PMI | AfricaIRS (AIRS) Project

Indoor Residual Spraying (IRS) Task Order six

AIRS TANZANIA PROJECT

ENTOMOLOGICALMONITORING OF 2016 IRS ACTIVITIES

FINAL REPORT

FEBRUARY 2017

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MAINLAND TANZANIA:
ENTOMOLOGICAL MONITORING OF 2016 IRS ACTIVITIES

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ACRONYMS

AIRS	Africa Indoor Residual Spraying
CBR	CDC Light Trap with Bottle Rotator
CDC	Centers for Disease Control and Prevention
CMCo	Community mosquito collectors
CS	Capsule Suspension
DC	District Council
DVCO	District Vector Control Officer
ELISA	Enzyme-Linked Immunosorbent Assay
GPS	Global Positioning System
IBD	Indoor biting densities
IRD	Indoor resting densities
IRS	Indoor Residual Spraying
LLIN	Long Lasting Insecticidal Net
LT	Light Trap
NIMR	National Institute for Medical Research
NMCP	National Malaria Control Program
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

Abt Associates supports the implementation of indoor residual spraying (IRS) for malaria control in Tanzania through the Africa Indoor Residual Spraying (AIRS) Project, funded by the United States Agency for International Development (USAID) under the President's Malaria Initiative (PMI). AIRS Tanzania conducted IRS from February–March 2016 in eight districts within the Lake Victoria region using pirimiphos-methyl (Actellic 300CS), a long-lasting organophosphateinsecticide. In addition, non-IRS districts were included as control sentinel sites. The project was implemented in collaboration with the National Malaria Control Program (NMCP) and the National Institute for Medical Research Tanzania (NIMR), Mwanza Centre.

Morphological identification of *Anopheles* from mosquito collection traps including CDC Light Trap (CDC-LT), CDC Collection with Bottle rotators (CBR), Prokopack aspirator, Pyrethrum Spray Catch (PSC) and Clay pot indicated that 94% were *An. gambiae*s.l., with the remaining 6% being *An. funestus* s.l. Furthermore, *An. gambiae* s.l. was the main vector species sampled by all collection methods in each district. *An. gambiae* s.l. was the predominant vector species in all the study sites throughout the year. Analysis of the samples by Polymerase Chain Reaction (PCR) revealed the following composition: *An. arabiensis* (55.4%), *An. gambiae* s.s.(6.5%), *An. funestus s.s.* (13.7%),*An. parensis* (4.7%) and *An. rivolum* (0.1%). Approximately twenty percent of the assayed samples (19.6%) could not be amplified by PCR. Overall, the sporozoite rate remained low at 1.7% with wide (0.9-4.7%) variation between sentinel sites.
A strong correlation was observed between *An. gambiae* s.l. biting rates and indoor resting densities (IRDs), and the mean rainfall was observed, suggesting that the risk of malaria transmission was highly dependent on rainfall patterns. In most sprayed sentinel sites the highest indoor CDC light trap catches of *Anopheles gambiae* s.l. were recorded in January and February (before spraying), with a marked decrease in biting rate occurring between March and May (after spraying). In one control area (Bukombe site) there was a large increase in biting rates in May which was not seen in all the sprayed sites. In general, *An. gambiae* s.l. biting rates decreased post IRS, although in Missenyi, where spraying was done in early February, a large biting peak was recorded between June and August. Indoor biting densities (IBD) also declined sharply between March and April in the unsprayed control sites of Bukombe and Busega. This may be attributed to heavy rainfall in March and April resulting in flushing out of larvae from breeding sites. Light traps collected mostly unfed mosquitoes (80.4%). In Musoma rural, there was more outdoor biting risk before people went to bed (18:00 – 22:00) compared to indoors. Subsequent indoor biting tended to occur mostly late at night, with some signs of biting continuing up until early morning between 04:00 – 06:00.

A comparison of the mean number of *Anopheles* mosquitoes collected by PSC and Prokopack aspirator, revealed that both Prokopack aspirator and PSC collected almost similar numbers of each of the *Anopheles* species, ($p>0.05$). The indoor resting density (IRD) of *An. gambiae* s.l. was greater in unsprayed sentinel sites (Bukombe site) than in the sprayed districts, except in Missenyi district where it was higher in the June to September period. This probably can be attributed to rainfall (55mm in May and 15.9mm in September), received in Missenyi district which occurred throughout the month.

Spray quality assurance was conducted through cone bioassays that exposed insectary-reared susceptible *An. gambiae* s.s. on sprayed walls of different surface types within the first 14 days from start of the operation. Results of the mean 24-hours mortality scores were found to range between 97.8-100%, a strong indicator that the spraying was of satisfactory quality. Further follow-up with monthly assays were conducted to monitor the insecticide decay rate. In September 2016 (6 months post spray), 24-hour mortality was still >80 percent at all sites on all sprayed wall surface types. Moreover, the residual efficacy of indoor residual spraying (IRS) with pirimiphos-methyl (Actellic CS 300) using a susceptible strain of *Anopheles gambiae* s.s. was generally between six to eight months

for mud and painted walls where residual efficacy for concrete, white wash and burnt brick walls was seven to eight months (according to World Health Organization (WHO) criteria of >80% mortality).

In summary, IRS has maintained sporozoite rates at low levels and this also confirms that IRS can maintain its residual efficacy for 6-8 months hence spraying with pirimiphos-methyl provides an attractive choice for malaria vector control in the area.

1.0 INTRODUCTION

Entomological monitoring is an integral component for any disease control intervention involving vector control. It provides important information that indicates whether interventions such as Indoor Residual Spraying (IRS) are appropriately applied and remain effective over the expected duration(during and after implementation), in accordance with the manufacturers claims(WHO, 2015). Since 2007, the National Institute for Medical Research (NIMR), Mwanza Centre has been conducting entomological monitoring in Kagera region where IRS was first introduced in Mainland Tanzania. From 2010, IRS activities were extended to Mwanza, Geita and Mara regions. In January 2013, NIMR Mwanza Centre extended its entomological monitoring activities to cover targeted districts under IRS intervention in the Lake Victoria basin.

From January 2016, Mwanza Centre continued to implement entomological monitoring activities to cover eight sites in eight districts targeted for spraying and two control sites (in two unsprayed districts) in the Lake Zone. The intervention districts are Missenyi, Bukoba rural, Ngara, Chato, Musoma rural, Butiama, Kwimba and Sengerema. The unsprayed control districts are Busega and Bukombe.

In 2016, the PMI AIRS Tanzania Project used a long-acting organophosphate formulation (pirimiphos-methyl 300CS) for IRS in the 8 districts. This report provides information on the entomological monitoring activities completed between January 1 and December 31, 2016 which were carried out in eight sprayed sentinel districts and two unsprayed sentinel districts. It also provides information on the pirimiphos-methyl 300CS residual efficacy in eight sprayed sentinel sites.

2.0 STUDY OBJECTIVES

2.1 MAIN OBJECTIVE

The main objective of the program was to evaluate the biological efficacy of p-methyl 300 CS on different sprayed wall surfaces and its entomological impact against malaria vectors post-IRS intervention in the Lake Victoria basin, Tanzania.

2.2 SPECIFIC OBJECTIVES

The specific objectives of the program were:

- To identify the species of malaria vectors in intervention and control areas
- To assess vector density, distribution and seasonality in the intervention and control sentinel sites
- To monitor vector feeding and resting behavior in designated sites across the intervention districts
- To provide quality assurance of the IRS programs
- To monitor the insecticide decay rate in designated sites across the intervention districts
- To rear and maintain a colony of susceptible *Anopheles gambiae* (Kisumu strain)

3.0 METHODS

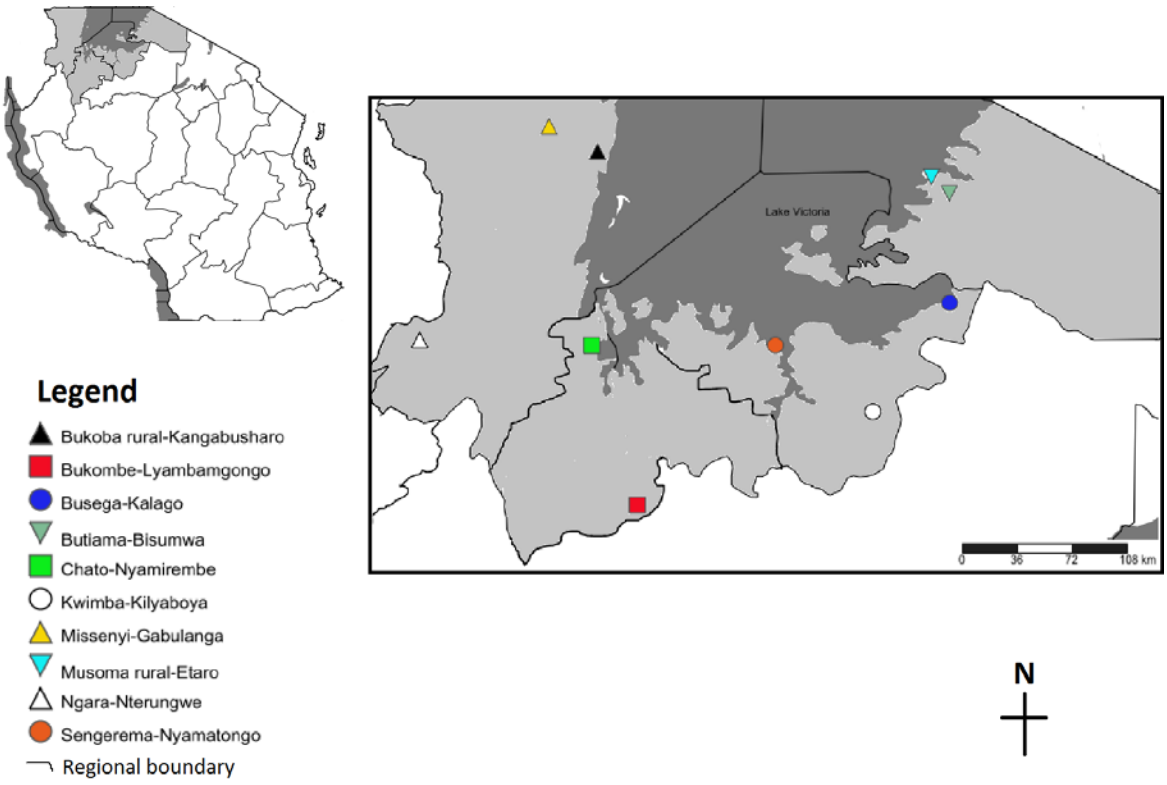
3.1 STUDY PERIOD AND AREA

Entomological data was collected before the IRS campaign in January 2016 and after the IRS campaign between February 2016 and December 2016 in the sentinel districts listed in Table 1. Geographical locations of the sites are shown in Figure 1.

Table 1: Data Collection Sites

Region	District	Site (village)	GPS coordinates	Date of spraying	Spray Status
Kagera	Missenyi	Gabulanga	1° 11.808'S 31°27.913'E	8th-10th Feb	Sprayed
	Bukoba Rural	Kangabusharo	1° 20.958'S 31°44.981'E	10th-11th Feb	Sprayed
	Ngara	Nterungwe	2°29.505'S 30°42.447'E	22nd Feb	Sprayed
Geita	Chato	Nyamirembe	2o 31.509'S 31°42.881'E	9th-14th March	Sprayed
	Bukombe	Lyambamgongo	3° 29.644'S 31°5.966'E		Non-Sprayed (Control)
Mwanza	Sengerema	Nyamatongo	2°31.453'S 32° 47.48'E	29th March	Sprayed
	Kwimba	Kilyaboya	2°55.609'S 33°21.733'E	21st-22nd March	Sprayed
Mara	Musoma Rural	Etaro	1°30.234'S 33°42.319'E	9th-10th March	Sprayed
	Butiama	Bisumwa	1°36.176'S 33°48.602'E	9th-11th March	Sprayed
Simiyu	Busega	Kalago	2°15.998'S 33°48.726'E		Non-Sprayed (Control)

Figure 1: Map of PMI Tanzania Mainland Entomological Surveillance Sites



3.2 PERSONNEL TRAINING

A total of 20 Community mosquito collectors (CMCo) were hired to help undertake the field work for the entomological surveillance in 10 sentinel sites. All hired Community mosquito collectors were given three days orientation training at the NIMR Mwanza Centre by the core surveillance team.

We conducted a three-day refresher training for both the CMCos and district vector control officers (DVCOs) in January 2016 to ensure that they all followed best practices in mosquito field collection and understood the AIRS Tanzania entomological monitoring standards. The training covered the following topics: introduction to mosquitoes, identification of mosquito breeding sites, operating CDC Light Traps (with and without bottle rotators), claypots, PSC, Prokopack aspirator, differentiating culicines from anophelines through morphological identification, identification of adult female *Anopheles* mosquitoes by species (at least differentiating *An. funestus* group from *An. gambiae* complex) and carrying out wall cone bioassays.

3.3 REARING OF SUSCEPTIBLE *ANOPHELES GAMBIAE* (KISUMU STRAIN)

A technician was hired for managing mosquito rearing and mass production at the NIMR-Mwanza insectary. The NIMR Mwanza insectary is divided into two main rooms, the adult and the larvae rooms. The adult room environment is maintained at 27 ± 1°C warmth, and 60-80% relative humidity. Adult mosquitoes in the adult room are exposed to a light/dark regimen of 12/12 hours over 24 hours day cycle. The larvae room environment is maintained at 30 ± 1°C warmth and 60-

80% relative humidity. The adult *An. gambiae* s.s. are reared in 30cm x 30cm x 30cm cages and fed with 10% glucose for daily nutritional maintenance. In order to lay eggs, adult females *An. gambiae s.s.* are fed on rabbit blood. Glass petri dishes containing water were provided to adult mosquitoes in rearing cages for oviposition purpose. After oviposition, the petri dishes containing eggs are introduced in white plastic trays containing water for hatching into larvae. Newly emerged larvae are fed with Tetramin® fish food in plastic trays where they develop through various stages into pupae. Pupae are collected, counted daily from trays and kept in small shallow water dishes and allowed to emerge inside the adult cage. Each cage is clearly labeled with the date of pupae collection.

Adult *Anopheles gambiae* s.s. (susceptible Kisumu strain) were reared and the numbers increased to meet the demand of field activities involving cone wall bioassays. Insectary-reared adult *An. gambiae s.s.* were used for cone wall bioassay testing in the selected sentinel sites every month. The two to five day old *An. gambiae* s.s. mosquitoes are mainly used for wall cone bioassay tests to evaluate the decay rate of insecticides on various wall surface types.

3.4 VECTOR DENSITY, SPECIES COMPOSITION, RESTING BEHAVIOR, AND SEASONALITY

We used two entomological sampling methods, CDC Light traps (indoors) and claypots, in ten sentinel sites (eight sprayed and two unsprayed sites) to collect adult mosquitoes flying indoors, potentially seeking a blood-meal and outdoor resting mosquitoes, respectively. In addition, three entomological sampling methods, CDC light trap with bottle rotators (CBR), PSC and Prokopack aspirators, were used in three sprayed sites to collect adult mosquitoes to help determine basic entomological indicators, including vector density, species composition, resting behavior and seasonality.

Throughout the monitoring period, 24 houses were sampled by CDC Light traps and Clay pot every month in each sentinel district. In addition, 10 houses were sampled by PSC, Prokopack aspirators and CDC light traps with bottle rotators by each team per site per month (Table 2).

Table 2: Mosquito Trapping Method and Number of Houses Monitored per Site per Month

Trapping method	Number of houses monitored per site per month			
	Jan	Feb	March	April-Dec
CDC LT	24	24	24	24
Claypot	24	24	24	24
Prokopack aspirator	-	-	-	10
PSC	-	-	-	10
CBR	-	-	-	10

3.4.1 CDC LIGHT TRAP METHOD (INDOOR BITING MOSQUITOES)

In each selected village in a district, two houses per night were selected for setting two CDC light traps on 28 consecutive days in a month. The CDC light trap was installed at about 1.5m above the floor next to the head of the sleeping person(s). The person(s) was requested to sleep under an untreated mosquito net(s) overnight. The trap consists of a fan with 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 source. CDC light traps were set to operate from 6.00pm to 6.00am to trap mosquitoes. Captured mosquitoes were transferred separately into labeled paper cups covered with a piece of netting material and taken for preliminary morphological identification in the field (Figure 2). Live mosquitoes from the trap were left to die and a count per trap was taken and summarized by species, sex and abdominal status.

Figure 2: Mosquito collection using indoor CDC Light trap in sentinel site

3.4.2 CLAY POT METHOD (OUTDOOR RESTING MOSQUITOES)

The clay pot method was used to collect outdoor resting mosquitoes. The pots were molded by local potters using clay soil available from the area. The clay pots were made of size 0.5m diameter with an opening 20cm wide. Each clay pot had a 2cm hole made at the bottom of the pot rendering them useless for storage of water as they allowed water to freely drain out. Each mosquito collector had four clay pots which were set up outdoors overnight near selected houses with different construction materials. The pots were set up from 6.00pm to 6.00am. The pots were positioned at an inclined angle to let mosquitoes enter and rest inside the dark inner wall surface of the pot (Figure 3). In the morning at 06.00am, the CMCos covered the opening using a piece of netting fabric with a small entry hole for inserting an aspirator to suck out mosquitoes and transfer them into a paper cup.

Figure 3: Mosquito collection using claypots in sentinel site

3.4.3 PYRETHRUM SPRAY CATCH AND PROKOPACK ASPIRATOR (INDOOR RESTING MOSQUITOES)

Pyrethrum spray catch and Prokopack aspirators were used to sample indoor resting mosquitoes from 10 houses over 16 days within each selected sentinel site per month. Mosquitoes were collected by PSC from 10 randomly selected houses within a sentinel site. Prokopack aspiration was also conducted in 10 randomly selected houses (the same houses that were used by PSC). The next two days, the houses sampled with Prokopack aspiration and PSC collection were alternated for mosquito collection by the two methods. Pyrethrins are rapidly degraded in the environment through direct photolysis with a half-life of less than 1 day according to US-EPA, therefore carry over effects of PSC are unlikely, the interval between PSC and Prokopack aspiration being two days. Collections using the two methods were conducted over four days in a week. Some of the houses were sampled more than once. The PSC and Prokopack aspiration were carried out in the morning between 6:00 a.m. and 8:00 a.m.

Pyrethrum extract0.1% (mixing 5ml of pyrethrum with 5 liters of kerosene) was applied using an agricultural sprayer used to knock down mosquitoes for the PSC activity. Before the PSC was performed, all occupants were asked to move out of the house. Also all foodstuffs were removed from the house and the windows and doors closed. White calico sheets were spread out to cover the floor and all horizontal surfaces in the rooms where PSC was to be conducted. Windows and other mosquito entry and escape routes around the house were sprayed first from the exterior followed by the interior of the house until the house was full of insecticide mist. The collectors then left the house with all doors and windows closed. Ten minutes later the house was opened and all mosquitoes knocked down by the insecticide were collected from the white sheets (Figure 4). The insecticide dissipates quickly but residents were asked to open the doors and windows and remain outside for 30 minutes after spraying. The mosquitoes were put in well-labeled moist petri dishes and taken to the field office where they were counted and sorted out morphologically by species, sex and abdominal status. Collected mosquitoes were differentiated as either *Anopheles* or *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 collected mosquitoes were preserved for later analysis using molecular assays to identify the sibling species and determine malaria infection rates using enzyme linked immunosorbent assays (ELISA).

Figure 4: Mosquito collection using pyrethrum spray collection (PSC) in sentinel site

3.4.4 CDC PROKOPACK ASPIRATOR (INDOOR RESTING MOSQUITOES)

The Improved Prokopack Aspirator Model 1419 was used for sampling of indoor resting mosquitoes. Aspiration of resting adults produce collections of both sexes and all physiological stages directly from their resting sites, allowing better estimations of species diversity, abundance, sex ratio, and physiological status (Silver, 2008).

At 23 ounces (650 g) it is light weight and highly maneuverable; with the included extension pole the unit can sample from ground level up to 13 feet (4 m) high. This enables collections to be made on upper walls, ceilings, and under furniture (Figure 5). Also included with the aspirator, is a 12 volt 12 amp hr gelled-electrolyte battery, the extension pole, five collection cups and lids with stainless steel mosquito mesh, a universal voltage automatic charger (100-240 VAC, 50/60 Hz, 5 amps per hr) permitting complete recharging in 2.5 hours. Total run time for a fully charged battery is four hours.

The Prokopack aspirator was used for indoor resting mosquito collection and operated on a 12 V dry-cell battery placed in a custom-made pouch and attached to a belt around the collector's waist. A total of 16 experimental collections were completed over a period of 4 weeks each month. On average, each collector sampled one household per day. Most households were sampled on two occasions per month. Aspirations were done indoors of all enrolled households starting at 06.00 hrs. and finishing around 08:00 hrs. All mosquito collectors were previously trained by the same supervisor and had comparable aspiration techniques. They were spot-checked on random occasions throughout the collection to make sure their technique was accurate. Walls and ceilings were systematically aspirated using progressive down- and upward movements along its entire length. Therefore, the time a collector spent aspirating was not pre-defined, but was dependent on the size of the room being sampled. The collection exercise was continued until no mosquitoes could be seen flying around, an indication that all resting mosquitoes have been collected from the room.

Figure 5: Showing individual operating Prokopack back-pack aspirator for mosquito collection

3.4.5 CDC LIGHT TRAP WITH BOTTLE ROTATORS *(INDOOR AND OUTDOOR BITING TIMES)*

With human landing catch (HLC) practice being restricted by Ethical Review Board (ERB) in Tanzania, CDC Light traps fitted with bottle rotators were used as a proxy to HLC to collect information related to vector feeding time and changes in the feeding behavior of mosquitoes. LT with rotating bottles were set in ten randomly selected houses per site. This exercise was conducted in three sentinel sites namely; Chato DC, Sengerema DC and Musoma rural DC, surveyed monthly from end of March to December 2016.

CDC light trap sampling was scheduled on nights near a new moon to minimize the effect of moonlight on the outdoor light-trap collection and to reduce bias when comparing species distribution across seasons. An estimate of the presence and period of moonlight was calculated using a lunar calendar based on the method described on the website http://www.timeanddate.com/calendar/moonphases.html. The indoor CDC Bottle Rotator (CBR) was set up in the sleeping area of the house while the outdoor CBR was set up just outside the house within a 10 meter radius around the house.It was assumed that the mosquitoes that entered a trap during any hour were those actively seeking hosts, and in most cases, would bite human hosts in the same hour and room/house if the bed net trap was absent. The indoor and outdoor human-biting fraction of the *Anopheles* mosquitoes (and time of biting) were determined and recorded throughout the whole sampling period in the selected sentinel sites.

CDC light trap with bottle rotators were set indoors with a person sleeping under an untreated net from 6:00 p.m. to 6:00 a.m. and outdoors from 6.00 p.m. to 10.00 p.m. (Figure 6). The bottle collectors exchanged their positions every two hours, enabling separate two hourly collections. Samples of *Anopheles gambiae* s.l. sibling species were preserved in a 1.5 ml Eppendorf tube in silica gel for further ELISA and molecular analysis. For the outdoors collection, the timing of collection ended at 10.00 p.m. due to the fact that people in these communities retire to bed and there are no people outdoors after 10 pm.

Figure 6: Mosquito collection using CDC bottle rotator in sentinel site

3.5 QUALITY OF SPRAY AND INSECTICIDE DECAY RATE

The tests for quality of spray and insecticide decay rate were done based on the World Health Organization protocol(WHO, 1998). The test cones were placed at two different heights (upper and lower at 2m and 1m heights, respectively) on sprayed wall surfaces. Control surfaces were artificially constructed of dried blocks of cement, mud, wooden, painted, burnt brick and whitewash surfaces. Batches of 10 mosquitoes, two to five days-old non-blood-fed female *Anopheles gambiae* (Kisumu strain), were introduced into each of the cones. The mosquitoes were left in the cones exposed to the (sprayed or unsprayed) surfaces for 30 minutes, after which they were transferred to clean paper cups.

Knockdown and mortality were observed and recorded 60 minutes post-exposure and after a 24-hour holding period, respectively. When mortality in the control site was scored between 5% and 20%, the results of the treated samples were corrected using Abbot's formula, and those above 20% were discarded.

Quality of spray had to be done within 14 days of IRS start date while decay rate involved monthly assays up to when mortality fell below 80% on two subsequent months. For quality spraying assessment, three houses of each wall surface commonly found in the area were randomly chosen for cone bioassay in each sentinel site. The most common materials used for construction of house walls in sentinel sites were mud, wood, concrete, white wash, painted and burnt brick. Two rooms were assayed in each house with two replicates in each room. Ten mosquitoes were exposed in each cone. For the longitudinal monitoring of decay rate, two houses representing specific wall surface were targeted (mud, cement, whitewash etc.). Only one room was assayed in each house with two replicates in each room.

3.6 VECTOR MOLECULAR CHARACTERIZATION

All vectors collected were identified to species morphologically (Gillies and Coetzee, 1987, Gillies and DeMeillon, 1968). Female anopheline 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 al., 1987), the abdomen of anopheline females is currently used in polymerase chain reaction (PCR) analysis to identify members of the *An. gambiae* s.l. and the *Anopheles funestus* s.l. groups (Scott et al., 1993) and for future genetic/molecular analysis.

A sub-sample of anopheline mosquito specimens was used for vector molecular characterization and sporozoite rate determination, specifically to conduct the following analyses:

- **Identification of sibling species of *An. gambiae* s.s.**

  A sub-sample of Anophelines were identified by species by using PCR (Scott et al., 1993).

- **Detection of the sporozoites**

  A sub-sample of Anophelines were assayed for detection of sporozoites using Enzyme-linked Immuno-sorbent assay (ELISA).

3.7 DATA ANALYSIS

Pyrethrum Spray Collection and Prokopack data was used to calculate the density of vectors in a room using the formula:

Vector Density = Total number of vectors collected by species /Total number of rooms surveyed.

Bites per person per night is obtained by the total number of anopheles mosquitoes collected by CDC light trap per month divided by the number of trap days collection in a month, which doesn't always have the same absolute value as human landing catches but will show the same trends..

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4.0 RESULTS AND DISCUSSION

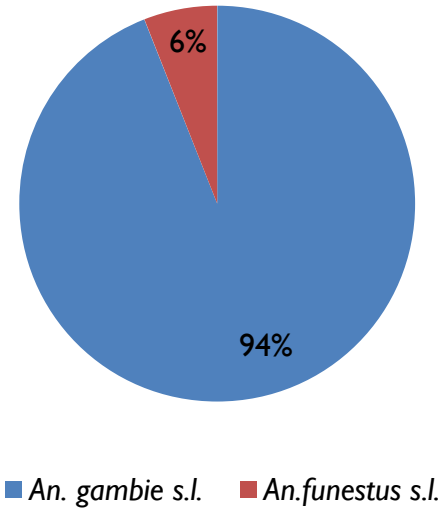
4.1 SPECIES COMPOSITION

A total of 14,012 female *Anopheles* mosquitoes were collected by all collection methods combined. Overall species composition by morphological identification was 94.0% *An. gambiae* s.l. and 6.0% *An. funestus s.l.* (Table 3 and Figure 7).

Table 3: Anopheles Species Composition by Morphological Identification

District	*An. gambiae s.l.* N (%)	*An. funestus s.l.* N (%)	Total *Anopheles* per district
Ngara	201(82)	44(18)	**245**
Missenyi	2392(99.4)	14(0.6)	**2406**
Bukoba Rural	297(100)	0	**297**
Chato	2434(90.2)	264(9.8)	**2,698**
Sengerema	1646(86)	269(14)	**1,915**
Kwimba	251(82.6)	53(17.4)	**304**
Musoma Rural	1466(99)	15(1)	**1,481**
Butiama	1172(92.4)	96(7.6)	**1,268**
Bukombe	2615(98.8)	31(1.2)	**2,646**
Busega	695(92.4)	57(7.6)	**752**
Total per Species	**13169(94.0)**	**843(6.0)**	**14012**

Figure 7: Overall Anopheles Species Composition by Morphological Identification



Furthermore, of the total 14,012 female *Anopheles* mosquitoes collected during the monitoring year: 10,818 (77.2%) were collected by CDC-LT, 1,682 (12.0%) by Claypots, 305 (2.1%) by PSC, 331(2.4%) from Prokopack aspirator, and 876 (6.3%) from CBR (Table 4).

An. gambiae s.l. was the most abundant vector species sampled by all collection methods in each district. The number of *An. gambiae s.l.* and *An. funestus* s.s. were higher in light traps set indoors compared to both indoor CBR and indoor resting collections by pyrethrum spray (PSC) across all districts. Claypot traps caught the least number of *Anopheles* mosquitoes (Table 4).

Table 4: Mean Density Per Night of Anopheles Species by Collection Method for each District

District	Collection Method	Sampling frequency (trap nights)	*An. gambiae s.l.* N (Mean per trap night)	*An. funestuss.l.* N (Mean per trap night)
Chato	Light trap (indoors)	336	835 (2.49)	240(0.71)
	Clay pot (outdoors)	336	721(2.15)	18(0.05)
	CBR (indoors & outdoors)	90	490(5.44)	6(0.07)
	Prokopack aspirator	128	192(1.5)	0
	PSC	128	196(1.53)	0
Musoma Rural	Light trap (indoors)	320	1019(3.18)	15(0.05)
	Clay pot (outdoors)	320	222(0.69)	0
	CBR (indoors &outdoors)	90	146(1.62)	0
	Prokopack aspirator	144	35(0.24)	0
	PSC	144	44(0.31)	0
Sengerema	Light trap (indoors)	315	1311(4.16)	101(0.32)
	Clay pot (outdoors)	315	87(0.28)	13(0.04)
	CBR (indoors &outdoors)	90	150(1.67)	84(0.93)
	Prokopack aspirator	144	66(0.46)	38(0.26)
	PSC	144	32(0.22)	33(0.23)
Ngara	Light trap (indoors)	336	184(0.55)	44(0.13)
	Clay pot (outdoors)	336	17(0.05)	0
Missenyi	Light trap (indoors)	336	2152(6.40)	10(0.03)
	Clay pot (outdoors)	336	240(0.71)	4(0.01)
Bukoba Rural	Light trap (indoors)	322	275(0.85)	0
	Clay pot (outdoors)	322	22(0.07)	0
Butiama	Light trap (indoors)	330	1172(3.55)	96(0.29)
	Clay pot (outdoors)	330	0	0
Kwimba	Light trap (indoors)	316	129(0.41)	53(0.17)
	Clay pot (outdoors)	316	122(0.39)	0
Bukombe	Light trap (indoors)	336	2503(7.45)	29(0.09)
	Clay pot (outdoors)	336	112(0.33)	2(0.01)

Busega	Light trap (indoors)	318	600(1.89)	50(0.16)
	Clay pot (outdoors)	318	95(0.30)	7(0.02)

CBR: CDC Bottle Rotator Trap; PSC: Pyrethrum Spray Catch

4.2 MOLECULAR ANALYSIS OF MOSQUITO SPECIES COMPOSITION AND SPOROZOITE RATES

A sub sample of 10,645 (76%) female *Anopheles* mosquitoes of the collected 14,012 were analyzed by ELISA for detection of *P. falciparum* sporozoites. Of those tested for sporozoites, a subsample of 5,120 were simultaneously subjected to species identification by PCR (Table 5). The PCR results confirmed the local vector population to be predominantly *An. arabiensis* (55.4%), *An. funestus* s.s.(13.7%) and *An. gambiae* s.s (6.5%), with very few *An. parensis* (4.7%) and *An. rivolorum* (0.1%). Approximately twenty (19.6) percent of the samples were not amplified. The non-amplification can be explained by :(1) There might be other species that were morphologically mis-identified as belonging to the *An. gambiae* complex or *An. funestus* group(2)Storage of samples.Some samples were stored for several months before testing and DNA quality may have degraded under inadequate storage conditions. This is the most probable explanation for most failed amplifications as most of the fresh samples amplified.
Sporozoite rates were found to vary across districts (0.9% – 4.7%), with an overall sporozoite rate of 1.7%(95%CI:1.5-2.0) (see Table 5). *An. gambiae* s.l. had a slightly higher sporozoite positive rate at 4.2% (95% CI: 3.5-5.0) (134/3176) than *An. funestus s.l.* at 3.5% (95% CI: 2.5-5.0) (34/947) although the difference was not statistically significant (P=0.27) (Table 6). Further analysis showed that members of *An gambiae* complex were leading with *An gambiae* s.s. at 9.6% followed by *An arabiensis* at 3.6%. The funestus group showed *An funestus* s.s. to lead with 3.7% followed by *An parensis* at 3.3% infection. No sporozoites were detected among the few *An. rivulorum* samples assayed

Table 5: Overall Species Identification by PCR and Sporozoite ELISA Results

	Species Identification PCR							ELISA Results (all species)		
District	*No. tested* N	*An. gambiae* s.s. *N (%)*	*An. arabiensis* N (%)	*An. funestus* s.s. N (%)	*An. parensis* N (%)	An.rivolorum	Did not amplify	No. Positive N	Total Tested N	Sporozoite rates % (95% CI)
Ngara	245	75(30.6)	126(51.4)	13(5.3)	0	0	31(12.7)	10	220	4.5 (2.2-8.2)
Missenyi	563	76(13.5)	307(54.5)	0	0	0	180(32.0)	22	1,357	1.6 (1.0-2.4)

Bukoba rural	70	21(30.0)	20(28.6)	0	0	0	29(41.4)	4	310	1.3 (0.4-3.3)
Chato	758	8(1.1)	466(61.5)	1(0.1)	98(12.9)	0	185(24.4)	21	1810	1.2 (0.7-1.8)
Sengerema	817	34(4.2)	563(68.9)	124(15.2)	15(1.8)	0	81(9.9)	13	1,422	0.9 (0.5-1.6)
Kwimba	279	1(0.4)	189(67.7)	7(2.5)	7(2.5)	3(1.1)	72(25.8)	9	386	2.3 (1.1-4.4)
Musoma rural	536	36(6.7)	143(26.4)	96(17.7)	107(19.8)	0	154(28.5)	31	1,416	2.2 (1.5-3.1)
Butiama	441	12(2.7)	50(11.2)	238(53.1)	0	0	141(31.5)	26	888	2.9 (1.9-4.3)
Bukombe	928	69(7.4)	579(62.4)	225(24.2)	12(1.3)	0	43(4.6)	38	2,250	1.7 (1.2-2.3)
Busega	483	1(0.2)	400(82.8)	1(0.2)	0	0	81(16.8)	8	586	1.4 (0.6-2.7)
Total	**5,120**	**333(6.5)**	**2,843(55.5)**	**705(13.8)**	**239(4.7)**	**3(0.1)**	**997(19.4)**	**182**	**10,645**	**1.7 (1.5-2.0)**

Note: *The results in figure 7 show 95% *Anopheles gambiae* s.l., but we're getting a larger number of other species in the molecular results. This may be attributed to the fact that morphological identification were done by CMCOs who are not yet completely mastered on anopheles species identification thus the difference between morphology and PCR identification. We will put more emphasize on morphological identification to species level by CMCOs through training at NIMR and also at the sentinel sites in year 2017.

Table 6: SPOROZOITE CARRIAGE BY MOSQUITO SPECIES AS IDENTIFIED BY PCR

Mosquito species	No. of samples analysed	Number of sporozoite positives	Sporozoite rate (%)
An. gambiae s.s.	333	32	9.6
An. arabiensis	2843	102	3.6
An. funestus s.s.	705	26	3.7
An. parensis	239	8	3.3
An. rivulorum	3	0	0
Unidentified by PCR	997	14	1.4

4.3 VECTOR SEASONALITY

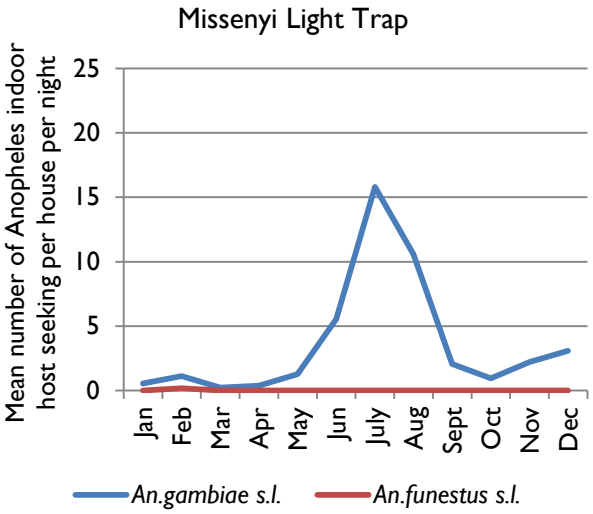
An. gambiae s.l. was the predominant vector species in all the study sites throughout the year. Two clear peaks of high vector densities were observed following periods of rainfall in Bukombe, unsprayed district, with the first peak being pronounced between December and February (following the short rains of October-December). This was followed by a second peak between May and July (following the longer rains in April-June) (see Figures 8).Furthermore, another unsprayed district (Busega) shows very small rises in density and it is mostly in November and again in January and February (see Figure 9).

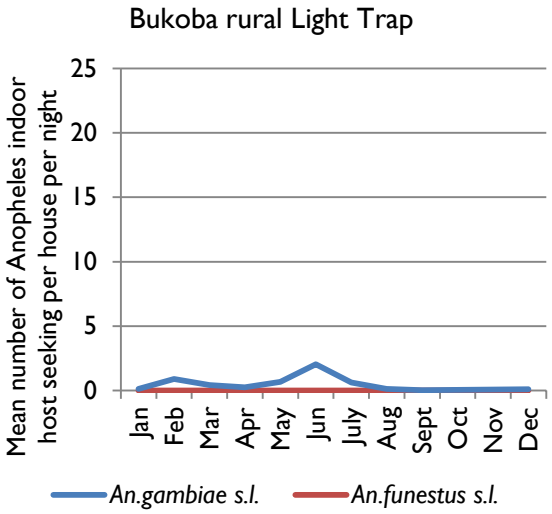
Indoor biting densities of *Anopheles gambiae* s.l. and *An. funestus* s.l. between January and December 2016 in all sentinel districts are presented in Figure 8 and Figure 9. The indoor biting density of *Anopheles funestus* group was far lower than that of *An. gambiae* s.l. However, the species was not found in several sites. The highest *An. funestus* s.l. biting rate per person per night was recorded during the month of May in Butiama and in June in Sengerema. In most sprayed sentinel sites the highest indoor CDC light trap catches of *Anopheles gambiae* s.l. were recorded in January and February (before spraying), with a marked decrease in biting rate occurring between March and May (after spraying). Nevertheless, the pattern in Missenyi, seems to completely defy what is seen in the other IRS sites. It also had the highest biting rate recorded among all sites. This may be attributed by the presence of breeding sites which is favoured by sugarcane plantation in the sentinel site.

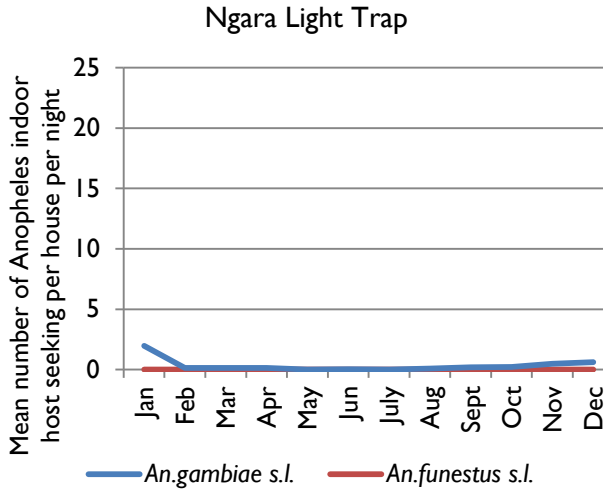
There were large differences in biting rates between sentinel sites before spraying with particularly high biting rates recorded in Butiama (7.81), Sengerema (7.60) and Musoma rural (5.55) in January-February. Biting rates were far lower in Bukoba rural (0.29), Missenyi (0.91), Kwimba (0.97) and Ngara (1.05) with fewer bites per person per night recorded in the same period. In general, *An. gambiae* s.l. biting rates decreased post IRS; likewise, indoor biting densities also declined sharply between March and April in the unsprayed control sites of Bukombe and Busega. This may be attributed to heavy rainfall received in March and April resulting in flushing out of larvae from breeding sites (rainfall data- see Annex 1) leading to low vector recruitment in the community.

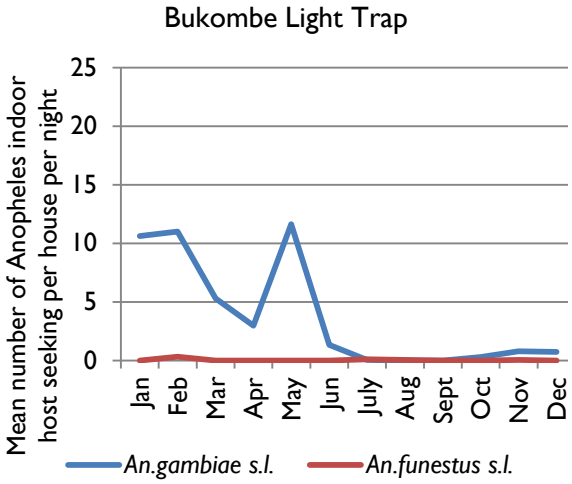
After the end of the long rainy season no increases or very small increases were noted in indoor biting rates in several sites: Ngara, Butiama, Kwimba, Chato and Busega sentinel sites (Figure 8 and Figure 9).However, indoor biting rates in May were far lower than in January and February in sprayed sites except for Missenyi. In Bukombe (control) there was a large increase in biting rates in May which was not seen in the sprayed sites (Figure 8). The contrary was observed in Busega (control site) where the biting rate remained low in May, estimated at 1 bite per person per night (Figure 9).

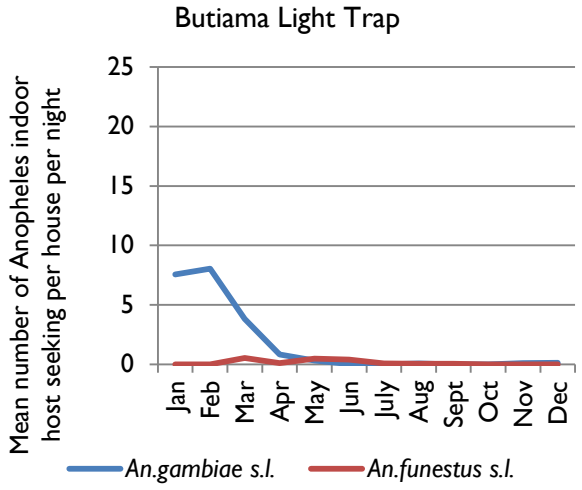
Figure 8: Monthly Indoor Biting Rates (CDC-LT) of *Anopheles* mosquitoes in six districts

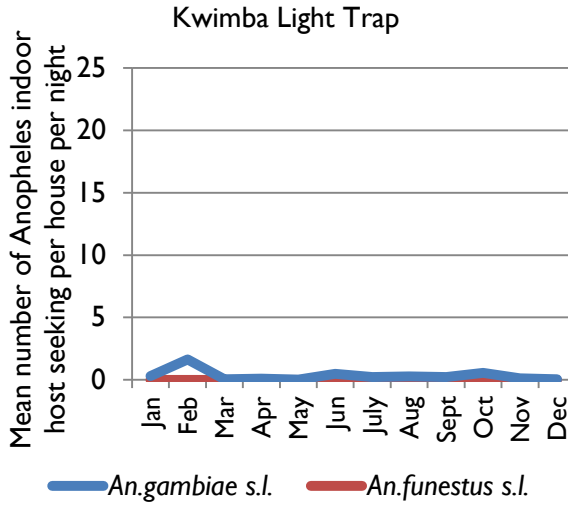






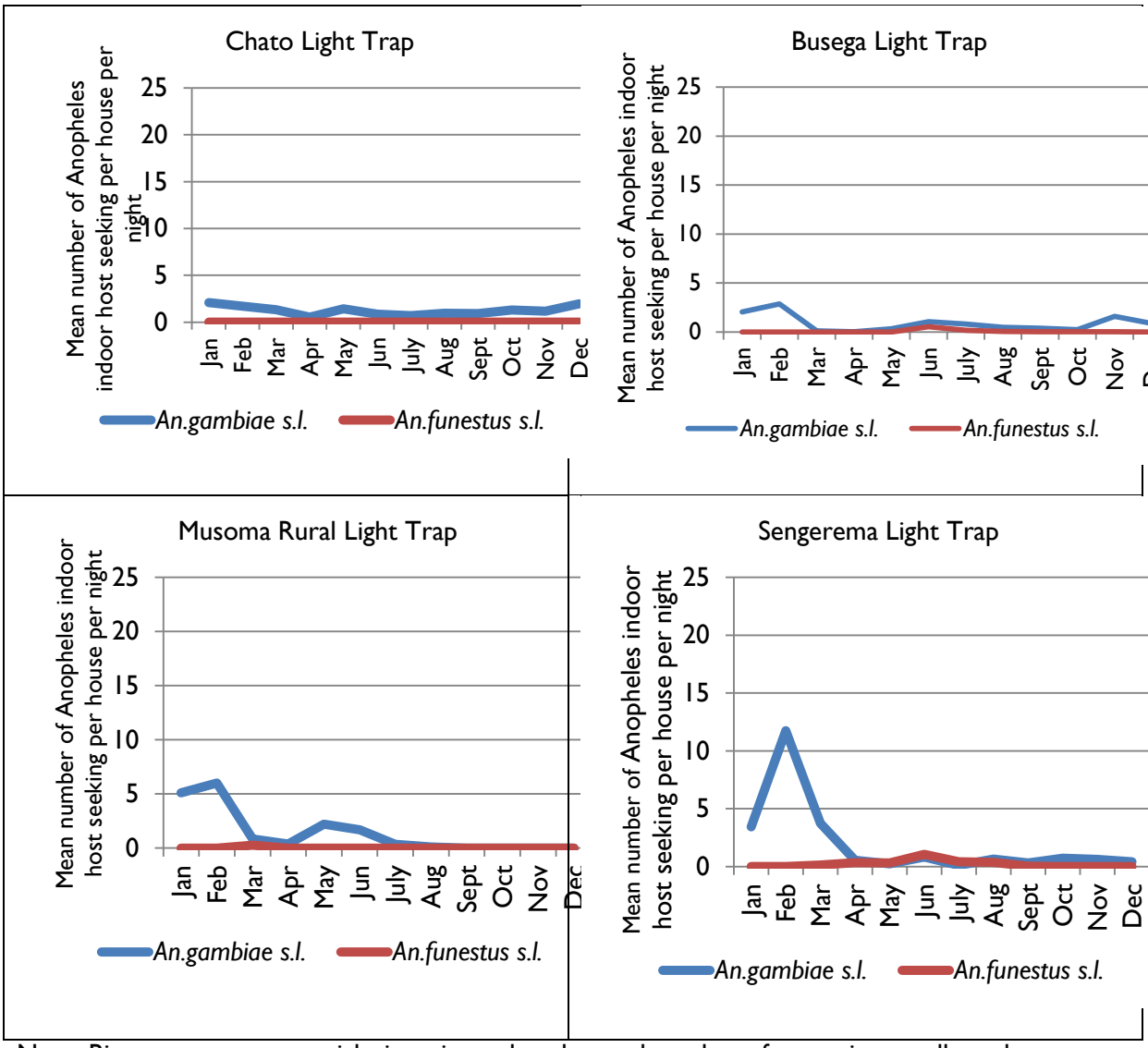






Note: Bites per person per night is estimated as the total number of mosquitoes collected per month divided by the number of collection trap days in a month.

Figure 9: Monthly Indoor biting rates (CDC-LT) of *An. gambiae* s.l. and *An. funestus* in four districts



Note: Bites per person per night is estimated as the total number of mosquitoes collected per month divided by the number of collection trap days in a month.

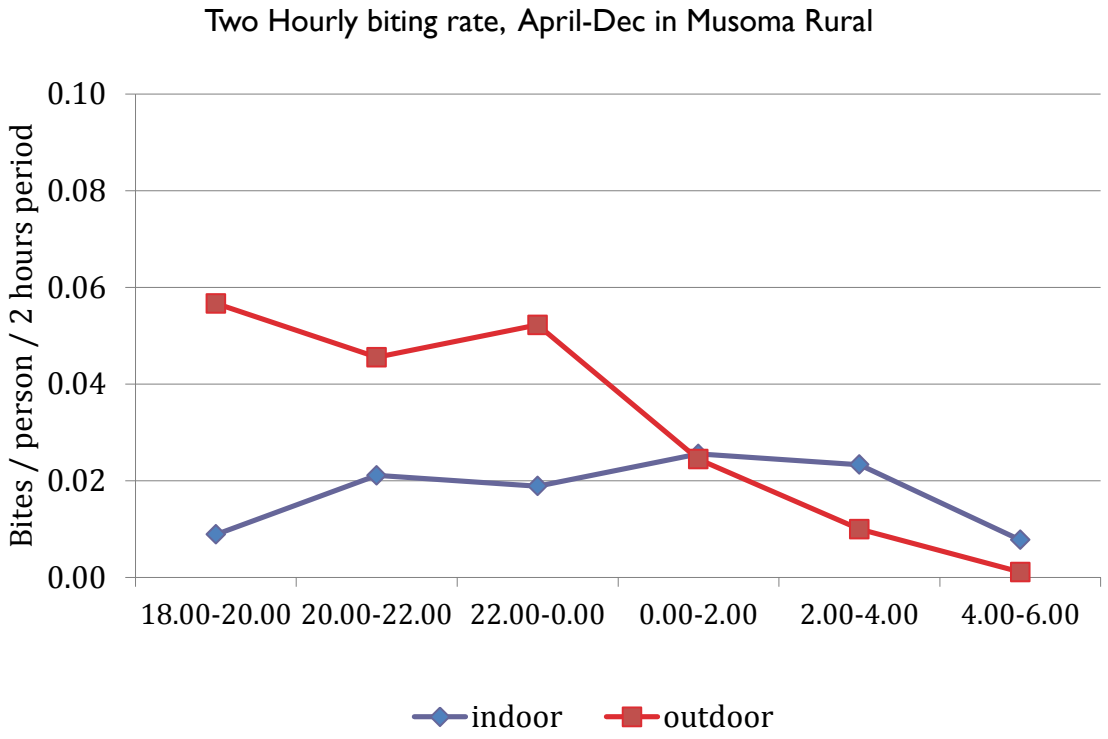
4.4 BITING TIMES OF An. GAMBIAE s.l. and An. FUNESTUS s.l. (INDOORS AND OUTDOORS)

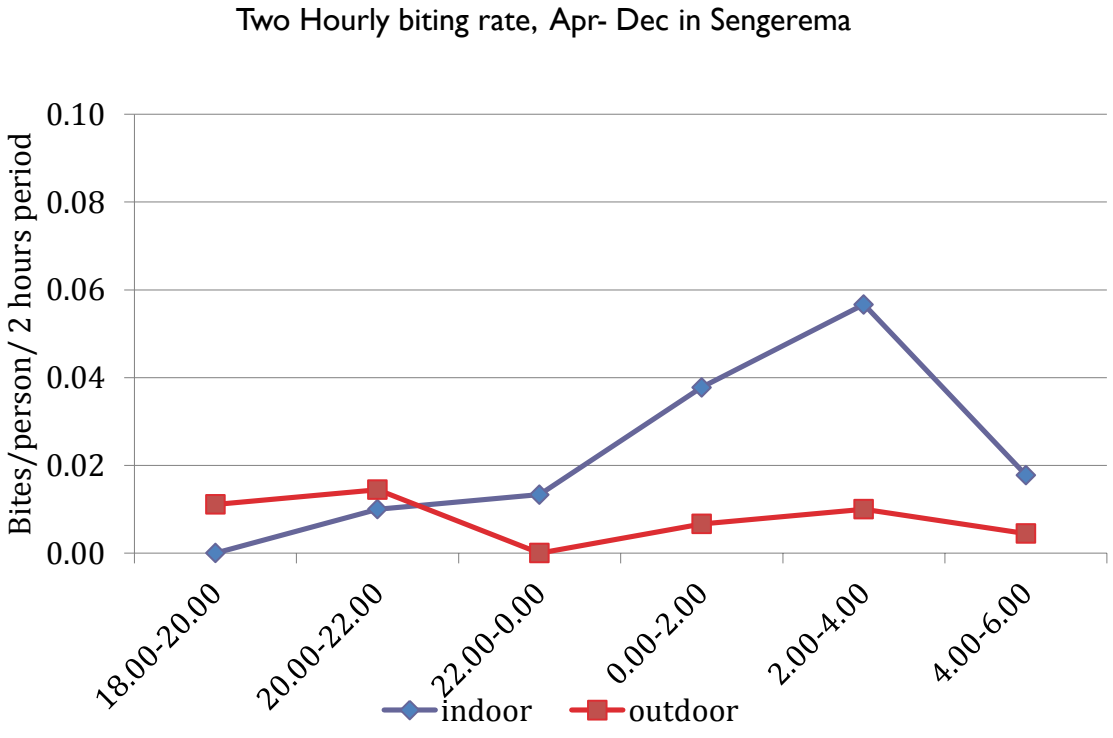
Trapping was conducted over ten nights each month in 3 selected sentinel sites (Musoma rural, Sengerema, Chato) using CDC light trap bottle rotators (indoors and outdoors) from April 2016(after spraying in the sentinel sites). No baseline data was collected before spraying.

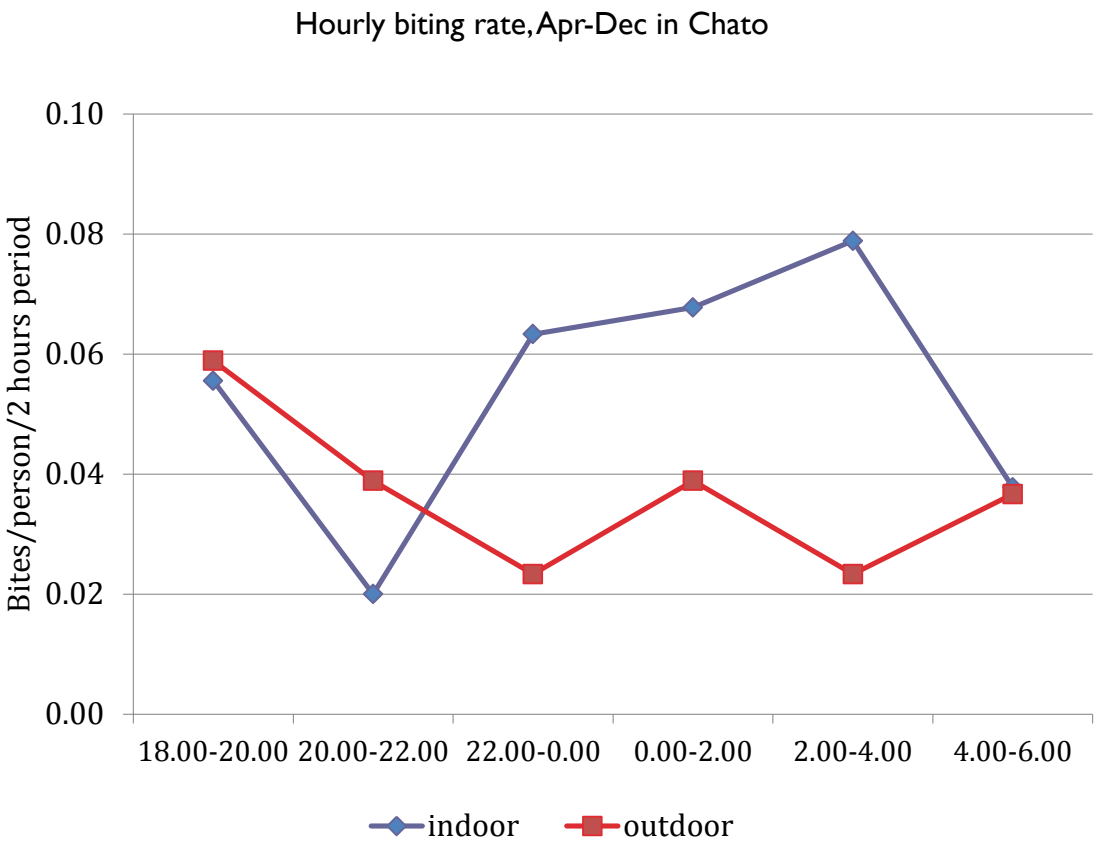
Overall, there was more outdoors biting risk occurring before people went to bed (18:00 – 22:00) compared toindoors during the same time period (see Figure10).Peak indoor biting was observed late at night in Chato and Sengerema (Figure 10), with some indication of early morning biting in Sengerema (04:00-06:00). In Musoma rural, indoor biting rates were fairly consistent throughout the night (Figure 10).

Meanwhile, for *An. gambiae* s.l. there was more outdoor biting risk before people went to bed (18:00 – 22:00) compared toindoors during the same time period (see Figure 10). Peak indoor biting was observed late at night in the three selected sentinel sites (Figure 10). Similarly, for *An. funestus* complex, there was more outdoor biting risk before people went to bed (18:00 – 22:00) compared to indoors during the same time period in Sengerema (see Figure 11). Indoor biting rates were fairly consistent throughout the night when people went to bed in Sengerema (Figure 11), with some indication of early morning peak indoor biting in Sengerema (04:00-06:00)(see Figure 11).

Figure 10: Biting Rates (Bites per Person per two Hours) of *Anopheles gambiae* s.l. collected by CDC Light trap bottle rotators

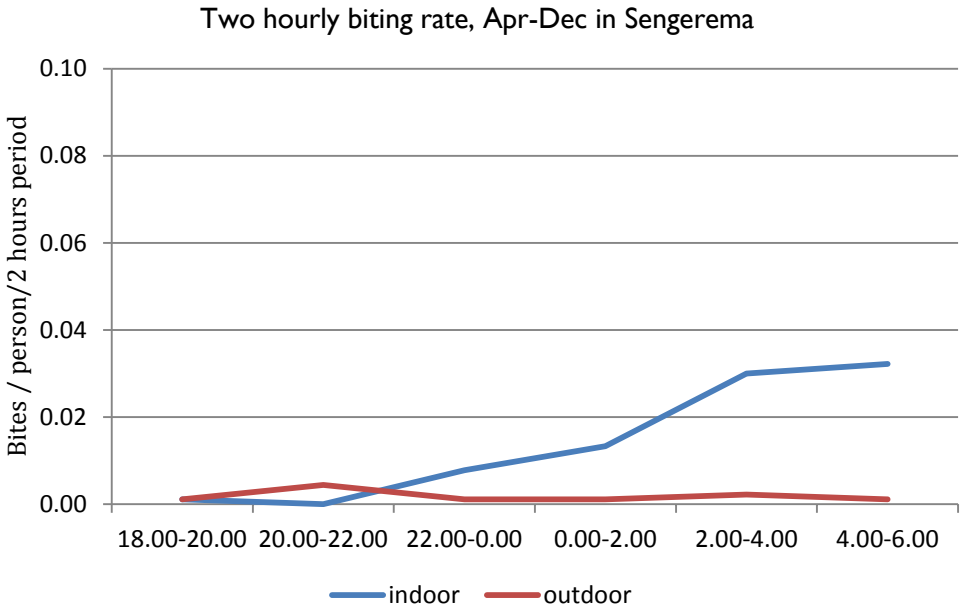

Two Hourly biting rate, April-Dec in Musoma Rural
Bites / person / 2 hours period
0.10
0.08
0.06
0.04
0.02
0.00
18.00-20.00
20.00-22.00
22.00-0.00
0.00-2.00
2.00-4.00
4.00-6.00
indoor
outdoor


Two Hourly biting rate, Apr- Dec in Sengerema
Bites/person/ 2 hours period
0.10
0.08
0.06
0.04
0.02
0.00
18.00-20.00
20.00-22.00
22.00-0.00
0.00-2.00
2.00-4.00
4.00-6.00
indoor
outdoor



Note: Bites per person per night is estimated as the total number of mosquitoes collected yearly divided by the number of collection trap days in a year.

Figure 11: Biting Rates (Bites per Person per two Hours) of *Anopheles funestus s.l.* collected by CDC Light trap bottle rotators



Note: Bites per person per night is estimated as the total number of mosquitoes collected yearly divided by the number of collection trap days in a year. The number of *An. funestus* collected was too low to present biting rates for the other sites.

4.5 FEEDING LOCATION

Feeding location sampling was conducted over ten nights of each month in the 3 selected sentinel sites (Musoma rural, Sengerema, Chato) using CDC light trap bottle rotators (indoors and outdoors) in April 2016(after spraying in the sentinel sites). No baseline data was collected before spraying.

Results obtained from the selected sentinel sites during the period March to December showed that 521 Anopheles were collected indoors and 355 were collected outdoors. Overall, there was more indoor biting risk (endophagic) compared to outdoors (exophagic) (Table 7). Furthermore, results reveal that observed mean of collected anopheles mosquito was higher in indoor compared to outdoor; this difference was found to be statistically significant (Mean=57.88, SD=13.6) and (Mean= 39.44, SD=17.96); t=5.29 and p=0.001.

Table 7: Human biting and indoor resting catches of female Anopheles mosquitoes in the three selected sentinel site for April -December sampling period

Mosquito species	**Indoor**	**Outdoor**	**Ratio**
	Musoma Rural		
Anopheles gambiae s.l.	72	74	0.49:0.51
Anopheles funestus s.l.	0	0	n.a.
Total	**72**	**74**	**0.49:0.51**
	Sengerema		
Anopheles gambiae s.l.	89	61	0.59:0.41
Anopheles funestus s.l.	65	19	0.77:0.23
Total	**154**	**80**	**0.66:0.34**
	Chato		
Anopheles gambiae s.l.	289	201	0.59:0.41
Anopheles funestus s.l.	6	0	100:0

Total	**295**	**201**	**0.59:0.41**

4.6 PYRETHRUM SPRAY COLLECTION (PSC) AND PROKOPACK ASPIRATOR RESULTS IN SELECTED SENTINEL SITES (INDOOR RESTING MOSQUITOES)

This trial compared PSC and Prokopack aspirator methods for the collection of indoor resting mosquito species to estimating densities per room.

A comparison of mean of Anopheles mosquitoes collected by PSC and Prokopack aspirator reveals that there was no statistically significant difference in the mean number of Anopheles species collected, P>0.05 (Table 8) by each method. However, the absolute number of Anopheles species collected by Prokopack aspirator wasfound to be higher than those of PSC. Therefore, theProkopack aspirator will be used in future collections of indoor resting mosquitoes.

Table 8: Comparison of Means of Anopheles Species in PSC and PROKOPACK ASPIRATOR Collections

Anopheles species	Collection Method	Sampling Efforts (# of collections)	Mean	Lower CI	Upper CI	P-Value
An. gambiae s.l.	Prokopack aspirator	416	0.70	0.19	1.22	0.5042
	PSC	416	0.65	0.17	1.17	
An. funestus s.l.	Prokopack aspirator	416	0.09	0.017	0.200	0.7336
	PSC	416	0.08	0.006	0.16	
Overall	Prokopack aspirator	416	0.18	0.16	0.203	0.447
	PSC	416	0.17	0.21	1.29	

4.7 ABDOMINAL STATUS OF COLLECTED MOSQUITOES

The percentage of unfed females was generally high, ranging between 60.8-80.4 percent, with few in the fed range between 14.5-29.5% and the remainder being gravid range between 5.1-11.3% (Table 9). Light traps collected mostly unfed mosquitoes (80.4%).This was anticipated as the plan is to divert host-seeking mosquitoes into light traps before being able to feed. Also, the other mosquito traps collected mostly unfed mosquitoes. Approximately, one-third of the mosquitoes of blood-feeding and gravid were collected by each of the method (PSC, CBR, Prokopack aspirator and Claypot), which were most likely resting shortly after blood-feeding (Table 9). Blood-fed were more likely to be collected outdoor by Clay pot (29.5 %) compared to other methods. However, the only statistically significant difference was obtained when compared withCDC-Light trap (14.5%); χ^2= 7.49, p<0.0001. The highest proportion of gravid females were collected by PSC (11.3%), followed by Clay pot (9.7%), prokopack aspirator (9.5%) and CDC-Light trap (5.1%) though the difference in the observed proportions were not statistically significant.

Table 9: Comparing Means of *Anopheles* by Abdominal Status and Collection Method

	Unfed	Blood-fed	Gravid
Total number collected	8,369	1511	534
Mean collected per trap night	2.56	0.46	0.16
Percentage of total	80.4%	14.5%	5.1%
Total number collected	1012	492	161
Mean collected per trap night	0.31	0.15	0.05
Percentage of total	60.8%	29.5%	9.7%
Total number collected	611	219	46
Mean collected per trap night	2.26	0.81	0.17
Percentage of total	69.7%	25%	5.3%
Total number collected	189	55	31
Mean collected per trap night	0.45	0.13	0.07
Percentage of total	68.7%	20%	11.3%
Total number collected	205	80	30
Mean collected per trap night	0.49	0.19	0.07
Percentage of total	65.1%	25.4%	9.5%

* The sorting on abdominal status was based on fed, unfed and gravid only.

4.8 QUALITY OF SPRAY AND INSECTICIDE DECAY RATE

4.8.1 QUALITY OF INSECTICIDE SPRAY

Pirimiphos-methyl (organophosphate) was sprayed in targeted districts. At the beginning of the IRS campaign, cone bioassays were done to assess the quality of spraying in the eight sprayed sentinel sites (Missenyi, Bukoba rural and Ngara districts in Kagera, Musoma rural and Butiama in Mara, Sengerema and Kwimba districts in Mwanza region and, Chato district in Geita). The assessment was important in ascertaining the efficacy and homogeneity of insecticide application, two main components of spray quality. *Anopheles gambiae* KISUMU strain, which is susceptible to the

insecticide, were reared at the NIMR Mwanza insectary and were exposed to wall assays for assessing the quality of spraying. Bioassays were performed at 3-14 days after the IRS start date, following WHO procedures. Cone bioassays were conducted in 15 sprayed structures in the selected village in each district within 3-14 days of spraying to assess the quality of spraying followed by subsequent monitoring on a monthly basis to determine the insecticide decay rate. In each district, three structures/houses of each sprayed wall surface substrate type (making a total of up to at least 15 structures) were sampled and used for the tests. The villages involved in the quality of spraying assessment are shown in Annex 2 & 3. The common wall surface types found in the villages included; mud, cement painted, whitewash and burnt brick.

The quality assurance tests conducted in the IRS targeted districts showed that the quality of spraying was good and homogeneous. The cone bioassay test results showed that within 3-14 days after spraying, the test mortality rates of susceptible mosquitoes exposed to the insecticide sprayed surfaces was ranging between 90.8-100% (Annex 2) across all wall surface types sprayed by different teams and spray operators. The lowest performance at 90.8% mortality was recorded on cement surface in Missenyi district.

There were no differences in test mortality rates of mosquitoes exposed to the sprayed walls at two different heights at baseline on different structures, which was ranging between 90.8-100% (Annex 2). This indicates that the spraying was homogeneous.

4.8.2 INSECTICIDE DECAY RATE OF ACTELLIC 300CS

Six months after spraying, the test mortality rates were scored as ≥85% in all sprayed sentinel sites. The performance of the insecticide sprayed was greater than the WHO defined 80% test mortality cut-off in all the 8 districts (Figures 12-19). Quality assurance tests conducted in the IRS targeted districts showed that the quality of spraying was good and homogeneous. Six months after spraying, the test mortality rates remained well above 85% in a few sites and at 100% at most sentinel sites, which is an indication of the long residual life of Actellic 300 CS. With IRS conducted in February, it is likely to be effective against mosquitoes emerging during the long rains but is unlikely to have much impact on the first mosquito peak expected between December and February (10-12 months after spraying). Only after nine months post spray were mortality rates dropping below 80%.

*In all figures below the arrow line indicate ≥80% mortality rate which recommended by WHO by cone wall bioassay.

Figure 12:WHO Cone Test Results, *An. gambiae* Kisumu Strain Mortality after 30 Minutes Exposure to Pirimiphos-methyl, Bukoba rural district

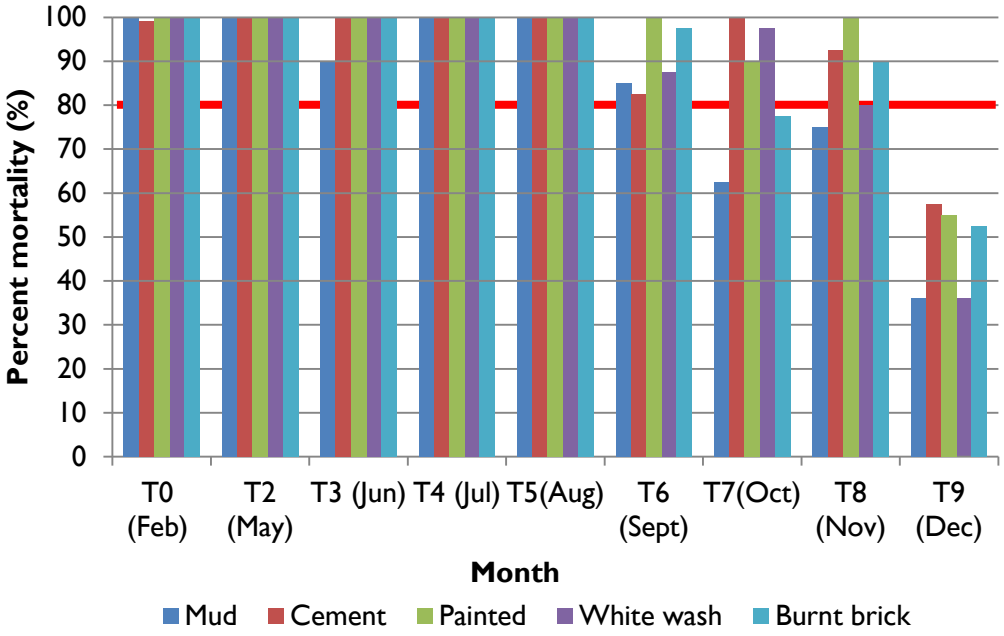


Figure 13: WHO Cone Test Results, *An. gambiae* Kisumu Strain Mortality after 30 Minutes Exposure to Pirimiphos Methyl, Missenyi district

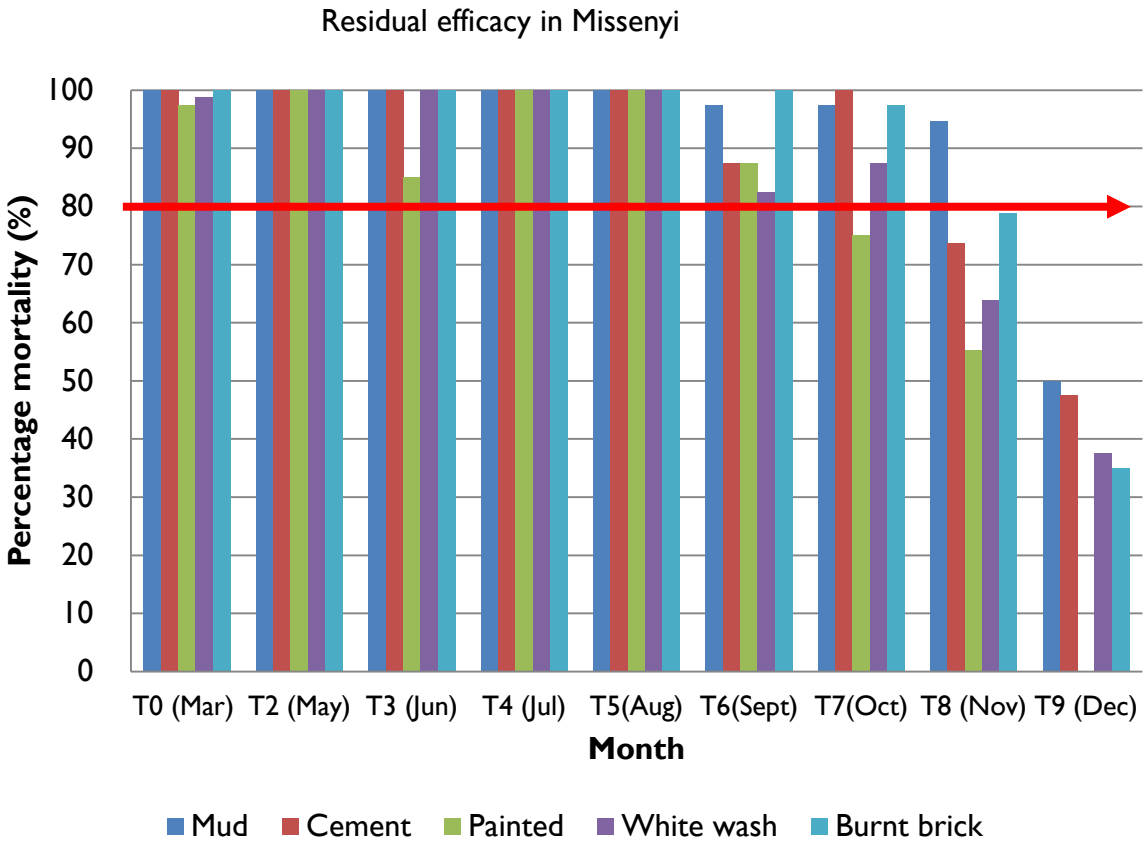


Figure 14: WHO Cone Test Results, *An. gambiae* Kisumu Strain Mortality after 30 Minutes Exposure to Pirimiphos-methyl, Bukoba rural district

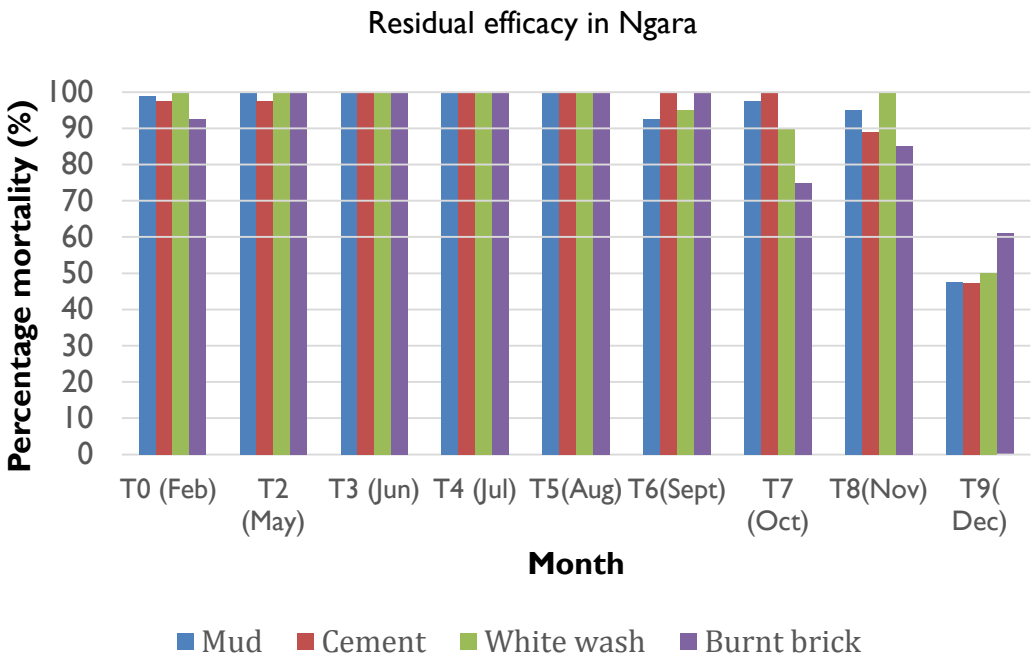


Figure 15: WHO Cone Test Results, *An. gambiae* Kisumu Strain Mortality after 30 Minutes Exposure to Pirimiphos Methyl, Chato district

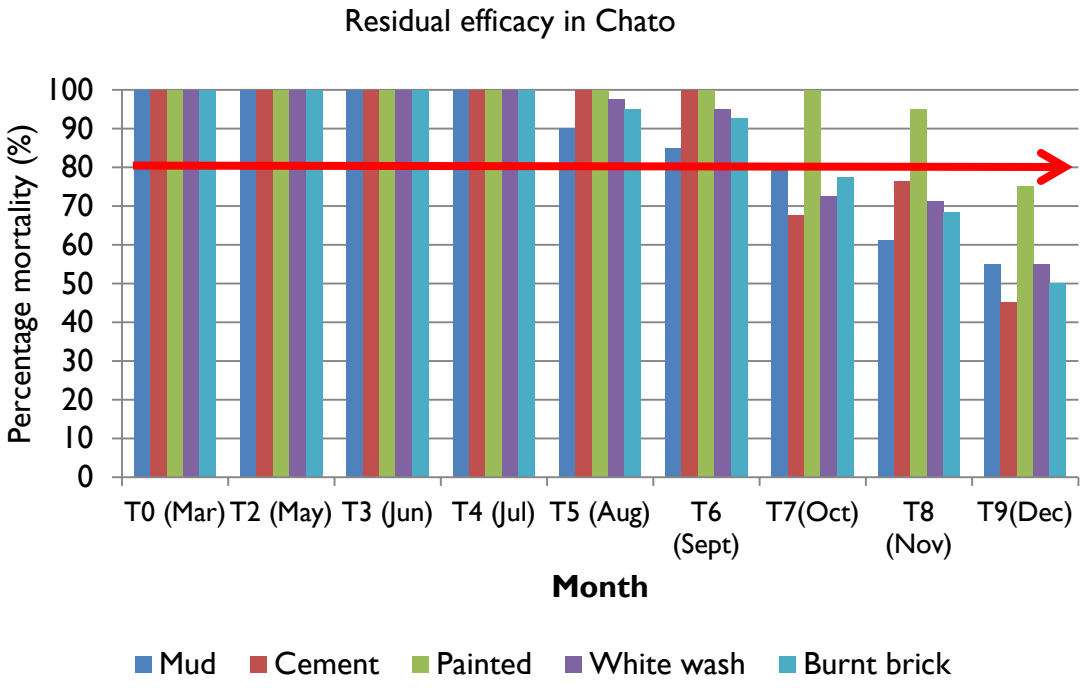
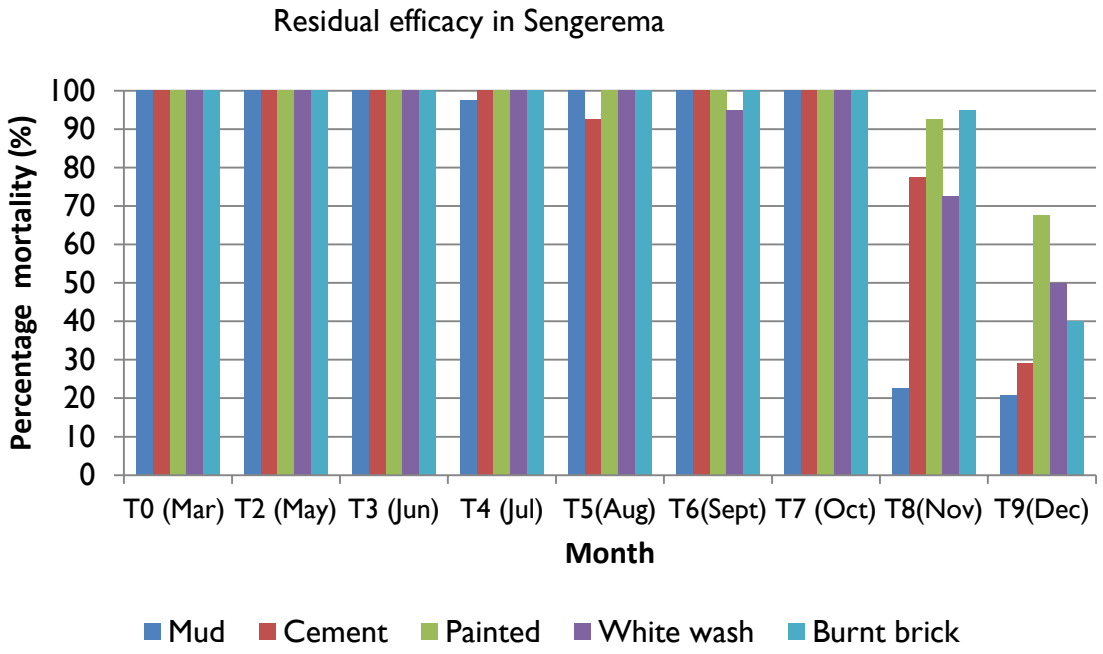


Figure 16: WHO Cone Test Results, *An. gambiae* Kisumu Strain Mortality after 30 Minutes Exposure to Pirimiphos Methyl, Sengerema district



*A drastic fall was observed beginning month eight with mud surface scoring as low as 22% mortality; while the rest of the surfaces scored between 72% and 95% mortality. At month nine, the highest mortality score was recorded on a painted wall surface at 67.5%.

Figure 17: WHO Cone Test Results, *An. gambiae* Kisumu Strain Mortality after 30 Minutes Exposure to Pirimiphos Methyl, Kwimba district

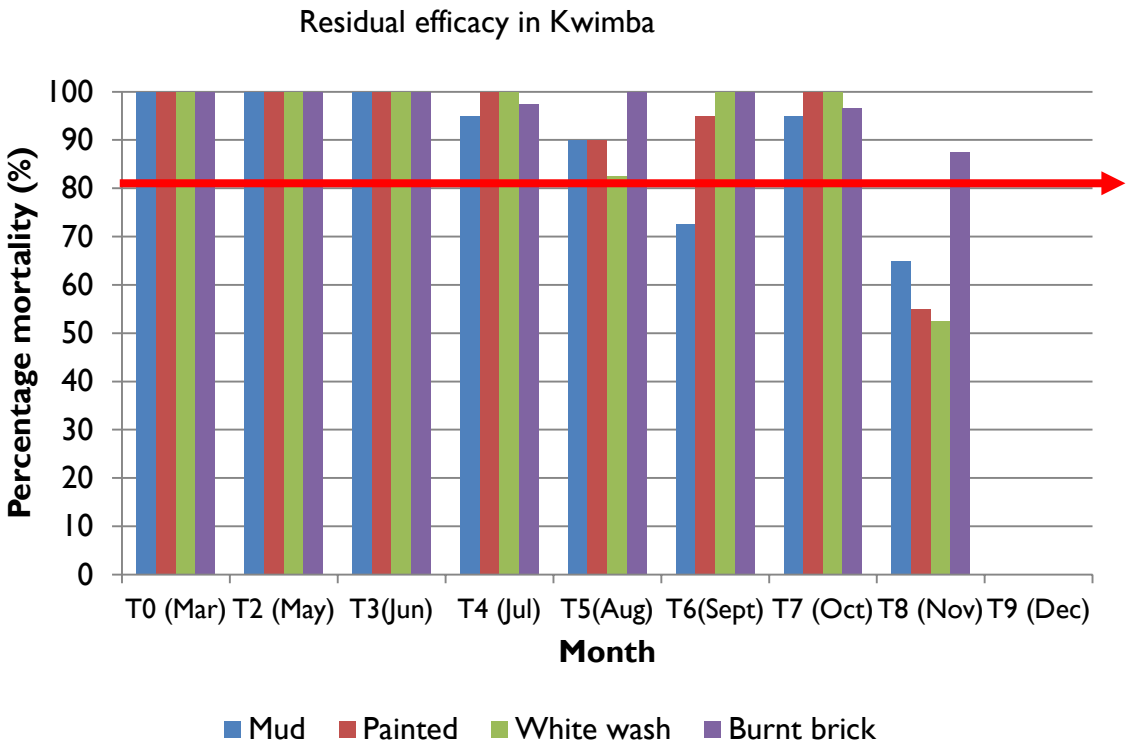


Figure 18: WHO Cone Test Results, *An. gambiae* Kisumu Strain Mortality after 30 Minutes Exposure to Pirimiphos Methyl, Musoma rural district

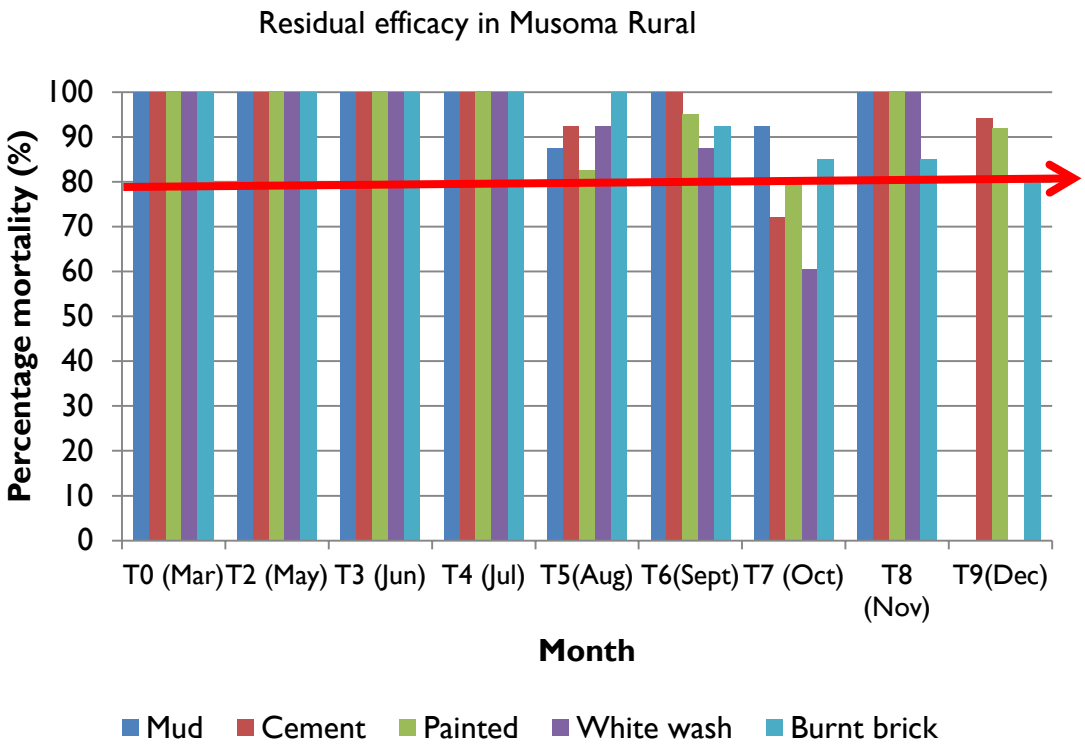
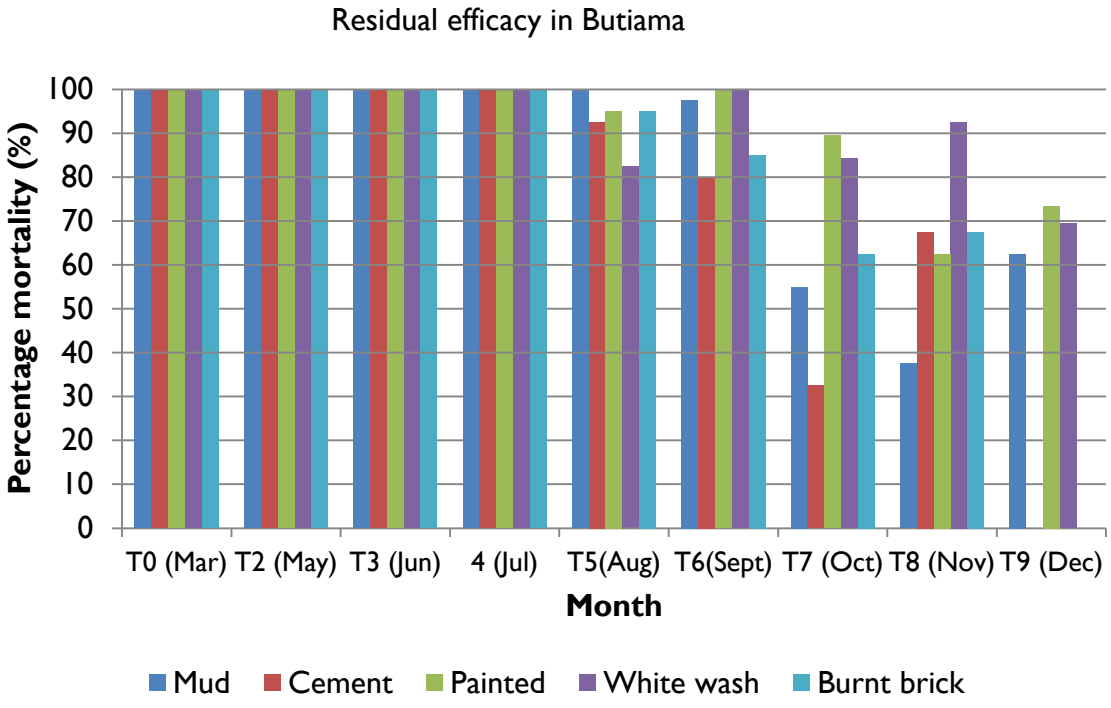


Figure19: WHO Cone Test Results, *An. gambiae* Kisumu Strain Mortality after 30 Minutes Exposure to Pirimiphos Methyl, Butiama district



*Results on burnt brick and cement sprayed surfaces were discarded due to high mortality in control surfaces

5.0 CONCLUSION

Monitoring of monthly indoor mosquito densities was conducted at 10 sentinel collection sites in 8 sprayed sites and 2 unsprayed sites. The results show *An. gambiae* s.l. to be the predominant malaria vector in all the collection sites. In the current study, we observed *An. gambiae s.l.* to be the predominant malaria vector in some of the sentinel districts. It is highly likely that the vector currently dominates much of the lake zone endemic region of Tanzania. The species identification results indicate that *An. arabiensis* is predominant over *An. gambiae s.s.*in the region although both species are found at low densities.

Monthly trends in malaria vector species composition and temporal distribution showed *An. gambiae* s.l. to dominate the vector population throughout the year. Two clear peaks of high vector densities in some of the sentinel sites were observed to correspond with periods of short (October-December) and long (April-June) rains. IRS in February is likely to be effective against vectors during the peak period following the long rains (May to July), which also happens to be the major transmission season. However, the operation appears unlikely to have much impact against the second minor transmission season that usually follows the short rains in December to February (10-12 months after spraying). The presence of *An. arabiensis* in abundance is most likely attributable to effect of the indoor based interventions in the area, including LLINs and IRS. *An. arabiensis* which is more opportunistic in its feeding has been associated more with zoophily and endophily (Githeko et al., 1996, Bayoh et al., 2010).

From analysis of sporozoite infection in the vector population, the overall sporozite rate was found to be low (1.7%). This may be possibly explained by: (1) presence of IRS programs in the areas; (2) mass campaign of distribution of LLIN in the country and (3) availability of improved diagnosis and effective treatment in health facilities. The highest sporozoite carriage was detected among *An gambiae* s.s (9.6%) followed by *An arabiensis* (3.6%),

Prokopack aspirator and PSC were both conducted in the same house to monitor proportions of vectors resting indoors by morning for each collection trap. The densities of both *An. funestus* and *An. gambiae s.l.* were highest in the Prokopack aspirator as compared to PSC traps.

Consistent with results from other studies in western Kenya (Bayoh et al., 2014), we observed high rates of late night indoor biting by *Anopheles* mosquitoes. A small proportion of biting occurred early in the evening before most individuals are protected by bed nets and 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. A study in Senegal recently reported broad daylight biting of *An. funestus* (Sougoufara et al., 2014).

In general, Pirimiphos-methyl IRS lasted for six to eight months. This insecticide has been reported to have a long acting period on sprayed wall (Chanda et al., 2013, Mashauri et al.), and therefore provides an attractive alternative to pyrethroids for IRS in Tanzania.

CHALLENGES

Delivery of laboratory supplies and reagents for PCR and ELISA assay has been a bit of a challenge. We received the supplies and reagents at the end of the year hence laboratory work on PCR and ELISA started late hence it may be one of the reasons of the samples not being amplified by PCR. Also, unexperienced CMCOs may have contributed to the observed discrepancies of the results of anopheles species identification by morphology and PCR.

ANNEX 1.

Rainfall data in sentinel sites in January- December 2016

Month	Average Precipitation (mm) per District									
	Musoma Rural	Butiama	Busega	Kwimba	Sengerema	Chato	Bukombe	Ngara	Bukoba Rural	Missenyi `
January	42.8	42.8	65.9	68.7	47.8	49.4	96	38.4	22.2	22.2
February	19.2	19.2	16.9	31.2	25.2	28.7	48.9	30.7	22.8	22.8
March	41	41	54.9	45.6	31.4	30.2	38.6	28.7	29.7	29.7
April	74.7	74.7	77	66.6	51.3	46.4	57.1	45.5	80	80
May	34.3	34.3	25.9	16.9	26.3	21.2	10.7	10.7	55.6	55.6
June	2.6	2.6	1.4	0.3	1.1	0.1	0.0	0.1	2.4	2.4
July	0.8	0.8	0.5	0.1	0.8	1.7	0.1	0.9	1.7	1.7
August	2.2	2.2	1	0	0.2	1.8	0.6	4.4	5.2	5.2
September	15.3	15.3	16	10.8	21.1	14.9	7	16.7	15.9	15.9
October	21.6	21.6	19.8	15.2	17.3	13.1	11.2	14	18	18
November	52.2	52.2	56.3	29	46.1	30.9	39.7	23.2	26	26
December	14.5	14.5	16.2	14.3	15.5	15	26.2	16.3	20.9	20.9

Source: http://iridl.ldeo.columbia.edu/maproom/Health/index.html

ANNEX 1.

Detailed results on Quality Assurance Tests, WHO Cone Bioassay in Pirimiphos-methyl Sprayed District

Test Date	District	Test Site	Wall surface Type	No of Houses tested	Number of Mosquitoes					
					Tested	**Knock Down 30 min**	**% Knock down 30 min**	**Dead after 24H**	**%Test Mortality**	**% Corrected Mortality**
10-11 Feb 2016	Missenyi	Buturage	Mud	3	120	26	21.7	120	100	
			Cement	3	120	40	33.3	109	90.8	
			White wash	3	120	18	15	120	100	
			Painted	3	120	47	39.2	120	100	
			Burnt brick	3	120	21	17.7	118	98.3	
12-13 Feb 2016	Bukoba Rural	Bulinda	Mud	3	120	10	8.3	120	100	
			Cement	3	120	26	21.7	120	100	
			White wash	3	120	11	9.2	120	100	
			Painted	3	120	42	35	120	100	
			Burnt brick	3	120	34	28.3	120	100	
15-16 Feb 2016	Ngara	Mukirehe	Mud	3	120	28	23.3	120	100	
			Cement	3	120	27	22.5	120	100	
			White wash	3	120	65	54.2	120	100	
			Painted	3	120	41	34.2	114	95	
			Burnt brick	3	120	3	2.5	119	99.2	
12-13 Mar 2016	Musoma Rural	Etaro	Mud	3	120	38	31.7	120	100	

			Cement	3	120	108	90	120	100	
			White wash	3	120	120	100	120	100	
			Painted	3	120	120	100	120	100	
			Burnt brick	3	120	115	95.8	120	100	
14-15 Mar 2016	Butiama	Bisumwa	Mud	3	120	70	58.3	120	100	
			Cement	2	80	80	100	80	100	
			White wash	2	80	80	100	80	100	
			Painted	1	40	28	70	40	100	
			Burnt brick	2	80	80	100	80	100	
16-17 Mar 2016	Sengerema	Irunda	Mud	3	120	120	100	120	100	
			Cement	3	120	103	85.8	120	100	
			White wash	3	120	120	100	120	100	
			Burnt brick	3	120	108	90	120	100	
21-22 Mar 2016	Kwimba	Ilunda	Mud	3	120	117	97.5	120	100	
			Cement	3	120	103	85.8	120	100	
			White wash	3	120	120	100	120	100	
			Burnt brick	3	120	108	90	120	100	
18-19 Mar 2016	Chato	Nyamirembe	Mud	3	120	75	62.5	120	100	
			Cement	3	120	73	60.8	120	100	
			White wash	3	120	89	74.2	120	100	
			Painted	3	120	120	100	120	100	
			Burnt brick	3	120	90	75	120	100	

ANNEX2.

Map of Lake Victoria regions, Tanzania QA Sentinel village locations

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