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THE PMI VECTORLINK PROJECT UGANDA

ADDENDUM TO UGANDA ENTOMOLOGICAL MONITORING ANNUAL REPORT, JANUARY 1 - DECEMBER 31, 2018

(INCORPORATING MOLECULAR ASSAY DATA)

Recommended Citation: The PMI VectorLink Project Uganda. 2019. Addendum to *Uganda Entomological Monitoring Annual Report, January 1 - December 31, 2018*. Rockville, MD, USA: 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: January 10, 2020

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TABLE OF CONTENTS

ACRONYMS ..... **iv**

Executive Summary ..... **v**

1. Introduction **6**

2. METHODOLOGY **8**

2.1 Pyrethrum Spray Catch 8
2.2 Human Landing Catch 8
2.3 Vector Susceptibility Testing 8
2.4 Selection of Samples for Molecular Assay 8
2.5 Advanced Molecular Assays 9
2.6 Vector Species Identification 9

3. RESULTS **10**

3.1 Molecular Assay Results 10
3.1.1 Identification of Vector Species 10
3.1.2 Detection and Identification of Malaria Parasites *P. falciparum* and *P. vivax* 13
3.1.3 Entomological Inoculation Rate Estimates 13
3.1.4 Detection of Resistance Markers kdr and Ace-1 13

4. DISCUSSION **14**

5. REFERENCES **15**

LIST OF TABLES

Table 1: Summary of PCR analysis of *An. gambiae* and *An. funestus* complexes from bionomics and PSC studies conducted in sentinel sites in various districts in Uganda, 2018 11

Table 2: Summary of PCR analysis of *An. gambiae* s.l. that survived and those that died during insecticide susceptibility studies using WHO tube bioassays in different surveillance sites in Uganda, May–June 2018 12

ACRONYMS

Ace -1	Acetylcholinesterase 1 gene
CDC	Centers for Disease Control and Prevention
ELISA	Enzyme-linked Immunosorbent Assay
HLC	Human Landing Collection
IRS	Indoor Residual Spray
ITNs	Insecticide treated nets
kdr	knockdown resistance gene
LLIN	Long-lasting Insecticide-treated Net
NMCP	National Malaria Control Program
PBO	Piperonyl butoxide
PCR	Polymerase Chain Reaction
PMI	President's Malaria Initiative
PSC	Pyrethrum Spray Catch
USAID	United States Agency for International Development
WHO	World Health Organization

EXECUTIVE SUMMARY

Indoor residual spraying (IRS) and insecticide treated nets (ITNs) remain the primary mosquito vector control interventions in many parts of the world, including sub-Saharan Africa, where malaria continues to be a major public health concern.

During the 2018 spray campaign, the U.S. President's Malaria Initiative (PMI) VectorLink Uganda Project conducted IRS with the organophosphate pirimiphos-methyl (Actellic® 300CS) in 15 districts in eastern and northern Uganda (Alebtong, Amolatar, Budaka, Bugiri, Butaleja, Butebo, Dokolo, Kaberamaido, Kibuku, Lira, Namutumba, Otuke, Pallisa, Serere and Tororo). Spraying in Alebtong, Amolatar, Dokolo, Kaberamaido and Otuke was funded by the Department for International Development, United Kingdom (DFID-UK), while spraying in the remaining 10 districts was funded by United States Agency for International Development (USAID)/PMI.

To inform vector control interventions, particularly IRS, the project conducted monthly entomological monitoring using the Centers for Disease Control and Prevention (CDC) light traps, human landing catches (HLCs), pyrethrum spray catches (PSCs), and cone wall bioassays (used only in sprayed areas) to assess the quality of spraying and determine the residual life of the insecticide sprayed. Insecticide susceptibility tests were carried out on pirimiphos-methyl (organophosphate), bendiocarb (carbamate), and three pyrethroids (alpha-cypermethrin, deltamethrin and permethrin) in two sprayed districts (Bugiri and Lira), two former IRS districts (Gulu and Kitgum) and six non-IRS districts (Hoima, Kamwenge, Katakwi, Nakaseke, Soroti and Wakiso).

VectorLink Uganda submitted the comprehensive Annual Entomological Monitoring Final Report in April 2019, before molecular assay results were available. This addendum supplements that report by presenting molecular assay results associated with samples collected in 2018.

Advanced molecular assays were performed on a proportion of samples collected from January to September 2018 in IRS intervention districts of Alebtong, Amolatar Budaka, Bugiri, Butaleja, Butebo, Dokolo, Kaberamaido, Kibuku, Namutumba, Otuke, Pallisa, Serere and Tororo, in longitudinal study districts of Apac, Bugiri, Otuke, Soroti and Tororo, and in susceptibility study districts of Bugiri, Gulu, Lira, Soroti.

Vector identification by polymerase chain reaction revealed a limited diversity of *Anopheles* species of the major vectors *Anopheles gambiae* s.l. which identified only *An. arabiensis* and *An. gambiae* s.s, and *An. funestus* s.l. which identified only *An. funestus* s.s. species. A test for accuracy in field morphological identification skills of the field team on samples collected by HLCs and by PSCs revealed 87% and 81.7% accuracy on all species, respectively. One-hundred percent accuracy was observed for samples collected using CDC light traps.

No assays were performed to detect malaria parasite sporozoites and for detection and identification of mutations on genetic resistance markers knockdown resistance (*kdr*) and Acetylcholinesterase-1 (*Ace-1)* genes.

1. INTRODUCTION

Indoor residual spraying (IRS) and insecticide-treated nets (ITNs) remain the primary mosquito vector control interventions in many parts of world, including sub-Saharan Africa, where the disease continues to be a public health concern.

Through the support of the U.S. President's Malaria Initiative (PMI), the Uganda IRS Project Phase I and Phase II implemented 10 rounds IRS in 10 districts in Northern Uganda. During the 2018 IRS campaign, PMI VectorLink Project Uganda sprayed in 15 target districts: Alebtong, Amolatar Budaka, Bugiri, Butaleja, Butebo, Dokolo, Kaberamaido, Kibuku, Lira, Namutumba, Otuke, Pallisa, Serere and Tororo.

Both Uganda Indoor Residual Spraying (IRS) Project Phase I and Phase II and the follow-on PMI VectorLink Uganda program supported the National Malaria Control Program (NMCP) to conduct entomological monitoring activities in Northern and Eastern Uganda to enhance capacity for entomological monitoring.

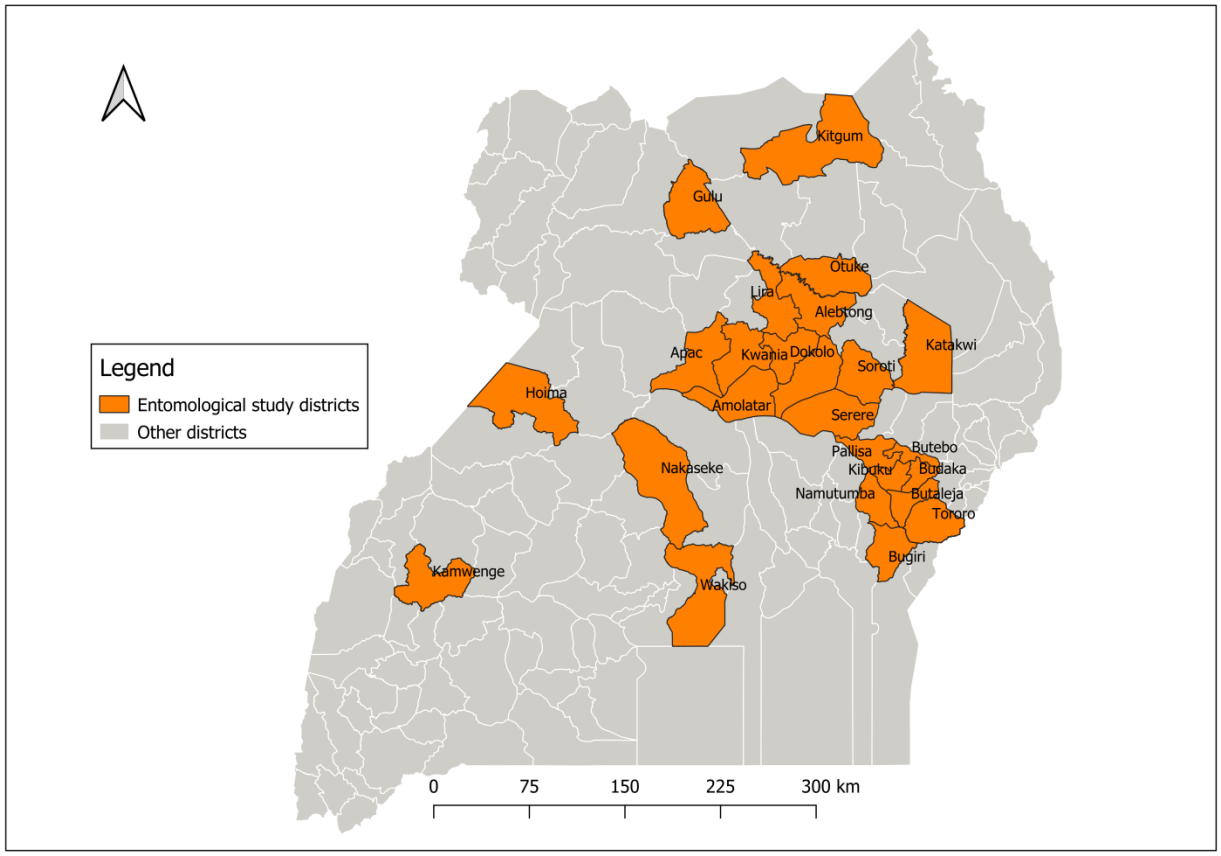
The PMI VectorLink Project carried out entomological monitoring activities in the indoor residual spraying (IRS) districts (Figure 1) and supported the National Malaria Control Division's entomological monitoring activities countrywide to enhance in-country capacity. Longitudinal entomological monitoring was conducted in three IRS intervention districts: Bugiri, Otuke and Tororo; Apac, a former IRS district; and Soroti, a district which was never sprayed were used as control districts. In all districts, entomological monitoring data was collected using pyrethrum spray catches (PSCs), Centers for Disease Control and Prevention (CDC) light traps and human landing collections (HLCs) indoors and outdoors. For susceptibility tests, trained village adult mosquito collectors used test tubes to collect adult *Anopheles funestus* s.l., while larval collections were conducted to collect *An. gambiae* s.l. Mosquito collectors would use torches to search inside houses for resting mosquitoes. Once identified, the test tube is placed over the mosquito which would then enter the test tube which is first closed with a finger/thumb and then with a ball of cotton wool which is pushed inside the tube to confine the mosquito. Up to three mosquitoes can be collected in one test tube.

The main objective of the entomology surveillance activities is to provide entomological data to inform decision making on IRS targeting and on choice of insecticide, and to measure impact on entomological indicators. The information generated from the surveillance includes vector identification, vector abundance and seasonality, vector biting patterns and location; measurement of spray quality; and monitoring the decay rate of insecticides sprayed on different types of wall surface. The project was also tasked with building NMCP capacity to do insectary management and associated field vector surveillance.

Gulu University, through Dr. Richard Echodu, Dean of the Faculty of Science, was contracted to conduct a molecular analysis of mosquitoes from various entomological monitoring studies carried out by the President's Malaria Initiative (PMI) VectorLink Project in collaboration with Uganda's Ministry of Health. In total, 916 *An. gambiae* s.l. and 140 *An. funestus* s.l. were collected from longitudinal surveillance, 134 *An. gambiae* s.l. and 9 *An. funestus* s.l. were collected from pre- and post-indoor residual spraying (IRS) PSCs, and 1,024 *An. gambiae* s.l. that were used for insecticide resistance tests were subsequently sent to Gulu University for molecular analysis for determination of species, mechanism of resistance and infection status. So far, there are laboratory results on species identification for 820 *An. gambiae* s.l. and 132 *An. funestus* s.l. from the longitudinal data, 116 *An. gambiae* s.l. and 5 *An. funestus* s.l. for pre-IRS PCSs, and 897 *An. gambiae* s.l. from the insecticide resistance tests. However, Gulu University has encountered challenges in carrying out the molecular analysis, especially with the reagents for *kdr* analysis, and no laboratory results related to the mechanism of resistance and infection status has been received to date. Mosquito species identification was conducted using polymerase chain reaction (PCR) procedures as described by Scott *et al.* (1993) for *An. gambiae* s.l. and Koekemoer *et al.* (2002) for *An. funestus* s.l.

This addendum report presents molecular data associated with the results presented earlier in the Entomological Monitoring Annual Report for 2017/18, which VectorLink Uganda submitted on April 1, 2019. It covers the period from January to September 2018 where activities were conducted under the PMI VectorLink Project.

Figure 1: PMI VectorLink Project Districts for Entomological Monitoring



Key: Districts where various entomological monitoring activities were conducted in 2018

No.	Entomological monitoring activity	Districts where conducted
1.	Bionomics studies	Bugiri, Otuke and Tororo, Apac and Soroti
2.	Pre-IRS PSCs to monitor baseline indoor resting vector densities	Alebtong, Amolatar, Budaka, Bugiri, Butaleja, Butebo, Dokolo, Kaberamaido, Kibuku, Lira, Namutumba, Otuke, Pallisa, Serere, and Tororo
3.	Post-IRS PSCs and cone wall bio-assays to monitor impact of IRS on indoor resting vector densities and quality of spraying	Alebtong, Amolatar, Budaka, Bugiri, Butaleja, Butebo, Dokolo, Kaberamaido, Kibuku, Lira, Namutumba, Otuke, Pallisa, Serere, and Tororo
4.	Cone wall bio-assays to monitor residual efficacy of Actellic 300CS	Kaberamaido, Lira, Pallisa, and Tororo
5.	Insecticide susceptibility studies	Bugiri, Gulu, Hoima, Kamwenge, Katakwi, Kitgum, Lira, Nakaseke, Soroti and Wakiso

2. METHODOLOGY

This section describes briefly each mosquito collection methods used. The various sampling methodologies were detailed in the Entomological Monitoring Annual Report submitted in April 2019.

2.1 PYRETHRUM SPRAY CATCH

In each selected sentinel district (Apac, Bugiri, Otuke, Soroti and Tororo), one village was selected for PSC. Twenty houses per village were chosen in each district, giving a total of 20 houses per district. The houses were selected randomly at different distances to cover the area selected in each village and the same houses were sampled each month. The samples were identified morphologically and preserved in 1.5 ml Eppendorf tubes containing silica gel. A sub-sample of the mosquitoes collected were sent for PCR analysis to the Gulu University Molecular laboratory.

2.2 HUMAN LANDING CATCH

HLCs were conducted in the intervention districts of Bugiri, Otuke and Tororo; and control districts of Apac, of Soroti. Two houses were sampled in each district in a selected village on two consecutive nights to obtain four person-nights of collection per district per month (2 houses x 2 collection nights = 4 person-nights). The same houses were sampled each month. Collected mosquitoes were killed using cotton soaked in diethyl methyl; identified; counted by species, location, and hour of collection; and preserved in 1.5 ml Eppendorf tubes with silica gel. A sub-sample of the mosquitoes collected by HLC were sent for PCR analysis at the Gulu University Molecular laboratory.

2.3 VECTOR SUSCEPTIBILITY TESTING

Immature malaria vectors were collected from different larval habitats in Bugiri, Gulu, Lira, Soroti districts from May to June 2018. Field-collected *An. gambiae* s.l. larvae were reared to adult stage in the insectary. Batches of sugar-fed and 3–5 days old 25 females, were subsequently subjected to World Health Organization (WHO) tube tests following the standard WHO 2016 protocol. These females were exposed to pirimiphos-methyl 0.25%, bendiocarb 0.1%, alphacypermethrin 0.05%, deltamethrin 0.05% and permethrin 0.75% on WHO impregnated filter papers for 60 minutes.

Intensity assays were conducted by exposing wild-caught vector mosquitoes to insecticide dosages of 5× and 10× the diagnostic concentrations of alphacypermethrin, deltamethrin and permethrin, according to the standard WHO bioassay method. All exposures were for one hour, and final mortality was scored after a 24-hour holding period during which a 10% sugar solution was made available to surviving mosquitoes.

Piperonyl butoxide (PBO) synergist assays were conducted using 3–5-day-old *An. gambiae* s.l. mosquitoes reared from field-collected larvae.

The exposure time was 60 minutes. Methodologies used for the above synergist assays, susceptibility tests, and strength of resistance were detailed in the Entomological Monitoring Annual Report of April 2019. Similar to other collections, a portion of samples from these tests were subjected to PCR assays at the Gulu University Molecular laboratory to identify sibling species.

2.4 SELECTION OF SAMPLES FOR MOLECULAR ASSAY

About 30% of annual samples to be assayed for each collection method in each district was pre-determined for molecular species identification during the planning phase of the activity. The number was estimated based on mosquito specimens available at the PMI VectorLink Project offices in Tororo and available budget.

A total of 2,416 *Anopheles* samples from various study sites and collection methods (longitudinal, insecticide susceptibility, and pre-and post IRS PSC studies) were selected randomly based on the number of monthly collections and number of mosquitoes used for susceptibility tests.

2.5 ADVANCED MOLECULAR ASSAYS

The advanced molecular assays performed by the Gulu University Molecular laboratory included the vector species identification only.

2.6 VECTOR SPECIES IDENTIFICATION

Following morphological identification of individual samples in the field, a selected proportion of malaria vectors (*An. gambiae* s.l. and *An. funestus* s.l.) were amplified by PCR and directly sequenced using deoxyribonucleic acid (DNA) barcoding (mtDNA COI) and ITS2 (nDNA) primers and protocols for species confirmation. These initial screens provided verified positive controls for the PCR-based species diagnostic assays for downstream identifications. Where species diagnostic primers revealed unexpected band sizes, ITS2 or COI sequences were performed to verify species identity (Koekemoer et al. 2002). The MR4 *An. gambiae* s.l. assay for species diagnosis (Scott et al., 1993) was found to be highly reliable. *An. arabiensis* samples were identified using this assay. Specimens amplified as *"An. gambiae"* using this assay were not further speciated into *An. gambiae* s.s. or *An. coluzzii* because Gulu University did not include the primer for the speciation of *An. gambiae* s.s. into *An. gambiae* s.s. or *An. coluzzii.*

3. RESULTS

3.1 MOLECULAR ASSAY RESULTS

A total of 2,416 samples were delivered for analysis at Gulu University Molecular laboratory. Of 1,073 specimens that were analyzed, 312 specimens failed to amplify or gave strange amplicon sequences for the specific assays due to poor quality/degraded DNA.

3.1.1 IDENTIFICATION OF VECTOR SPECIES

Molecular assays performed on the samples morphologically identified *An. gambiae* s.l. and *An. funestus* s.l.

3.1.1.1. PCR analysis results of mosquito samples from longitudinal data collection

A summary of the PCR molecular analysis for the speciation of the *An. gambiae* s.l. and *An. funestus* s.l. from longitudinal surveillance is given in Table 1

Molecular analysis results of 132 mosquitoes morphologically identified as *An. funestus* s.l. from the longitudinal bionomics studies in the non-IRS district of Soroti, which used PSCs, indoor and outdoor human landing catches (HLCs), and CDC light trap catches as sampling methods, indicated that 114 were *An. funestus* s.s., 1 was *An gambiae* s.s., 8 were *An. arabiensis,* and 9 samples did not amplify. *An. funestus* s.s. was the only identified member of the *An. funestus* s.l. in Soroti. No other species in this complex was identified in the district. *An. arabiensis* was the most commonly identified member of the *An. gambiae* s.l. in all five longitudinal bionomics study districts of Apac, Bugiri, Otuke, Soroti, and Tororo (Table 1). However, in some of the districts, several discordant results were observed between morphological identification conducted by the Ministry of Health staff who conducted the bionomics studies and the molecular identification; this was especially true in Soroti and Otuke districts. Molecular identification showed that 19.1% (103/539) of mosquitoes morphologically identified as *An. gambiae* s.l. by ministry staff were actually *An. funestus* s.s., and 6.8% (9/132) of mosquitoes morphologically identified as *An. funestus* s.l. were *An. arabiensis* (12/136) and *An gambiae* s.s. (1/136). The original misidentification clearly indicates the need for capacity building in morphological identification of *Anopheles* mosquitoes among the ministry staff who conduct these studies.

Of the 132 morphologically identified *An. funestus* s.l. in Soroti, 114 (86.36%) were identified as *An. funestus* s.s., 8 (6.06%) *An. arabiensis*, 1(0.76%) *An. gambiae* s.s. and 9 (6.82%) could not be identified due to poor quality of DNA. For the 108 morphologically identified *An. gambiae* s.l. from HLCs indoor and outdoor studies in non-IRS districts, 38 (35.2%) were identified as *An. arabiensis,* 11 (10.2%) as *An. gambiae* s.s., 35 (32.4%) as *An. funestus* s.s., and 24 (22.2%) could not be identified due to poor quality of DNA. While for the 181 morphologically identified *An. gambiae* s.l. from HLCs indoor and outdoor studies in IRS districts, 160 (88.40%) were identified as *An. arabiensis,* 7 (3.87%) as *An. gambiae* s.s., 5 (2.76%) as *An. funestus* s.s., and 9 (4.97%) could not be identified due to poor quality of DNA (Table 1). For the 121 morphologically identified *An. gambiae* s.l. from PSC studies in IRS districts, 95 (98.51%) were identified as *An. arabiensis,* 1 (0.83%) as *An. gambiae* s.s., 1 (0.83%) as *An. funestus* s.s., and 24 (19.83%) could not be identified due to poor quality of DNA (Table 1).

3.1.1.2 PCR analysis results of mosquito samples from insecticide resistance test

Molecular analysis of the *An. gambiae* s.l. found that *An. gambiae* s.s. was the most commonly identified member of the *An. gambiae* s.l. used in susceptibility tests in the non-IRS district of Gulu, while *An. arabiensis* was the most commonly identified member of the *An. gambiae* complex used in tests in the IRS districts of Bugiri and Lira and in the non-IRS district of Soroti (Table 2). There were no mis-identification of mosquitoes species sampled from insecticide resistance tests (% accuracy= 100%).

An. gambiae s.s. was the most commonly identified member of the *An. gambiae* s.l. that survived exposure to deltamethrin insecticide in tests in Gulu district and among those that died after exposure to permethrin in Gulu and Soroti districts and to bendiocarb and pirimiphos-methyl in Gulu district. *An. arabiensis* was the most commonly identified member of the *An. gambiae* complex that survived exposure to deltamethrin in tests in Lira and Soroti districts. *An. arabiensis* was the most commonly identified member of the *An. gambiae* s.l. that died after exposure to alpha-cypermethrin insecticide in Bugiri, Lira, and Soroti districts. *An. arabiensis* was the most commonly identified member of the *An. gambiae* s.l. that died after exposure to permethrin in Bugiri and Lira districts, and after exposure to bendiocarb and pirimiphos-methyl in tests in Bugiri, Lira and Soroti districts (Table 2).

Table 1: Summary of PCR analysis of *An. gambiae* and *An. funestus* complexes from bionomics and PSC studies conducted in sentinel sites in various districts in Uganda, 2018

District	Sampling method and location	Field Team Morphological species ID result	Total # of mosquitoes sent for analysis	Total analyzed	PCR Results				% accuracy
					An. gambiae s.s.	*An. arabiensis*	Sample did not amplify	*An. funestus* s.s.	
A. Longitudinal studies:									
Apac	HLCs Indoors	*An. gambiae* s.l.	14	7	1 (14.3%)	5 (71.4%)	1 (14.3%)	0 (0.0%)	100%
Bugiri	PSCs	*An. gambiae* s.l.	80	25	3 (12.0%)	20 (80.0%)	2 (8.0%)	0 (0.0%)	100%
Bugiri	HLCs Indoors	*An. gambiae* s.l.	22	22	1 (4.5%)	21 (95.5%)	0 (0.0%)	0 (0.0%)	100%
Bugiri	HLCs Outdoors	*An. gambiae* s.l.	23	5	0 (0.0%)	5 (100%)	0 (0.0%)	0 (0.0%)	100%
Otuke	PSCs	*An. gambiae* s.l.	39	39	4 (10.3%)	10 (25.6%)	16 (41.0%)	9 (23.1%)	61%
Otuke	HLCs Outdoors	*An. gambiae* s.l.	16	12	1 (8.3%)	9 (75.0%0	2 (16.7%)	0 (0.0%)	100%
Otuke	HLCs Indoors	*An. funestus* s.l.	8	5	0 (0.0%)	4 (80.0%)	1 (20%)	0 (0.0%)	0%
Otuke	LTCs	*An. gambiae* s.l.	25	20	1 (5.0%)	15 (75.0%)	4 (20.0%)	0 (0.0%)	100%
Soroti	PSCs	*An. gambiae* s.l.	419	419	45 (10.7%)	101 (24.1%)	220 (52.5%)	53 (12.6%)	73.4%
Soroti	HLCs Indoors	*An. gambiae* s.l.	22	20	1 (5.0%)	8 (40.0%)	5 (25.0%)	6 (30.0%)	60%
Soroti	HLCs Indoors	*An. funestus* s.l.	126	126	1 (0.8%)	3 (2.4%)	8 (6.3)	114 (90.5%)	96.6%
Soroti	HLCs Outdoors	*An. gambiae* s.l.	81	81	9 (11.1%)	25 (30.9%)	18 (92.2%)	29 (35.8%)	54%
Tororo	PSCs	*An. gambiae* s.l.	33	28	0 (0.0%)	24 (85.7%)	4 (14.3%)	0 (0.0%)	100%
Tororo	HLCs Indoors	*An. gambiae* s.l.	61	61	5 (8.2%)	46 (75.4%)	5 (8.2%)	5 (8.2%)	91.1%
Tororo	HLCs Outdoors	*An. gambiae* s.l.	81	81	0 (0.0%)	79 (97.5%)	2 (2.5%)	0 (0.0%)	100%
Tororo	HLCs Outdoors	*An. funestus* s.l.	6	1	0 (0.0%)	1 (100%)	0 (0.0%)	0 (0.0%)	0%
Total		***An. gambiae* s.l.**	**916**	**820**	**71 (8.7%)**	**368 (44.9%)**	**279 (34.0%)**	**102 (12.4)**	**81.10%**
		***An. funestus* s.l.**	**140**	**132**	**1 (0.8%)**	**8 (6.1%)**	**9 (6.8%)**	**114 (86.4%)**	**92.70%**

District	Sampling method and location	Field Team Morphological species ID result	Total # of mosquitoes sent for analysis	Total analyzed	PCR Results				% accuracy
					An. gambiae s.s.	*An. arabiensis*	Sample did not amplify	*An. funestus* s.s.	
B. Pyrethrum spray collections:									
Alebtong	Pre-IRS PSC	*An. gambiae* s.l.	3	3	0 (0.0%)	3 (100%)	0 (0.0%)	0 (0.0%)	100%
Amolatar	Pre-IRS PSC	*An. gambiae* s.l.	2	2	0 (0.0%)	1 (50.0%)	1 (50.0%)	0 (0.0%)	100%
Budaka	Pre-IRS PSC	*An. gambiae* s.l.	3	1	0 (0.0%)	0 (0.0%)	1 (100%)	0 (0.0%)	NA
Butaleja	Pre-IRS PSC	*An. gambiae* s.l.	40	37	0 (0.0%)	30 (81.1%)	7 (18.9%)	0 (0.0%)	100%
Butebo	Pre-IRS PSC	*An. gambiae* s.l.	15	12	0 (0.0%)	6 (50.0%)	5 (41.7%)	1 (8.3%)	85.7%
Dokolo	Pre-IRS PSC	*An. gambiae* s.l.	5	4	0 (0.0%)	3 (75.0%)	1 (25.0%)	0 (0.0%)	100%
Kaberamaido	Pre-IRS PSC	*An. gambiae* s.l.	13	11	0 (0.0%)	9 (81.8%)	2 (18.2%)	0 (0.0%)	100%
Kubuku	Pre-IRS PSC	*An. gambiae* s.l.	7	1	0 (0.0%)	1 (100%)	0 (0.0%)	0 (0.0%)	100%
Kibuku	Pre-IRS PSC	*An. funestus* s.l.	9	5	0 (0.0%)	4 (80.0%)	1 (20.0%)	0 (0.0%)	0%
Otuke	Pre-IRS PSC	*An. gambiae* s.l.	7	7	0 (0.0%)	7 (100%)	0 (0.0%)	0 (0.0%)	100%
Pallisa	Pre-IRS PSC	*An. gambiae* s.l.	30	30	0 (0.0%)	24 (80.0%)	6 (20.0%)	0 (0.0%)	100%
Serere	Pre-IRS PSC	*An. gambiae* s.l.	2	1	1 (100%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	100%
Tororo	Pre-IRS PSC	*An. gambiae* s.l.	7	7	0 (0.0%)	7 (100%)	0 (0.0%)	0 (0.0%)	100%
Total	**Pre-IRS PSC**	***An. gambiae* s.l.**	**143**	**121**	**1 (0.8%)**	**95 (78.5%)**	**24 (19.8%)**	**1 (0.8%)**	**99.00%**

Note: Under each row, figures refer to number and percent of the total mosquito samples analyzed

Table 2: Summary of PCR analysis of *An. gambiae* s.l. that survived and those that died during insecticide susceptibility studies using WHO tube bioassays in different surveillance sites in Uganda, May–June 2018

Study district	Insecticide	Survivors				Dead			
		An. gambiae s.s.	*An. arabiensis*	Negative (Didn't amplify)	Total *An. gambiae* s.l.	*An. gambiae* s.s.	*An. arabiensis*	Negative (Didn't amplify)	Total *An. gambiae* s.l.
Bugiri	Alpha-cypermethrin	3 (6.7%)	37 (82.2%)	5 (11.1%)	45	3 (4.5%)	56 (84.8%)	7 (10.6)	66
Gulu	Alpha-cypermethrin	13 (31.7%)	16 (39.0%)	12 (29.3)	41	16 (26.23%)	21 (34.43%)	24 (39.34%)	61
Soroti	Alpha-cypermethrin	3 (5.2%)	54 (93.1%)	1 (1.7%)	58	15 (19.2%)	56 (71.8%)	7 (9.0%)	78
Bugiri	Deltamethrin	*ND	ND	ND	ND	1 (14.3%)	4 (57.1%)	2 (28.6%)	7
Lira	Deltamethrin	1 (6.7%)	9 (60.0%)	5 (33.3%)	15	0 (0.0%)	3 (50.0%)	3 (50.0%)	6
Soroti	Deltamethrin	0 (0.0%)	74 (100%)	0 (0.0%)	74	1 (3.7%)	6 (22.2%)	20 (74.1%)	27
Gulu	Deltamethrin	12 (75.0%)	3 (18.75%)	1 (6.25%	16	4 (14.81%)	13 (48.15%)	10 (37.04%)	27
Gulu	Permethrin	ND	ND	ND	ND	68 (70.1%)	23 (23.7%)	6 (6.2%)	97
Soroti	Permethrin	ND	ND	ND	ND	54 (62.1%)	30 (34.5%)	3 (3.4%)	87

Study district	Insecticide	Survivors				Dead			
		An. gambiae s.s.	*An. arabiensis*	Negative (Didn't amplify)	Total *An. gambiae* s.l.	*An. gambiae* s.s.	*An. arabiensis*	Negative (Didn't amplify)	Total *An. gambiae* s.l.
Lira	Permethrin	ND	ND	ND	ND	9 (28.1%)	23 (71.9%)	0 (0.0%)	32
Bugiri	Permethrin	ND	ND	ND	ND	5 (12.2%	35 (85.4%)	1 (2.4%)	41
Gulu	Bendiocarb	ND	ND	ND	ND	9 (52.9%)	6 (35.3%)	2 (11.8%)	17
Lira	Bendiocarb	ND	ND	ND	ND	0 (0.0%)	20 (100%)	0 (0.0%)	20
Bugiri	Bendiocarb	ND	ND	ND	ND	0 (0.0%)	2 (100%)	0 (0.0%)	2
Lira	Pirimiphos-methyl	ND	ND	ND	ND	1 (5.6%)	17 (94.4%)	0 (0.0%)	18
Soroti	Pirimiphos-methyl	ND	ND	ND	ND	1 (4.5%)	21 (95.5%)	0 (0.0%)	22
Bugiri	Pirimiphos-methyl	ND	ND	ND	ND	1 (5.0%)	19 (95.0%)	0 (0.0%)	20
Gulu	Pirimiphos-methyl	0 (0.0%)	1 (100%)	0 (0.0%)	1	13 (68.4%)	6 (31.6%)	0 (0.0%)	19
Total		**32 (12.8%**	**194 (77.6%)**	**24 (9.6%)**	**250**	**201 (31.1%)**	**361 (55.8%)**	**85 (13.1%)**	**647**

Note: Under each row, figures refer to number and percent of the total mosquito samples analyzed

3.1.2 Detection and Identification of Malaria Parasites *P. falciparum* and *P. vivax*

Detection and identification of malaria parasites was not conducted by Gulu University Molecular laboratory.

3.1.3 Entomological Inoculation Rate Estimates

Since no ELISA studies were conducted by Gulu University Molecular laboratory to determine the Sporozoite rates, no entomological inoculation rates could be established.

3.1.4 Detection of Resistance Markers kdr and Ace-1

No assays for detection of *kdr* and *Ace-1* genes were performed by the Gulu University Molecular laboratory.

4. DISCUSSION

For the species identification performed at the Gulu University Molecular laboratory using molecular assays, *An. arabiensis* was found to be the dominant malaria vector of the *An. gambiae* complex followed by *An. gambiae* s.s., more so in the IRS districts, while *An. funestus* s.s. was the only species of *An. funestus* s.l. recorded from five longitudinal surveillance districts of Apac, Bugiri, Otuke, Soroti, and Tororo. Molecular analysis of the *An. gambiae* s.l. found that *An. gambiae* s.s. was also the most commonly identified member of the *An. gambiae* s.l. used in susceptibility tests in the non-IRS district of Gulu, while *An. arabiensis* was the most commonly identified member of the *An. gambiae* complex used in tests in the IRS districts of Bugiri and Lira and in the non-IRS district of Soroti. This was in contrast to what was happening prior to IRS in some of these districts. Historically, *An. arabiensis* has been encountered less frequently than *An. gambiae* s.s. in Apac district, with *An. funestus* s.s., *An. gambiae* s.s. and *An. arabiensis* contributing to 87.8%, 12.2% and 0% of annual entomological inoculation rate respectively; prior to introduction of IRS. In Tororo, *An. gambiae* s.s., *An. arabiensis and An. funestus* s.s. contributed to 80.0%, 1.5% and 18.5% of annual entomological inoculation rate respectively between June 2001 and May 2002 (Okello et *al.* 2006). Another study also confirmed that *An. gambiae* s.s. was the major vector in Tororo district (Okia et al. 2016) prior to IRS. While in this study *An arabeinbsis* was found to be more abundant than *An gambiae* or *An. fuenstus* s.s. in both Apac and Tororo districts. IRS might have had more impact on vectors that are resting and feeding indoors like *An gambiae* or *An. fuenstus* s.s. and led to shift in species composition. *An. arabiensis* seemed to have less affected by vector control that target indoors most likely to due to the behavioral plasticity this vector exhibits.

However, in some of the districts, several discordant results were observed between morphological identification conducted by the Ministry of Health staff who conducted the bionomics studies and the molecular identification; this was especially true in Soroti and Otuke districts. Overall, molecular species identification showed that 19.1% (103/539) of mosquitoes morphologically identified as *An. gambiae* s.l. by ministry staff were actually *An. funestus* s.s., and 10.23% (13/127) of mosquitoes morphologically identified as *An. funestus* s.l. were *An. arabiensis* (12/127) and *An. gambiae* s.s. (1/127). Most of the morphological species mis-identifications were recorded in Otuke and Soroti districts for both *An. gambiae* s.l. and *An. funestus* s.l.. In Otuke district, 9/23 (39.13%) of mosquitoes collected using PSC and morphologically identified as *An. gambiae* s.l. in the field turned out to be *An. funestus* s.s with PCR analysis and 4/4 mosquitoes reported as *An. funestus* s.l. by the ministry staff were all found to be *An. arabiensis* with PCR analysis. Similarly, 88/277 (31.76%) of mosquitoes reported as *An. gambiae* s.l. by the field ministry staff from Soroti were found to be *An. funestus* s.s with the molecular analysis. Lastly, 4/118 (3.4%) mosquitoes reported as *An. funestus* s.l. by the ministry field staff were identified as *An. gambiae* s.s. (1/118) and *An. arabiensis* (3/118) with molecular work. The original misidentification clearly indicates the need for capacity building in morphological identification of *Anopheles* mosquitoes among the ministry staff who conduct these studies.

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