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THE PMI VECTORLINK PROJECT

UGANDA

ANNUAL ENTOMOLOGY REPORT

DECEMBER 1, 2017 –DECEMBER 31, 2018

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ACRONYMS AND ABBREVIATIONS

Ace -1	Acetylcholinesterase 1 gene
b/p/h	Bites per Person per Hour
b/p/n	Bites per Person per Night
CDC	Centers for Disease Control and Prevention
ELISA	Enzyme-linked Immunosorbent Assay
HLC	Human Landing Catch
IRS	Indoor Residual Spray
KD	Knock Down
kdr	knockdown resistance gene
LLIN	Long-lasting Insecticide-treated Net
m/t/n	Mosquitoes per Trap per Night
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 long-lasting insecticide-treated bednets (LLINs) remain the primary mosquito vector control interventions in many parts of world, including sub-Saharan Africa, where malaria continues to be a major public health concern.

During the 2018 spray campaign, the 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 USAID/PMI.

To guide proper targeting of 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). 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).

The project tested clothianidin, a new insecticide molecule recommended by WHO for IRS from the neonicotinoid class, in 14 sites (Apac, Bugiri, Bugweri, Dokolo, Gulu, Kaberamaido, Katakwi, Kibuku, Lira, Otuke, Pallisa, Serere, Soroti and Tororo), while chlorfenapyr, another new insecticide molecule from the pyrrole insecticide class, was tested in 10 sites (Apac, Bugiri, Bugweri, Dokolo, Gulu, Kaberamaido, Katakwi, Kibuku, Serere and Tororo).

Mosquito collections using the methods listed above demonstrated the presence of highly diverse anopheline fauna, including both the main vectors *Anonpheles funestus* s.l. and *An. gambiae* s.l., and other potential vectors and non-vectors such as *An. ardensis, An. coustani, An. hancocki, An. maculipalpis, An. obscurus, An. paludis, An. pharoensis, An. pretoriensis, An. squamosus* and *An. ziemanni.*

Our findings highlight high levels of heterogeneity and diversity in mosquito vector species composition and behavior in the monitored areas. However, the role of the various anopheline mosquitoes in malaria transmission apart from *An. gambiae* s.l. and *An. funestus* s.l. needs to be investigated in the study districts.

Malaria vectors, *An. gambiae* s.l. and *An. funestus* s.l. were collected both indoors and outdoors. In some districts the densities were higher outdoors than indoors with the reverse being observed in other districts. *An. funestus* s.l. indoor biting collections were higher in Apac (54%), Otuke (67%) and Soroti (77%).For other anopheline mosquito species, outdoor collections were higher for *An. gambiae* s.l. than indoor collections in all districts except Apac district.

All the three sampling methods (CDC light traps, PSCs and HLCs) used in longitudinal mosquito collections yielded a total of 14,560 *Anopheles* mosquitoes from the five sentinel sites (Apac, Bugiri, Otuke, Soroti and Tororo districts). Morphological identification of the mosquitoes revealed that 10,594 (72.8 percent) were *An. funestus* s.l., 3,333 (22.9 percent) were *An. gambiae* s.l., 663 (4.3 percent) were other anopheline mosquitoes such as *An. ziemanni, An. coustani, An. ardensis, An. maculipalpis, An. squamosus, An. obscurus,* and *An. pharoensis.* Soroti (control) had the highest percentage of all *An. funestus* s.l. collected, 93.89 percent, followed by Apac at 4.3 percent, Otuke at 1.45 percent, Bugiri at 0.21 percent and last by Tororo at 0.06 percent (Table 2).

Both *An. gambiae* s.l. and *An. funestus* s.l. remained susceptible to pirimiphos-methyl, the insecticide used in the 2018 IRS campaign. Synergist tests with piperonyl butoxide (PBO) against *An. gambiae* s.l. indicated that the main resistance mechanism involved was monoxygenases, since pre-exposure to 4 percent PBO-treated

papers fully or partially restored susceptibility in resistant populations of *An. gambiae* s.l. Both *An. gambiae* s.l. and *An. funestus* s.l. were susceptible to clothianidin and chorfenapyr, the new next generation insecticides. However, both *An. gambiae* s.l. and *An. funestus* s.l. were resistant to pyrethroids (alpha-cypermethrin, deltamethrin and permethrin).

Cone wall bioassays performed for IRS quality assurance assays exhibited 100 percent mortality across different wall surface types, indicating that the spray operation achieved optimal quality spraying. The residual efficacy of the Actellic® 300CS used during the spray campaign remained effective for four to six months after the spray campaign (testing continuing in two districts).

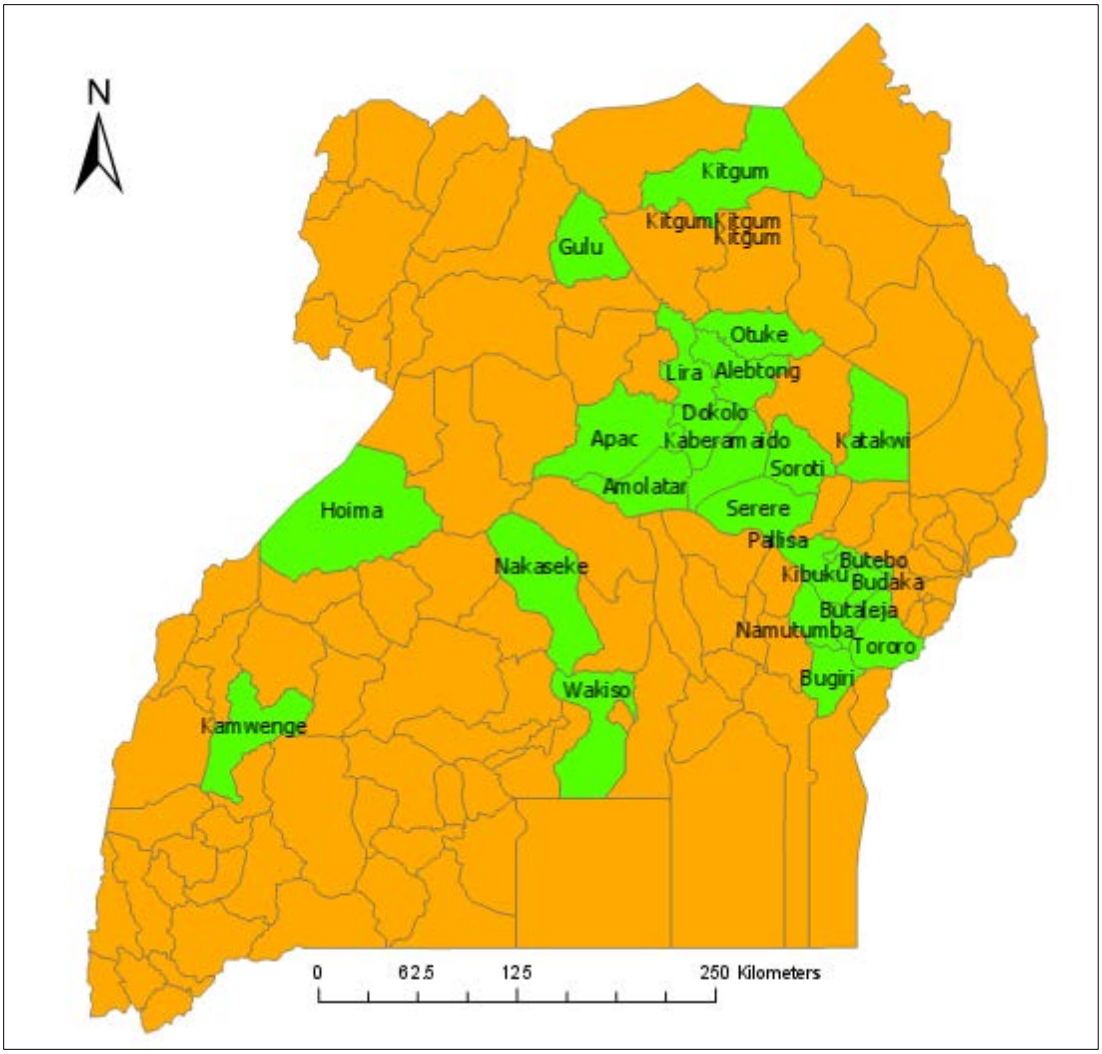
1. INTRODUCTION

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. Most of Uganda experiences a bi-modal rainfall pattern. March to May constitutes the first major rainfall season in Uganda, while September to November is the second rainfall season over most parts of the country. June to August is generally part of the dry season over most parts of south western, central, Lake Victoria basin and some parts of eastern region but a continuation of rainfall season for much of the northern Uganda. December to February is a dry season over most parts of the country, except for some areas around Lake Victoria, Western and South-Western which sometimes receive isolated rainfall in December. For the eastern region IRS districts, the first rains are usually experienced between late February to mid-March and peaking around late April, with cessation of rains expected around late May/mid-June except during El Nino years. For the northern region IRS districts, the first rains are usually experienced from mid-March with cessation of rains expected around late June/early July, except during El Nino years. Seasonality of malaria transmission is associated with rainfall pattern with increases 2-4 weeks after the start of rains and decreasing during the dry seasons.

Entomological monitoring activities help to supplement epidemiological data essential in guiding proper targeting of IRS; evaluate the susceptibility level of the local vectors to different insecticides and determine the underlying mechanisms; inform selection of insecticides; ensure the quality of spraying; monitor the impact of IRS on vector density, vector behavior, and composition; and determine parity rates and monitor the residual life of different insecticides on different types of wall surfaces. This entomological monitoring annual report covers the period from December 1, 2017 to December 31, 2018 under PMI VectorLink.

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 and hence used as a control district. In all these 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 using test tubes were used to collect adult *Anopheles funestus* s.l., while larval collections were conducted to collect *An. gambiae* s.l.

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

Longitudinal Monitoring (Bionomics Studies)

VectorLink Uganda collected adult mosquitoes on a monthly basis from February through December 2018 using PSCs, CDC light traps and HLCs in five sentinel sites: Bugiri, Otuke and Tororo (current IRS districts), Apac (a former IRS district, sprayed in February–March 2017 by the Ministry of Health), and Soroti (a non-IRS district used as the control district). Table 1 summarizes the longitudinal monitoring methods.

Table 1: Longitudinal monitoring adult mosquito collection methods

Collection Methods	Time	Frequency	Sample
PSCs	6:00 am to 10:00 am	Two days per site per month	Twenty houses per site (10 houses each day for two days)
HLCs	6:00 pm to 6:00 am	Two consecutive nights per site per month	Two houses per site
CDC LTs	6:00 pm to 6:00 am	Two consecutive nights per site per month	Two houses per site indoors only, using one CDC LT per house indoor

2.1. Behavior and Density

2.1.1. Pyrethrum Spray Catch

In each district where PSCs were conducted (Bugiri, Otuke and Tororo, Apac and Soroti), 20 houses in one village were selected. PSCs were conducted from 6:00 am to 10:00 am, once per month over two days in each district from February to December 2018. The same houses were visited each month. KillIt (commercial nomenclature) aerosol was used to knock down the mosquitoes. It contains the pyrethroids d-Tetramethrin 0.135% w/w, d-Allethrin 0.06% w/w and cypermethrin 0.46% w/w. One-roomed sleeping grass-thatched houses were selected for mosquito collection using PSCs. The room was closed for 5-10 minutes after spraying with Killt, and then the knocked-down mosquitoes were collected using forceps into a labeled petri dish. The samples were identified morphologically[1] and preserved in 1.5 ml Eppendorf tubes with a hole pierced in it and kept in a plastic container containing silica gel for further identification using the Polymerase Chain Reaction (PCR) technique, and a set of samples collected by this method during the mentioned period were sent for PCR at the Gulu University Biosciences Research Laboratories for further analysis.

2.1.2. Human Landing Catches

HLCs were conducted in Bugiri, Otuke, Tororo, Apac and Soroti. Two houses were sampled in each selected village on two consecutive nights to obtain four person-nights of collection per district per month (two houses x two collection nights = four person-nights). In all districts, two human volunteers (trained adult mosquito collectors) were positioned, one inside the house and the other outside, to collect mosquitoes. Collections were conducted from 6:00 pm to 6:00 am. For each hour of collection, collectors collected mosquitoes for 55 minutes and rested for 5 minutes, during which they exchanged positions. During the time

[1] Gillies MT and Coetzee M. 1987. A supplement to the Anophelinae of Africa south of the Sahara (Afrotropical Region). South African Institute for Medical Research, 55: 33–81.

of collection, the collectors sat quietly on a small chair and exposed part of their legs (up to the knees) and arms; when they felt landing mosquitoes, they turned on a torch and collected the mosquitoes using a mouth aspirator. Collected mosquitoes were transferred into labeled paper cups assigned for each hourly collection. Collected mosquitoes were subsequently killed using cotton soaked in diethyl ether, identified, counted by species, location, and hour of collection, and preserved in 1.5 ml Eppendorf tubes with a hole pierced in it and kept in a plastic container containing silica gel. A subset of samples from these collections was sent to the Gulu University Biosciences Research Laboratories for PCR analyses. Data obtained from HLCs were used to directly determine human biting rates (HBR). HBR was calculated by dividing the number of mosquitoes of single species/ species complex (*An. gambiae* s.l. and/or *An. funestus* s.l.) collected by number of person nights.

2.1.3. CDC Light Trap

CDC light traps were installed in two houses in three intervention districts (Bugiri, Otuke and Tororo), a former IRS district sprayed in February – March 2017 by the Ministry of Health (Apac) and control district (Soroti). In all these five study districts, data was collected for two consecutive nights, from 6:00 pm to 6:00 am, resulting in four trap nights per month for each district.

The traps were set up in the bedrooms beside the bed with humans sleeping under untreated bed nets, at the bed's footrest, about 1.5 m above the floor. After each night of collection, diethyl ether was used to kill the mosquitoes in the paper cups, and the mosquitoes were identified and preserved in 1.5 ml eppendorf tubes for future species identification based on PCR. The same houses were used each month. Data was collected from February 2018 to December 2018. A subset of samples from these collections was sent to the Gulu University Biosciences Research Laboratories for molecular analyses.

2.1.4. Vector Susceptibility Testing

Insecticide susceptibility studies using the World Health Organization (WHO) tube bioassays was conducted to determine insecticide susceptibility status, insecticide resistance intensity and resistance mechanisms of major malaria vectors, *An. gambiae* s.l. and *An. funestus* s.l. to insecticides recommended by the WHO for use in public health. The three classes of insecticides tested included: carbamates (bendiocarb 0.1%), pyrethroids (alpha-cypermethrin 0.05%, deltamethrin 0.05% and permethrin 0.75%) and organophosphates (pirimiphos-methyl 0.75%). These studies were conducted in 10 districts spread out throughout Uganda and thus somewhat representative between May and October 2018

The project also conducted insecticide susceptibility studies of *An. gamabiae* s.l. to the next generation insecticides clothianidin, a neonicotinoid,and chlorfenapyr, a pyrrole, in the districts of Bugiri, Gulu, Lira, Soroti, Apac, Bugweri, Katakwi Kaberamaido, Kibuku Otuke, Pallisa, Serere and Tororo between May and October 2018.

Field-collected larvae of *An. gambiae* s.l. were reared to adult stage in the field insectaries established in the study districts. Batches of 20-25 females, sugar-fed and three to five day old mosquitoes were subsequently subjected to WHO tube tests following the standard WHO 2016[2] protocol. Adult *An. funestus* s.l. mosquitoes collected in early mornings using test tubes were immediately used for susceptibility testing. These females were exposed to pirimiphos-methyl 0.25%, alpha-cypermethrin 0.05%, permethrin 0.75%, bendiocarb 0.1% and deltamethrin 0.05% on WHO impregnated filter papers for 60 minutes. Knockdown was scored at 60 minutes immediately after the exposure period, at which time all mosquitoes were gently transferred to holding tubes. Mortality was recorded at 24 hours after exposure. Where control mortality scored higher than 5 percent but below 20 percent, Abbott's correction was applied to test mortalities and those above 20

[2] World Health Organization (WHO). 2016. Test Procedures for Insecticide Resistance Monitoring in Malaria Vector Mosquitoes, 2nd Edition. Geneva: WHO.

percent led to tests being discarded (Abbott [3]1925). Susceptibility levels of *An. gambiae* s.l. and *An. funestus* s.l. were evaluated based on WHO criteria (WHO 2016). WHO classifies 24-hour mortality rates higher than 98 percent as susceptible, between 90 percent and 97 percent as suggestive of resistance and requiring further investigation, and below 90 percent as resistant.

Intensity assays were conducted by exposing wild caught vector mosquitoes to insecticide dosages of 5× and 10× the diagnostic concentrations of alpha-cypermethrin, deltamethrin and permethrin, according to the standard WHO bioassay method (WHO 2016). 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.

The synergist assays were conducted using mosquitoes reared from field-collected larvae. Four bioassay exposures were done as follows: In the first group of replicates, the mosquitoes were exposed to the insecticide only (alpha-cypermethrin, delatmethrin or permethrin), the second group was exposed to 4% piperonyl butoxide (PBO) only, the third group to 4% PBO followed by an insecticide (alpha-cypermethrin, delatmethrin or permethrin), and the last group was exposed to the Acetone solvent (control). All replicates were exposed for 60 minutes and mortality was recorded 24 hours after exposure, according to the WHO (2016) protocol. This process was repeated three times based on the standard procedure.

For clothianidin susceptibility tests, freshly treated filter papers (treated at 13.2 mg active ingredient per paper) were inserted into plastic cylinders and tested according to standard WHO susceptibility test protocols and according to the Africa Indoor Residual Spraying Project (AIRS)[4] standard operating procedure 001. The exposure time was 60 minutes. Afterward, mosquitoes were transferred into holding cylinders with filter paper treated only with distilled water and provided with lightly moistened cotton wool containing 10% sugar solution that was changed daily. Knock-down was recorded halfway through the test at 30 minutes and at the end of the test at 60 minutes. Mortality was recorded on days 1, 2, 3, 4, 5, and 6, and final mortality on day 7 after exposure. A negative control was tested at the same time and mortality recorded on days 1 through 7. The test was conducted with *An. gambiae* s.l. collected from several breeding sites in villages in 14 different districts (Apac, Bugiri, Bugweri, Dokolo, Gulu, Kaberamaido, Katakwi, Kibuku, Lira, Otuke, Pallisa, Serere, Soroti and Tororo) between May and October 2018. For each district, four replicates of 25 mosquitoes were tested (total of 100 sugar-fed females) with clothianidin papers, and two replicates were used at the same time with the negative control papers (impregnated only with distilled water). In addition to the negative control described above, a positive control was done by similarly exposing a laboratory-reared susceptible *An. gambiae* s.s. Kisumu strain.

For chlorfenapyr susceptibility tests, 250ml glass Wheaton bottles were treated at 100 µg/bottle concentration of chlorfenapyr according to AIRS[5] standard operating procedure 002, and *An. gambiae* s.l. and *An. funestus* s.l. were tested according to the CDC[6] bottle assay protocols. A 250ml glass bottle treated with 1ml of acetone solvent was used as the negative control. Testing of bottles was done within 24h of treatment. The exposure time was 60 minutes. Knock-down was recorded halfway through the test at 30 minutes and at the end of the test at 60 minutes while mosquitoes were still in the bottles. After the end of exposure, mosquitoes were released into clean cages and then gently aspirated into labelled paper cups covered with untreated netting and provided with lightly moistened cotton wool containing 10% sugar water. Mortality for both species was recorded days 1, 2, and final mortality on day 3 after exposure. The test was conducted with *An. gambiae* s.l. collected from several breeding sites in nine different districts (Apac, Bugiri, Bugweri, Dokolo, Gulu, Kaberamaido, Katakwi, Serere, Soroti and Tororo districts) between May and October 2018. For each district, four replicates of 25 mosquitoes were tested (total of 100 sugar-fed females) with clothianidin papers, and two replicates were used at the same time with the negative control papers (impregnated only with distilled water). In addition to the negative control described above, a positive control was done by similarly exposing a laboratory-reared susceptible *An. gambiae* s.s. Kisumu strain.

[3] Abbott WS. 1925. A method of computing the effectiveness of an insecticide. *Journal of Economic Entomology* 18: 265-267.
[4] Clothianidin test protocol (03 08 2017
[5] Chlorfenapyr test protocol (03 08 2017)
[6] Guideline for Evaluating Insecticide Resistance in Vectors Using the CDC Bottle Bioassay.

All the above susceptibility tests were conducted to the extent possible under the recommended optimal conditions, at temperatures around 27°C +-2°C and 70–80 percent relative humidity. Similar to other collections, a portion of samples from these tests were sent to the Gulu University Biosciences Research Laboratories for PCR assays to identify sibling species and detect presence of knockdown (*Kdr*) and acetylcolinesterase-1 (*Ace-1*) genes and to determine sporozoite rates.

2.1.5. IRS Quality Assays and Insecticide Decay Rate Monitoring

Standard WHO cone bioassay tests were performed in one site in each of the 15 spray districts (Alebtong, Amolatar, Budaka, Bugiri, Butaleja, Butebo, Dokolo, Kaberamaido, Kibuku, Lira, Namutumba, Otuke, Pallisa, Serere, and Tororo) within one week of the start of spraying to assess the quality of spraying. Routine wall bioassays were subsequently monitored monthly in four districts of Kaberamaido, Lira, Pallisa and Tororo up to December 2018 or until mortality dropped to below 80 percent for two consecutive months. Three houses of different wall types (plastered and painted, plain brick and mud-walled), were randomly selected in each study village. The same houses were used each month. Cones were placed at heights of 0.5 m, 1.0 m, and 1.5 m above the floor. Cones lined with self-adhesive tape were fixed on the sprayed walls for the assay. The control cone was affixed on a wall lined with a paperboard with adhesive in an unsprayed house to avoid any potential airborne effect. Two- to five-day-old female mosquitoes were used for the tests. Susceptible *An. gambiae* s.s. Kisumu strain mosquitoes were introduced into the plastic cones in batches of 10 and left exposed on the sprayed surface for 30 minutes at different heights. Numbers of mosquitoes knocked down at the 30th minute were recorded. At the end of the 30-minute exposure period, the mosquitoes were carefully collected and transferred to paper cups and provided with 10% sugar solution soaked on cotton wool pads placed on top of the paper cups covered with net with final mortality recorded after 24 hours holding period post-exposure.

Tests for the airborne effect of pirimiphos-methyl (Actellic® 300CS) were conducted with mosquitoes placed inside a mosquito net cage and hung 10 cm away from the sprayed wall surface at a height of 1.0 m above the floor with knock-down recorded at 30 mins and 60 mins after exposure. The mosquitoes were then transferred into clean paper cups that were kept for a 24-hour holding period. Dead and live mosquitoes were counted after 24 hours, and the percentage mortality was calculated for each house and recorded according to WHO protocol. *An. gambiae* s.s. (Kisumu strain) was used for quality and decay rate, and fumigant effect assessment.

3. RESULTS

3.1 Anopheline Species Collected by Different Methods

3.1.1 Longitudinal Monitoring

During the reporting period, in Bugiri, Otuke, and Tororo (current IRS districts), Apac (a former IRS district, sprayed in February–March 2017 by the Ministry of Health), and Soroti (a non-IRS district used as the control), a total of 14,560 female anopheline mosquito species were collected using the three collection methods (PSCs, CDC light traps (LTs), and HLCs) and morphologically identified.

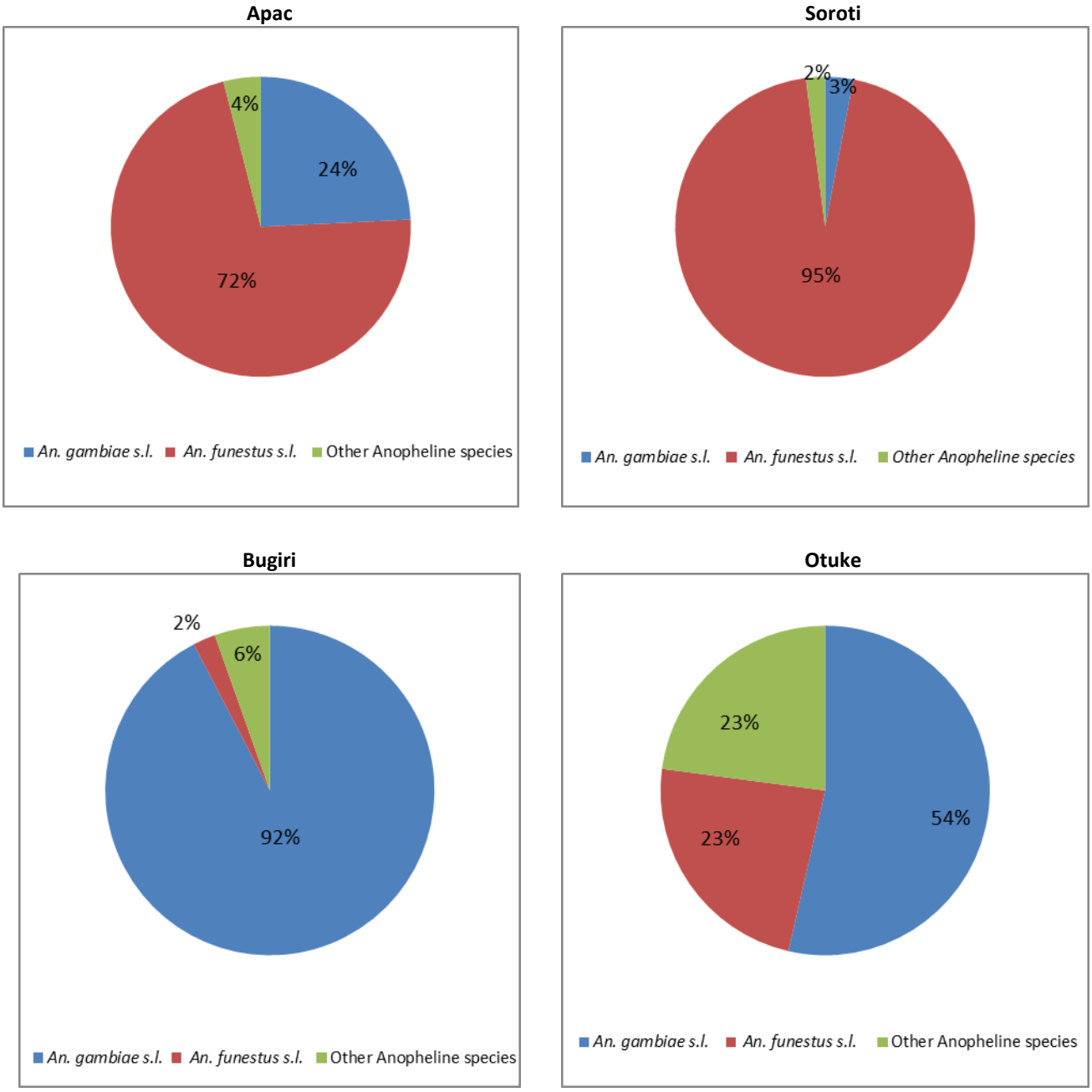
- A total of 3,333 *An. gambiae* s.l. were collected: 881 (26.4%) using PSCs, 1,605 (48.2%) using CDC LTs, and 847 (25.4%) using HLCs.
- A total of 10,594 *An. funestus* s.l. were collected: 5,781 (54.6%) using PSCs, 2,165 (20.4%) using CDC LTs, and 2,648 (25.0%) using HLCs.
- *An. funestus* s.l. was the most abundant (72.8%) *Anopheles* species collected, followed by *An. gambiae* s.l. (22.9%). Other anopheline species like *An. coustani, An. pharoensis, An. pretoriensis, An. squamosus,* and *An. ziemanni* comprised the remaining 4.3% (Figure 2). However, vector distribution differed by study site.

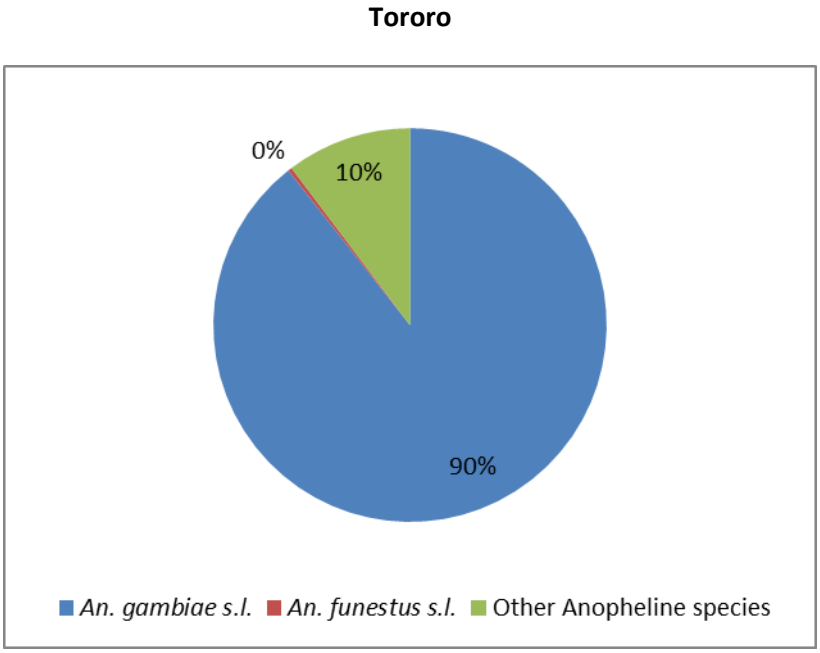
Table 2 summarizes the number of mosquitoes collected, by district and species, from February to December 2018.

Table 2: Number of female anopheline mosquitoes collected in each district by all three collection methods

Mosquito species	Apac	Bugiri	Otuke	Soroti	Tororo	Total	%
An. gambiae s.l.	157	918	352	317	1,589	3,333	22.9
An. funestus s.l.	465	22	154	9,947	6	10,594	72.8
Other Anopheline species	26	54	152	218	183	633	4.3
Total per district	**648**	**994**	**658**	**10,482**	**1,778**	**14,560**	**100**

Figure 2: *Anopheles* species composition by study site, February to December 2018





3.1.2 Pyrethrum Spray Catch

PSC collections yielded 6,691 *Anopheles* mosquitoes (Table 3 and Figure 3). By species they were 5,781 (86.40%) *An. funestus* s.l., 881 (13.17%) *An. gambiae* s.l., and 29 (0.43%) other anopheline species. Most *Anopheles* vectors were caught in the unsprayed (control) site. *An. gambiae* s.l. predominated catches in the intervention areas (Bugiri, Otuke and Tororo), while a greater proportion of *An. funestus* s.l. was caught in the former IRS district (Apac) and control area (Soroti).

Table 3: Number of mosquitoes by species collected using PSC in the study districts

	Apac	Bugiri	Otuke	Soroti	Tororo	Total
An. gambiae s.l.	76	366	72	117	250	845
An. funestus s.l.	355	14	81	5330	1	5781
Other Anophelines	0	0	6	21	2	29
Total per district	**431**	**380**	**159**	**5468**	**254**	**6691**

Figure 3: Indoor Resting Densities of *An. gambiae* s.l. and *An. funestus* s.l. in five districts before and after IRS Intervention

Figure 3A: *An. gambiae* s.l.

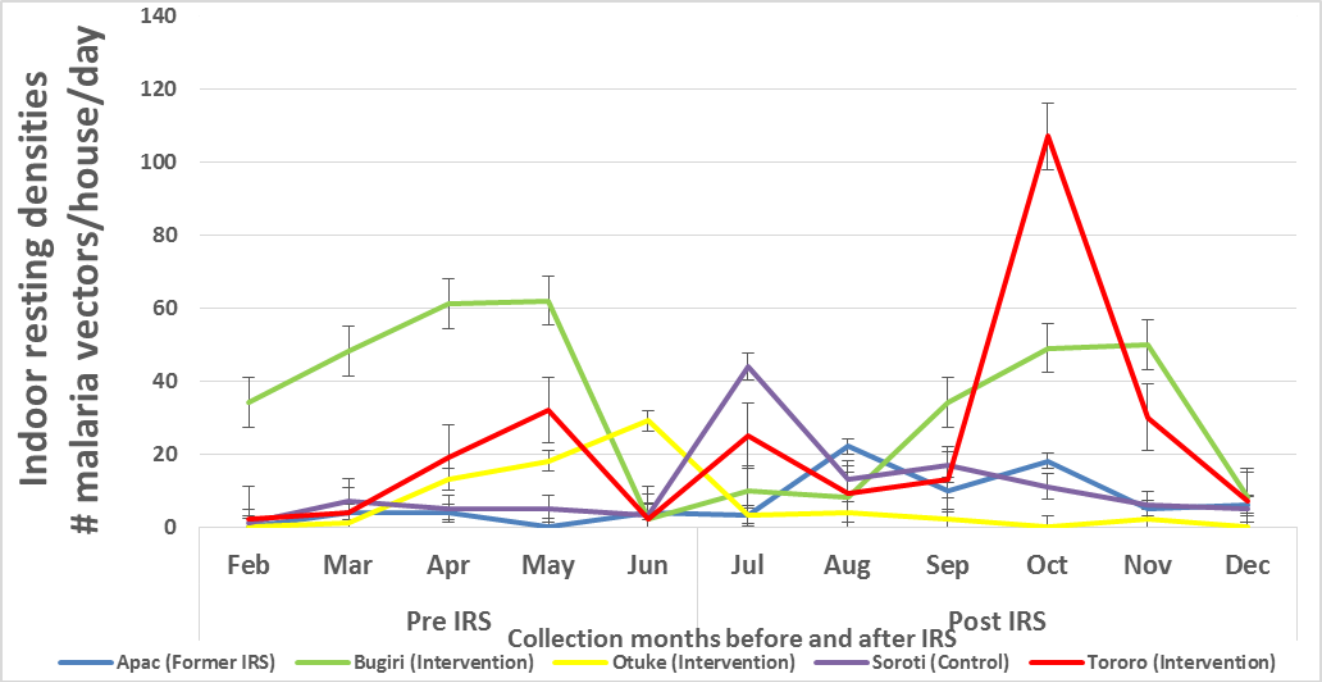
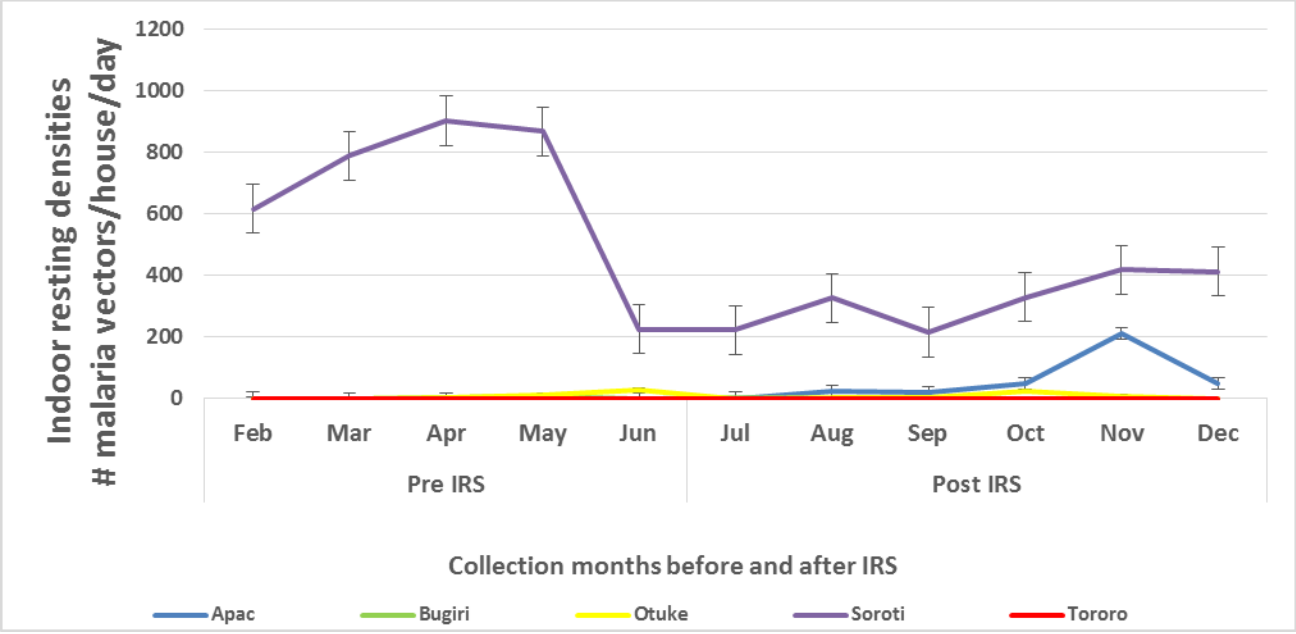


Figure 3B: *An. funestus* s.l.



3.1.3 Human Landing Catches

A total of 4,108 *Anopheles* mosquitoes were collected using HLCs from February 2018 to December 2018. The species identified morphologically from this collection were: *An. funestus* s.l. (2,778), *An. gambiae* s.l. (864), other anopheline species such as *An. ardensis, An. coustani, An. hancocki, An. maculipalpis, An. obscurus, An. paludis, An. pharoensis, An. pretoriensis, An. squamosu*s and *An. ziemanni* (466). *An. gambiae* s.l. was the most abundant mosquito species collected in the intervention areas (Bugiri, Otuke and Tororo), while *An. funestus* s.l. was the most abundant mosquito species collected in the former IRS district (Apac) and control area (Soroti) - Table 4. Otuke, Soroti and Tororo districts in that order appeared to have the highest anopheline species diversity.

All districts demonstrated a different pattern of *An. funestus* s.l. and *An. gambiae* s.l. human biting rates (HBRs) both indoors and outdoors and before and after IRS, which began in April 2018 for Bugiri and in June 2018 for Otuke and Tororo (Table 5 and Figures 4A – 4E). In the IRS (intervention) districts, the mean HBR dropped to between 0.21 to 0.92 and 0.50 and 1.17 bites per person per night (b/p/n) indoors and outdoors respectively for *An. gambiae* s.l. and between 0.00 to 0.13 and 0.00 and 0.08 b/p/n indoors and outdoors respectively for *An. funestus* s.l.. Soroti (control) had the highest mean *An. funestus* s.l. HBR both indoors and outdoors throughout the monitoring period.

Table 4: Mosquito species collected by HLC indoors and outdoors in five study districts in Uganda from February to December 2018

Anopheline species	Apac			Bugiri			Otuke			Soroti			Tororo			Total		Grand Total
	Indoors	Outdoors	Total	Indoors	Outdoors	Total	Indoors	Outdoors	Total	Indoors	Outdoors	Total	Indoors	Outdoors	Total	Indoors	Outdoors	
An. gambiae s.l.	29	31	60	76	78	154	22	48	70	68	81	149	187	244	431	382	482	865
An. funestus s.l.	41	33	74	3	2	5	10	5	15	2063	621	2684	0	0	0	2117	661	2778
Other Anophelines	11	5	16	13	36	49	15	58	73	50	119	169	53	106	159	142	324	466
Total per district	**81**	**69**	**150**	**92**	**116**	**208**	**47**	**111**	**158**	**2,181**	**821**	**3,002**	**240**	**350**	**590**	**2,641**	**1,467**	**4,109**

Table 5: Indoor and outdoor mean HBR for *An. gambiae* s.l. and *An. funestus* s.l., estimated using HLC, expressed as mean bites per person per night from all collection rounds, by district, before and after spraying

District	*Anopheles gambiae* s.l. (b/p/n)				*Anopheles funestus* s.l. (b/p/n)			
	Indoors		Outdoors		Indoors		Outdoors	
	Pre-spray	Post-spray	Pre-spray	Post-spray	Pre-spray	Post-spray	Pre-spray	Post-spray
Bugiri	3.25	0.86	3.69	0.68	0.13	0.04	0.13	0.0
Otuke	0.85	0.21	1.85	0.50	0.35	0.13	0.15	0.08
Tororo	8.25	0.92	11.05	1.17	0.00	0.00	0.05	0.00
Apac*	0.60	0.71	0.30	1.04	0.05	1.67	0.0	1.38
Soroti**	1.25	1.79	1.95	1.75	46.50	47.21	13.85	14.33

*Former IRS district. The pre- and post-spray estimates are based on the period when spraying was done in intervention districts: pre-spray was three months before spraying and post-spray was all the months after spraying up, to July 2018.

**Unsprayed control district. The pre- and post-spray estimates are based on the period when spraying was done in intervention districts: pre-spray was three months before spraying and post-spray was all the months after spraying up, to July 2018.

Figure 4: Monthly indoor and outdoor HBRs for *An. funestus* s.l. and *An. gambiae* s.l. in four intervention districts combined and one control district, before and after IRS intervention.

Figure 4A: Monthly indoor and outdoor HBRs for An. funestus s.l. and An. gambiae s.l. in four intervention districts combined and one control district, before and after IRS intervention.

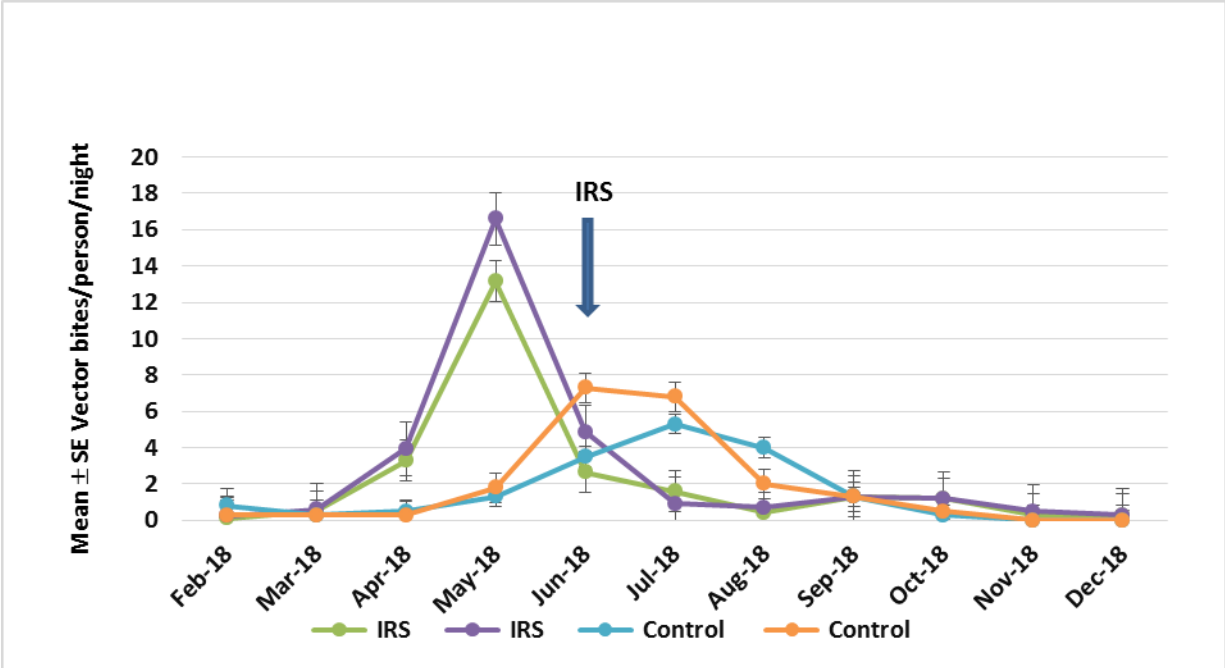


Figure 4B. Monthly An. funestus s.l. indoor HBR for current IRS combined, IRS withdrawn and control districts

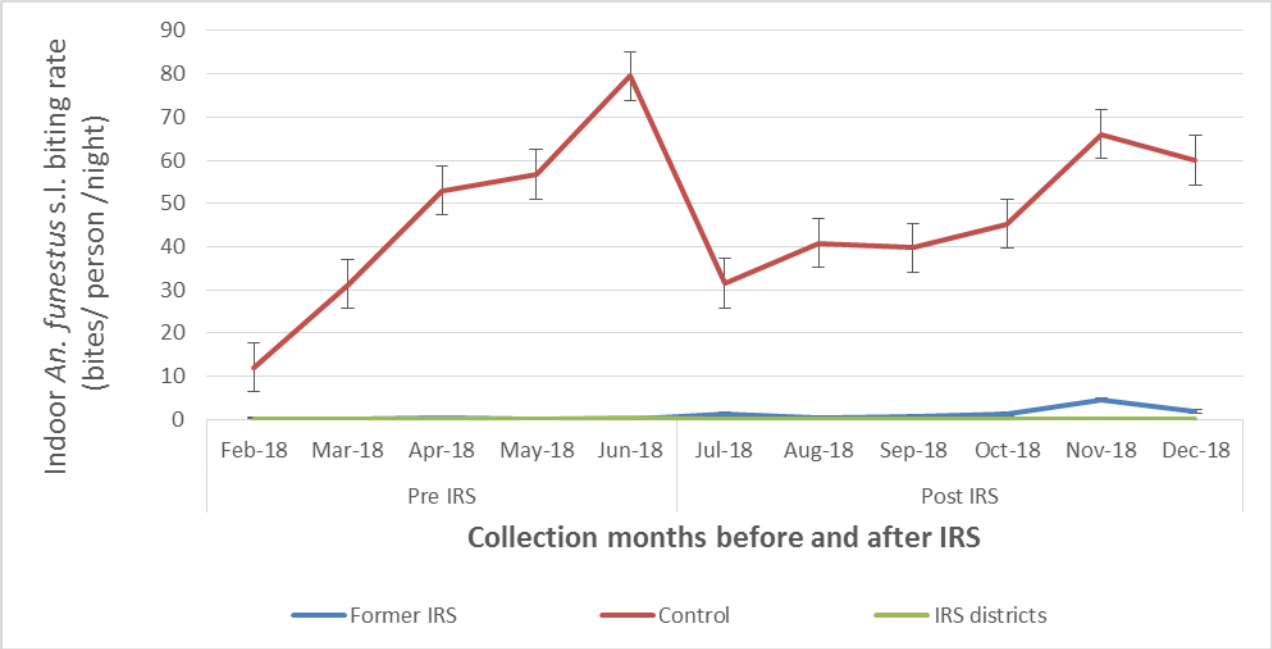


Figure 4C. Monthly An. funestus s.l. outdoor HBR in current IRS districts combined, IRS withdrawn and control districts

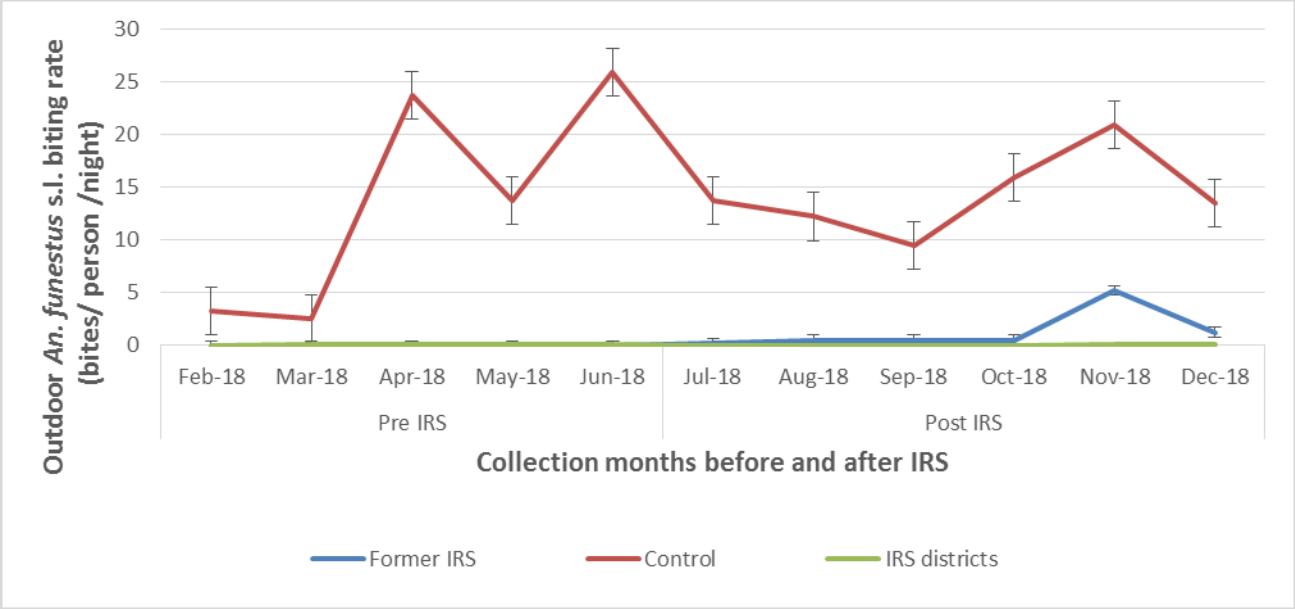


Figure 4D. Monthly An. gambiae s.l. outdoor HBR in current IRS districts combined, IRS withdrawn and control districts before and after IRS.

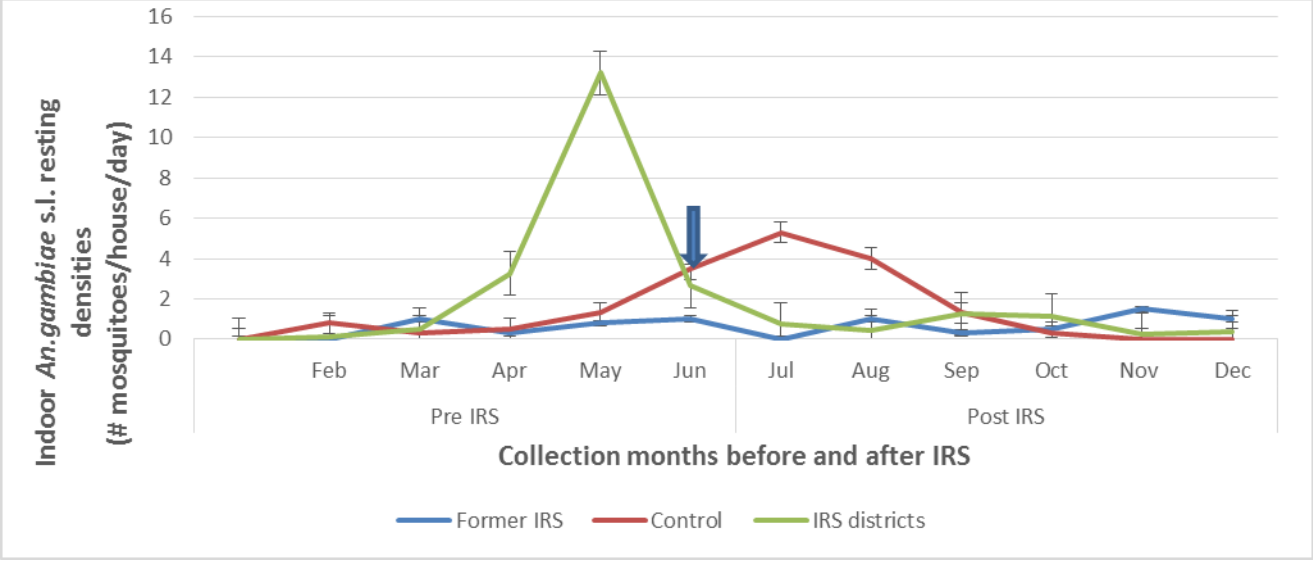
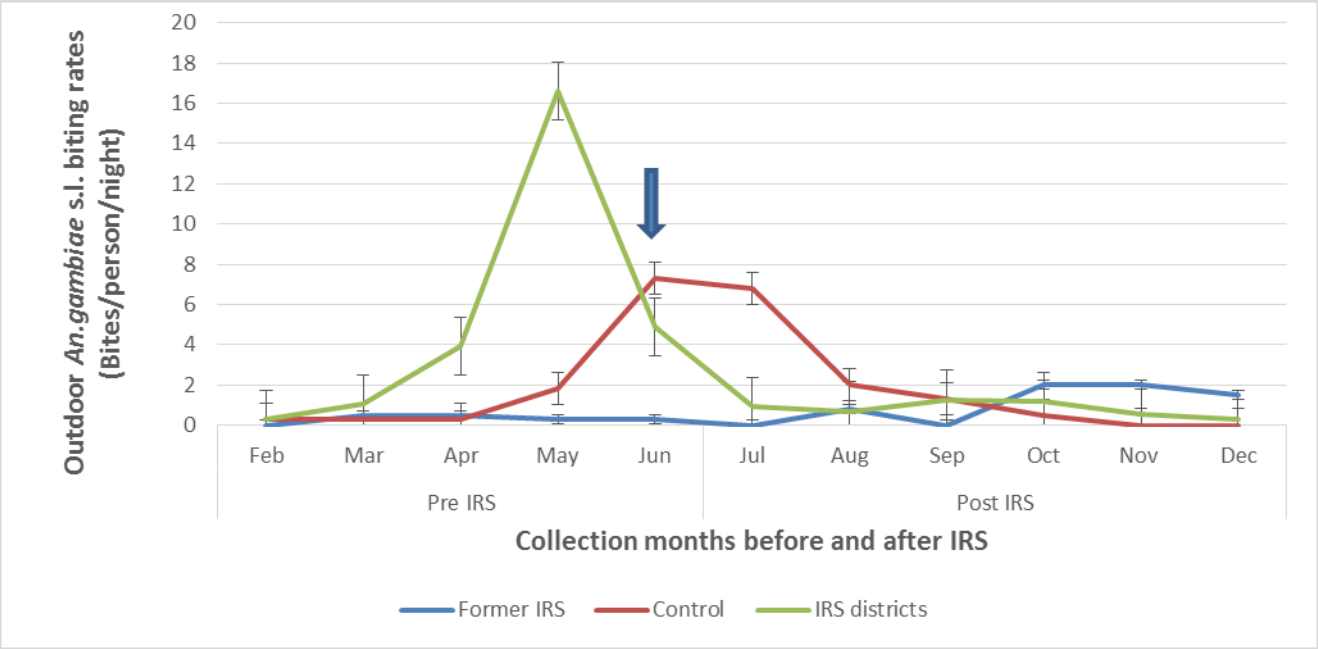


Figure 4E Monthly An. gambiae s.l. outdoor HBR in current IRS districts combined, IRS withdrawn and control districts before and after IRS



Annex 1 Table A shows the mean indoor and outdoor HBR for *An. gambiae* s.l. and *An. funestus* s.l. before and after spraying. Soroti, the control district, had the highest HBRs for *An. funestus* s.l. indoors and outdoors throughout the monitoring period. Following spraying, decreases in HBR were observed in the intervention districts. There was a drop in both *An. gambiae* s.l. and *An. funestus* s.l. HBR observed both indoors and outdoors in the intervention districts (Bugiri, Otuke and Tororo), while an increase was observed for both *An. gambiae* s.l. and *An. funestus* s.l. both indoors and outdoors in the former IRS district (Apac) and in the control district (Soroti). The low HBRs observed in the intervention areas could indicate the potential impact of spraying Actellic® 300CS against both predominant malaria vectors.

Table 6 shows numbers of each mosquito species collected in intervention areas, former IRS and control areas indicating for each the total person-nights and subsequent biting rate expressed as bites per person per night (b/p/n.) *An. funestus* s.l. and *An. gambiae* s.l. were observed to contribute to 94 percent, 89 percent and 71 percent of the bites in the control, former IRS and intervention areas, respectively.

Table 6: Mosquito species collected by HLC and their mean biting rates in intervention, former IRS and control areas, February to December 2018

Species Collected	Intervention Area			Former IRS District			Control Area		
	Total Numbers Collected	Total Person Nights	B/P/N	Total Numbers Collected	Total Person Nights	B/P/N	Total Numbers Collected	Total Person Nights	B/P/N
An. gambiae s.l.	655	132	4.96	60	44	1.36	149	44	3.39
An. funestus s.l.	20	132	0.25	74	44	1.68	2684	44	61.00
Other Anopheline Species	281	132	2.13	16	44	0.36	169	44	3.84

In all the study districts, the indoor biting activity of *An. funestus* s.l. was close to zero from 6:00 to 9:00 pm except for Soroti where it appears to be active throughout the night. In Soroti increasing biting activity occurs after midnight and peaks both indoors and outdoors between 4:00 a.m. and 6:00 a.m. (9 b/p/h). Biting

activity of *An. funestus* s.l. is higher indoors than outdoors wherever it occurs. The biting activity of *An. gambiae* s.l. follows a similar biting pattern with higher indoor biting activity than outdoors in all the study districts (Figures 5A-5D). *An. funestus* s.l. HBR was higher in the control area than in intervention areas, both indoors (3.91 versus 0.02 b/p/h) and outdoors (1.81 versus 0.01 b/p/h). Generally, the hourly HBRs of *An. gambiae* s.l. was low in all sites. The highest HBRs recorded was 0.8 bites per person per hour in Tororo (Figure 5C) unlike An. *funestus* s.l. which reaches 9 bites per person per hour (Figure 5A) in the control site.

Figure 5: Hourly HBRs of *An. funestus* s.l. in Apac, Bugiri, Otuke, Soroti and Tororo as Determined through HLCs

Figure 5A. *An. funestus* s.l. Indoor

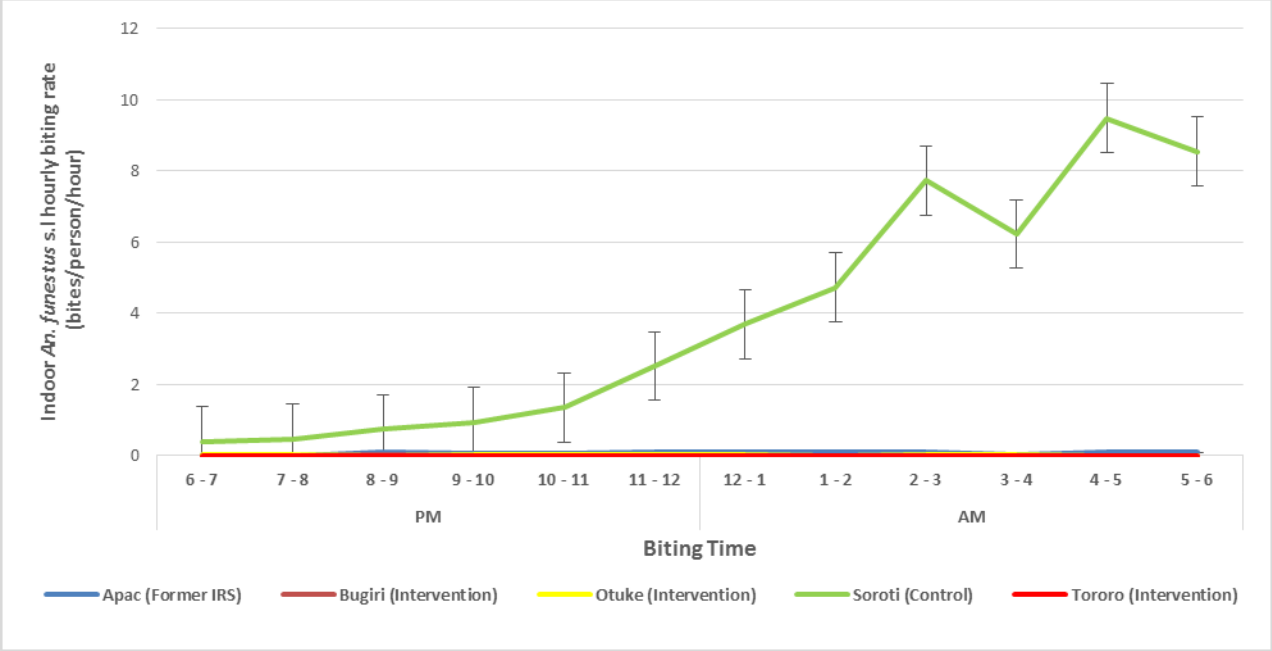


Figure 5B. *An. funestus* s.l. Outdoor

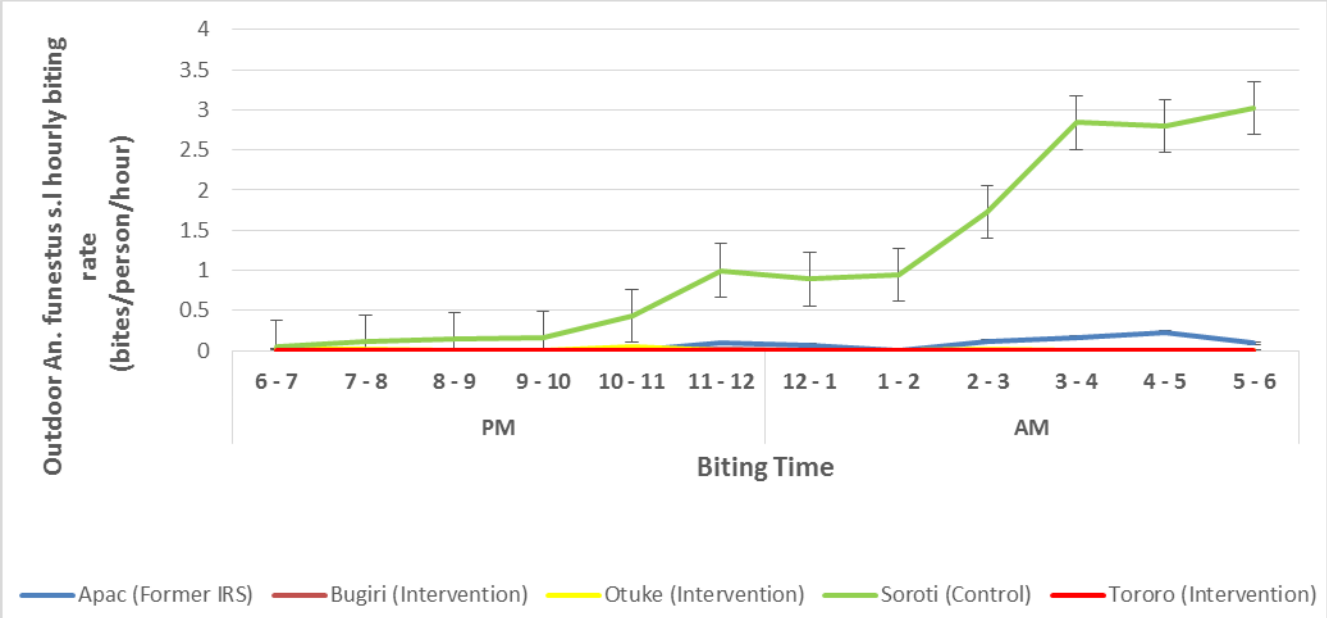


Figure 5C. ***An. gambiae*** **s.l. Indoor**

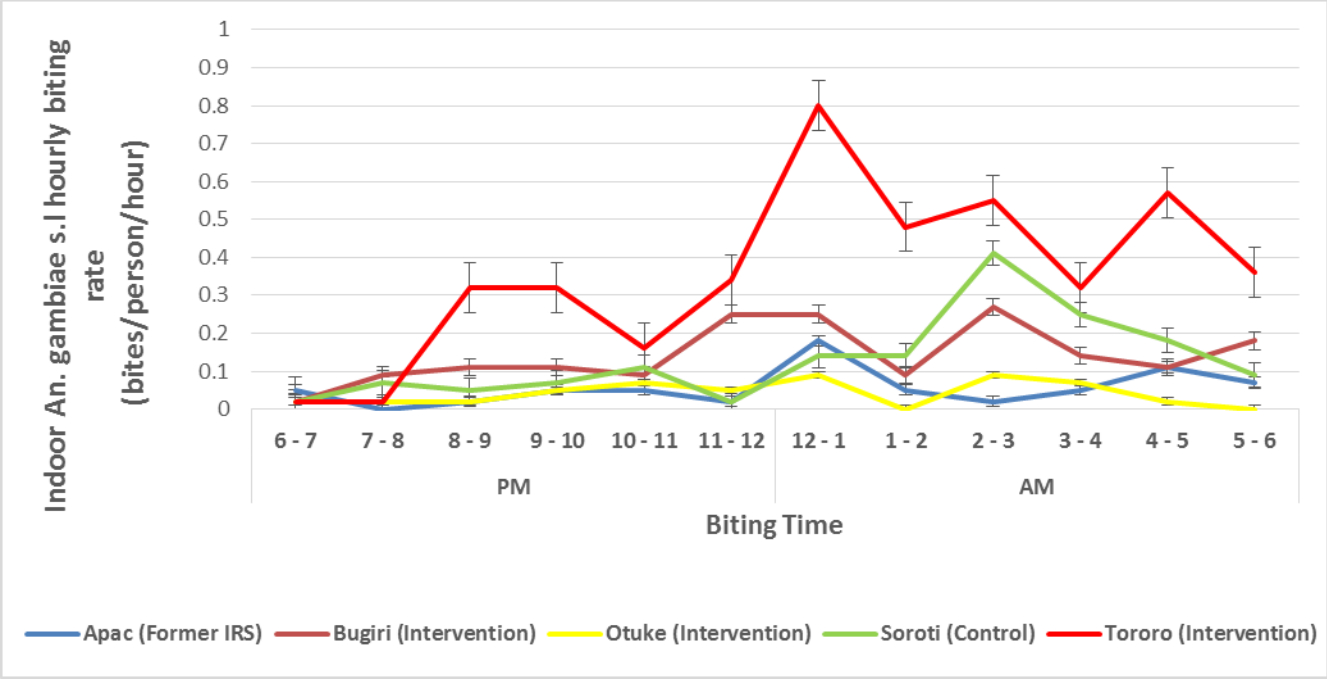
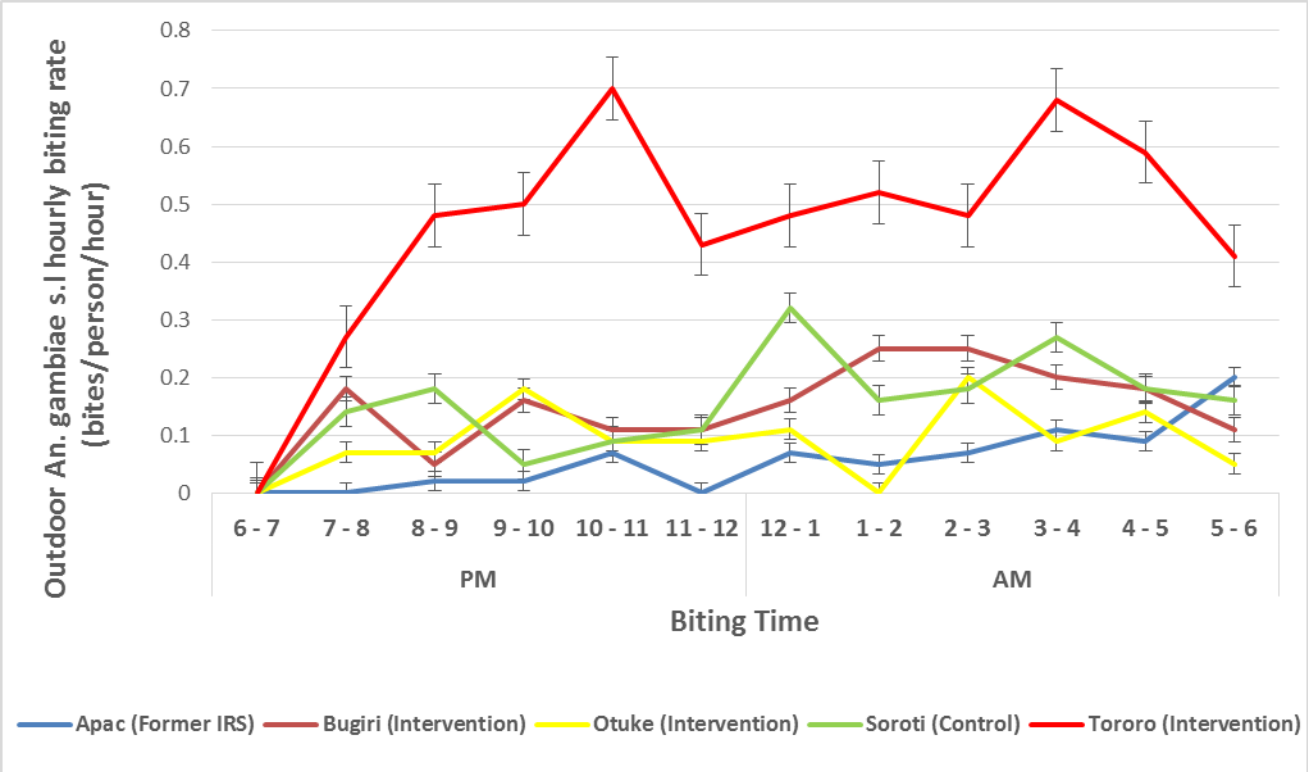


Figure 5D. ***An. gambiae*** **s.l. Outdoor**



3.1.4 CDC Light Trap (CDC-LT)

The CDC light trap collections yielded a total of 3,770 *Anopheles* mosquitoes from the five sentinel sites. Table 7 shows the monthly and total collections per district. Morphological identification of the mosquitoes revealed that 2,035 (53.98%) were *An. funestus* s.l., 1,588 (42.12%) were *An. gambiae* s.l., 147 (3.90%) were other anopheline mosquitoes like *An. ziemanni, An. coustani, An. ardensis, An. maculipalpis, An. squamosus, An. obscurus, An. pharoensis*. Soroti (control) had the highest percentage of all *An. funestus* s.l. collected, 94.99 percent, followed by Otuke at 2.85 percent.

In terms of mean collections, *An. funestus* s.l. was most abundant in Soroti (with mean collection of 43.93 mosquitoes per trap per night over the 11 collection months), followed by Otuke (with mean collection of 1.32 mosquitoes per trap per night over the 11 months). In Apac district, where IRS was discontinued in 2014 but re-sprayed by the Ministry of Health in February – March 2017, both *An. gambiae* s.l. and *An. funestus* s.l. were collected in low densities over most of the monitoring period. Peak density, 4.50 and 1.25 mosquitoes per trap per night (m/t/n), respectively for *An. funestus* s.l. (recorded in November 2018) and *An. gambiae* s.l. (recorded in August and October 2018). The mean density was 1.49 m/t/n. *An. gambiae* s.l. densities were similarly very low over most of the collection period, with a mean density of 0.18 m/t/n (Figures 6A and 6B).

A sub-set of the mosquitoes collected from longitudinal studies are being analyzed for speciation of the *An. gambiae* and *An. funestus* complexes, the presence of knock-down resistance (*Kdr*), determination of sporozoite rates.

Table 7: Number of Anopheles mosquitoes collected from the five sentinel sites using CDC light trap collections

	Apac	Bugiri	Otuke	Soroti	Tororo	Total
An. gambiae s.l.	21	398	210	51	908	1588
An. funestus s.l.	36	3	58	1933	5	2035
Other Anophelines	10	5	75	29	28	147
Total per district	67	406	343	2013	941	3770

Figure 6: Indoor CDC Light Trap Density per Trap Per Night in Apac, Bugiri, Otuke, Soroti and Tororo Districts

Figure 6A. ***An. funestus*** **s.l.**

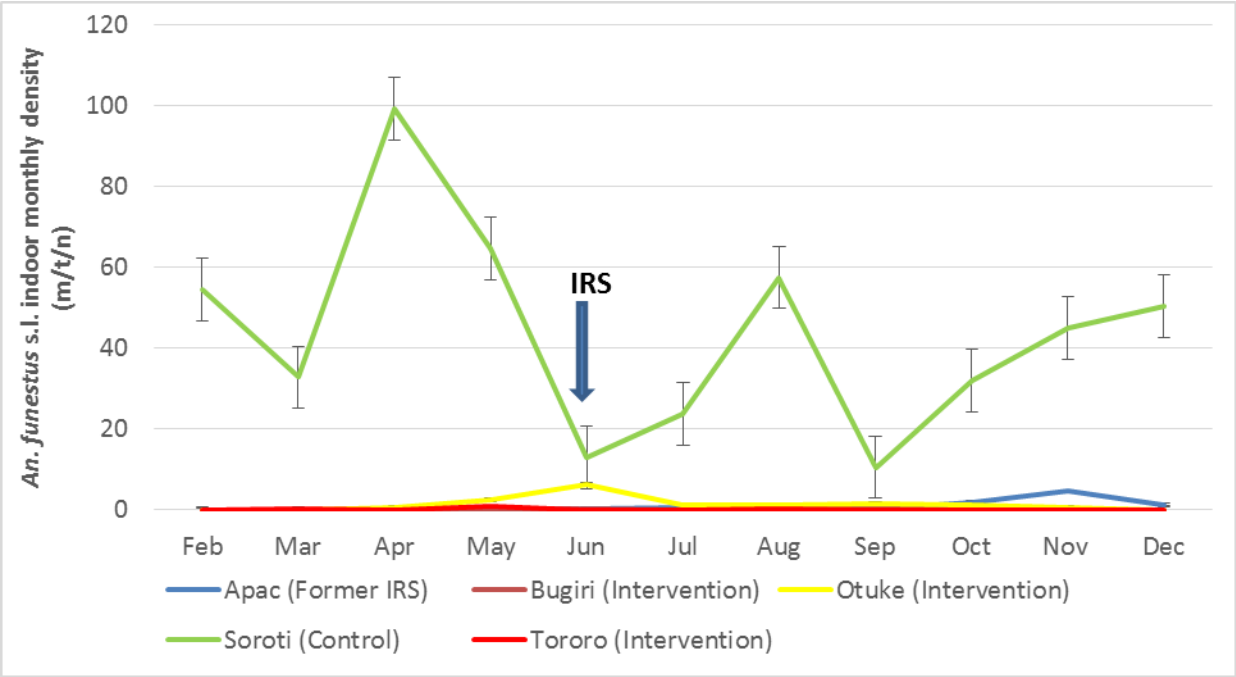
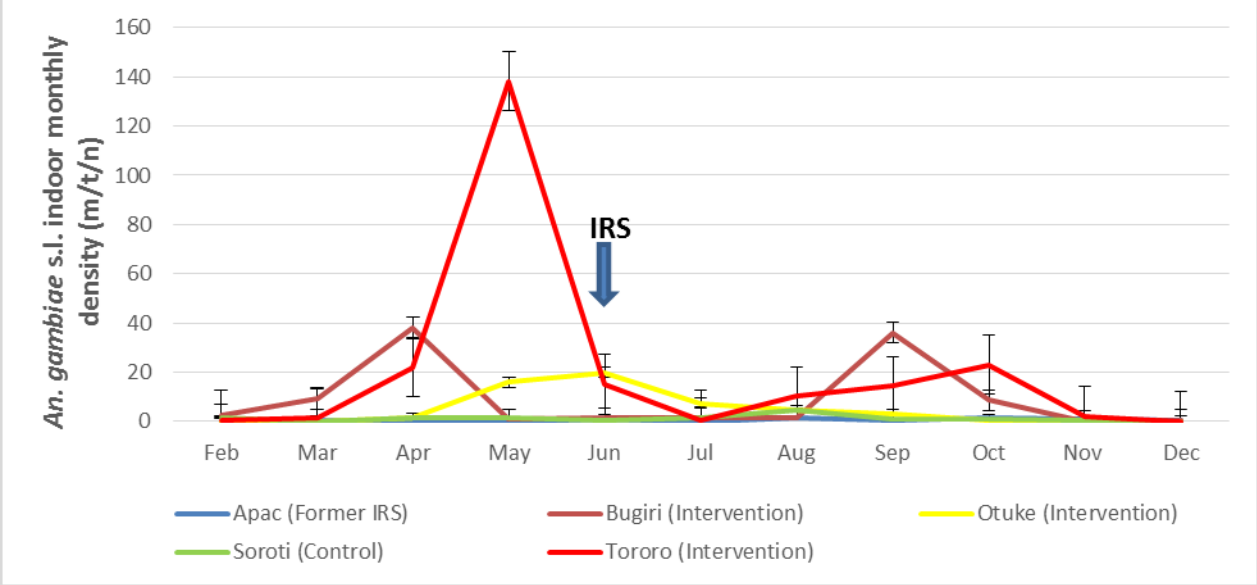


Figure 6B. ***An. gambiae*** **s.l.**



3.2 Cone Wall Bioassay Tests

During the spray operations, WHO cone bioassays were conducted in one site per each of the 15 spray districts (Alebtong, Amolatar, Budaka, Bugiri, Butaleja, Butebo, Dokolo, Kaberamaido, Kibuku, Lira, Namutumba, Otuke, Pallisa, Serere, and Tororo) within one week of the start of spraying to assess the quality of spraying with pirimiphos-methyl (Actellic 300CS). Thereafter, the residual efficacy and fumigant effect of Actellic 300CS was monitored in four districts (Kaberamaido, Lira, Pallisa, and Tororo) on a monthly basis on three types of wall surfaces: plaster painted, plain brick, and mud and wattle, which constitute most wall surfaces in the IRS districts. The susceptible *An. gambiae* s.s. (Kisumu strain) was used for quality and decay rate assessment.

3.2.1 Quality of Spraying

The results for the spray quality bioassays (conducted within one week after spraying) showed high spray quality (100% mosquito mortality) in all 15 spray districts.

3.2.2 Insecticide Decay Rate

Cone bioassay test results indicate that Actellic 300CS remained effective with 100% test mortality rates on all three surfaces at six months after spraying and then its effectiveness started waning (Table 8).

Table 8: Wall Bio-Assay Results in Four Insecticide Decay Rate Monitoring Sites, December 2018

Time	% Mortality of *An. gambiae* s.s. (Kisumu strain)																Overall Mean
	Pallisa				Kaberamaido				Lira				Tororo				
	Painted	Plain Brick	Mud	Mean	Painted	Plain Brick	Mud	Mean	Painted	Plain Brick	Mud	Mean	Painted	Plain Brick	Mud	Mean	
T0	100 (30/30)	100 (30/30)	100 (30/30)	100	100 (30/30)	100 (30/30)	100 (30/30)	100	100 (30/30)	100 (30/30)	100 (30/30)	100	100 (30/30)	100 (30/30)	100 (30/30)	100	100
T1	100 (30/30)	100 (30/30)	100 (30/30)	100	100 (30/30)	100 (30/30)	100 (30/30)	100	100 (30/30)	100 (30/30)	100 (30/30)	100	100 (30/30)	100 (30/30)	100 (30/30)	100	100
T2	100 (30/30)	100 (30/30)	100 (30/30)	100	100 (30/30)	100 (30/30)	100 (30/30)	100	100 (30/30)	100 (30/30)	100 (30/30)	100	100 (30/30)	100 (30/30)	100 (30/30)	100	100
T3	100 (30/30)	100 (30/30)	100 (30/30)	100	100 (30/30)	100 (30/30)	100 (30/30)	100	100 (30/30)	100 (30/30)	100 (30/30)	100	100 (30/30)	100 (30/30)	100 (30/30)	100	100
T4	100 (30/30)	100 (30/30)	100 (30/30)	100	100 (30/30)	100 (30/30)	100 (30/30)	100	100 (30/30)	100 (30/30)	100 (30/30)	100	100 (30/30)	100 (30/30)	100 (30/30)	100	100
T5	100 (30/30)	100 (30/30)	100 (30/30)	100	100 (30/30)	100 (30/30)	100 (30/30)	100	100 (30/30)	100 (30/30)	100 (30/30)	100	90.0 (27/30)	76.7 (23/30)	70,0 (21/30)	78.9	94.8
T6	100 (30/30)	100 (30/30)	100 (30/30)	100	100 (30/30)	100 (30/30)	100 (30/30)	100	100 (30/30)	100 (30/30)	100 (30/30)	100	83.3 (25/30)	83.3 (25/30)	60,0 (18/30)	75.6	93.8
T7	83.3 (25/30)	70.0 (22/30)	60.0 (18/30)	72.2	TBD	TBD	TBD		TBD	TBD	TBD						
T8	73.3 (22/30)	60.0 (18/30)	43.3 (11/30)	56.7	TBD	TBD	TBD		TBD	TBD	TBD						

Pallisa, T0 =April 2018; Kaberamaido, Lira, Tororo, T0 = June 2018; TBD = To be Done

Key: T0 is the test done within 2 weeks after spraying an area; T1, T2, T3 etc represent the test results of studies conducted monthly after spraying an area i.e. 1, 2, 3 etc months post-spraying

3.2.3 The Airborne Effect

Tests for airborne effect of Actellic 300CS were conducted alongside T_1 to T_8 in Pallisa district and T_6 in Kaberamaido, Lira and Tororo districts (Table 9).

Table 9: Fumigant effect of Actellic 300CS spraying on the knockdown and mortality of *An. gambiae* s.s. by district in Pallisa (T8) and Kaberamido, Lira and Tororo (T6), December 2018

District	Spray date	Type of wall surface	Number of *An. gambiae* s.s tested in sprayed houses	Number knocked down after 30 minutes (%)	Number knocked down after 60 minutes (%)	Number (%) dead after 24 hours
Pallisa	April 2018	Plaster Painted	20	0 (0%)	9 (45%)	10 (50%)
		Plain Brick	20	0 (0%)	9 (45%)	9 (45%)
		Mud and Wattle	20	0 (0%)	7 (35%)	8 (40%)
Tororo	June 2018	Plaster Painted	20	0 (0%)	18 (90%)	18 (90%)
		Plain Brick	20	0 (0%)	17 (85%)	17 (85%)
		Mud and Wattle	20	0 (0%)	17 (85%)	17 (85%)
Lira	June 2018	Plaster Painted	10	0 (0%)	2 (20%)	10 (100%)
		Plain Brick	10	0 (0%)	2 (20%)	10 (100%)
		Mud and Wattle	10	0 (0%)	3 (30%)	10 (100%)
Kaberamaido	June 2018	Plaster Painted	10	2 (20%)	5 (50%)	10 (100%)
		Plain Brick	10	0 (0%)	3 (30%)	9 (90%)
		Mud and Wattle	10	0 (0%)	0 (0%)	9 (90 %)

3.3 WHO Susceptibility Testing

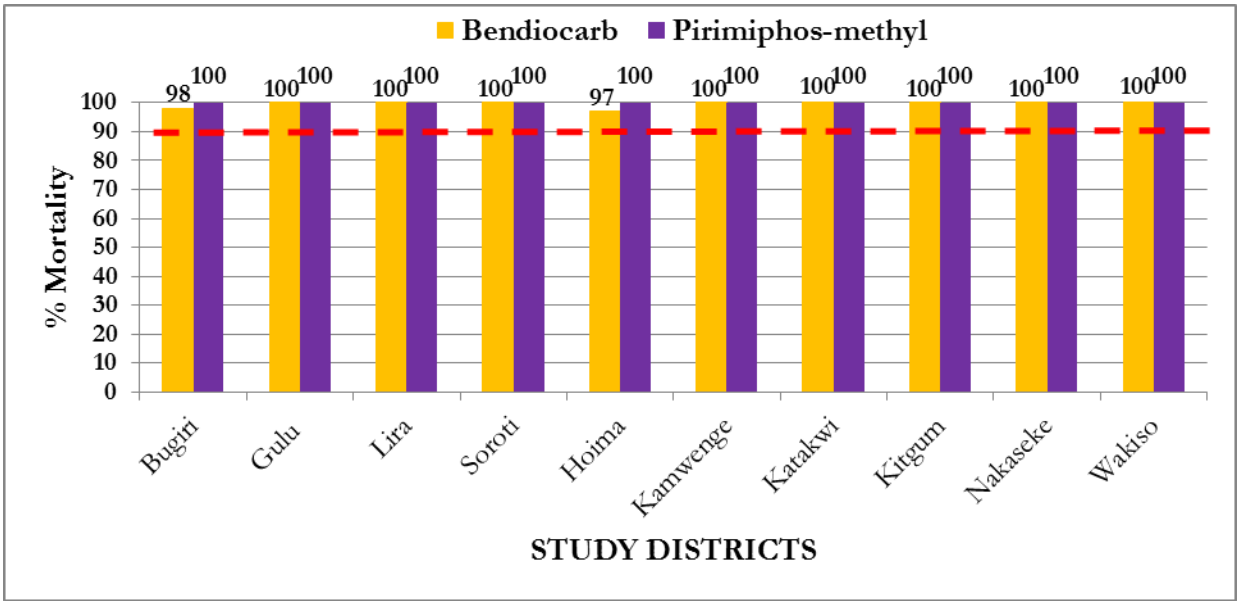
Susceptibility testing was conducted in Gulu, Lira, Soroti and Bugiri districts in May to June 2018 and in Hoima, Kamwenge, Katakwi, Kitgum, Nakaseke and Wakiso districts in September to October 2018. Tests conducted in Hoima, Katakwi, Kitgum, Lira and Wakiso could not cover all planned target insecticides due to a shortage of mosquitoes in the field.

The WHO 2016 standard susceptibility test method was used to test the main malaria vector collected, *An. gambiae* s.l. No *An. fu*nestus s.l. larvae were collected, but *An. fu*nestus s.l. adults were collected and immediately tested in Katakwi district.

An. gambiae s.l. was found susceptible (98-100 percent mortality) to pirimiphos-methyl in all the 10 study districts, while *An. gambiae* s.l. was susceptible to bendiocarb in Bugiri, Gulu, Lira, Kamwenge, Nakaseke, Soroti and Wakiso districts but had possible resistance detected in Hoima (97 percent mortality) – Annex 1 Table B and Figure 7A. *An. gambiae* s.l. was found resistant to alpha-cypermethrin, deltamethrin and permenthrin in all the 10 study districts where they were tested. Resistance varied from 0percent for alphacypermethrin in Kamwenge district to 85 percent for deltamethrin in Bugiri (Figures 7B). *An. funestus* s.l. was found susceptible (98-100 percent mortality) to both pirimiphos-methyl and bendiocarb in Katakwi district, while *An. funestus* s.l. was found resistant to alpha-cypermethrin, deltamethrin and permethrin in Katakwi district (Figure 7C).

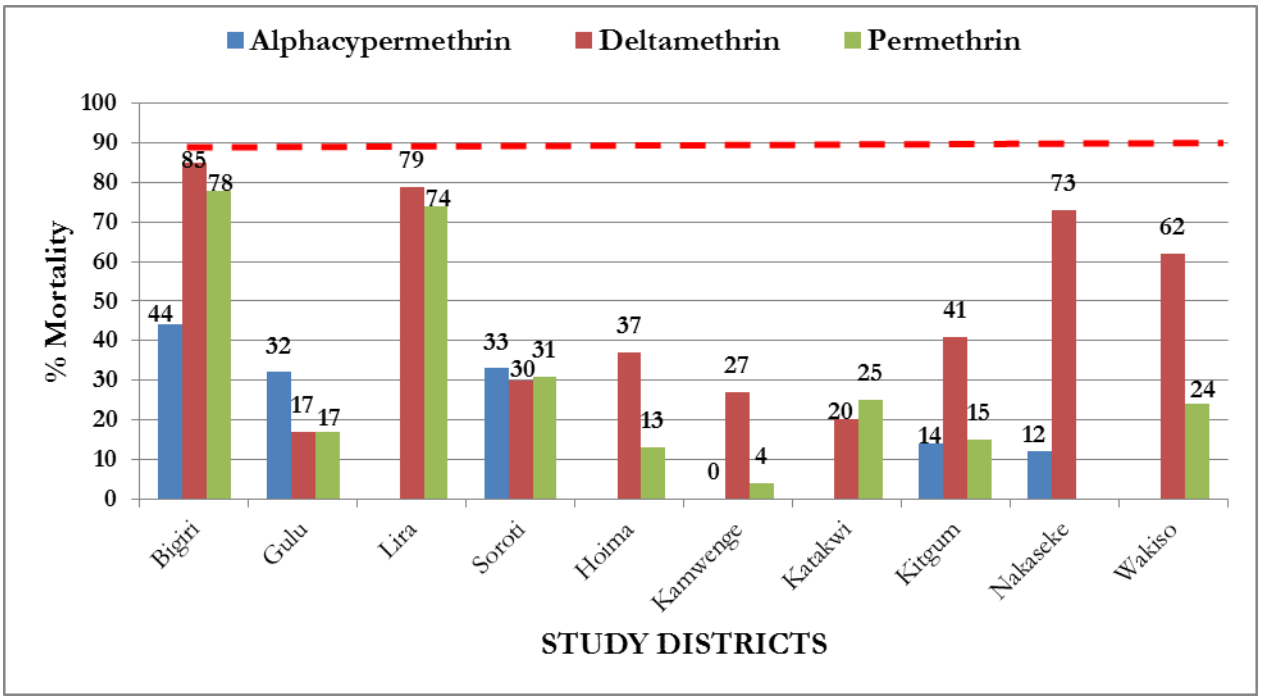
Figure 7: 24 hr Mortality of Adult *An. gambiae* s.l. From Larval Collections and *An. funestus* s.l. Collected using Test Tube Collections, Exposed to a Range of Insecticides at Respective Diagnostic Concentrations (May-October 2018)

Figure 7A. *An. gambiae* s.l.



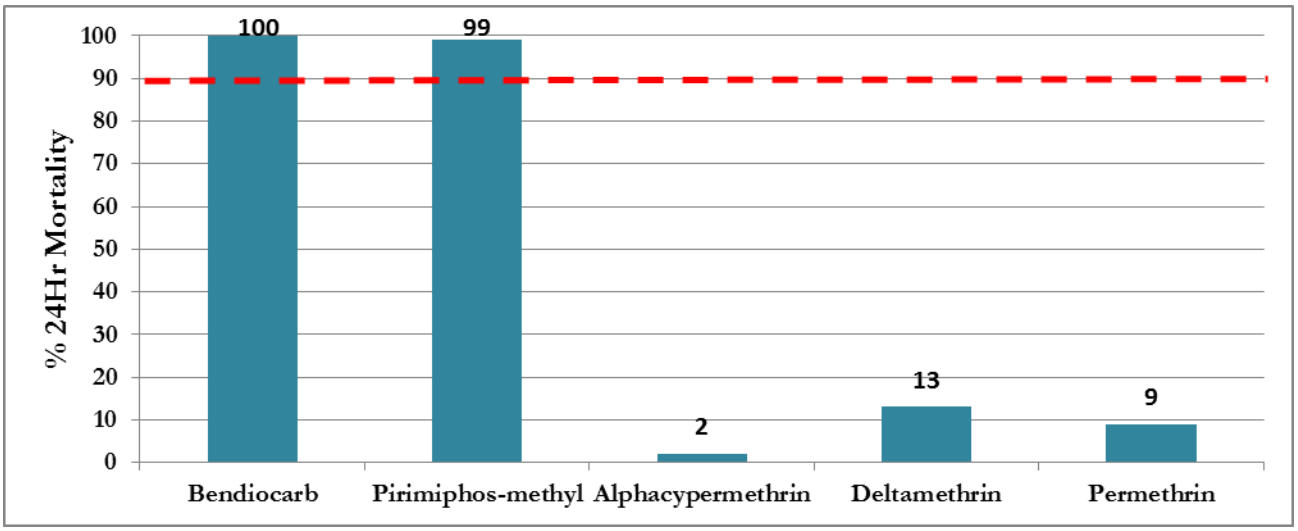
Red line indicates mortality below 90% are resistant mosquitoes

Figure 7B. *An. gambiae* s.l.



Red line indicates mortality below 90% are resistant mosquitoes

Figure 7C. *An. funestus* s.l. in Katakwi district



3.3.1 Determination of the Intensity of Resistance AND SYNERGIST ASSAYS USING WHO Tube Tests

Bioassays for intensity of resistance were conducted where *An. gambiae* resistance was detected with the discriminating concentrations (24 hr mortality < 90%) of the respective insecticides using both the CDC bottle assay method in Bugiri, Gulu and Lira districts and WHO tube assay method in Kamwenge, Katakwi, Kitgum and Nakaseke districts.

Results of exposure to 5× alpha-cypermethrin and deltamethrin in CDC bottle bioassays showed that the intensity of resistance was low in Bugiri, Gulu and Lira (100% mortality) and moderate for permethrin in Bugiri (97% mortality) and Gulu (96% mortality (Figure 8). Results of resistance intensity studies of *An. gambiae* s.l. using the WHO tube assay in the four districts of Kamwenge, Katakwi, Kitgum and Nakaseke showed high intensity resistance to all three pyrethroids: alpha-cypermethrin, deltamethrin and permethrin (Figure 9).

Figure 8: 24h percent mortality of adult *An. gambiae* s.l. following exposure to multiples of respective diagnostic concentrations using CDC bottle bioassays, June 2018

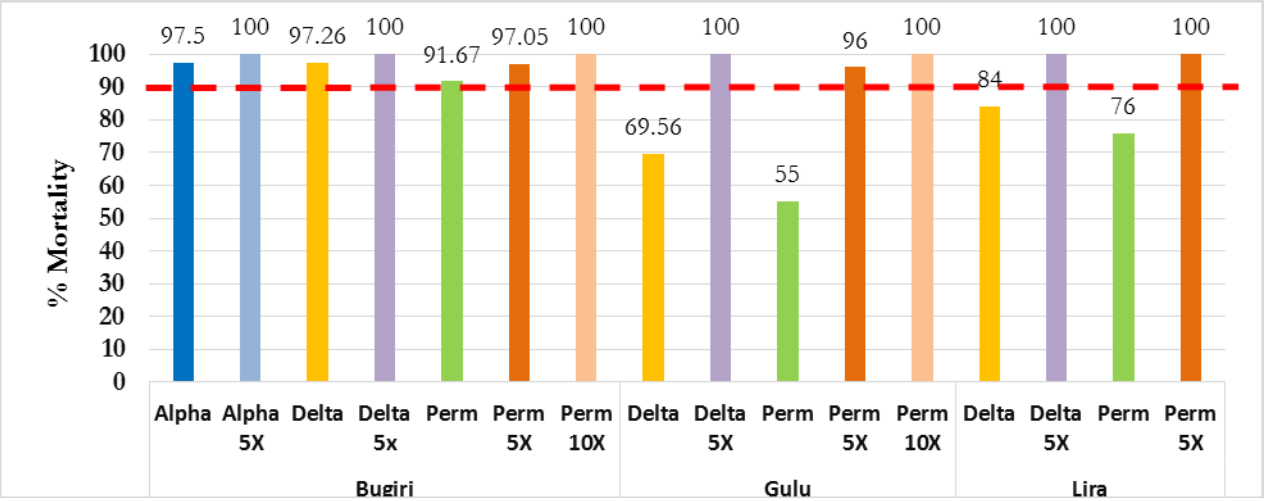
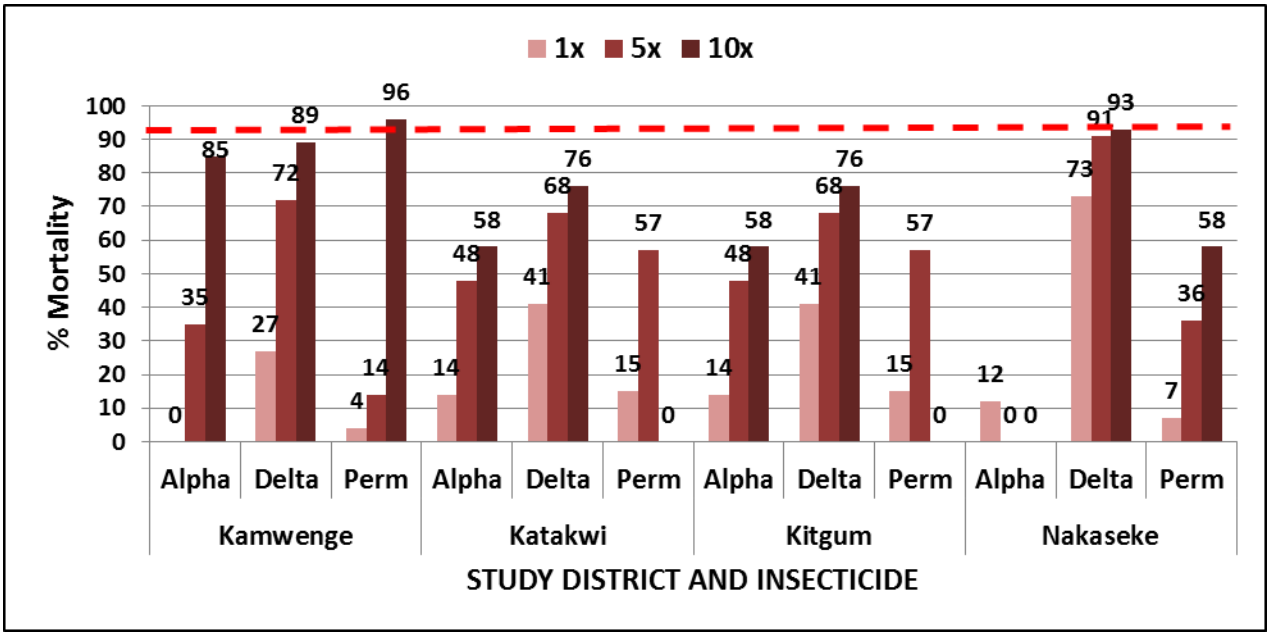


Figure 9: Percent mortality of *An. gambiae* s.l. after exposure to different concentrations of alpha-cypermethrin, deltamethrin and permethrin using WHO papers in four districts in Uganda, October 2018



Key: Alpha = alphacypermethrin; Delta = Deltamethrin; Perm = Permethrin
Red line indicates mortality below 90% are resistant mosquitoes

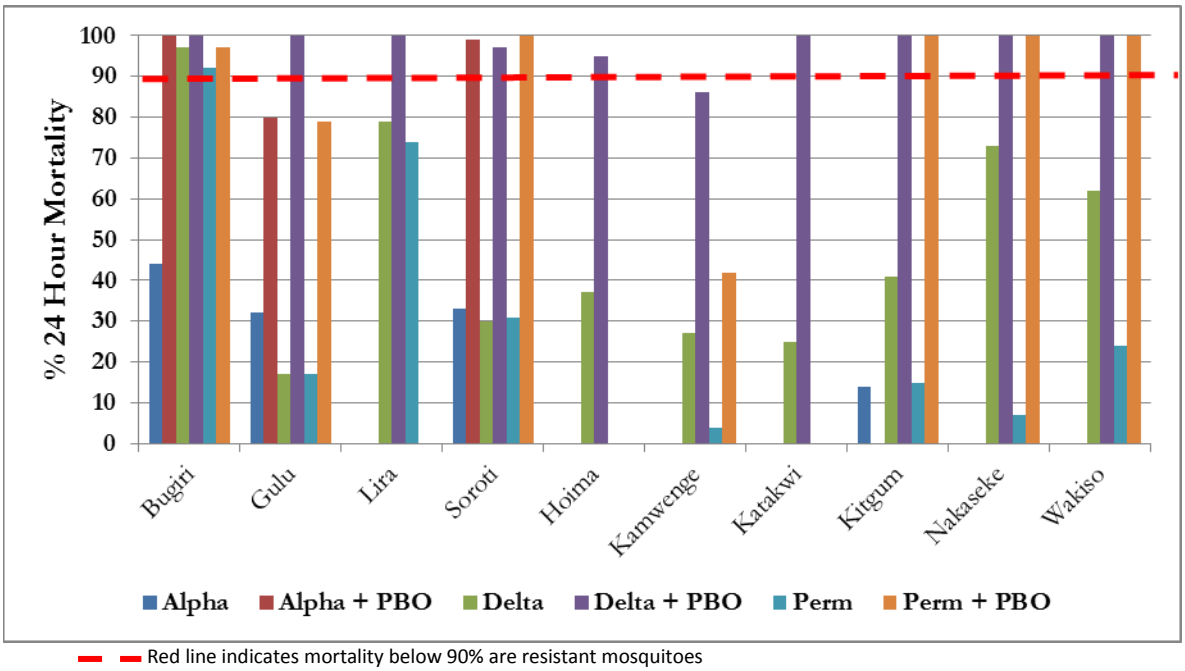
The synergist assay using piperonyl butoxide (PBO) restored *An. gambiae* s.l. susceptibility to:

- alpha-cypermethrin in Bugiri and Soroti but not in Gulu where it was tested,
- deltamethrin in Bugiri, Gulu, Katakwi, Kitgum, Lira, Nakaseke and Wakiso but not in Hoima, Kamwenge and Soroti and,

- permethrin in Kitgum, Nakaseke, Soroti and Wakiso but not in Bugiri, Gulu and Kamwenge districts where it was were tested (Figure 10).

Full restoration of the efficacy of the insecticides suggests that monoxygenases are the only form of metabolic resistance prevailing in the area. Partial restoration of the efficacy of the insecticides suggests that, though monoxygenases are the main form of metabolic resistance prevailing in the area, other forms of resistance mechanisms are also present and need to be investigated.

Figure 10: Synergist assay mortality results in *An. gambiae* s.l. from 10 districts in Uganda upon exposure to alpha-cypermethrin, deltamethrin, and permethrin only or with 4% PBO pre-exposure June –October 2018



Key: Some tests not done in some districts due to inadequate mosquito samples

Table 10: Synergist assay mortality results in *An. gambiae* s.l. from 10 districts in Uganda upon exposure to alpha-cypermethrin, deltamethrin, and permethrin only or with 4% PBO pre-exposure June –October 2018

Insecticide	Bugiri	Gulu	Lira	Soroti	Hoima	Kamwenge	Katakwi	Kitgum	Nakaseke	Wakiso
Alphacypermethrin	44	32	-	33	-	0	-	14	12	-
Alphacypermethrin + PBO	100	80	-	99	-	-	-	100	-	-
Deltamethrin	97	17	79	30	37	27	25	41	73	62
Deltamethrin + PBO	100	100	100	97	95	86	100	100	100	100
Permethrin	92	17	74	31	-	4	-	15	7	24
Permethrin + PBO	97	79	-	100	-	42	-	100	79	100
Key:	**Confirmed Resistance**				**Possible Resistance**			**Susceptible**		

3.4 Clothianidin and Chlorfenapyr Susceptibility Test Results

The clothianidin and chlorfenapyr susceptibility test were done according to the PMI AIRS protocol with the standard operating procedures SOP 001 and SOP 002 respectively.

The clothianidin tests were conducted on samples collected in Apac, Bugiri, Bugweri, Dokolo, Gulu, Kaberamaido, Katakwi, Kibuku, Lira, Otuke, Pallisa, Serere, Soroti and Tororo districts. *An. gambiae* s.l. was found to be fully susceptible (100 percent mortality) to clothianidin in all the 14 study districts in Uganda after a seven-day holding period. (Figure 11). The chlorfenapyr tests were conducted on samples collected in Apac, Bugiri, Bugweri, Dokolo, Gulu, Kaberamaido, Katakwi, Serere and Soroti districts (Figure 12). *An. gambiae* s.l. was found to be fully susceptible (99-100 percent mortality) to chlorfenapyr in all the nine study districts in Uganda after a three-day holding period. Both clothianidin and chlorfenapyr tests were conducted on the wild *An. gambiae* s.l. and the laboratory standard susceptible *An. gambiae* s.s. Kisumu strain which was also killed in all the tests. *An. funestus* s.l. from Awoja and Aleere villages in Gweri sub-county, Soroti district were fully susceptible (100 percent mortality) to both clothianidin and chlorfenapyr.

Figure 11: Clothianidin Susceptibility Test Results on Wild *An. gambiae* s.l. (Percentage Mortality at 7th Day Post-Exposure)

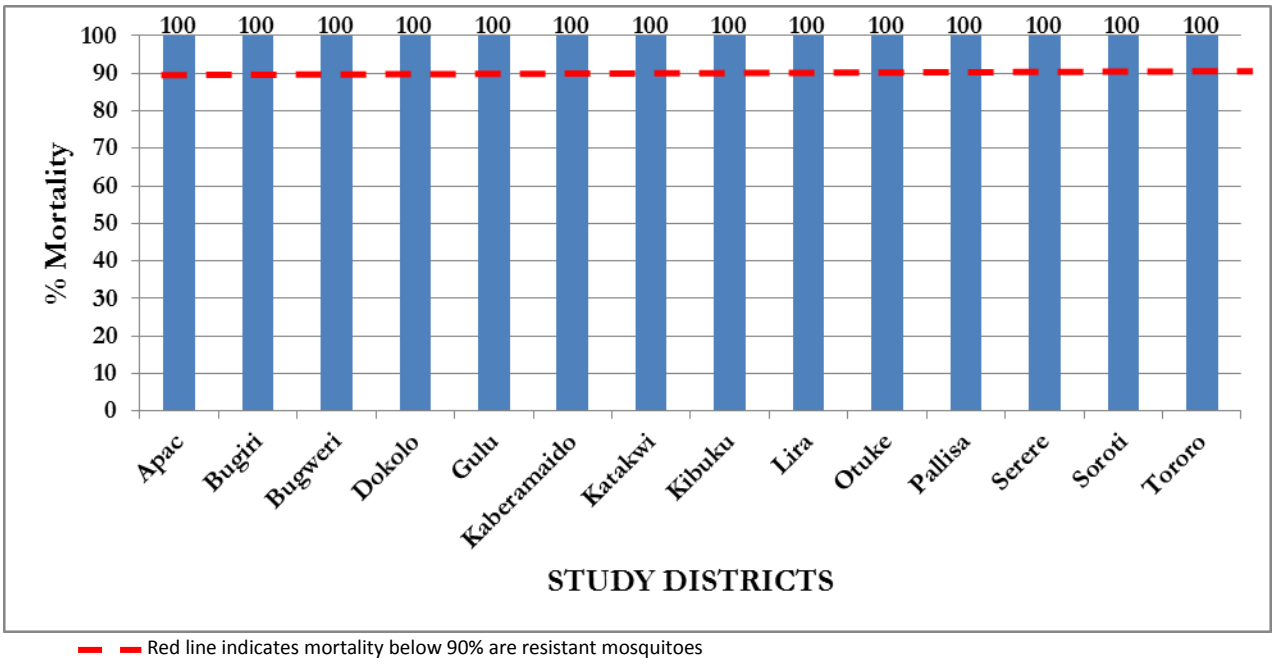
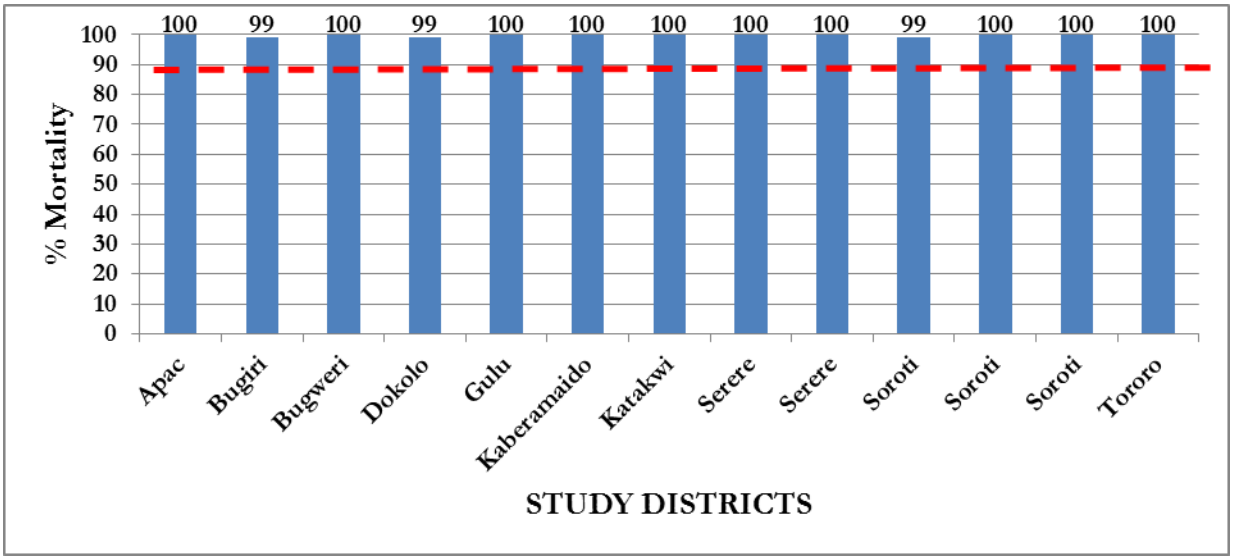


Figure 12: Chlorfenapyr Susceptibility Test Results on Wild *An. gambiae* s.l. (Percentage Mortality at 3rd Day Post-Exposure)



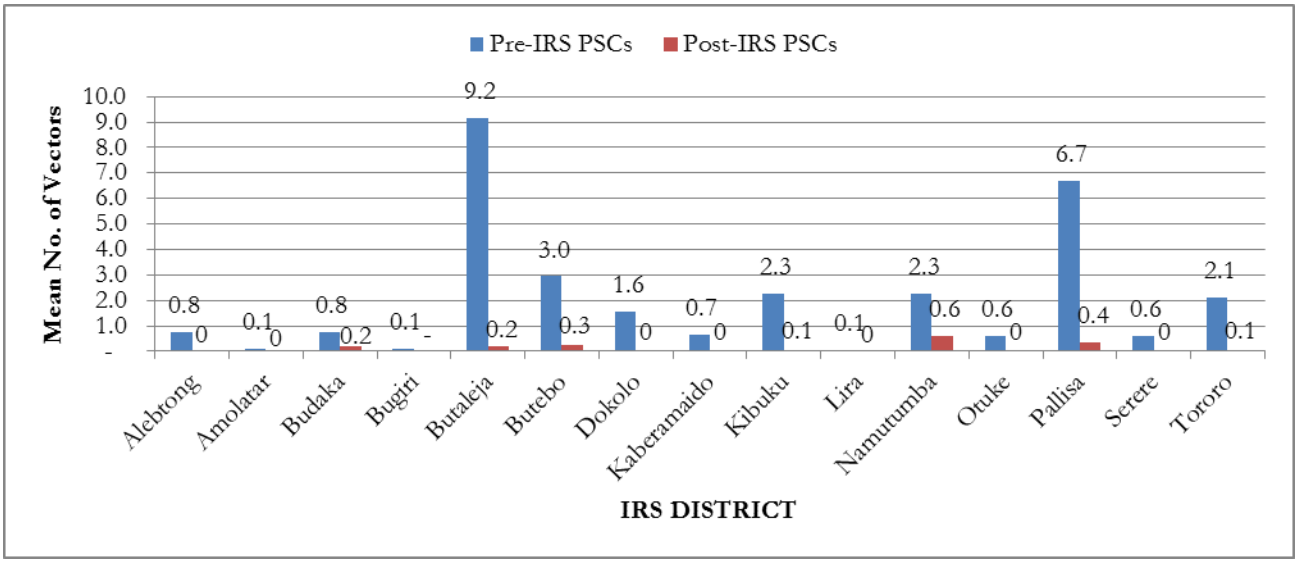
Note: Where a district is repeated, mosquito samples were collected from different locations and tested

Red line indicates mortality below 90% are resistant mosquitoes

3.5 Pre- and Post-IRS PSCs in the Current 15 IRS Districts, March–June 2018

The project collected a total of 605 female malaria vectors (555 *An. gambiae* s.l. and 50 *An. funestus* s.l.) during the pre-IRS PSCs in March and June 2018 compared to 34 female malaria mosquitoes (28 *An. gambiae* s.l. and 6 *An. funestus* s.l.) during the post-IRS PSCs in April and June 2018 in the 15 current IRS districts (Figure 13). The data shows the indoor vector resting densities decreased following IRS.

Figure 13: Mean number of vectors per house caught during pre- and post-PSCs in IRS districts, 2018



4. DISCUSSION, LESSONS LEARNED, AND CHALLENGES

Results of PSCs show that *An. funestus* s.l. was the most abundant species collected while resting indoors in the control and former IRS districts. In contrast, *An. gambiae* s.l. predominated the indoor HLCs in the IRS intervention districts. This may indicate that the more endophillic and endophagic *An. funestus* s.l. responds faster to IRS than *An. gambiae* s.l. which may also include the more exophilic and exophagic *An. arabiensis* species.

HLCs demonstrated that Soroti control area had highest mean *An. funestus* s.l. HBR both indoors and outdoors. *An. gambiae* s.l. was found at higher densities in the control, albeit at much lower densities than *An. funestus* s.l. Furthermore, the HLCs revealed significant differences between outdoor and indoor biting densities for both vector species. Observed biting activity was generally consistently higher indoors than outdoors for *An. gambiae* s.l., although outdoor HBRs were sometimes higher than indoor HBRs in a few months especially in the IRS districts. *An. gambiae* s.l. HBR were highest immediately preceding the start of spraying in the IRS districts. Biting activity of *An. funestus* s.l. was consistently higher indoors than outdoors especially in the control district of Soroti. Regarding the overnight biting patterns observed, biting starts in early evening, between 6:00 pm and 8:00 pm at most sites, and generally peaks after midnight, towards morning hours of between 4:00 am and 6:00 am

Similar to PSC and HLC data, CDC light trap collections showed *An. funestus* s.l. (72.8%) as the most abundant species, followed by *An. gambiae* s.l. (22.9%). The CDC light trap collections indoors demonstrated that the study areas have a variety of anopheline species, specifically eight different species (*An. funestus* s.l., *An. gambiae* s.l., *An. coustani, An. coustani, An. ardensis, An. maculipalpis, An. squamosus and An.pharoensis* which were the most abundant) across the sampled sites.

The WHO cone wall bioassay results obtained in five of 15 sprayed districts showed that the spray quality of the 2018 spray campaign with Actellic® 300CS in all the districts was satisfactory at all monitored sites. The monitoring for insecticide decay rate showed that the longest the insecticide stayed effective on sprayed surfaces was between four months (in Tororo) and six months (in Pallisa), although testing is continuing in two districts.

An. gambiae s.l. was found susceptible (98-100 percent mortality) in all the 10 study districts, while *An. gambiae* s.l. was susceptible to bendiocarb in Bugiri Gulu, Lira, Kamwenge, Nakaseke, Soroti and Wakiso districts but with possible resistance detected in Hoima (97 percent mortality). Vector susceptibility to pirimiphos-methyl and bendiocarb was the same as in previous years. *An. gambiae* s.l. was found resistant to alpha-cypermethrin, deltamethrin and permenthrin in all the 10 study districts where they were tested. Resistance to pyrethroids varied from 0% for alpha-cypermethrin in Kamwenge district to 85 percent for deltamethrin in Bugiri.

The observed widespread resistance to pyrethroids has become common in sub-Saharan Africa, particularly following extensive roll-out of LLINs that started about a decade ago, in order to achieve universal coverage. The impact of pyrethroid resistance on the efficacy of LLINs needs to be assessed. It also points to the need to deploy next generation LLINs like the PBO-synergized LLINs to combat insecticide resistance to pyrethoids and extend the usefulness of LLINs in malaria control. Continued lack of resistance to pirimiphos-methyl is good news for Uganda, although there is need to rotate it with another next generation insecticide with other modes of action against malaria vectors as we have used it for three consecutive years now. The full susceptibility of both *An. gambiae* s.l. and *An. funestus* s.l. to both clothianidin and chlorfenapyr observed in various study areas indicates that Uganda has other effective next generation insecticides that can be rotated with Actellic 300CS in IRS as an alternative to manage insecticide resistance in the country.

In areas where resistance was detected with diagnostic doses of pyrethroids, , intensity assays were conducted (if enough mosquitoes were available to carry out the tests). Results of intensity assays with 5× and 10× concentrations for alpha-cypermethrin, deltamethrin, and permethrin in *An. gambiae* s.l. in seven districts

showed variable results ranging from low intensity of resistance to moderate and high intensity of resistance. This has to be monitored regularly and in wider geographical ranges to determine areas where attention is needed to mitigate the impact of insecticide resistance on malaria vector control through deployment of next generation IRS and LLIN products.

Results of synergist assays conducted in various districts suggest the presence of metabolic resistance mainly due to monoxygenases, although other resistance mechanisms appear to also play a minor role in some study districts. We therefore expect better malaria vector control with PBO synergized LLINs than pyrethroid-only LLINs which were deployed in some districts of Uganda during the last universal LLINs distribution coverage in 2017 to 2018. There is, however, need to conduct similar tests in various parts of Uganda in order to map the extent of this and other resistance mechanisms such as esterases and glutathione-s-transferases in the country. Molecular assays are ongoing at Gulu University laboratories in Northern Uganda. Some analysis has been conducted but no report has been received from Gulu University. A separate addendum to this report will be prepared and submitted after the assays are completed.

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6. ANNEX 1

Table A: Mean indoor and outdoor vector biting rates per person per day for *An. funestus* s.l. and *An. gambiae* s.l. Indoors and Outdoors by district, February-December 2018

District	Feb		Mar		Apr		May		Jun		Jul		Aug		Sep		Oct		Nov		Dec	
	A.g	A.f	A.g	A.f	A.g	A.f	A.g	A.f	A.g	A.f	A.g	A.f	A.g	A.f	A.g	A.f	A.g	A.f	A.g	A.f	A.g	A.f
Apac																						
HLC indoors	0	0	2	0	0.5	0.5	1.5	0	2	0	0	2.5	2	1	0.5	1.5	1	2.5	3	9	2	3.5
HLC outdoors	0	0	1	0	1	0	0.5	0	0.5	0	0	0.5	1.5	1	0	1	4	1	4	10.5	3	2.5
Bugiri																						
HLC indoors	0.5	0.5	3	0.5	11	0	11.5	0	0.5	0	1.5	0	1	0	4	0	2	0.5	1.5	0	1.3	0
HLC outdoors	1.5	0	3.5	0.5	5	0	19.5	0.5	1	0	0.5	0	1	0	2.5	0	2.5	0	1	0	1	0
Otuke																						
HLC indoors	0	0	0	0	1	0.5	3	0.5	4.5	2.5	1	0	0.5	0.5	1	1	0	0	0	0	0	0
HLC outdoors	0	0	0	0	0	0.5	7	0.5	11.5	0.5	3	0	0.5	0	1	0	0.5	0	0.5	0.5	0	0.5
Soroti																						
HLC indoors	1.5	24	0.5	65.5	1	101	2.5	113.5	7	159	10.5	63	8	81.5	2.5	79.5	0.5	90.5	0	132	0	120
HLC outdoors	0.5	6.5	0.5	5	0.5	47.5	3.5	27.5	14.5	52	13.5	27.5	4	24.5	2.5	19	1	32	0	42	0	27
Tororo																						
HLC indoors	0	0	0	0	7.5	0	64.5	0	10.5	0	2	0	1	0	2.5	0	5	0	0	0	0.5	0
HLC outdoors	0	0	0	0	18.5	0	73	0	16.5	0	2	0	2.5	0	4	0	4	0	1.5	0	0	0

Table B: Percent 24-hour holding mortality of *Anopheles gambiae s.l.* after exposure to seven public health insecticides, May – October 2018 (results for adults reared from larvae)

	Bugiri		Gulu		Lira		Soroti		Hoima		Kamwenge		Katakwi		Kitgum		Nakaseke		Wakiso	
	No. Tested	% Mortality	No. Tested	% Mortality	No. Tested	% Mortality	No. Tested	% Mortality	No. Tested	% Mortality	No. Tested	% Mortality	No. Tested	% Mortality	No. Tested	% Mortality	No. Tested	% Mortality	No. Tested	% Mortality
Carbamate:																				
Bendiocarb	50	98	100	100	100	100	100	100	100	97	83	100	*	*	*	*	100	100	100	100
Organophosphate:																				
Pirimiphos-methyl	107	100	100	99	100	100	116	100	100	100	102	100	104	100	126	100	100	100	100	100
Pyrethroid:																				
Alphacypermethrin	103	44	100	32	*	*	103	33	*	*	103	0	101	20	103	14	100	12	*	*
Deltamethrin	100	85	100	17	100	79	106	30	100	37	97	27	110	25	100	41	100	73	100	62
Permethrin	100	78	100	17	100	74	96	31	80	13	95	4	102	10	101	15	100	07	100	24

Key:	Confirmed resistance		Possible resistance		Susceptible

* = Testinng was not done due to inadequate mosquito samples.