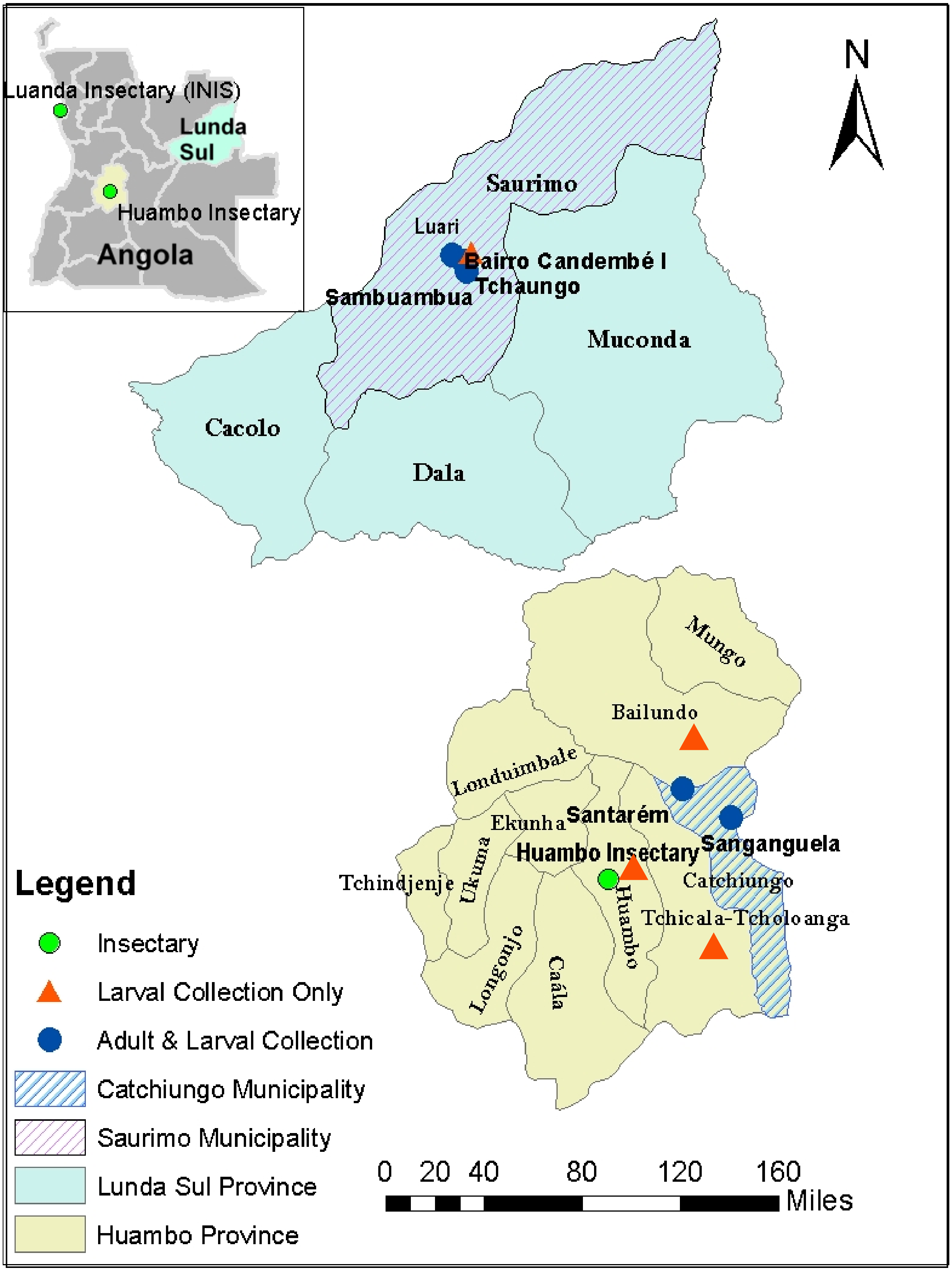
This report presents results from entomological monitoring conducted between January and March 2020 and outlines the key project achievements including development of infrastructure and personnel.

2. METHODOLOGY

2.1 STUDY SITES

From January to March 2020, entomological monitoring was conducted in two provinces—Huambo and Lunda Sul. Huambo Province was primarily chosen based on the existing facilities (insectary) and presence of technicians that previously received training under AIRS and supported by DNSP. Although sampling was not possible due to COVID-19, we chose Luanda Province as a site primarily to increase visibility and enhance the relationship with NMCP for sustainability of activities. Both Lunda Sul and Huambo provinces have a considerable malaria burden based on case data reported in the District Health Information Software 2 (DHIS2) platform. Due to the short duration of mosquito trapping, one municipality was chosen within each province. The project selected Saurimo Municipality in Lunda Sul Province and Catchiungo Municipality in Huambo Province given they had the highest number of malaria cases reported in DHIS2 for 2019. Specific villages were then selected within the municipality following in person meetings with the municipality chief medical officer who had local level case data and provided introductions to village leaders for project sensitization. Figure 1 shows the locations of the municipalities and villages within each of the two provinces.

Figure 1: Map of Year 1 VectorLink Angola Provinces and Study Sites



2.1.1 HUAMBO PROVINCE

Huambo Province is located on an inland plateau (Bié Plateau) at an elevation of approximately 1,700 meters above sea level. Malaria is meso-endemic and stable in the area, with *P. falciparum* causing more than 90% of malaria cases (Figure 2). Annual rainfall ranges from 1,750 to 1,250 mm in northern and central areas of Huambo Province, with significant rainfall between October and April and a dry season between May and September (Figure 3). In Huambo Province, the predominant malaria vectors are *An. gambiae* and *An. funestus*, which are highly anthropophilic and endophagic species and the most successful vectors of malaria.

Prior to collections, VectorLink restored the insectary located in Huambo (originally constructed in 2014), which provided a space for staff training and data collection. While the insectary had been well-maintained, minor refurbishments were done to prepare the space. The team also worked closely with the provincial and municipal health authorities to coordinate preparations for the arrival of the team and the start of field work.

Collections of adult *Anopheles* mosquitoes were performed in two sentinel villages; namely Sanganguela and Santarem in the municipality of Catchiungo (approximately 1.5 hours' drive from Huambo town). Larval collections were performed in the municipalities of Huambo, Bailundo, and Tchicala within the province of Huambo (denoted with red triangles in Figure 1).

Figure 2: Map of Angola Showing Estimated Malaria Transmission Risk by Province and Location of Lunda Sul and Huambo Provinces

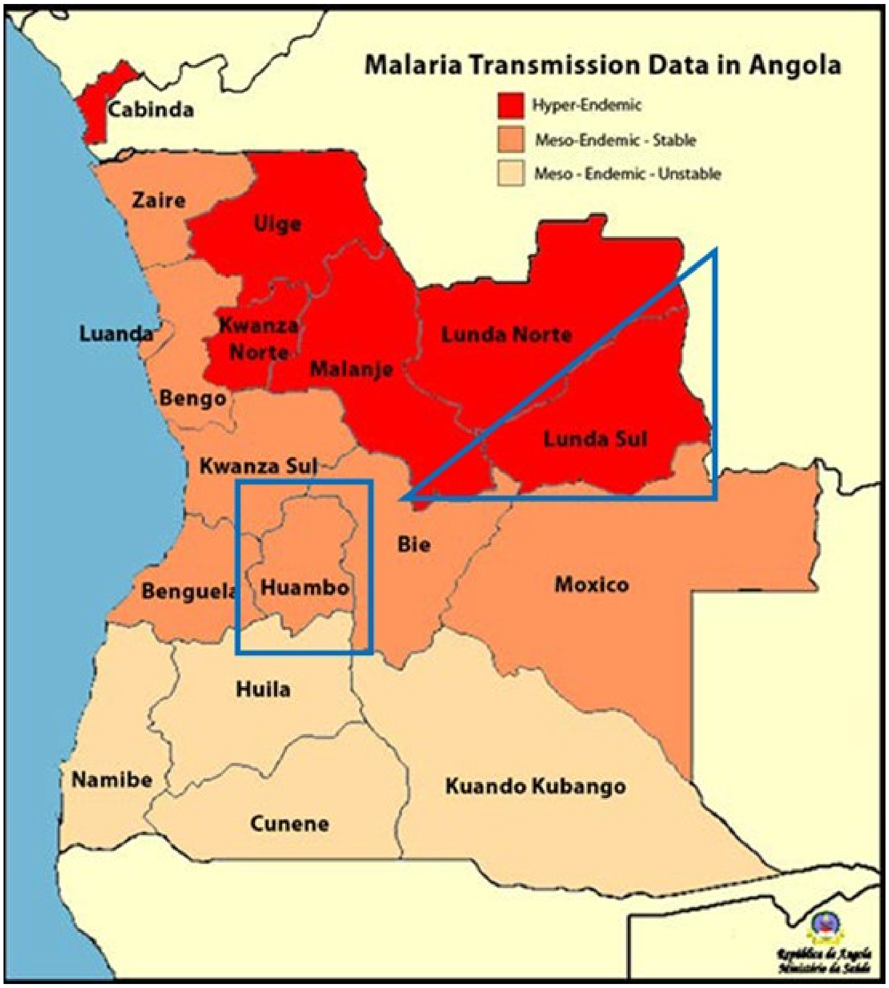
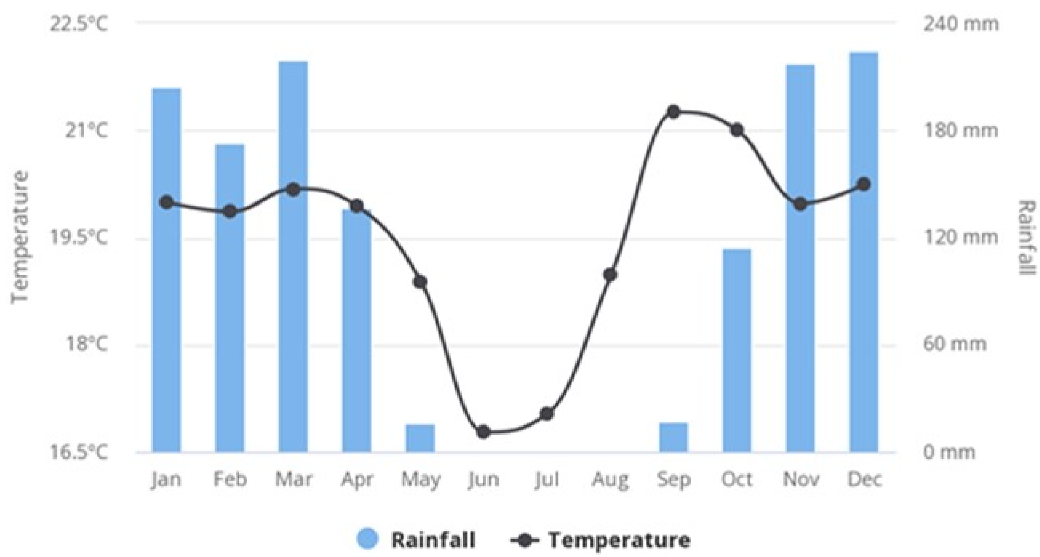


Figure 3: Mean Temperature and Rainfall for Huambo Province for 1991-2016



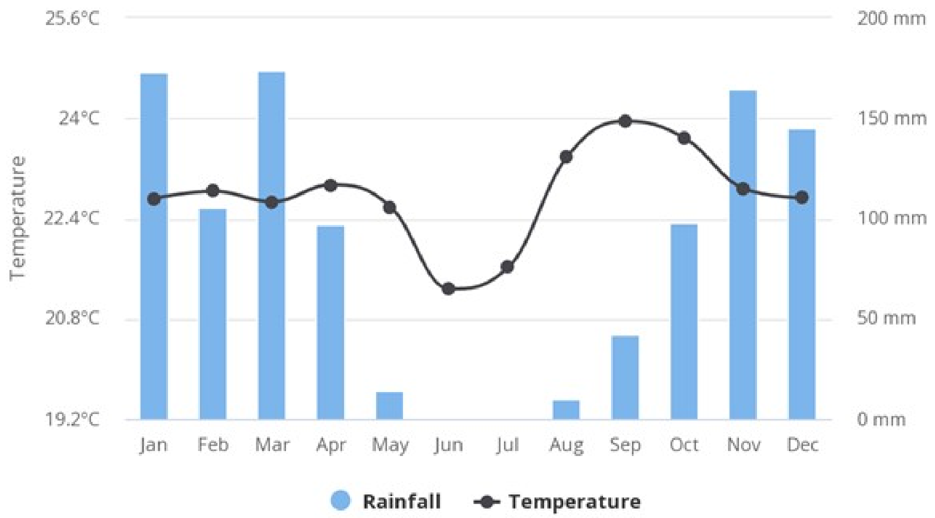
Source: Climatic Research Unit (CRU) of University of East Anglia (UEA).

2.1.2 Lunda Sul Province

Lunda Sul Province is located in eastern Angola, with the provincial capital of Saurimo located approximately 946 kilometers (588 miles) east of the national capital, Luanda. It is bordered to the north by Lunda Norte Province, to the east by the Democratic Republic of the Congo, to the south by Moxico Province, to the southwest by Bié Province, and to the west by Malanje Province (Figure 2). Lunda Sul province is hyper-endemic for malaria, with high levels of transmission taking place (Figure 2). Municipalities include Cacolo, Dala, Muconda, and Saurimo (Figure 1). The main road from Luanda to the mining city Lubumbashi in the Democratic Republic of the Congo traverses the province from west to east. Lunda Sul Province has a population of 516,077 (2014 census). The province is dominated by dry savannah land; only in the Kasai River valley are there remnants of tropical rainforest. The Kasai River forms the eastern and southern frontier of Lunda Sul Province and is the main river of the province, though the Kwango River is also a major river in the province. The climate is predominantly tropical with stable mean temperature ranging between 21-24°C throughout the year. The rainy season takes place between October and April followed by a dry season between May and September (Figure 4).

Collections of adult *Anopheles* mosquitoes were performed in three sentinel villages—Tchaungo, Sambuambua, and Candembe 1—in the municipality of Saurimo in the province of Lunda Sul (Figure 1). All three sentinel sites were easily accessible and were within 30 minutes' drive of Saurimo town. Larval collections occurred mostly in Candembe 1 village within Saurimo Municipality and were conducted four days per week.

Figure 4: Mean Temperature and Rainfall for Lunda Sul Province for 1991-2016



Source: Climatic Research Unit (CRU) of University of East Anglia (UEA).

2.1.3 LUANDA PROVINCE

Due to restrictions from the COVID-19 pandemic, it was not possible to conduct entomological monitoring scheduled for March/April in Luanda Province. Luanda was chosen for monitoring based on several factors, including the fact that it has mesoendemic malaria transmission risk (Figure 2). Inclusion of Luanda also supports improved central level entomology capacity and strengthens the engagement of the NMCP for sustainability. As part of this long-term strategy, investment was made to establish an insectary in Luanda located at INIS.

2.2 OVERVIEW OF TRAPPING METHODS AND FREQUENCY

Table 1 shows the sampling frequency and timing of all collection methods. Entomological monitoring was conducted in Sanganguela and Santarém villages in Huambo Province from January 24 to February 14, 2020 and from February 23 to March 21, 2020 in Tchaungo, Sambuambua and Candembe 1 villages in Lunda Sul Province. Trapping involved use of indoor CDC LTs and Prokopack aspirators, while susceptibility tests were conducted using WHO tube tests. All tests were conducted according to VectorLink Standard Operating Procedures (SOPs) (https://pmivectorlink.org/resources/tools-and-innovations/). *Anopheles* mosquitoes collected during the entomological monitoring were identified morphologically using the key of Gillies & Coetzee, 1987 and preserved in silica gel in individualized Eppendorf tubes labelled with mosquito data and stored at -20°C until further molecular laboratory analyses can take place at INIS in Luanda.

Table 1: Summary of Collection Methods and Trapping Frequency

Type of Collection	Time	Frequency	Capture Effort
Indoor CDC LTs	6:00 p.m. to 7:30 a.m.	Four nights per site per week	12 houses per night (48 houses per week) for 3 weeks
Indoor Prokopack aspiration	6:00 a.m. to 7:00 a.m.	Four days per site per week	10 houses per day (40 houses per week) for 3 weeks
Larval collections	11:00 a.m. to 2:00 p.m. (9:00 a.m. to 5:00 p.m. when not adult mosquito trapping)	Four days per week	n/a

2.3 INDOOR CDC LIGHT TRAP

Adult *Anopheles* mosquitoes were collected for four days per week by indoor CDC LTs. CDC LTs were used to monitor densities of host seeking mosquitoes inside 48 houses per week per site. A single 6V CDC LT was hung in each house in the sleeping area, approximately 1.5 meters from the ground, adjacent to an existing, occupied pyrethroid bed net (Figure 5B).

2.4 INDOOR PROKOPACK ASPIRATION

To monitor the number of indoor resting mosquitoes, mechanical Prokopack aspiration was conducted early in the morning in ten houses per morning by sweeping the aspirator close to the wall, ceiling and furniture of the house for approximately 15 minutes per house (depending on size of the house) (Figure 5A, 6A). *Anopheles* mosquitoes were collected four days per week by indoor mechanical Prokopack aspirations in houses that were not used for indoor CDC LTs.

Figure 5: Photographs from Entomological Monitoring Activities in Huambo Province

A- Prokopack aspirator being carried by mosquito brigade staff, B- Removing CDC light trap, C- Tying up mosquito collection bag, D- Morphological identification at the Huambo insectary, E- *An. gambiae* s.l. from Sanganguela, F- Community sensitization in Sanganguela.

Figure 6: Sensitization of Village Elders to Trapping Techniques

A- Prokopack aspiration demonstration, B- CDC LT demonstration, C- Informed consent before Prokopack aspiration, D- Hanging CDC LT inside a bedroom.

2.5 Insecticide Susceptibility Tests

Insecticide susceptibility tests using World Health Organization (WHO) tubes were scheduled to be conducted to determine vector susceptibility to pyrethroids (permethrin, deltamethrin, alpha-cypermethrin), pyrethroid resistance intensity, and to determine whether piperonyl butoxide (PBO) synergist pre-exposure increases pyrethroid mortality. All bioassays were conducted according to WHO 2016 guidelines. Larval collections were performed in the municipalities of Huambo, Bailundo, and Tchicala within the province of Huambo; and in three villages in Saurimo Municipality (Luari, Tchaungo, and Candembe 1) in Lunda Sul Province (Figure 7A and 7B). Larval sites around Candembe 1 were most productive for *An. gambiae* s.l. Morphological identification of adult mosquitoes was done quickly before testing while alive, and corroborated after testing when dead. The primary morphological features used to identify *An. gambiae* s.l. were: 1) no lateral tufts projecting from abdomen, 2) pale interruption on vein 1 of the wing, 3) speckled legs, 4) white bands on legs at tarsomeres, and 5) palps with three white bands. Figure 8 shows the team conducting the WHO tube tests.

Figure 7: Larval Collections of *An. gambiae* s.l. in Candembe I Village in Lunda Sul Province (A, B) and Subsequent Larval Rearing (C) and Morphological Identification of Adults (D)

Figure 8: Photographs of WHO Tube Tests in Saurimo, Lunda Sul Province

A- Preparing materials for WHO tube test of a pyrethroid, B- Conducting WHO tube tests using PBO synergists and pyrethroids, C- Preparing materials for WHO tube test, D- Recording knock-down of mosquitoes shortly after the end of exposure to insecticide.

2.6 DATA MANAGEMENT

Field data was collected on paper forms approved by PMI in anticipation of use with VectorLink Collect. As VectorLink Collect was not ready to be used during data collection, all completed data forms were scanned and saved within Google Drive to ensure no loss of data until VectorLink Collect was online. To prepare weekly progress reports summarizing data collected that week, an Excel database was used to enter the number of each mosquito species collected per trap. Another Excel file was used to link each sample with household information using a unique household identification number.

Following the conclusion of field collections, the Entomological Coordinator worked with the VectorLink home office Monitoring and Evaluation (M&E) team to be trained on use of the new VectorLink Collect database, a centralized database that VectorLink developed on the DHIS2 platform and is currently rolling out across the 24 countries. The database will house all entomological surveillance data collected by the project.

In April 2020, the VectorLink M&E Specialist conducted a virtual training to prepare a small team of PSI/Angola staff team to use VectorLink Collect to enter entomological data collected from Huambo and Lunda Sul provinces. The teams will also use this database to enter, store, and analyze all data collected from sentinel sites. With continued remote support from home office M&E specialist, the VectorLink Angola Entomological Coordinator will support data cleaning and perform descriptive analysis and interpretation of entomology data.

3. RESULTS

3.1 MALARIA VECTOR SPECIES COMPOSITION

A total of 266 *Anopheles* were collected using overnight indoor CDC LTs and early morning indoor Prokopack aspirations in Huambo Province. When broken down by trapping method, 93.6% of *Anopheles* were collected by CDC light traps (n=249), compared to Prokopack aspiration (6.4%, n=17). All 17 *Anopheles* collected by Prokopack aspiration were *An. funestus* s.l., whereas CDC LT collections consisted of 69.9% *An. funestus* s.l. (n=174), 17.7% *An. gambiae* s.l. (n=44), 5.2% *An. coustani* (n=13), 6.8% *An. squamosus* (n=17), and 0.4% *An. rufipes* (n=1) (Figure 10).

Figure 9: Overall *Anopheles* Species Composition from Huambo Province (Sanganguela and Santarém villages) by Indoor CDC Light Trap (top) and Indoor Prokopack Aspiration (bottom); January 20-February 9, 2020

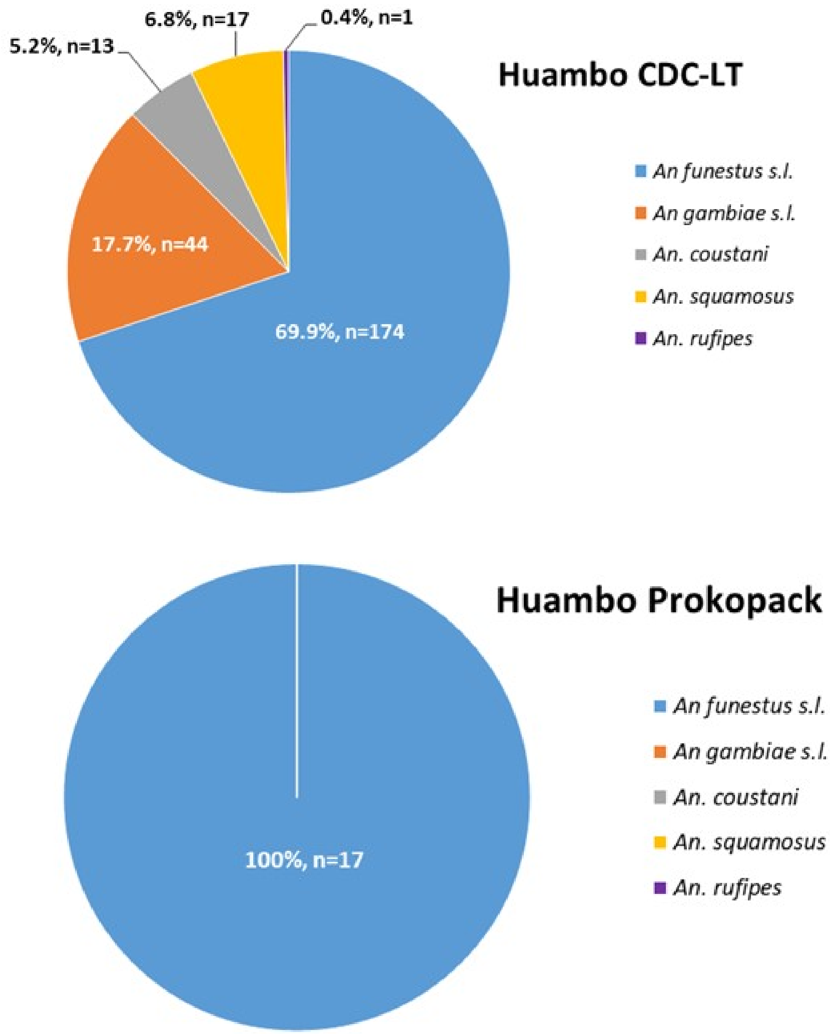
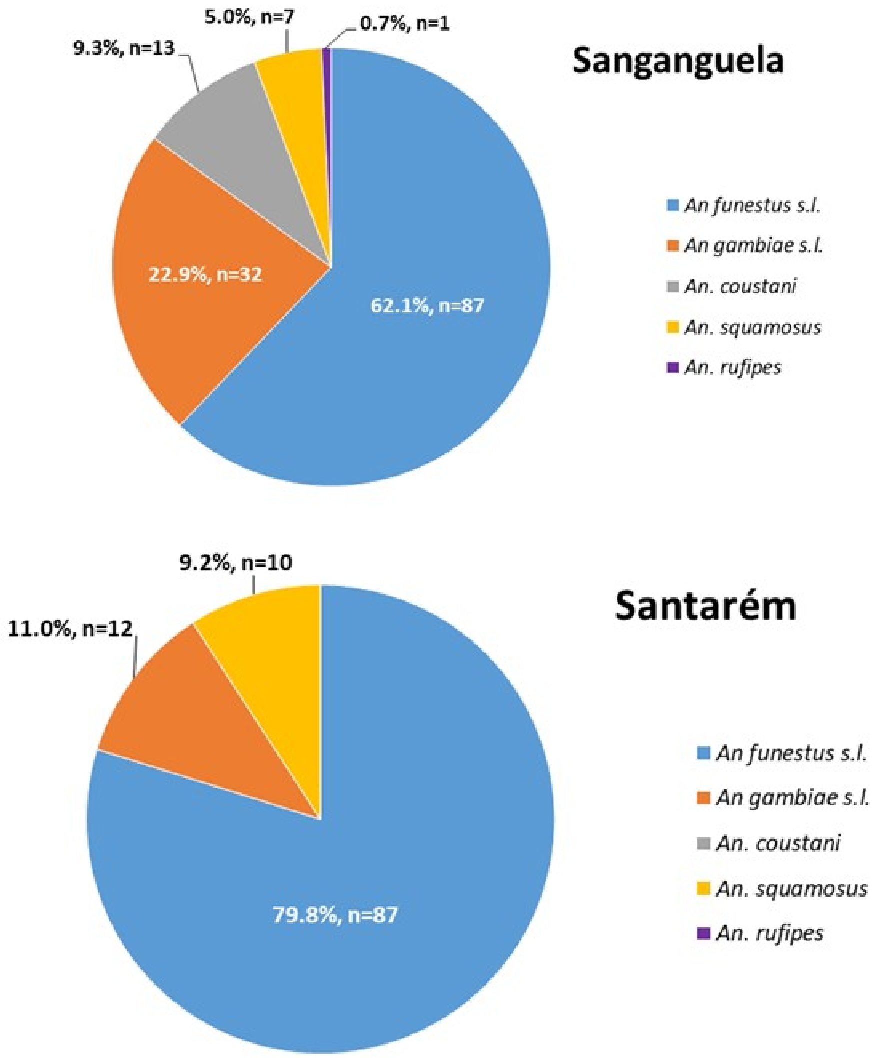


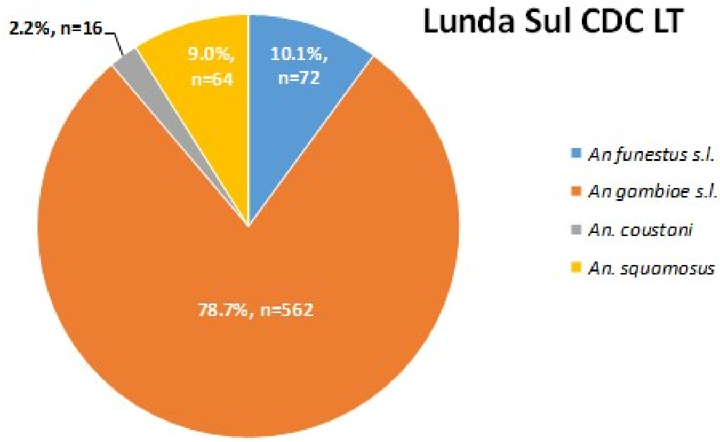
Figure 11 shows the species composition from CDC LT collections separately for each site (Sanganguela and Santarém) in Huambo Province. *An. funestus* s.l. was the most common *Anopheles* species collected in both sites (62.1% and 79.8%, respectively), with smaller proportions of *An. gambiae* s.l. and *An. squamosus*. Despite the distance between the two sites (about 70 kilometers), the species composition was similar in both locations.

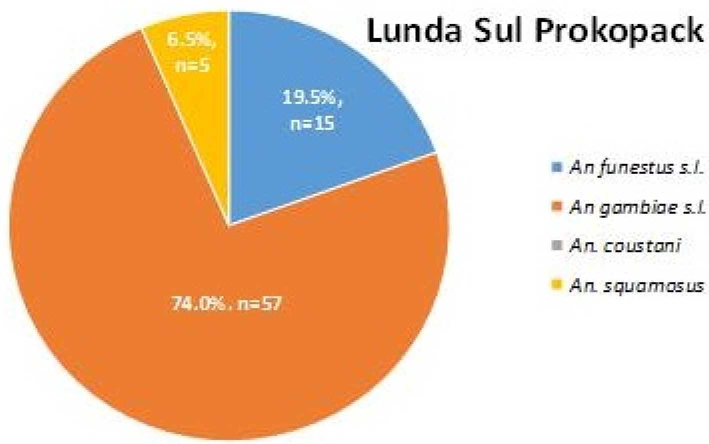
Figure 10: *Anopheles* Species Composition from Indoor CDC Light Traps in Sanganguela (top) and Santarém (bottom) in Huambo Province



In Lunda Sul Province, a total of 791 *Anopheles* were collected during the collection period using both indoor CDC LTs and indoor Prokopack aspiration, comprising 78.3% (n=619) *An. gambiae* s.l., 11.0% (n=87) *An. funestus* s.l., 8.7% (n=69) *An. squamosus*, and 2.0% (n=16) *An. coustani*. As shown in Figure 12, when broken down by trapping method, the vast majority of *Anopheles* were collected by CDC LTs (90.3%, n=714), compared to Prokopack aspiration (9.7%, n=77).

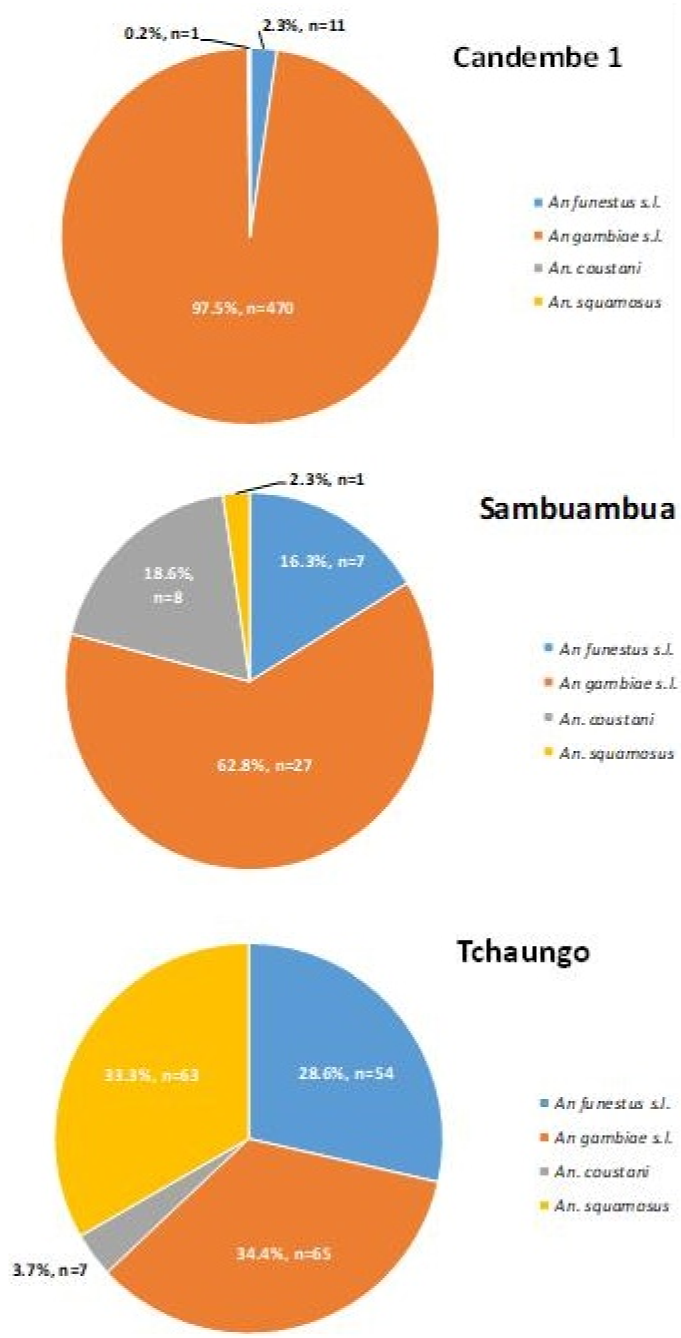
Figure 11: Overall *Anopheles* Species Composition from Tchaungo, Sambuambua, and Candembe 1 Villages in Lunda Sul Province by Indoor CDC Light Trap (top) and Indoor Prokopack Aspiration (bottom); February 23-March 21, 2020





When broken down further by village for CDC LT collections, Candembe 1 had little species diversity with primarily (97.5%) *An. gambiae* s.l. In Sambuambua, *An. gambiae* s.l. was the most common species collected (62.8%), with a smaller proportion of *An. funestus* (16.3%) and *An. coustani* (18.6%). In Tchaungo, there was a more even distribution of a diversity of species, with *An. gambiae* s.l. (34.4%), *An. funestus* s.l. (28.6%), and *An. squamosus* (33.3%) collected (Figure 12). It is worth noting that the number of houses used for trapping was different per village, with 132 trap nights in Candembe 1 village, 21 in Sambuambua, and 24 in Tchaungo. More trapping was done in Candembe 1 as it was a much larger village and it was a productive site.

Figure 12: *Anopheles* Species Composition from Indoor CDC Light Traps in Candembe 1 (top), Sambuambua (middle), and Tchaungo (bottom)



3.2 Malaria Vector Densities

Table 2 shows the mean density of the two major vector species *An. funestus* s.l. and *An. gambiae* s.l. in CDC LT and Prokopack collections from two sites in Huambo Province. The mean density of *An. funestus* s.l. per CDC LT was higher in Santarém (1.81 per trap) than Sanganguela (0.81 per trap). Overall, the mean number collected in Huambo Province by Prokopack aspiration was extremely low at 0.13 *An. funestus* s.l. compared to a mean of 1.12 collected by CDC LT, equating to eight times greater catch by CDC LT.

Table 2: Summary of *An. funestus* s.l. and *An. gambiae* s.l. Collected by CDC Light Trap and Prokopack Aspirator in Sanganguela and Santarém Villages, Catchiungo Municipality, Huambo Province

	Sanganguela	Santarém	Total
An. funestus s.l.			
Total collected by CDC LT	87	87	**174**
Total trap nights	108	48	**156**
Mean mosquitoes collected per trap night	0.81	1.81	**1.12**
Total collected by Prokopack	5	12	**17**
Total trap nights	92	40	**132**
Mean collected per house	0.05	0.3	**0.13**
An. gambiae s.l.			
Total collected by CDC LT	32	12	**44**
Total trap nights	108	48	**156**
Mean mosquitoes collected per trap night	0.30	0.25	**0.28**
Total collected by Prokopack	0	0	**0**
Total trap nights	92	40	**132**
Mean collected per house	0.00	0.00	**0.00**

Table 3 shows the mean density of the two major vector species from CDC LT and Prokopack collections from three sites in Lunda Sul Province. The mean density of *An. funestus* s.l. per CDC LT was higher in Tchaungo (2.25 mosquitos per trap) than Sambuambua (0.33 mosquitos per trap) and Candembe 1 (0.08 mosquitos per trap). The density of *An. gambiae* s.l. in CDC LTs was highest in Candembe 1 (3.56 mosquitos per trap) and lowest in Sambuambua at 1.29 mosquitos per trap. Overall, the mean number of *An. gambiae* s.l. collected by Prokopack aspiration in Lunda Sul was low, at a mean of 0.40 mosquitos per house compared with 3.18 mosquitos per CDC LT, equating to eight times greater catch by CDC LT.

Table 3: Summary of *An. gambiae* s.l. and *An. funestus* s.l. Collected by CDC Light Trap and Prokopack Aspirator by Village in Saurimo Municipality, Lunda Sul Province

	Tchaungo	Sambuambua	Candembe 1	Total
An. funestus s.l.				
Total collected by CDC LT	54	7	11	**72**
Total trap nights	24	21	132	**177**
Mean mosquitoes collected per trap night	2.25	0.33	0.08	**0.41**
Total collected by Prokopack	9	1	5	**15**
Total trap nights	12	21	110	**143**
Mean collected per house	0.75	0.05	0.05	**0.10**
An. gambiae s.l.				
Total collected by CDC LT	65	27	470	**562**
Total trap nights	24	21	132	**177**

	Tchaungo	Sambuambua	Candembe 1	Total
Mean mosquitoes collected per trap night	2.71	1.29	3.56	**3.18**
Total collected by Prokopack	8	6	43	**57**
Total trap nights	12	21	110	**143**
Mean collected per house	0.67	0.29	0.39	**0.40**

3.3 INSECTICIDE SUSCEPTIBILITY TESTS

Despite considerable effort made over 13 days of larval collections in Bailundo and Huambo areas, the team had difficulty finding suitable larval sites around Huambo Province due to persistent daily rainfall, low temperatures, and sandy soil that drains quickly. Another contributing factor could be the long term government larviciding, which consisted of treating large water pools with the larvicide *Bacillus thuringiensis israelensis* (Bti). There were several larval sites where *Anopheles* larvae were collected (Figure 13). However, after emerging to adults, they were identified as being *An. coustani* and *An. squamosus.* As these species are either secondary or non-vector species, they were not used for susceptibility testing (the species of interest for susceptibility testing are *An. funestus* s.l. and *An. gambiae* s.l.). Despite great efforts, it was not possible to conduct susceptibility testing in Huambo Province during the four-week period of data collection. For training purposes and local capacity building, bioassays with permethrin and deltamethrin were carried out using a limited number of the collected secondary vector species (*An. coustani* and *An. squamosus).*

Figure 13: Larval Collections in Huambo Province

A- Larval collections being conducted on the outskirts of Huambo town, B-*Anopheles* larvae collected that were morphologically identified as *An. coustani* and *An. squamosus.*

Susceptibility testing was conducted in Lunda Sul Province with all mosquitoes morphologically identified as *An. gambiae* s.l. Tests with permethrin and deltamethrin showed that *An. gambiae* s.l. were resistant to both insecticides (Figure 14). Further tests showed that pre-exposure to PBO 4% resulted in an increase in mortality for permethrin (to 69%), although mortality remained below the 90% WHO threshold (Figure 15). Pre-exposure to PBO 4% followed by deltamethrin resulted in 100% mortality, compared to 64% for deltamethrin alone (Figure 16). Further replicates were planned but had to be cancelled due to the COVID-19 implemented restrictions. Therefore, the synergist data should be considered as preliminary until more replicates can be conducted.

Figure 14: Percent Mortality (24h) of *An. gambiae* s.l. after Exposure in WHO Tube Tests to Permethrin (0.75%) and Deltamethrin (0.05%)

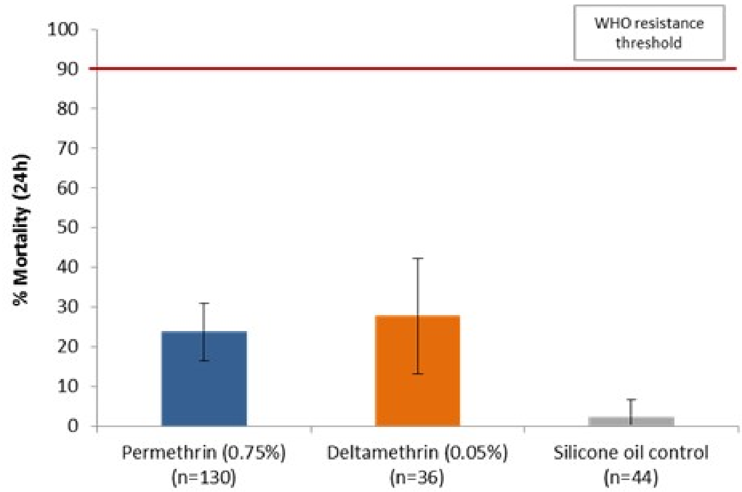


Figure 15: Percent Mortality (24h) of *An. gambiae* s.l. after Exposure in WHO Tube Tests to Permethrin (0.75%) and PBO (4%) + Permethrin (0.75%)

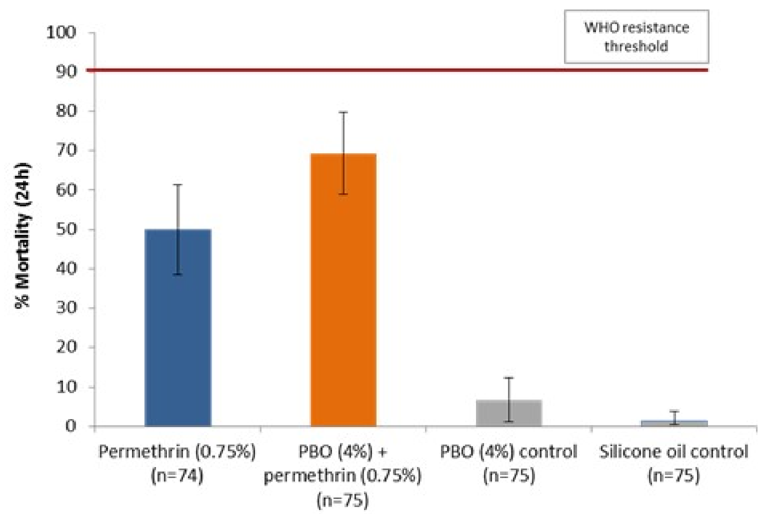
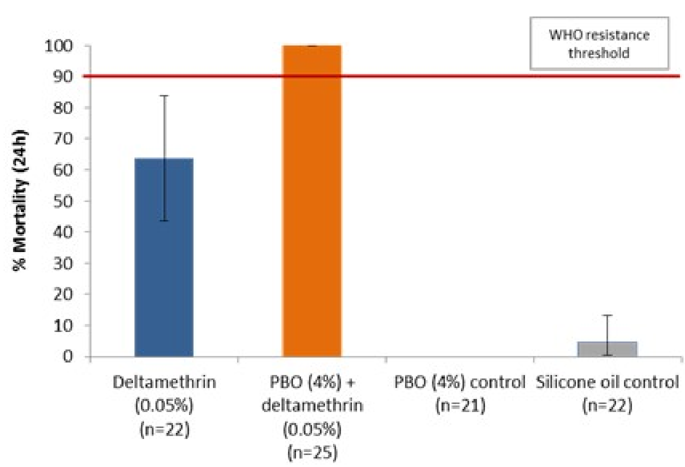


Figure 16: Percent Mortality of *An. gambiae* s.l. after Exposure in WHO Tube Tests to Deltamethrin (0.05%) and PBO (4%) + Deltamethrin (0.05%)



4. LOCAL CAPACITY AND INFRASTRUCTURE DEVELOPMENT

4.1 HUAMBO PROVINCE

Four entomology technicians (Silvestre Fernando, Vicente Chipepa, Luís Gonçalves, and Inácio Chilala) that had several years of prior entomology experience received refresher training. One Veterinarian (Mauro da Costa) and four staff from the Catchiungo mosquito brigade (Herculano Mussungo, José Tulumba, Almeida da Cunha, and Alberto Bapolo), received three days of basic training (Figure 17). The following topics were covered:

1. Brief introduction to the VectorLink Project and vector control approaches;
2. Introduction and practical set-up of an indoor CDC light trap according to project SOPs;
3. Introduction and practical set-up of a Prokopack aspirator according to SOPs;
4. Data collection forms for indoor CDC light trap, Prokopack and for WHO bioassays;
5. Morphological identification of primary and secondary *Anopheles* species using the key of Gillies and Coetzee (1987).

Figure 17: Training Photographs from Huambo

A- Morphological identification training using key of Gillies and Coetzee (1987), B- Training on CDC light trap.

4.2 LUNDA SUL PROVINCE

All field data collection was conducted in close collaboration with the Saurimo mosquito brigade. Before conducting data collection four mosquito brigade staff were trained under the supervision of George Olang (Consultant), Cátia Marques (Entomology Coordinator) and Arlete Troco (NMCP). The training lasted four days and covered the following topics (Figure 18):

1. Brief introduction to malaria and vector control approaches;
2. Introduction and practical set-up/use of indoor CDC light trap and indoor Prokopack aspirator;
3. Introduction and practice of WHO susceptibility bioassays;
4. Step-by-step review of all data collection forms for CDC light trap, Prokopack, and WHO bioassays;
5. Morphological identification of primary and secondary *Anopheles* species;
6. Step-by-step review of SOPs.

Figure 18: Training of Mosquito Brigade Staff and Technicians on the Use of the CDC Light Traps (A, C) and Prokopack Aspirator (B) in Lunda Sul Province

4.3 LUANDA PROVINCE

VectorLink collaborated with INIS in Luanda to develop local capacity for entomological monitoring. A new insectary was established by renovating an existing building to prepare separate rooms for rearing of *Anopheles* larvae and adults, as well as a room for processing field mosquito samples and performing microscopy for morphological identification (Figure 19). One Field Epidemiology Training Program (FETP) fellow (Luzala Garcia) received training in Huambo and conducted CDC LT, Prokopack aspiration, and larval collections in both Huambo and Lunda Sul. The Entomology Coordinator based in Luanda was recruited by the project and facilitated all field data collection and training. All *Anopheles* species collected through indoor CDC LTs and by Prokopack aspiration have been stored individually in Eppendorf tubes and will be used later in 2020 for laboratory analyses at INIS in Luanda for identification by polymerase chain reaction (PCR) and to determine malaria sporozoite infection rates in mosquitoes. All mosquito samples were individually labelled, with the corresponding information for each sample recorded in an Excel database. Eppendorf tubes were stored in Ziploc bags for each date of collection and location. In Year 2, INIS staff will receive training with support from PMI headquarters before starting molecular analysis of samples.

Figure 19: Insectary at INIS in Luanda Following Refurbishment

5. DISCUSSION

The main malaria vector species collected in Huambo Province were *An. funestus* s.l. while in Lunda Sul Province it was *An. gambiae* s.l., although there was local variation by village. Indoor CDC LT was the method that collected the majority of *Anopheles* in both Huambo and Lunda Sul. Early morning Prokopack aspiration collected the same species as CDC LT but added very few *Anopheles* to the daily total numbers collected. The relatively low number of *An. funestus* s.l. and *An. gambiae* s.l. collected early morning by Prokopack aspiration, compared to overnight CDC LTs, suggests that vector species exited houses very early (before sunrise) and did not rest indoors during the daytime. Therefore, we recommend that future trapping efforts should be primarily focused on indoor CDC LT collections.

The generally low total number of *Anopheles* collected in Huambo Province is likely due to several factors, including the heavy rain and cold weather during the collection period and the high altitude of the collection sites (1,750 meters above sea level). Moreover, ongoing vector control activities in the area (larviciding applications of Bti as well as fogging and spraying with pyrethroids) could have played a role in the low number of vectors during the capture period. Despite considerable efforts by the team, the low number of *An. gambiae* s.l. larvae and *An. funestus* adults collected made it impossible to conduct insecticide resistance testing in Huambo Province. More collections are needed to know the true pattern of the vector populations in Huambo Province (species composition and abundance of each). Given the investment in human resources and infrastructure made, the team recommends that surveillance activities continue in Huambo Province in the future.

Larval collections were more successful in Lunda Sul than in Huambo due to warmer temperatures, more suitable topography, and a soil type that is more favorable to standing water. In addition, no larviciding activities have been carried out in Lunda Sul since 2017. WHO susceptibility tests showed that *An. gambiae* s.l. were resistant to permethrin (mean mortality 33.3% (68/204)) and deltamethrin (mean mortality 41.4% (24/58)). While not a surprising result as insecticide resistance is a growing problem throughout PMI countries, the finding of pyrethroid resistance may mean that ITNs are not providing optimal control in Lunda Sul. Preliminary bioassays indicated an increase in mortality with pre-exposure to PBO followed by permethrin or deltamethrin. In the next work plan period, we will focus on conducting more replicates of susceptibility testing, including synergist assays in more provinces in order to provide NMCP with valuable information regarding choice of insecticides for ITNs and IRS. Previous data collected have indicated the importance of *An. funestus* found indoors in several provinces. Additional capacity building and surveillance is needed to collect adult *An. funestus* and conduct bioassays to ascertain resistance status of this primary vector species.

During data collection, we had great support from NMCP, the DPS, other health authorities, the mosquito brigades, and community leaders. There is a need to continue to work closely with these stakeholders to integrate *Anopheles* surveillance and insecticide susceptibility testing into the vector control activities of the province. There is also a need for further entomological capacity building of entomology technicians and provincial mosquito brigade staff. The team made great progress in building basic capacity and there is a workforce that can continue basic entomological monitoring in Year 2 with further VectorLink training and support. NMCP considers that continuous capacity building on NMCP staff and DPS provincial offices will improve ownership and sustainability of entomological activities in Angola. NMCP is committed to have dedicated staff to participate in training, data collection, supervision, and analysis, as well as to advocate for the importance of entomological activity within the higher levels of MOH.