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AIRS KENYA ANNUAL ENTOMOLOGICAL MONITORING REPORT

DECEMBER 2015 TO NOVEMBER 2016

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Abt Associates Inc. | 4550 Montgomery Avenue | Suite 800 North | Bethesda, Maryland 20814 | T. 301.347.5000 | F. 301.913.9061 | www.abtassociates.com

AIRS KENYA
ANNUAL ENTOMOLOGICAL MONITORING REPORT

DECEMBER 2015 – NOVEMBER 2016

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ACRONYMS

ACT	Artemisinin-based Combination Therapy
AIRS	Africa Indoor Residual Spraying
CDC	Centers for Disease Control and Prevention
COP	Chief of Party
DDT	Dichlorodiphenyltrichloroethane
ELISA	Enzyme-Linked Immunosorbent Assay
F&A	Finance and Administration
HLC	Human Landing Catch
IRS	Indoor Residual Spraying
ITN	Insecticide Treated Net
KDR	Knock Down Rate
KEMRI	Kenya Medical Research Institute
LLIN	Long Lasting Insecticidal Net
M&E	Monitoring and Evaluation
MOH	Ministry of Health
MOP	Malaria Operational Plan
MOU	Memorandum of Understanding
NMCP	National Malaria Control Program
PCR	Polymerase Chain Reaction
PMI	United States President's Malaria Initiative
PPE	Personal Protective Equipment
PSC	Pyrethrum Spray Catch
RDT	Rapid Diagnostic Test
STTA	Short-Term Technical Assistance
TO	Task Order
USAID	United States Agency for International Development
WHO	World Health Organization

EXECUTIVE SUMMARY

Surveillance of mosquito vectors is critical in the evaluation of malaria control efforts. The Kenya National Malaria Control Programme (NMCP) in collaboration with development partners has been scaling up mosquito control by distribution of LLINs and application of IRS in different parts of the country, especially the malaria hyperendemic Lake Victoria regions of western Kenya. Application of indoor residual spraying requires constant monitoring of the vector populations for insecticide resistance and temporal changes in vector bionomics. AIRS Kenya performed baseline vector surveillance for insecticide resistance, vector densities and behavior in Migori and Homa Bay counties of western Kenya. Monthly collections were performed by pyrethrum spray catch (PSC), indoor CDC light traps and window exit traps. Malaria vector species composition comprised of 85% *An. funestus* s.s., 13% *An. arabiensis* and 1% *An. gambiae* s.s. Other species identified morphologically were 1.5% *An. coustani*, 0.04% *An. maculipalpis,* 0.07% *An. pharoensis and* 0.17% *An. rufipes.* Vector densities varied by collection method, time and location, where light traps collected significantly more *An. gambiae* s.l., *An. funestus* and *An. coustani* as compared to PSC ($p<.0001$). *An. funestus* was the dominant vector throughout the study period with two high density peaks associated with high rainfall periods between December to February and May to July. Significantly more fed ($p<.0001$) and half gravid ($p=0.003$) *Anopheles* were collected indoor by PSC as compared to window exit trap. Unfed and gravid female were more likely to be found in the exit trap as compared to PSC ($p<.0001$) and ($p<.0001$) respectively. Human landing catches indicated that *An. funestus* biting began before 18:00 hours both indoors and outdoors but peaked between 4:00 am and 6:00 am, with indoor biting continuing until 7:00am. A total of 5,073 samples were analyzed for sporozoite ELISA with 3.28% overall sporozoite rate. Only *An. funestus* were positive for sporozoite infection. Both adult collected *An. funestus* and larval collected *An. arabiensis* were fully susceptible to pirimiphos methyl and bendiocarb but resistant to deltamethrin and permethrin. Also, both East (L1014S) and West (L1014F) *kdr* mutation occurred in *An. arabiensis* at low frequencies, 0.0036 and 0.0027 respectively. The households participating in the entomological surveillance have high long lasting insecticide treated net (LLIN) coverage and use with over 97% of people reporting sleeping under a net the previous night and an average of 1 LLIN per 1.74 people. The results indicate that *Anopheles* mosquitoes spend time resting indoors to digest blood-meals and only exit either when unfed for host-seeking or when gravid to search for oviposition sites. IRS with pirimiphos methyl is expected to have impact against pyrethroid resistant *An. funestus* that currently dominated the vector population in the region. The data generated provides important baseline information for subsequent evaluation of IRS in Migori County, western Kenya. Continued monitoring of vector densities, behavior and evaluation of insecticide residual activity in the months following spraying would be critical in understanding the duration of action of the pirimiphos methyl in this setting.

1. BACKGROUND

A substantial decline in global malaria morbidity and mortality has been realized following the scale-up and use of long lasting insecticidal nets (LLINs) and indoor residual spraying (IRS). Globally, new malaria infections have declined within the past fifteen years by an estimated 37% with an overall drop in estimated malaria deaths by 60%[1]. In western Kenya, ITNs have previously been shown to reduce indoor occurrence of *An. funestus* by up to 94.5% with an estimated overall transmission reduction of *Plasmodium falciparum* by 90%[2]. While countries have been successful in achieving rapid improvements in malaria control, the burden is still unacceptably high, particularly in rural Africa[3]. A few countries in Sub-Saharan Africa account for 80% of malaria cases and 75% of deaths globally[1]. In Kenya, the malaria endemic Lake Victoria region of western Kenya remains the most important source of malaria transmission nationally[4, 5].

Enhanced vector control with combinations of tested, proven strategies has been recommended in the move towards malaria elimination[6]. Analysis of data from different countries of sub-Saharan Africa showed IRS to be effective in decreasing prevalence of malaria in a community by approximately 62%[7]. Results from malaria hyperendemic region of Uganda reported significantly lower parasite prevalence in children living in IRS districts as compared to non-sprayed neighboring districts[8]. Furthermore, implementation of IRS in combination with LLINs has been reported to offer a greater protective efficacy to further reduce malaria transmission in areas with persistent perennial malaria[9]. In Kenya, NMCP previously implemented IRS in malaria hotspots in highland districts and expanded to cover some lowland endemic districts. Spraying was last conducted with deltamethrin in 2012, with 460,447 structures sprayed. Currently, plans are underway through PMI-AIRS to spray in March 2017 with pirimiphos-methyl CS to provide protection during the long rainy season.

The effectiveness of core malaria interventions is threatened by increases in the distribution and strength of insecticide resistance in mosquitoes[10-12]. Of greatest concern is resistance to pyrethroids, the only class of insecticides currently approved for use on ITNs and widely applied in IRS. The global community has consequently recommended resistance management practices requiring the application of multiple insecticides and different biochemical modes of action (MOA) in rotations, mosaics, mixtures, or a combination of multiple interventions[13]. Given the widespread pyrethroid resistance in the lake endemic region of western Kenya, NMCP has developed an insecticide resistance management strategy involving the rotation of different classes of insecticides every 2 years in endemic and epidemic prone areas (where LLIN coverage is above 80%)[14]. Recent data from Uganda and western Kenya observed carbamate and organophosphate susceptibility of *An. funestus*, hence offering an alternative solution to resistance management in the region[11]. Therefore, spraying with pirimiphos-methyl CS in Migori County in 2017 is expected to be an effective measure.

Implementation of IRS for insecticide resistance management must be conducted in parallel with enhanced entomological surveillance coupled with efficient data management to inform programmatic decisions[15]. With the worsening situation of pyrethroid resistance in malaria vectors in Africa south of the Sahara, and reports of resistance to organophosphates and carbamates, and DDT[15], it is critical that the countries establish and implement national insecticide resistance monitoring plans that incorporate ongoing rotation of insecticides with different MOA. Additionally, sustained application of insecticide based interventions indoor have been reported to not only reduce the indoor resting vector densities[2], but also have exito-repellant effect deterring entry of mosquitoes into houses[16, 17], or cause exiting behavior in the vectors[18]. Increasing outdoor vector populations may be harder to control and densities harder to quantify given the vast outdoor environment over which they are dispersed. Furthermore, dispersion of mosquitoes to the outdoor environment due to insecticide-based interventions indoors may lead to serious under estimation of

the vector population by indoor based vector collection methods. Therefore, application of suitable vector collection tools for both indoor and outdoor mosquito populations is needed. Given the clear potential of LLINs and IRS to alter vector population dynamics, it is required that their continuous application be accompanied with robust monitoring of local vector populations.

The current study has collected baseline data to monitor the impact of IRS in 2017 with pirimiphos-methyl in a region where 95% of sampled households own at least 1 LLIN, but where there is widespread pyrethroid resistance in malaria vectors. Entomological collections for vector surveillance were initially conducted in Rongo, Awendo and Uriri sub-counties in Migori county and Homa Bay and Ndhiwa in Homa Bay county. Recently, Nyatike, Kuria East, Kuria West and Suna West sub counties in Migori County have been included in the entomological monitoring in a scale up for 2017 IRS. The entomological indicators monitored include vector density and behavior with regards to time, location of biting, resting and exiting and insecticide resistance monitoring.

1.1 Main Objective

To collect baseline entomology data prior to determining the effectiveness of IRS and monitor the insecticide resistance patterns of local vector populations in Migori County, western Kenya.

1.2 Specific Aims

1. To implement vector surveillance to determine malaria vector biting rates and indoor resting densities in Migori County before implementation of IRS.
2. To monitor the levels and mechanisms of insecticide resistance of local malaria vector populations in western Kenya.

2. METHODOLOGY

2.1 STUDY SITE

FIGURE 1: MAP OF SELECT SENTINEL SITES IN WESTERN KENYA

Monitoring was conducted in two sites in Rongo, Awendo and Uriri sub-counties of Migori County as well as one site in Homa Bay and Ndhiwa sub-counties in Homa Bay County from December 2015. An additional site in Nyatike, Kuria West, Kuria East and Suna West was included in entomological monitoring in July 2016 in preparations for IRS in 2017. All sites are in a malaria hyperendemic region of western Kenya close to Lake Victoria. Rongo, Awendo and Uriri sub-counties are the intervention sites earmarked for IRS with pirimiphos-methyl CS in 2017, while Homa Bay, Ndhiwa, Kuria East and Kuria West sub-counties will remain unsprayed sites in 2017. The residents are mainly of the Luo ethnic group with a few sites in Uriri occupied by the Luhyha community and both Kuria West and Kuria East are occupied by the Kuria community. The residents are mostly subsistence farmers with a few large-scale sugar-cane plantations. Crops produced for subsistence include maize, sorghum, and vegetables, and a few residents keep cattle. Residents mostly live in small houses, clustered into family social units of relatives called compounds. The compounds are separated by fences, farm lands and scattered shrubs and trees with various dirt roads and footpaths. The landscape is interspersed with gentle slopes, valley bottoms forming small swamps and some relatively flat surfaces.

Mosquito collections were performed monthly by pyrethrum spray catch (PSC), CDC light trap, and window exit traps. Between December 2015 and April 2016, 10 houses were sampled by PSC and 10 others by light trap every month by two teams in each sub county. However, between May and November, 5 houses were sampled by PSC and 10 others by light traps by each team per site per month (to allow time for morphological identification in the field) (Table 1). Different houses were sampled every month by randomizing a sentinel compound and sampling neighboring houses that consent. Window exit traps were installed only in houses that were designated for PSC to compare indoor resting and exiting ratios. During every collection effort, a questionnaire was administered to each house hold to capture various household characteristics including; roof type, wall type, whether eaves are open or not, cooking in the house, number of individuals indoors the previous night, presence of LLINs, number sleeping under an LLIN the night before the visit, and presence of cattle. A unique code was generated by tablets for each household sampled. The code was then written on the door and used to label all samples collected from that house. Human landing catches were also conducted.

TABLE 1: MOSQUITO TRAPPING METHOD AND NUMBER OF HOUSEHOLDS MONITORED PER SITE PER MONTH

Trapping Method	Number of Housesholds Monitored per Site per Month											
	Dec	Jan	Feb	March	April	May	June	July	Aug	Sept	Oct	Nov
PSC	20	20	20	20	20	5	5	5	5	5	5	5
CDC LT	20	20	20	20	20	10	10	10	10	10	10	10
Window exit trap	7	7	7	7	7	5	5	5	5	5	5	5

2.2 INDOOR BITING RATES (CDC LIGHT TRAP)

A single CDC light trap was deployed by dusk in the selected house and mosquitoes were collected from the trap the next morning following the deployment. The trap was positioned approximately 1.5m from the floor, next to a person sleeping under a bed net that was already in use by members of the household (the majority being LLINs). The trap consists of a fan and a collection bag attached to it. Mosquitoes attempting to feed upon the person under the net generally fly around the net trying to gain access and are then sucked into the trap when they approach the light. A piece of damp cotton wool was added into the light trap bag to improve survival of the trapped mosquitoes (for later parity dissections). The trapped mosquitoes were removed, placed in labeled paper cups and taken to a makeshift laboratory in the field for analysis. Live mosquitoes from the trap were killed by chloroform and a count per trap was taken and summarized by species, sex and abdominal status. Live, unfed female *Anopheles* mosquitoes were used in parity dissections.

2.3 Indoor Resting Densities (Pyrethrum Spray Catch)

Pyrethrum spray catches were conducted by using 0.025% Pyrethrum EC and 0.1% piperonyl butoxide sourced from the Pyrethrum Board of Kenya and mixed in kerosene before being sprayed with a hand-pump sprayer (reference #2). The houses were visited in the morning between 06:00 and 11:00 hours and sheets laid on the floors and over furniture that cannot be removed (from May collections were reduced to 5 and conducted between 06:00 – 09:00). All food, people and animals were removed from the house and the windows and doors closed. A collector first sprayed along the eaves and on any open space around the windows or gaps in the wall from the outside then proceeded inside and sprayed towards the walls and ceiling. After spraying, the houses remained closed for 10-15 minutes. After this period the sheets were retrieved from the house and examined for any mosquitoes that had fallen on them. The insecticide dissipates quickly but residents were asked to open the doors and windows and remain outside for 30 minutes after spraying. A count of all collected mosquitoes was taken for every house sampled and recorded by species, sex and abdominal status. Collected mosquitoes were differentiated as either *Anopheles* of *Culicine* and were further separated by sex. All female mosquitoes were further separated by abdominal status and categorized as fed, unfed, gravid or half gravid. The samples were preserved in labeled vials containing 70% ethanol and transported to the laboratory for further processing. Identification of species, sex and abdominal status was done in the field laboratory.

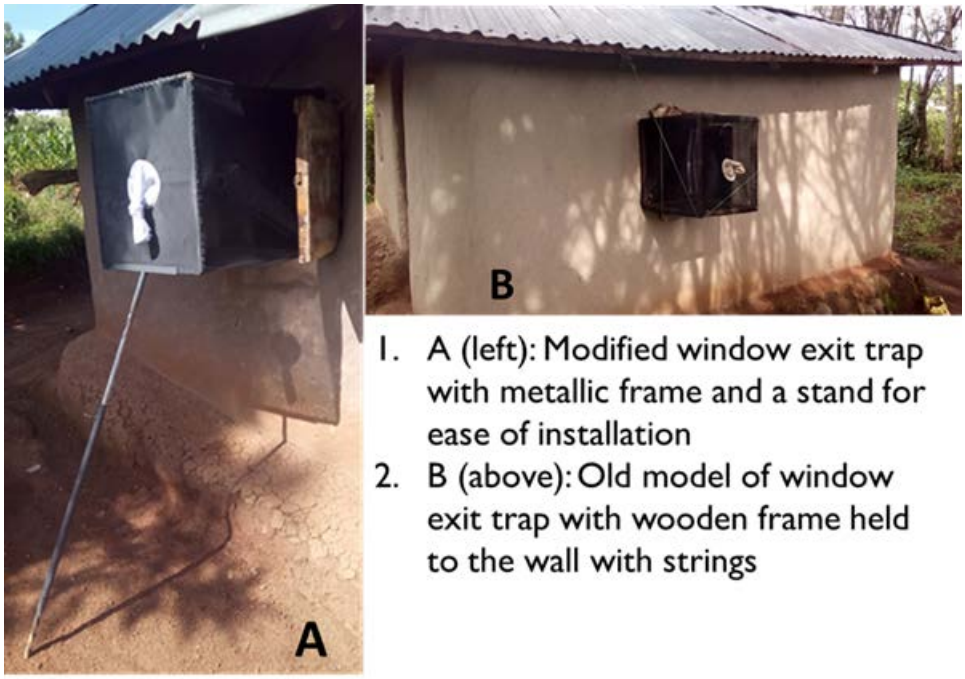
2.4 Vector Biting Behavior (Human Landing Catch)

To determine whether biting occurs indoors or outdoors and the peak biting time for the different malaria vector species, human landing catches (HLC) were conducted in February 2016, in five houses in Ndhiwa, Rongo, Awendo and Uriri for five nights. During HLC, four collectors were deployed per house per night for both the indoor and outdoor stations. The indoor station was set up in the living area of the house while the outdoor station was set up just outside the house within five meters of the front door. The collection period was from 5pm to 7am broken into two shifts of 7 hours each, so that one person manned each station for half the collection period (5pm to midnight) before being replaced by the other. Each collector sat on a stool and exposed his lower legs for the mosquitoes to land on. The collector monitored mosquitoes as they landed on his legs and captured them with an aspirator. Mosquitoes were then placed in a paper cup and provided with sugar solution. A new cup was used each hour to assess time of biting[19].

The mosquito collectors conducting the human landing catches were volunteers recruited from the community, consented and provided with requisite training. They were screened for malaria using a malaria rapid diagnostic test (RDT) one week before they commenced collection and four weeks after the collection ended. Those who were malaria positive were treated per the national guidelines with an ACT and were offered mefloquine prophylaxis two weeks after treatment with repeat doses once every week. Collectors who were malaria negative were offered malaria prophylaxis one week before collections begun, with repeat doses every week for six weeks. All collectors were followed for any malaria related symptoms for a period of six weeks and were encouraged to take a malaria test in the nearby health facility. During the collection nights, the collectors were supervised via random spot checks throughout the night by a mobile supervision team. Each collector was paid daily allowance for the collection period.

2.5 VECTOR EXITING BEHAVIOR (WINDOW EXIT TRAPS)

To determine whether vectors exited from houses before morning, we installed a single window exit trap in the same houses where PSC were performed from the month of January 2016. Window exit traps were initially made of a wooden frame covered with mosquito netting and are approximately one meter (1m) in length, width and height. These had a V-shaped entry point that extended the whole length of the trap (Figure 2). The traps were later modified with metallic frame and a funnel-shaped entry point to limit escape of mosquito from the trap after entry. The modified trap was fitted with a metallic stand for support and ease of installation (Figure 2). They were fitted over windows on the outside of the house. Mosquitoes enter through a cone shaped fitting which impedes them from escaping from the trap and back into the house. Mosquitoes were aspirated from the traps the following morning and placed in labelled paper cups. The mosquitoes were sustained on 10% sugar solution socked in cotton wool while being transported to make-shift lab in the field for further analysis.



1. A (left): Modified window exit trap with metallic frame and a stand for ease of installation
2. B (above): Old model of window exit trap with wooden frame held to the wall with strings

FIGURE 2: MODIFIED AND ORIGINAL WINDOW EXIT TRAP

2.6 INSECTICIDE RESISTANCE MONITORING (WHO CYLINDER TESTS AND CDC BOTTLE BIOASSAYS)

Mosquito collections for insecticide resistance monitoring were performed in March with additional sampling in April, May and June to raise sufficient numbers for the tests. Larval stages of mosquito were collected from all the sentinel sites. Collections were performed using larval dippers and sieves. *Anopheles* larvae were separated from the other aquatic organisms and sorted into different larval instars. The larval samples were maintained in a room with a portable space heater while in the field and were fed on either fish meal or spirulina. Pupae developing from the larvae were collected daily and placed in pupal cups. The pupal cups were then introduced into paper cups labelled with the collection site and provided with a wet cotton wool soaked in 70% sugar solution. The emerging adults from the pupae were trapped in the paper cups and sustained on the provided sugar pad. The emergent adults were marked with the date of emergence and raised to three day old adults for insecticide resistance tests.

FIGURE 3: FIELD WORKERS COLLECTING *ANOPHELES* LARVAE FROM A POND

Adult mosquitoes were also collected for insecticide resistance tests. Collections were performed by hand aspirators inside houses. The collected mosquitoes were placed in paper cups and labelled with collection site and date. All collected mosquitoes were transported to a holding room in the field and were monitored for 24 hours before insecticide resistance tests were performed.

Insecticide resistance status was assessed using WHO test-tube bioassay using diagnostic concentrations of deltamethrin (0.05%), permethrin (0.75%), bendiocarb (0.1%) and pirimiphos-methyl (0.25%). The WHO bioassay was done using 2-5 days old *An. funestus* s.l. or *An. gambiae* s.l. emerging from collected larvae or by direct exposure of collected adults. At least 100 mosquitoes of each species were exposed to each insecticide at a time. Knock-down was monitored every 10 minutes for 60 minutes. The samples were then transferred to a clean paper cup, provided with cotton wool soaked in sugar solution and held for 24 hours. Mortality was scored 24 hours after exposure. Dead mosquitoes were separated, and then live mosquitoes were frozen. The individual mosquitoes were placed in barcoded 1.5 ml Eppendorf tubes and marked either as dead or live. Samples were stored frozen awaiting molecular analysis to determine mechanisms of resistance. Insecticide resistance intensity assay was assessed by CDC bottle assay technique which involved coating 250ml bottles with varying concentrations of insecticide; x1, x2, x5 or x10 the diagnostic concentration. The bottles ware air dried overnight and 2-5 day old mosquitoes were exposed in the treated bottles with mortality monitored every 15 minutes for 1 hour (the diagnostic time was 30 mins).

2.7 Laboratory Analysis of Collected Vectors

All vectors collected were identified to species morphologically[20, 21]. The physiological status was determined by observation of the abdomen, and the ovaries of non-blood fed and non-gravid mosquitoes collected alive were dissected to determine parity status[22]. Female mosquitoes were divided into three parts for various procedures; head and thorax was used for determination of sporozoite rate by enzyme linked immunosorbent assay (ELISA) techniques (Wirtz et at 1987), the abdomen of blood-fed and half-gravid females were kept for blood-meal host determination and the remainder of the specimen was used in polymerase chain reaction (PCR) analysis to identify members of the *An. gambiae* s.l. and the *Anopheles funestus* s.l. groups[23] and for future genetic/molecular analysis. All mosquitoes morphologically identified as *An. gambiae s.l.* were analyzed by PCR for species differentiation. For *An. funestus s.l.,* all samples were initially analyzed (December 2015 to March 2016), in the subsequent months, April to September 2016, only 20% of *An. funestus s.l.* were tested for species identification in each month.

2.8 Ethical Considerations

Ethical approval for this study was granted by the Kenya Medical Research Institute, protocol number SSC 2776. Study participants taking part in the human landing collections of mosquitoes were duly consented, trained and protected from potential malaria infection during the collection exercise. Their participation was fully voluntary. During monthly mosquito collections, a verbal consent was sought from the household head before every collection exercise.

2.9 Data Management and Analysis

Data collection was done on tablets which are designed with buttons, drop down menus, and data quality checks to limit entry errors in the field. Each house sampled was allocated a unique code and a study number. The two codes were also allocated to mosquitoes from each house and were used to track the samples through all the laboratory procedures. Individual mosquitoes were labelled with pre-printed barcodes and linked to the field data by the collection identification which is automatically assigned in the database. Additional tests on individual mosquitoes, including sporozoite ELISAs and species identification by PCR were linked by the unique barcode label. Data entry was done on pre-tested data entry screens designed to limit data errors through drop down menus and automatic data checks. For data sharing, all data was merged into a single file and checked to ensure a proper merge. Poisson regression was used in data analysis controlling for clustering of mosquitoes at house hold level.

3. RESULTS

3.1 SPECIES COMPOSITION

A total of 6,967 *Anopheles* mosquitoes were collected by all collection methods combined, 5,958 of which were *Anopheles* females, the rest were males. Only female mosquitoes were considered in the analysis. Overall species composition by morphological identification was 85% *An. funestus* s.l., 13% *An. gambiae s.l.,* 1.0% *An. coustani*, 0.2% *An. rufipes*, 0.1% *An. pharoensis* and 0.04% *An. maculipalpis* (Figure 4 and Table 2).

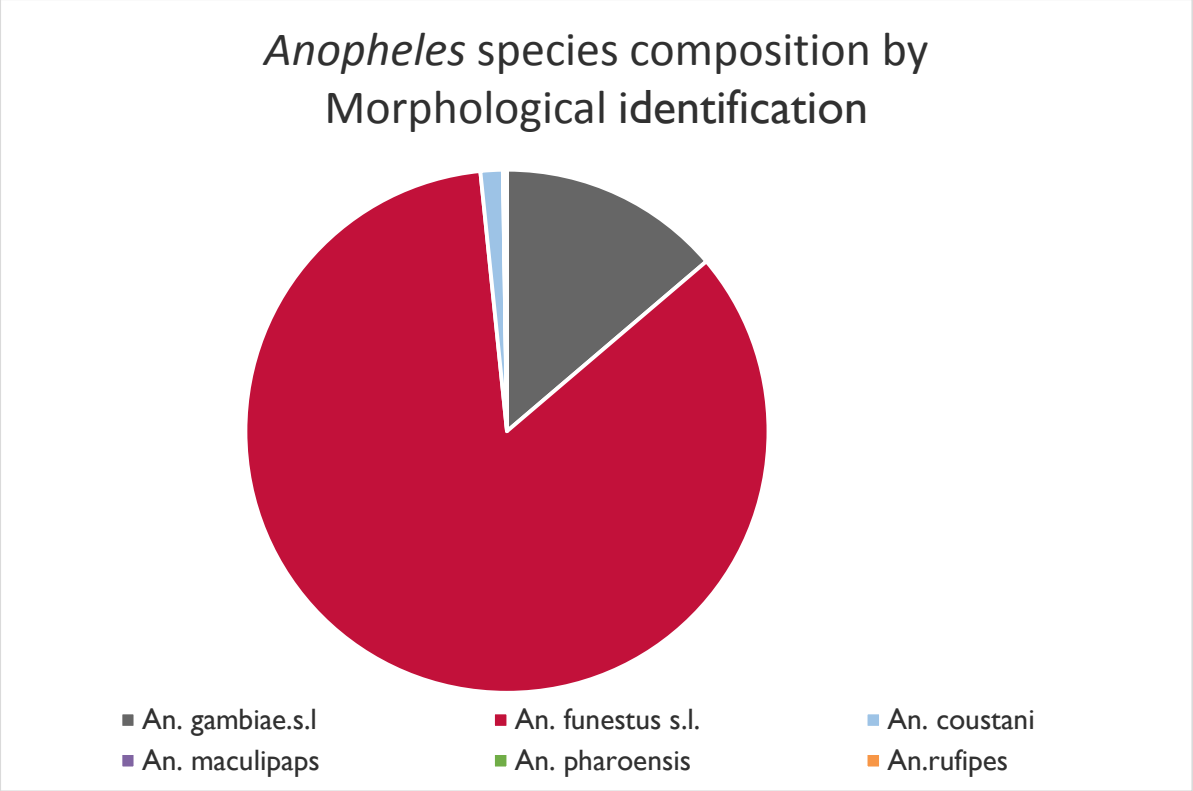


FIGURE 4: OVERALL *ANOPHELES* SPECIES COMPOSITION BY MORPHOLOGICAL IDENTIFICATION

TABLE 2: *ANOPHELES* SPECIES COMPOSITION BY MORPHOLOGICAL IDENTIFICATION

County	Sub County	*An. gambiae s.l.*		*An. funestus s.l.*		*An. coustani*		*An. maculipalpis*		*An. pharoensis*		*An. rufipes*		Total *Anopheles* per Sub County
		N	(%)	N	(%)	N	(%)	N	(%)	N	(%)	N	(%)	
Migori	Awendo	193	(18)	815	(78)	39	(4)	0		0		0		**1, 047**
Homa Bay	Ndhiwa	106	(15)	610	(84)	9	(1)	0		0		0		**725**
Homa Bay	Homa bay	95	(15)	519	(83)	11	(2)	0		0		1	(<1)	**626**
Migori	Rongo	178	(11)	1358	(87)	17	(1)	0		0		0		**1553**
Migori	Uriri	169	(9)	1,613	(90)	6	(<1)	2	(<1)	4	(<1)	8	(<1)	**1802**
Migori	Nyatike	72	(63)	42	(37)	0		0		0		0		**114**
Migori	Kuria	3	(4)	74	(96)	0		0		0		0		**77**
Migori	Suna	4	(29)	10	(71)	0		0		0		0		**14**
Total per Species		**820**	**(13)**	**5041**	**(85)**	**82**	**(1)**	**2**	**(<1)**	**4**	**(<1)**	**9**	**(<1)**	**5,958**

*Percentages are rounded to the nearest whole number.

3.2 Molecular Analysis of Mosquito Species Composition and Sporozoite Rates

A sub sample of 3332 (56%) female *Anopheles* mosquitoes of the collected 5,958were analyzed by PCR for species identification, while 5,640 (95%) samples were analyzed by ELISA for detection of sporozoites in the mosquito vector. The PCR results confirmed the local vector population to be predominantly *An. funestus* s.s. (93%) and *An. arabiensis* (7%), with very few *An. gambiae* s.s. (0.06%). Sporozoite rates were different across the counties with an overall sporozoite rate of 3.28% in the study area (Table 3). *An. funestus* s.l. had a much higher sporozoite positive rate at 3.71% (95% CI: 3.2-4.3) (162/4362) than *An. gambiae* s.l. at 0.15% (95% CI: <0.1-0.4) (1/680).

TABLE 3: OVERALL SPECIES IDENTIFICATION BY PCR AND SPOROZOITE ELISA RESULTS

Sub County	Species Identification PCR			ELISA Results *An. funestus* s.s.		
	***An. funestus* s.s. N**	***An. arabiensis* N**	***An. gambiae* s.s. N**	**No. positive N**	**No. Tested N**	**Sporozoite Rates % (95% CI)**
Awendo	636	46	0	28	937	2.99 (1.8-4.0)
Ndhiwa	275	45	0	29	668	4.34 (2.6-5.7)
Homa Bay	203	37	0	26	590	4.41 (2.5-6.5)
Rongo	909	64	2	47	1508	2.86 (2.2-4.0)
Uriri	1063	37	0	47	1641	3.12 (2.1-3.7)
Nyatike	10	45	0	1	89	1.12 (0.1-10.8)
Kuria	1	0	0	0	15	0
Suna	1	4	0	1	13	7.69
Total	**3098**	**232**	**2**	**179**	**5,461**	**3.28 (2.7-3.7)**

3.3 MONTHLY SPOROZOITE RATES AND ENTOMOLOGICAL INOCULATION RATE (EIR) FOR *AN. FUNESTUS*

Monthly sporozoite rates and EIR were calculated for *An. funestus*. The sporozoite rates were generally low with a peak between March and May while the highest EIR was recorded in May with about 3 bites per person per night.

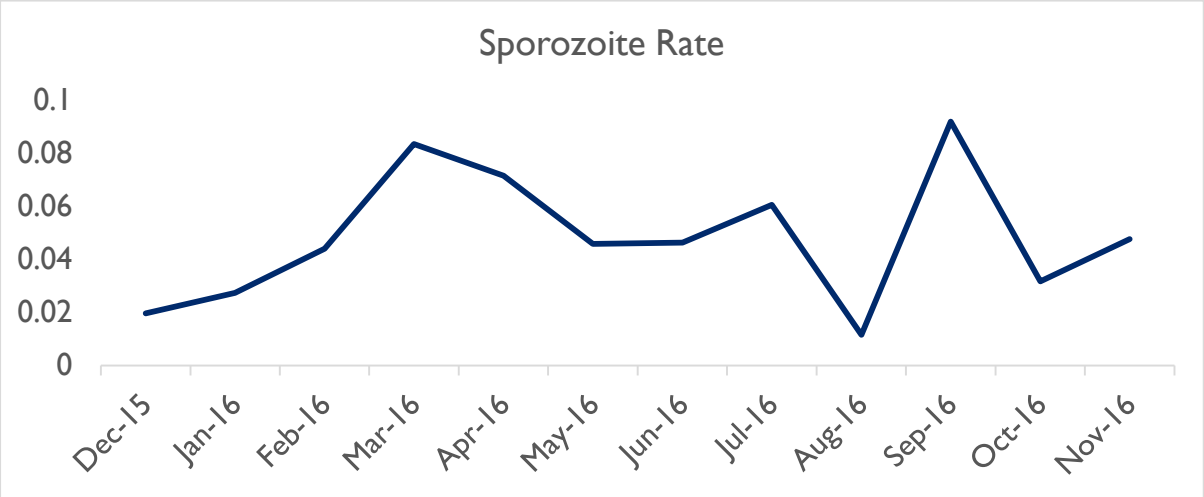


FIGURE 5 A: MONTHLY SPOROZOITE RATES FOR *AN. FUNESTUS*

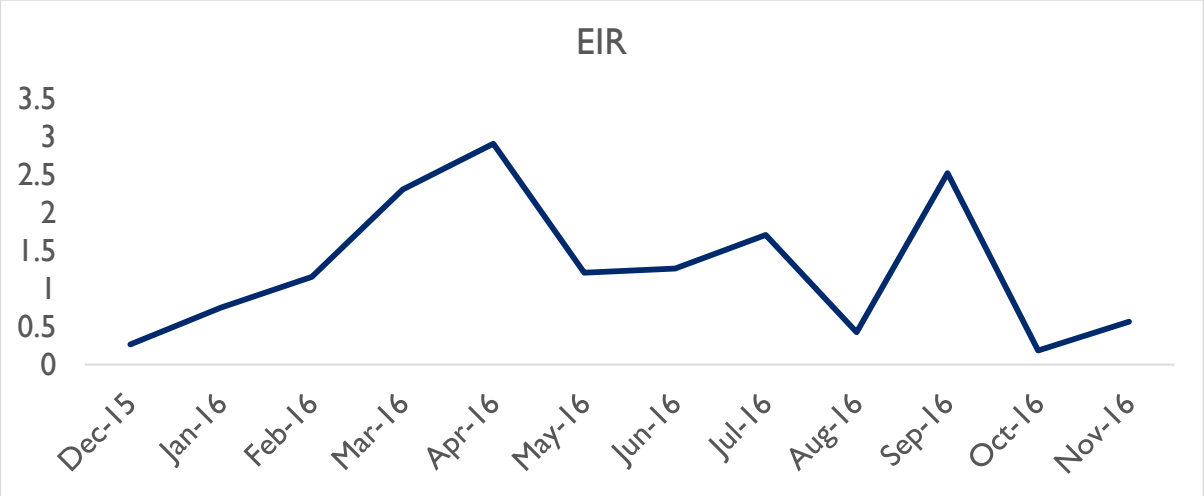


FIGURE 5 B: MONTHLY ENTOMOLOGICAL INOCULATION RATE (EIR)

3.4 VECTOR SEASONALITY

An. funestus was the predominant vector species in all the study sites throughout the year. Two clear peaks of high vector densities were observed following periods of rainfall, with the first peak in December to February (following the short rains of October-December) followed by a second in May to July (following the longer rains in April-June) (Figures 6 and 7). IRS in February is likely to be effective against mosquitoes emerging during the long rains but is unlikely to have any impact on the first peak between December and February (10-12 months after spraying).

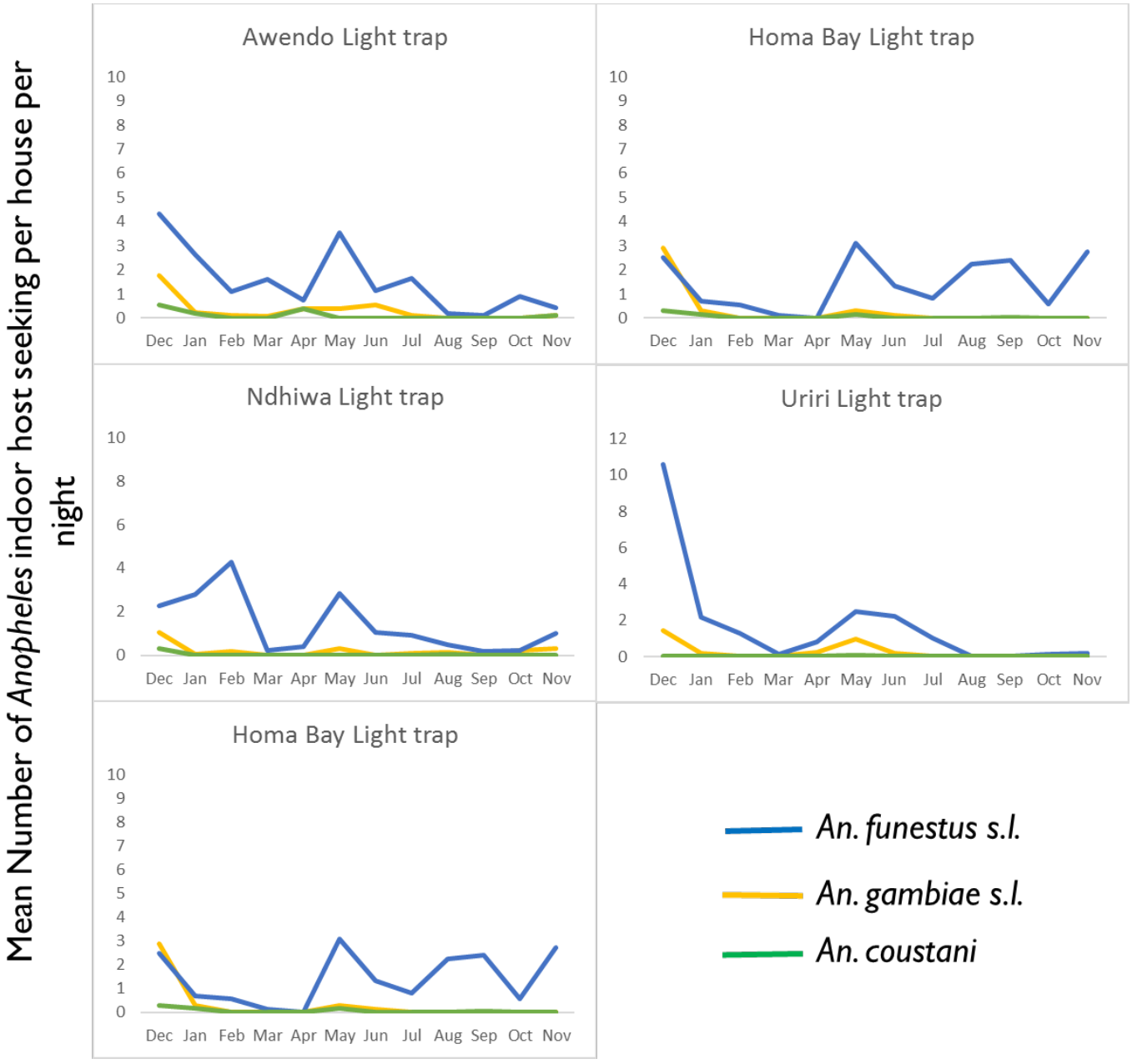


FIGURE 6: MONTHLY INDOOR BITING RATES (CDC-LT) OF *AN. FUNESTUS*, *AN. GAMBIAE S.L.* AND *AN. COUSTANI* IN FIVE SUB COUNTIES

FIGURE 7: MONTHLY INDOOR RESTING DENSITIES (PSC) OF *AN. FUNESTUS*, *AN. GAMBIAE* S.L. AND *AN. COUSTANI* IN FIVE SUB COUNTIES

An. funestus s.s. was the main vector species sampled by all collection methods in each Sub County. The proportions of *An. gambiae s.l.* and *An. funestus* s.s. were comparable in light trap indoor biting and indoor PSC resting collections across all Sub Counties. Window traps caught a small proportion of vectors that exited before morning, while most were caught resting indoors. This indicates that IRS should be an effective intervention against both *An. funestus* s.s. and *An. arabiensis*. The few *An. coustani* that were collected were in light traps, with even fewer captured by PSC and window exit trap (Table 4).

TABLE 4: MEAN DENSITY PER NIGHT OF *ANOPHELES* SPECIES BY COLLECTION METHOD FOR EACH SUB COUNTY

Sub County	Collection Method	Sampling Frequency (trap nights)	*An. gambiae s.l* N (mean per trap night)	*An. funestus s.s.* N (mean per trap night)	*An. coustani* N (mean per trap night)
Awendo	Light trap	267	111 (0.42)	497 (1.86)	37 (0.14)
	PSC	210	72 (0.33)	255 (1.21)	2 (0.01)
	Window Exit Trap	107	12 (0.11)	63 (0.59)	0
Ndhiwa	Light trap	259	52 (0.20)	332 (1.28)	9 (0.03)
	PSC	163	46 (0.28)	183 (1.12)	0
	Window Exit Trap	123	8 (0.07)	95 (0.77)	0
Homa Bay	Light trap	201	43 (0.21)	301 (1.50)	10 (0.05)
	PSC	138	49 (0.36)	202 (1.46)	1 (0.01)
	Window Exit Trap	83	3 (0.04)	16 (0.19)	0
Rongo	Light trap	260	112 (0.43)	615 (2.37)	16 (0.06)
	PSC	213	61 (0.28)	671 (3.13)	1 (0.00)
	Window Exit Trap	110	5 (0.05)	72 (0.65)	0
Uriri	Light trap	345	132 (0.38)	876 (2.54)	5 (0.01)
	PSC	247	27 (0.12)	647 (2.62)	0
	Window Exit Trap	154	10 (0.07)	90 (0.58)	1 (0.01)
Nyatike	Light trap	77	16(0.21)	22 (0.29)	0
	PSC	39	56 (1.44)	18 (0.46)	0
	Window Exit Trap	36	0	2 (0.06)	0
Kuria	Light trap	74	1 (0.01)	11 (0.15)	0
	PSC	40	0	4 (0.10)	0
Suna	Light trap	46	3 (0.07)	2 (0.04)	0
	PSC	19	1 (0.05)	5 (0.26)	0
	Window Exit Trap	21	0	3 (0.14)	0

3.5 BITING TIMES (HUMAN LANDING CATCH)

A total of 1,069 *Anopheles* mosquitoes were collected by HLC in 5 houses per night for five nights of collections in four sentinel sites, a total of 100 trap nights. Of these, 965 were morphologically identified as *An. funestus* s.l., 61 *An. gambiae* s.l. and 43 *An. coustani*. Biting by *An. funestus* s.l. was observed to begin early in the evening both indoors and outdoors but the peak was late at night, after midnight and continued at a high rate until 07:00 am. In 2017 monitoring of biting times will extend to 11:00am to detect any day time biting risk. As only 61 *An. gambiae* s.l. and 43 *An. coustani* were collected, it is not possible to conclude on their biting patterns (Figure 8).

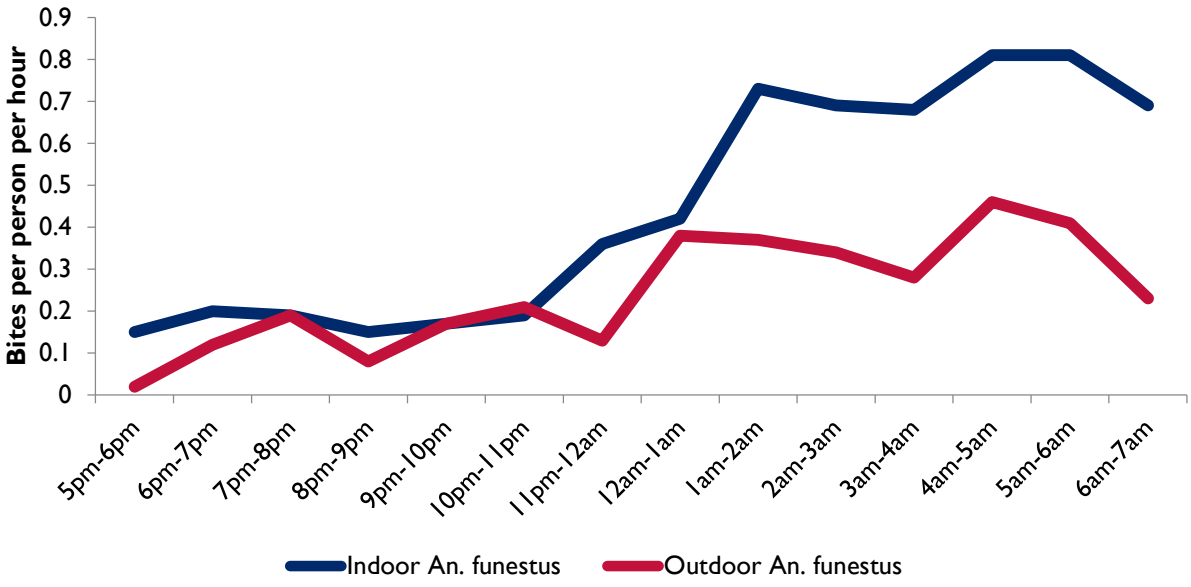


FIGURE 8: BITING RATES (BITES PER PERSON PER HOUR) OF *ANOPHELES FUNESTUS* S.S. COLLECTED BY HUMAN LANDING CATCHES

3.6 ABDOMINAL STATUS AND EXITING BEHAVIOR

Light traps collected mostly unfed mosquitoes (83%). This was expected as the concept is to divert host-seeking mosquitoes into light traps before being able to feed. PSC on the other hand collected mostly blood-fed females (76%), which were presumably resting shortly after blood-feeding. While in window exit traps unfed females were most frequent (64%) followed by gravid (20%). Half gravid females were rarely collected in window exit traps (Table 5).

TABLE 5: COMPARING MEANS OF *ANOPHELES* BY ABDOMINAL STATUS AND COLLECTION METHOD

	Unfed	Blood-fed	Half Gravid	Gravid
CDC-light trap (1,529 trap nights)				
Total number collected	2660	409	24	91
Mean collected per trap night	1.74	0.27	0.02	0.06
Percentage of total	84%	13%	<1%	3%
Pyrethrum spray catch (1,069 trap nights)				
Total number collected	207	1,703	247	130
Mean collected per trap night	0.19	1.59	0.23	0.12
Percentage of total	9%	74%	11%	6%
Window exit trap (634 trap nights)				
Total number collected	245	59	3	74
Mean collected per trap night	0.39	0.09	0.00	0.12
Percentage of total	64%	15%	<1%	19%

An analysis of probability of collecting *Anopheles* mosquitoes in either window exit trap or PSC for houses where the two collection methods were paired was performed by Poisson regression. Significantly more *An. funestus* s.s. and *An. gambiae s.l.* were found in the PSC compared to the Window exit trap ($p = 0.001$ for *funestus* and $p = 0.004$ for *gambiae*). However, gravid and unfed *Anopheles* were significantly more in the window exit trap compared to PSC ($P<0.0001$ for each comparison). Blood-fed and half gravid females were significantly more likely to be collected indoor by PSC collection than window exit trap ($p < 0.0001$ for blood fed and $p = 0.003$ for half-gravid)

(Table 6). A comparison of proportions of the different *Anopheles* species by PSC and light traps, revealed that light traps consistently sampled significantly more of each of the species, P<.0001, (Table 7).

TABLE 6: COMPARISON OF PROPORTIONS OF *ANOPHELES* MOSQUITOES IN PSC AND WINDOW EXIT TRAP FOR 524 TRAP NIGHTS IN HOUSES WHERE THE TWO METHODS WERE PAIRED

***Anopheles* Species**	**Collection Method**	**N**	**Mean**	**r_ratio**	**Lower_ci**	**Upper_ci**	**P Value**
Anopheles funestus female	PSC	955	1.82	1.36	1.48	1.68	0.004
	Window Exit Trap	322	0.61	1.00	1.00	1.00	
Anopheles gambiae female	PSC	96	0.18	1.67	1.06	2.65	0.03
	Window Exit Trap	35	0.07	1.00	1.00	1.00	
Anopheles Gravid	PSC	130	0.25	0.37	0.24	0.55	<.0001
	Window Exit Trap	258	0.49	1.00	1.00	1.00	
Anopheles fed	PSC	1435	2.74	4.95	3.24	7.57	<.0001
	Window Exit Trap	190	0.36	1.00	1.00	1.00	
Anopheles half gravid	PSC	163	0.31	7.07	2.77	18.09	<.0001
	Window Exit Trap	11	0.02	1.00	1.00	1.00	
Anopheles Unfed	PSC	174	0.33	0.15	0.11	0.21	<.0001
	Window Exit Trap	828	1.58	1.00	1.00	1.00	

TABLE 7: COMPARISON OF MEANS OF *ANOPHELES* SPECIES IN PSC AND LIGHT TRAP COLLECTIONS

***Anopheles* Species**	**Collection Method**	**Number of Sampling Efforts**	**Mean**	**R-ratio**	**Lower CI**	**Upper CI**	**P-Value**
An. gambiae s.l.	Light trap	1179	0.17	1.37	1.02	1.84	0.04
	PSC	815	0.19	1.00	1.00	1.00	
An. funestus s.l.	Light trap	1179	1.54	1.42	1.18	1.70	0.0002
	PSC	815	1.66	1.00	1.00	1.00	
An. coustani	Light trap	1179	0.03	13.86	4.11	46.70	<.0001
	PSC	815	0.003	1.00	1.00	1.00	

3.7 INSECTICIDE RESISTANCE TESTING

Due to difficulties finding larval breeding sites for *An. funestus* we were not able to raise sufficient numbers in all the sites for the required tests. *An. arabiensis* raised from field collected larvae showed full susceptibility to pirimiphos-methyl and bendiocarb but moderate resistance to deltamethrin and permethrin was observed across all study sites (Figure 9).

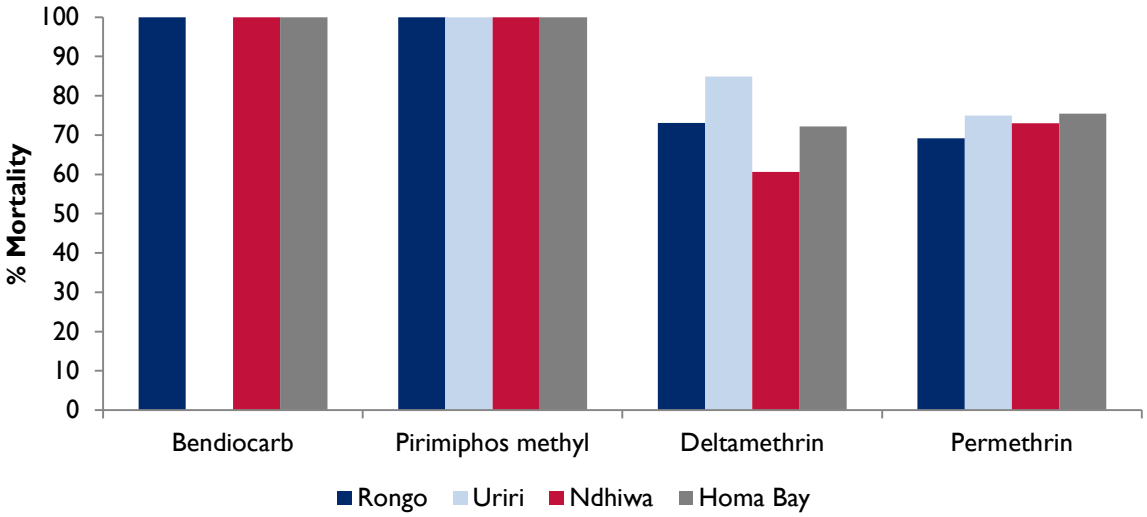


FIGURE 9: 24H MORTALITY OF *AN. ARABIENSIS* RAISED FROM FIELD COLLECTED LARVAE TESTED IN WHO CYLINDER TESTS ON EXPOSURE WITH TO BENDIOCARB 0.1%, PIRIMIPHOS-METHYL 0.25%, DELTAMETHRIN 0.05% AND PERMETHRIN 0.75%.

From direct exposure of adult collected *An. funestus* to the four classes of insecticides, full susceptibility to pirimiphos methyl was observed in Awendo, Rongo and Uriri (Figure 10). Similarly, susceptibility to bendiocarb was observed in adult collected *An. funestus* from Rongo and Uriri with probable low frequency resistance in Awendo. Low frequency resistance with about 86% mortality to deltamethrin and permethrin was observed in Awendo. Further resistance testing of *An. funestus* adults will be conducted before spraying in 2017 to confirm resistance status at all sites.

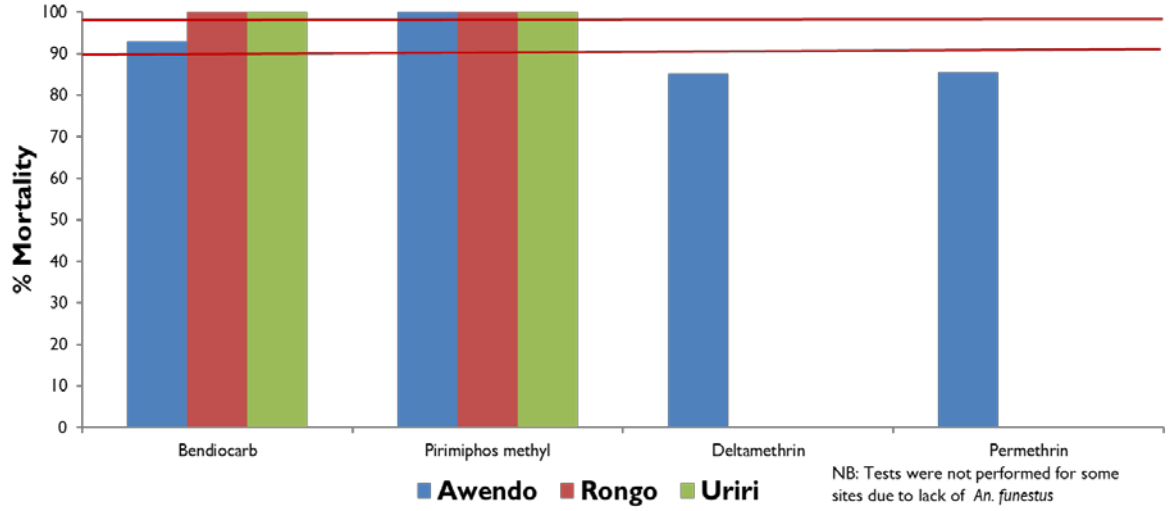


FIGURE 10: 24H MORTALITY OF ADULT COLLECTED *AN. FUNESTUS* FOLLOWING EXPOSURE IN WHO CYLINDER TESTS TO BENDIOCARB 0.1%, PIRIMIPHOS-METHYL 0.25%, DELTAMETHRIN 0.05% AND PERMETHRIN 0.75%.

Adult *An. funestus* from Awendo sub county were exposed to 1x, 2x, 5x and 10x times the diagnostic dose of deltamethrin and permethrin. There was 100% survival at the diagnostic dose for permethrin (1x) while about 60%, 35% and 10% survived 2x, 5x and 10x doses respectively at 30 minutes diagnostic time. Also in Awendo, 60%, 30%, 28% and 3% of adult collected *An. funestus* survived 1x, 2x, 5x and 10x doses of deltamethrin respectively. *An. arabiensis* raised from field collected larvae in Ndhiwa and Homa bay counties were exposed to 1x, 2x, 5x and 10x permethrin. In both sites, about 80% survival was observed with 1x which reduced with higher doses of insecticide at 30 minutes diagnostic time. About 2% of the exposed vectors survived 10x in Ndhiwa while 100% were killed by the same dose in Homa bay (Figure 11).

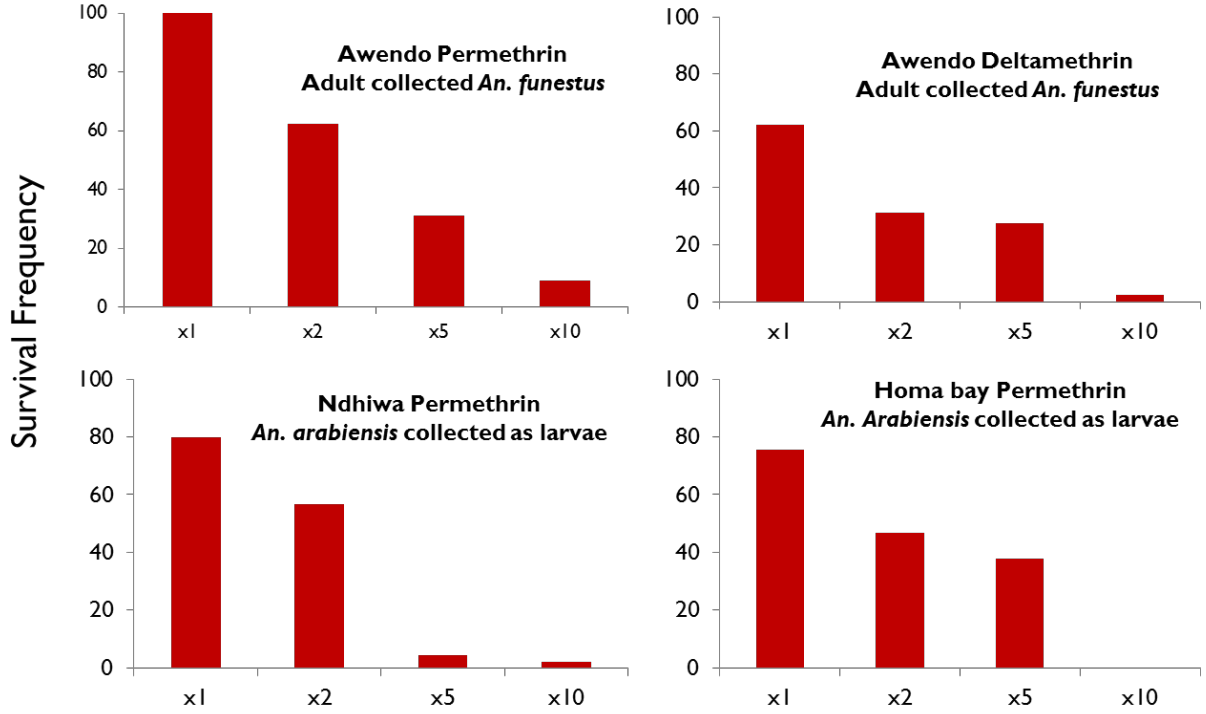


FIGURE 11: RESISTANCE OF ADULT COLLECTED *AN. FUNESTUS* FROM AWENDO AND *AN. ARABIENSIS* RAISED FROM LARVAE COLLECTED IN NDHIWA AND HOMA BAY AT 30 MINUTES DIAGNOSTIC TIME ON EXPOSURE TO VARYING DOSES OF INSECTICIDE

An. arabiensis that were used in susceptibility tests were further analysed for East (L1014S) and West (L1014F) African knock-down resistance (*kdr*) mutation. Both *kdr* East and West were observed in the vector population at low frequencies of 0.0036 and 0.0027 respectively. *An. funestus* were not tested for presence of *kdr* mutations.

3.8 AGE GRADING

Parity dissection of all live mosquitoes collected from light traps and window exit traps showed over 80% parous for *An. funestus* (Figure 12). The rates were consistently high for *An. funestus* across the collection period.

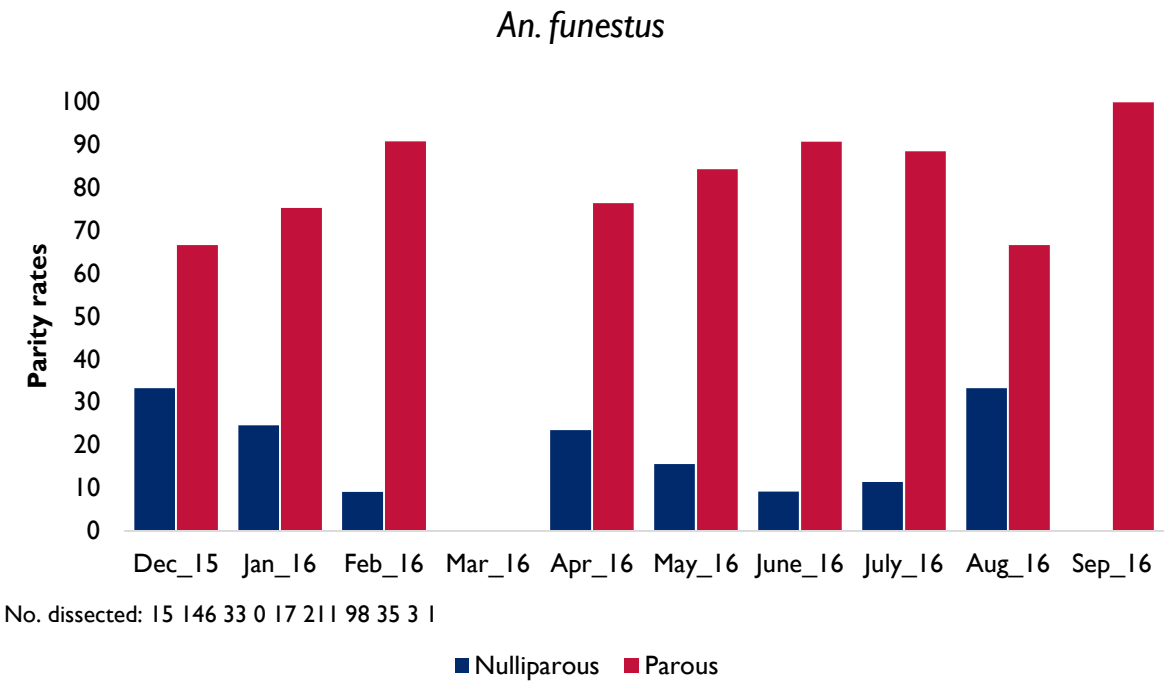


FIGURE 12: PARITY RATES BY MONTH FOR *AN. FUNESTUS* COLLECTED IN CDC-LIGHT TRAPS AND WINDOW EXIT TRAPS

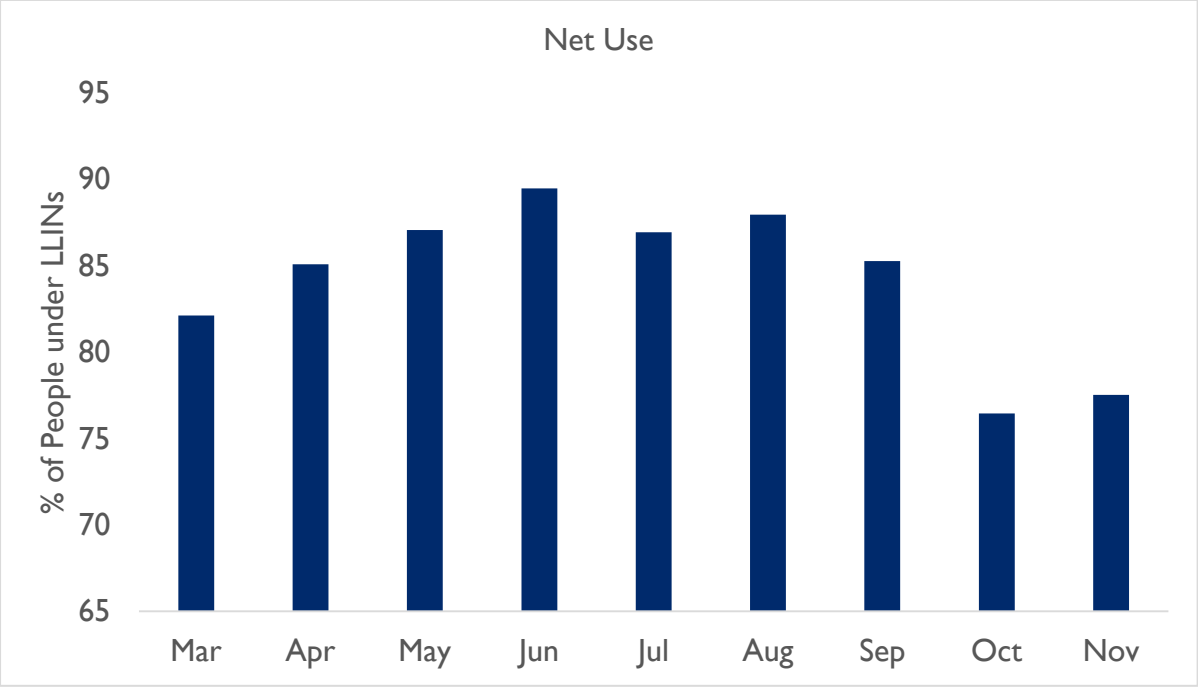
3.9 LONG LASTING INSECTICIDAL NET COVERAGE, AND USE

Houses were surveyed for net presence and reported use at every mosquito collection effort, with a total of 2,884 houses included over the reporting period. Most houses surveyed had at least one LLIN (83.3% - 97.1%) in all study sites. Net use was equally high with a low of 66.0% in Suna East and high of 99.1% in Rongo indicating to have slept under an LLIN the previous night before collection (Table 8). These results are not from a random sample of households and therefore not comparable to results from Kenya Malaria Indicators Survey (KMIS). Trends in net use over time (Figure 13) show net use to be generally high across most of the months and to peak around periods of high rains. Use of LLINs before March 2016 are not reported here since due lack of data.

TABLE 8: PRESENCE AND USE OF LLINS

Sub County	Total Houses Sampled	At Least 1 Net Present	Total Number of People	People Who Reported Sleeping Under a Net the Previous Night	Total No. of Nets Found in Houses	Mean Number of People per Net (Coverage)
Awendo	557	540 (97)	1,512	1,496 (99)	822	1.84
Ndhiwa	478	462 (97)	950	917 (97)	677	1.40
Homa bay	364	345 (95)	1,023	983 (96)	534	1.92
Rongo	554	538 (97)	1,590	1585 (100)	792	2.01
Uriri	710	671 (95)	1,886	1865 (99)	1063	1.77
Nyatike	85	76 (89)	106	80 (75)	111	0.95
Kuria	84	70 (83)	106	70 (66)	83	1.28
Suna	53	48 (91)	53	35 (66)	74	0.72
TOTAL	**2,885**	**2,750 (95)**	**7,226**	**7,031 (97)**	**4,156**	**1.74**

FIGURE 13: PERCENTAGE OF PEOPLE UNDER LLINS PER MONTH



3.10 HOUSEHOLD CHARACTERISTICS

The houses sampled mostly had iron sheet roof (96.1%), plastered mud walls (73.4%) and open eaves (74.6%).

TABLE 9: HOUSEHOLD CHARACTERISTICS

House Characteristic	Category	Frequency	Percentage (%)
Roof Type	Grass thatch	107	3.7
	Iron sheet	2,772	96.1
Wall type	Bricks	116	4.0
	Cement	245	8.5
	Rough mud	231	8.0
	Painted cement	165	5.7
	Plastered mud	2,115	73.4
	Others	10	0.4
Eaves	Closed	514	17.8
	Open	2,150	74.6

4. DISCUSSION

Monitoring of monthly indoor mosquito densities was conducted originally at eight sentinel collection sites in five sub-counties, Rongo, Awendo and Uriri in Migori County and Homa Bay and Ndhiwa in Homa Bay County from December 2015 and June 2016. In preparation for 2017 IRS, the sites were reorganized and scaled up to twelve, six in the spray area and six controls in July 2016. The results show *An. funestus* s.s. to be the predominant malaria vector in all the collection sites. *An. funestus* s.s. was previously nearly eliminated from Asembo bay region with the introduction of insecticide treated net[2], but recent studies have reported the re-emergence of this vector species in the region[24]. In the current study, we observed *An. funestus s.s.* to be the predominant malaria vector in Migori and Homa bay counties. It is highly likely that the vector currently dominates much of the lake endemic regions of western Kenya. Resistance to pyrethroid insecticides may also be a factor that contributed to greater densities of *An. funestus* s.s. The species identification results indicate that *An. arabiensis* is predominant over *An. gambiae* s.s. in the region although both species are at low densities.

Monthly trends in malaria vector species composition and temporal distribution showed *An. funestus* to dominate the vector population throughout the year. Two clear peaks of high vector densities in the region were observed to correspond with periods of short (October-December) and long (April-June) long rains. IRS in February will be effective against vectors during the two peak periods following long rains and short rains only if the residual efficacy of the pirimiphos methyl is found to last 10-12 months. The proliferation of *An. funestus* in both dry and wet season in the region compared to *An. arabiensis* is most likely attributable to nature of larval habitats, resulting from topography and human activities. A typical *An. funestus* larval habitat is a large, permanent or semi-permanent body of fresh water with emergent vegetation, such as swamps, large ponds and lake edges[26]. Our observation is that the area is characterized by wetlands and valley bottoms creating permanent or semi-permanent swamps. Also, abandoned fish ponds, pits from brick making and low flowing streams are common in the region. These could possibly contribute the high proliferation of *An. funestus* throughout the year.

From analysis of sporozoite infection in the vector population, only *An. funestus* s.s. was incriminated among other *Anopheles* species. Thus, the vector is not only important in terms of population abundance, but it is the main driver for malaria transmission in the region. *An. funestus* s.s. has been observed to be mostly endophilic and anthropophagic unlike *An. arabiensis* which is more opportunistic in its feeding and has been associated more with zoophily and endophily[27, 28]. No sporozoite infection was detected in *An. coustani* complex in this study even though a few of these were collected in indoor light raps and tested for sporozoite infection.

Indoor CDC light traps, PSC and window exit traps were used routinely in the monthly density monitoring. The results show that most of the mosquitoes were trapped while host seeking as compared to proportions either resting or exiting. All light traps were installed next to occupied LLINs normally used by households. This suggests that those mosquito collection methods that are implemented next to a host or mimic a host environment would be more appropriate in monitoring vector population densities. *An. coustani* were mostly collected in indoor light trap, evidence that the vector exhibits endophagic traits. Even though the vector has never been found with sporozoite before in this region, its indoor activities require continued monitoring.

Window exit traps and PSC were both conducted in the same house to monitor proportions of vectors resting indoors by morning against those exiting for each house overnight. The densities of both *An. funestus* and *An. gambiae s.l.* were highest in the PSC as compared to window exit traps. However, comparison of means of different physiological status of the mosquitoes between the two traps provides important information on mosquito behavior in relation to physiological status. The

results reveal that the mosquitoes are more likely to rest indoor when fed or half gravid while unfed and gravid mosquitoes were mostly found in the exit traps. Thus, after a successful blood meal, the vectors take time indoors to mature their eggs before exiting to lay when gravid. This trait increased the probability that a mosquito will acquire a lethal dose of insecticide on sprayed walls in case of IRS. Hence IRS with a non-exito-repellant insecticide is likely to have a great potential for vector control in the region. The main cause for exiting in this vector population is likely either, a search for alternative blood meal host or oviposition as evidenced by mostly unfed and gravid mosquitoes caught in the exit trap.

Consistent with results from other studies in western Kenya[19], we observed high rates of late night indoor biting by *An. funestus* s.s. A small proportion of biting occurred early in the evening before most individuals are protected by bed nets and a high rate of biting indoors between 4am and 6am when some people are getting out of bed. Provision of IRS in addition to bed nets may thus ensure more protection against bites that occur indoors when people are away from the protection of their bednets. While our sampling stopped at 7:00 am, the trend indicates that biting may continue later in the morning and will be monitored up to 11am in future. A study in Senegal recently reported broad daylight biting of *An. funestus*[29]. While such a biting behavior has not been reported in western Kenya before, the current data suggest a possibility of early morning biting by *An. funestus* s.s. both indoors and outdoors.

Exposure of adult collected *An. funestus* and larval collected *An. arabiensis* to insecticide treated WHO papers shows full susceptibility to pirimiphos methyl against both vectors. This insecticide has been reported to have a long acting period on sprayed walls[30], and therefore provides an attractive alternative to pyrethroids for IRS in western Kenya. Pyrethroid resistance in malaria vectors is widespread in western Kenya[31, 32]. We observed resistance to both deltamethrin and permethrin in all study sites. Exposure of mosquitoes to increasing doses of deltamethrin and permethrin showed a worrying situation with mosquitoes surviving up to 10 times the diagnostic dose of the insecticide. The intensity assay showed very high resistance to deltamethrin and permethrin while the WHO susceptibility tests showed only moderate resistance to the insecticide. This contrasts sharply with the WHO assay. It is not clear however if it is a problem with diagnostic dose, or the way tests were run. Additional tests have been arranged for with positive control with lab susceptible colony for each test. Further analysis of *An. arabiensis* that were used in the resistance testing for both East and West *kdr* mutation, showed a co-occurrence East (L1014S) and West (L1014F) in the same vector population. However, the mutant allele frequencies were quite low when compared to the wild type. The moderate pyrethroid resistance observed in the WHO susceptibility tests and high resistance intensity in the CDC bottle intensity assay is contradictory and suggests that further validation of the bottle bioassays is required. The low *kdr* frequency requires further investigation but probably indicates that metabolic resistance is important.

A survey of LLIN presence and use in the households where mosquito collections were performed showed very high net coverage and self-reported compliance rates. Use of insecticide treated nets has been widely reported to reduce human-vector contact and reduce vector numbers indoors[2, 33-35]. We however observed considerably high rates of indoor resting especially when mosquitoes are fed or half gravid, coupled with high parity rates of over 80%. There is clearly a need for additional control measure indoor to further limit vector-human contact indoors. Consequently, IRS with non-pyrethroid insecticides, particularly an organophosphate which has been shown to be effective against pyrethroid resistant mosquitoes, will be a valuable addition to vector control in the region. It is expected that spraying of houses with pyrimiphos methyl will markedly reduce vector densities indoor and alter vector population structures. The data so far collected therefore provide sufficient baseline information for evaluation of IRS with pirimiphos methyl for control of mosquitoes and reduction of malaria burden the region.

4.1 CHALLENGES

Collection of *An. funestus* larvae for resistance monitoring assay has been a bit of a challenge but the greatest hurdle has been raising the few larvae we collect to adult stage for the tests. Training to develop technical skills for raising *An. funestus* larvae is required. As a result, resistance testing took several months. The refusal rate was relatively high for PSC due to the odor of kerosene and particularly for fitting of window exit traps due to cold draughts and also many houses with no functional windows.

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ANNEX I: QUESTIONNAIRE FOR MOSQUITO COLLECTION

1) Date_____

2) House Code_____

3) Eaves

a) Open b) Partially open c) Closed

4) Type of roof

a) Grass thatch b) Iron sheet c) Fired clay d) Other__

5) Type of walls

a) Mud b) Plastered mud c) Brick d) Cement

e) Painted cement f) Wood g) Other_____

6a) Number of long-lasting insecticide treated nets in house_____ (if zero, skip to question 7)

6b) Number of people who slept under long-lasting insecticide treated nets last night_____

7a) Number of conventional nets treated in last 12 months in house _____ (if zero, skip to question 8)

7b) Number of people who slept under conventional nets treated in last 12 months last night __

8a) Number of untreated nets treated in house _____ (if zero, skip to question 9)

8b) Number of people who slept under untreated nets last night_____

9) Number of people who did not sleep under a net last night_____

10) Did you burn mosquito coil in the house last night?

a) Yes b) No

11) Did you use any insecticide spray cans like doom last night?

a) Yes b) No

12a) Presence of Livestock within the compound last night

a) Yes b) No

12b) Presence of livestock in the house at night?

a) Yes b) No

13) Did the residents cook inside the house last night or this morning?

a) Yes b) No

14) Has anyone sprayed the walls of this house with an insecticide in the last 12 months?

a) Yes b) No

15) How many months ago was the house sprayed _____

16) Who sprayed your house?

a) Government worker/Program b) Private company c) Household member d) Don't Know

e) Other_____

17) Mosquito collection method used

a) PSC b) Light Trap c) Window Exit Trap d) Drum Collection