



PRESIDENT'S MALARIA INITIATIVE



# TANZANIA MAINLAND 2017 ENTOMOLOGICAL MONITORING ANNUAL REPORT

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TANZANIA MAINLAND 2017  
ENTOMOLOGICAL MONITORING  
ANNUAL REPORT

JANUARY – SEPTEMBER 2017

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# Acronyms

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AIRS	Africa Indoor Residual Spraying Project
CBR	CDC Light Trap with Bottle Rotator
CDC	Centers for Disease Control and Prevention
CI	Confidence Interval
CS	Capsule Suspension
ELISA	Enzyme-Linked Immunosorbent Assays
IRD	Indoor Resting Density
IRS	Indoor Residual Spraying
LT	Light Trap
NIMR	National Institute for Medical Research
PCR	Polymerase Chain Reaction
PMI	President's Malaria Initiative
USAID	United States Agency for International Development
WHO	World Health Organization

# Acknowledgments

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# Executive Summary

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To determine the impact of indoor residual spraying (IRS) with Actellic® 300CS, the President's Malaria Initiative (PMI)/Africa Indoor Residual Spraying (AIRS) project in Tanzania used a variety of mosquito sampling techniques in its continued entomological surveillance in the Lake Victoria region. These methods included Centers for Disease Control and Prevention (CDC) indoor light traps, indoor and outdoor collection bottle rotatorr, outdoor clay pots, and Prokopack aspirators. Monthly mosquito collections and initial identification of mosquito species were carried out by a group of trained community mosquito collectors under the supervision of the National Institute for Medical Research (NIMR) Mwanza Centre.

The data reported here were collected from 14 sentinel field sites, 10 of which received IRS and four of which were non-sprayed control sites, in the nine-month period of January to September 2017. A week following IRS application, baseline World Health Organization (WHO) cone wall bioassays showed high insecticide efficacy, scoring 100 percent mortality in all rooms tested to assess IRS quality. Six months post spraying, the test mortality rates were  $\geq 80$  percent on most sprayed surfaces except painted, white-wash, and cement wall surfaces in Ngara, Kwimba, Chato, Nyang'hwale, and Bukoba Rural districts. However, eight months post spraying, the test mortality was  $\leq 80$  percent in all the sentinel sites except mud and burnt brick surfaces in Chato, Musoma Rural, and Sengerema districts. This gave a mean approximate IRS longevity of six to eight months.

A total of 5,845 female *Anopheles* mosquitoes were analyzed by polymerase chain reaction (PCR) for species identification, while 6,025 samples were analyzed by enzyme-linked immunosorbent assays (ELISA) for detection of *Plasmodium falciparum* sporozoites in the mosquito head and thorax. The PCR results confirmed the local vector population to be predominantly *An. arabiensis* (57.6%), *An. funestus* s.s. (12.6%), *An. parensis* (7.4%), and *An. gambiae* s.s. (5.0%). Approximately 17 percent (17.4%) of the samples were non-amplified by PCR.

There was no significant difference in the density of *An. arabiensis* in sprayed sites compared to non-sprayed sites ( $p=0.2575$ ), whereas six times the number of *An. funestus* s.s. were found in non-sprayed sites compared to sprayed sites ( $p=0.0088$ ). It appears likely that a species shift from *An. funestus* to *An. parensis* has occurred in the sprayed sites, with a probable reduction in larval competition between the two species following the successful control of endophilic *An. funestus* following IRS.

Sporozoite rates were found to vary widely across the sentinel districts ranging from 0 percent to 3.9 percent with a mean sporozoite rate of 1.7 percent (95% CI: 1.4–2.1) in the study area. Moreover, the sporozoite rate was 1.1 percent (95% CI: 0.8–1.5) (35/3175) in the sprayed sentinel sites compared with 2.4 percent (95% CI: 1.9–3.1) (69/2850) in the non-sprayed sentinel sites.

Overall, *An. funestus* s.s. had the highest sporozoite rate compared to other species, at 4.3 percent (2.8–6.2) in non-sprayed sites. Moreover, *An. arabiensis* had a higher sporozoite rate in non-sprayed sites (2.0%; 95% CI: 1.4–2.9) compared to sprayed sites (0.8%; 95% CI: 0.5–1.3) (P=0.003).

Blood-meal analysis indicated that *An. arabiensis* showed opportunistic feeding behavior, feeding on both human and animal sources. Despite this behavior, the anthropophily index was reasonably high with 59 percent of blood meals containing human blood (including mixtures with animal blood).

In general, IRS with Actellic 300CS appears to have been successful in keeping vector densities relatively low and reducing sporozoite rates compared to non-sprayed sites.

# 1. Introduction

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The indoor residual spraying (IRS) program in Tanzania is a joint U.S. Government and Government of Tanzania initiative and is funded by the U.S. President's Malaria Initiative (PMI), which aims to reduce the impact of malaria in sub-Saharan African countries. Abt Associates, through the PMI-funded Africa Indoor Residual Spraying (AIRS) project, is in its second year of implementation in the targeted districts in the Lake Zone. The PMI AIRS project supports Tanzania's National Malaria Control Program to facilitate the planning and implementation of the IRS program to reduce the incidence of malaria in the targeted districts.

Entomological monitoring, a crucial component of any malaria control program, continued to be carried out in 2017 to assess the efficacy of the IRS operations, inform the selection of insecticides and target spray areas, and monitor the behavioral and ecological response of vector species to the IRS intervention. PMI AIRS Tanzania continued to partner with the Mwanza Centre of the National Institute for Medical Research (NIMR) to provide advanced technical support and quality assurance for the IRS operations. Specifically, NIMR Mwanza Centre is fully responsible for all field-based entomological data collection as well as performance of the basic and advanced laboratory analyses associated with the entomological surveillance.

In 2017, NIMR Mwanza supported PMI AIRS Tanzania in implementing the following entomological monitoring activities:

1. Identify the species of malaria vectors in intervention and control areas.
2. Assess vector density, distribution, and seasonality in the intervention and control sentinel sites.
3. Monitor vector feeding and resting behavior in designated sites across the intervention districts.
4. Provide quality assurance of IRS through cone wall bioassays.
5. Rear and maintain a colony of susceptible *Anopheles gambiae* (Kisumu strain) in the NIMR Mwanza insectary unit.

This report provides information on the entomological monitoring activities completed between January 1 and September 30, 2017, in areas of 10 districts sprayed with pirimiphos-methyl (Actellic® 300CS) as well as in four control districts. Moreover, for clarity on decay rate, the information covers data from the start of spraying to October 31, 2017.

## 2. Methodology

### 2.1 Districts and sentinel villages for the 2017 campaign

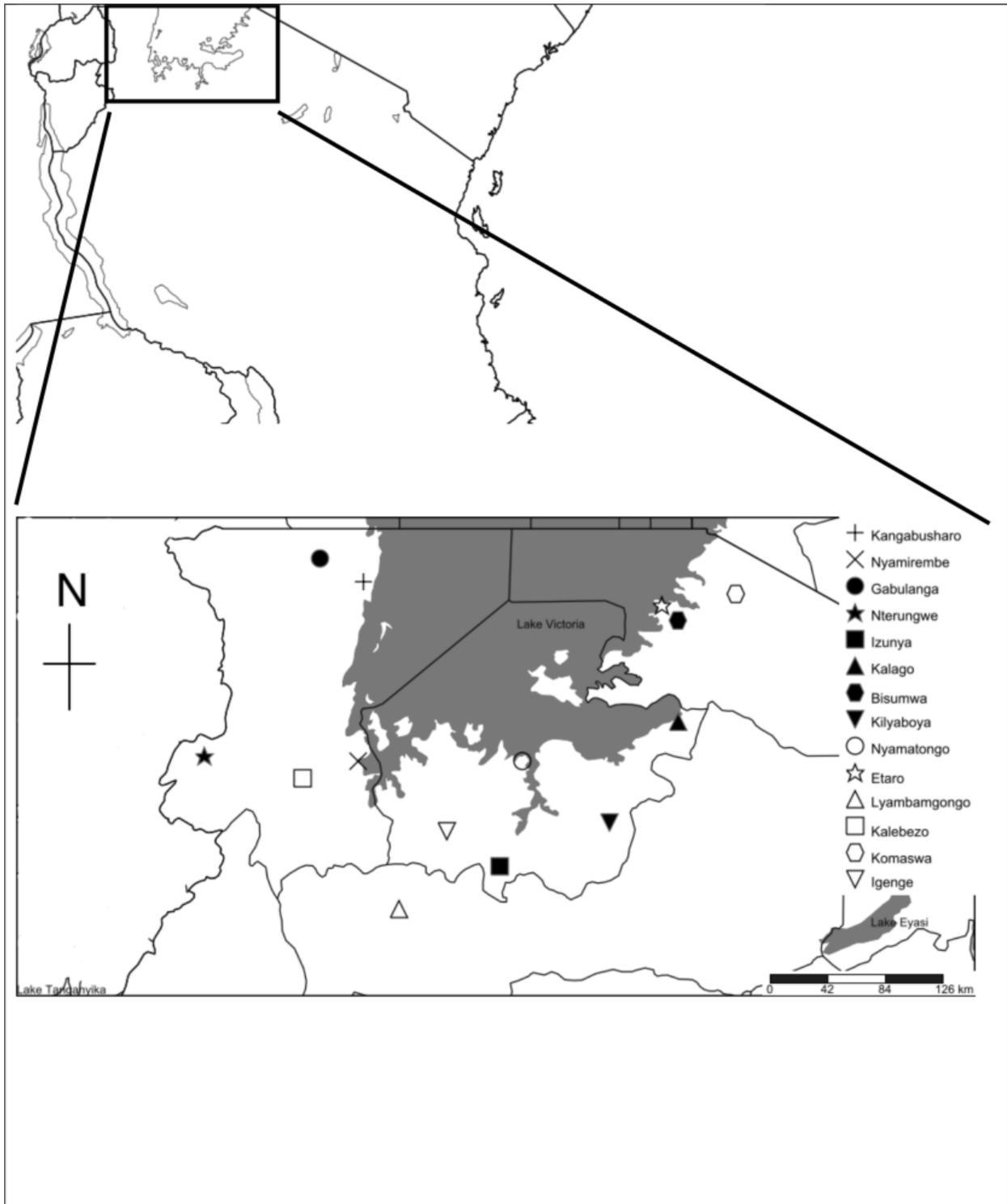
Entomological data were collected in 2017 in 14 villages, one per district that served as sentinel sites (Table 1). Geographical locations of the sites are shown in Figure 1. A summary of activities carried out in each sentinel site during the reporting period is indicated in Table 2. It should be noted that collection of mosquitoes in all sentinel sites started in January 2017 with the exception of Biharamulo, Geita Town Council, and Tarime sentinel districts, where the collection started in March 2017.

**TABLE 1: DATA COLLECTION SITES, 2017**

Region	District	Site (village)	GPS coordinates	Date of spraying	Spray status
Kagera	Missenyi	Gabulanga	1°11.808'S 31°27.913'E	26 Jan	Sprayed
	Bukoba Rural	Kangabusharo	1°20.958'S 31°44.981'E	27 Jan	Sprayed
	Ngara	Nterungwe	2°29.505'S 30°42.447'E	25–26 Jan	Sprayed
	Biharamulo	Kalebezo	2°64.385'S 31°35.587'E	Non-sprayed	Non-sprayed (Control)
Geita	Chato	Nyamirembe	2°31.509'S 31°42.881'E	25–26 Jan	Sprayed
	Nyang'hwale	Izunya	3°12.840'S 32°38.576'E	28–29 Jan	Sprayed
	Geita Town Council	Igence	2°98.705'S 32°29.600'E	15 June	Sprayed
	Bukombe	Lyambamgongo	3°29.644'S 31°5.966'E	Non-sprayed	Non-sprayed (Control)
Mara	Musoma Rural	Etaro	1°30.234'S 33°42.319'E	9–10 March	Sprayed

	Butiama	Bisumwa	1°36.176'S 33°48.602'E	9–11 March	Sprayed
	Tarime	Komaswa	1°43.335'S 34°19.470'E	Non-sprayed	Non-sprayed (Control)
Mwanza	Sengerema	Nyamatongo	2°31.453'S 32°47.48'E	29 March	Sprayed
	Kwimba	Kilyaboya	2°55.609'S 33°21.733'E	21–22 March	Sprayed
Simiyu	Busega	Kalago	2°15.998'S 33°48.726'E	Non-sprayed	Non-sprayed (Control)

**FIGURE 1: GEOGRAPHICAL LOCATIONS OF THE PMI AIRS TANZANIA MAINLAND OPERATION DISTRICTS, 2017**



**TABLE 2: SUMMARY OF ACTIVITIES CARRIED OUT IN EACH SENTINEL VILLAGE**

<b>Health district</b>	<b>Sentinel villages</b>	<b>Entomological activities</b>
<b>Kagera region</b>		
Ngara	Nterungwe	CDC LT indoors, clay pot outdoors, decay rate tests
Missenyi	Gabulanga	LT indoors, clay pot outdoors, decay rate tests
Bukoba Rural	Kangabusharo	CBR indoors/outdoors*, indoor Prokopack, parity rates, LT indoors, clay pot outdoors, decay rate tests
Biharamulo	Kalebezo	CBR indoors/outdoors*, indoor Prokopack, parity rates, LT indoors, clay pot outdoors, decay rate tests.
<b>Geita region</b>		
Chato	Nyamirembe	CBR indoors/outdoors, indoor Prokopack, parity rates, LT indoors, clay pot outdoors, decay rate tests
Nyang'hwale	Izunya	LT indoors, clay pot outdoors, decay rate tests.
Geita Town Council	Igence	LT indoors, clay pot outdoors, decay rate tests.
Bukombe	Lyambamgongo	CBR indoors/outdoors*, indoor Prokopack, parity rates, LT indoors, clay pot outdoors.
<b>Mara region</b>		
Musoma Rural	Etaru	CBR indoors/outdoors, indoor Prokopack, parity rates, LT indoors, clay pot outdoors, decay rate tests
Butiama	Bisumwa	LT indoors, clay pot outdoors, decay rate tests
Tarime	Komaswa	CBR indoors/outdoors*, indoor Prokopack, parity rates, LT indoors, clay
<b>Mwanza region</b>		
Sengerema	Nyamatomgo	CBR indoors/outdoors, indoor Prokopack, parity rates, LT indoors, clay pot outdoors, decay rate tests
Kwimba	Kilyaboya	LT indoors, clay pot outdoors, decay rate tests
<b>Simiyu region</b>		
Busega	Kalago	CBR indoors/outdoors*, indoor Prokopack, parity rates, LT indoors, clay

Note: CDC LT=Centers for Disease Control and Prevention light trap, CBR=CDC light traps with bottle rotators

\*The activities started in August 2017 due to delayed delivery of mosquito sampling equipment.

## 2.2 Personnel training

In engaging field activities for entomological surveillance, 28 local community mosquito collectors were recruited to undertake the fieldwork in 14 sentinel sites (two for each site). All recruits were trained for one week at NIMR Mwanza Centre by the core surveillance team.

We conducted refresher training for both the community mosquito collectors and district vector control officers in January 2017 to ensure that they all followed best practices in field mosquito collection, and understood the AIRS Tanzania entomological monitoring standards. The training covered the following topics: introduction to mosquitoes; identification of mosquito breeding sites; operation of CDC light traps (with and without bottle rotators), clay pots, and the Prokopack aspirator; differentiation of culicines from anophelines through morphological identification; identification of adult female *Anopheles* mosquitoes by species (at least differentiating *An. funestus* group from *An. gambiae* complex); dissection of mosquitoes to assess parity status; and carrying out of wall cone bioassays. Furthermore, on-site training was provided by the core NIMR surveillance team during monthly entomological monitoring.

## 2.3 Rearing of susceptible *An. gambiae* (Kisumu strain)

A technician was hired to manage mosquito rearing and production at the NIMR Mwanza insectary. This insectary has two main rooms: the adult and the larvae rooms. The adult room is maintained at  $27 \pm 1^\circ\text{C}$  and 60–80 percent relative humidity. Adult mosquitoes in the adult room are exposed to a light/dark regimen of 12/12 hours over a 24-hour day/night cycle. The larvae room environment is maintained at  $30 \pm 1^\circ\text{C}$  and 60–80 percent relative humidity. The adult *An. gambiae* s.s. are reared in 30cm x 30cm x 30cm cages and fed with 10 percent glucose solution for maintenance. In order to lay eggs, adult females *An. gambiae* s.s. are fed on rabbit blood. Glass petri dishes containing water are 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<sup>®</sup> 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 *An. 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 primarily used for cone wall bioassay tests to evaluate the decay rate of insecticides on various wall surface types.

## 2.4 Vector density, species composition, resting behavior, and seasonality

A total of four entomological sampling methods were used. The traps include: CDC light traps, clay pots, Prokopack aspirators, and CDC light trap with bottle rotator (CBRs). The CDC light traps (indoors), and clay pots were set in the 14 sentinel sites (both sprayed and non-sprayed

sites) to collect adult mosquitoes flying indoors, potentially seeking a blood meal, and outdoor resting mosquitoes, respectively. In addition, two entomological sampling methods, CBRs and Prokopack aspirators, were used in eight sentinel sites (four sprayed sites and four non-sprayed sites) to collect adult mosquitoes to help determine basic entomological indicators, including vector density, species composition, resting behavior, feeding and biting behavior, and seasonality. In the field, the project team morphologically identified the collected specimens (by genus/species) and quantified them. Sub-samples of host-seeking females were dissected for determination of the parity rate. Blood-fed females were individually preserved in micro-tubes for determination of blood-meal source. All captured females were individually conserved in micro-tubes for laboratory analysis (specific identification, infection, etc.).

### FIGURE 2: METHODS OF MOSQUITO SAMPLING

CDC light trap (top left), clay pot (top right), Prokopack aspirator (bottom left), and CBR (bottom right)



#### **2.4.1 CDC light trap method (indoor biting mosquitoes)**

In each selected village in a district, two houses per night were selected for the setting up of two CDC light traps on 28 consecutive days in a month. Briefly, in the selected houses, the CDC light trap was installed 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 CDC light traps were set to operate from 18:00 to 6:00 the next morning to trap mosquitoes. Captured mosquitoes were transferred separately into labeled paper cups covered with netting (Figure 2, top left).

#### **2.4.2 Clay pot method (outdoor resting mosquitoes)**

The clay pot method was used to collect outdoor resting mosquitoes. Local potters, using clay soil available from the area, molded the pots. The clay pots had a diameter of 0.5m and an opening of 20cm. They also had a 2cm hole at the bottom, which allowed water to freely drain out thus rendering them useless for storage of water. Each community mosquito collector was given four clay pots, which the collectors positioned outdoors overnight near selected houses made of different construction materials. The pots were set up from 18:00 to 6:00 the next morning. They were positioned at an inclined angle to let mosquitoes enter and rest inside the dark inner wall surface of the pot (Figure 2, top right). At 6:00 am, the mosquito collectors covered the opening with a piece of netting that had a small hole for inserting an aspirator to suck out mosquitoes and transfer them into a paper cup.

#### **2.4.3 Prokopack aspirator (indoor resting mosquitoes)**

The Improved Prokopack Aspirator Model 1419 (Figure 2, bottom left) was used to sample indoor resting mosquitoes from 10 houses over 20 days within each selected sentinel site per month. Mosquitoes were collected by Prokopack aspiration from 10 randomly selected houses within a sentinel site. Two houses were sampled each day with Prokopack aspiration. Collections using the Prokopack aspirator were conducted over five days in a week. Some of the houses were sampled more than once. Aspiration was carried out in the morning between 6:00 am and 8:00 am. Aspiration of resting adults produced 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). Data on the number of people who slept in the house the previous night, the type of house and wall surface, and the numbers of long-lasting insecticidal nets present were recorded.

The mosquitoes were put in well-labeled moist petri dishes and taken to the field office where they were sorted out morphologically by species. The abdominal status of all female anophelines that were collected was noted, and mosquitoes were sorted into the following categories: gravid, semi-gravid, unfed, and blood-fed females. The collected mosquitoes were preserved for later analysis using molecular assays to identify the sibling species and determine

malaria infection rates. The preserved mosquitoes will also be subjected to enzyme-linked immunosorbent assays (ELISA) to identify the source of the blood meal.

#### **2.4.4 CDC light trap with bottle rotators (indoor and outdoor biting times)**

Because Tanzania's Ethical Review Board restricts the use of the human landing catch method, the AIRS team used CBRs as a proxy to collect information on vector feeding time and changes in feeding behavior. CBRs were set in 10 randomly selected houses per site.

Trapping was conducted over 10 nights each month in four selected sprayed sentinel sites (Musoma Rural, Sengerema, Chato, and Bukoba Rural) and four non-sprayed sentinel sites (Biharamulo, Bukombe, Busega, and Tarime) using CBRs (indoors and outdoors). Collections started in March 2017 in three sites (Musoma Rural, Sengerema, and Chato) and in August 2017 in five sites (Bukoba Rural, Biharamulo, Bukombe, Busega, and Tarime). The later start date was due to the need for new CBR equipment in the five sites.

CBRs sampling was scheduled on nights near a new moon to minimize the effect of moonlight on the outdoor 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>. 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.

CBRs were set indoors with a person sleeping under an untreated net from 6:00 p.m. to 6:00 a.m. and outdoors from 18:00 pm to 6:00 (Figure 2, bottom right). The bottle collectors exchanged their positions every hour, enabling separate one-hour collections. Samples of anophelines were preserved in a 1.5 ml Eppendorf tube in silica gel for further ELISA and molecular analysis.

#### **2.5 Insecticides sprayed**

Pirimiphos-methyl (Actellic CS300) at a target dosage of 1g/m<sup>2</sup> was sprayed in the districts of Ngara, Missenyi, Bukoba Rural, Chato, Nyang'hwale, Geita Town Council, Sengerema, Kwimba, Butiama, and Musoma Rural. Annex A notes spraying and wall bioassay testing dates for each district.

#### **2.6 Effectiveness of indoor residual spraying**

Spray effectiveness in each IRS district was determined in two sprayed residential rooms (1 sitting room and 1 bedroom) of each surface wall type (five surface wall types per village) chosen in the treated villages, with one untreated control wall surface. The choice of rooms in the villages was done randomly and the selected sitting and bedrooms were repeatedly tested each month during the monitoring period.

Cone bioassays were performed in each room according to World Health Organization (WHO) standard protocols. Female mosquitoes of a susceptible strain of *An. gambiae* s.s. maintained at the insectary (NIMR Mwanza Centre) were used for this purpose. Two cones were placed on each wall and 10 mosquitoes were exposed in each cone. The location of the cones on the walls changed slightly each month because it was noted that the tape used to attach the cones removed part of the wall surface when the cone was removed. For the negative controls, one cone was fixed to a locally made, untreated block of similar surface wall type. The mortality of test mosquitoes was recorded 24 hours after exposure, with Abbott's correction implemented if mortality was between 5 percent and 20 percent in the negative controls. The IRS application was considered effective if the mortality was greater than 80 percent, as described by the WHO. A summary of control mortality during monitoring of insecticide decay rate in sentinel sites is shown in Annex B.

## 2.7 Laboratory analyses

From a sub-sample of *An. gambiae* s.l. collected, infective females were detected by the method of ELISA circumsporozoite (ELISA CSP), described by Burkot et al. (1984) and slightly modified by Wirtz et al. (1987).

The molecular identification of *An. gambiae* sibling species was performed on a sub-sample of *Anopheles* females collected from the sentinel sites. The molecular identification was performed by polymerase chain reaction (PCR) according to the protocol described elsewhere (Scott et al. 1993 and Wilkins et al. 2006) for *An. gambiae* s.l. and *An. funestus* s.l., respectively.

From *An. gambiae* s.l. collected by CDC light trap, Prokopack aspirator, and CBR, the origin of blood meals was determined by the direct ELISA method described by Beier et al. (1988). The blood-meal analysis started in June after optimization to estimate the source of blood meal of the main vector collected from structures occupied by humans only, animals only, and humans and animals mixed. To obtain mosquito samples, the team used the mosquito collection technique in the sentinel sites. The mosquitoes were identified, labeled, and transported to the NIMR Mwanza Centre laboratory for blood-meal source determination using ELISA.

## 2.8 Data analysis methods

- Prokopack collection data were used to calculate the density of vectors in a room using the formula:
  - $Vector\ density = Total\ number\ of\ vectors\ collected\ by\ species / Total\ number\ of\ rooms\ sampled.$
- *Bites per night* was obtained by the total number of mosquitoes collected per night using CDC LT collection data during the collection period
- *Sporozoite rates* = the proportion of *Anopheles* found positive for the presence of circumsporozoite proteins.
- *Parity rate* reflects the proportion of parous from the total number of ovaries dissected.

# 3. Results and Discussion

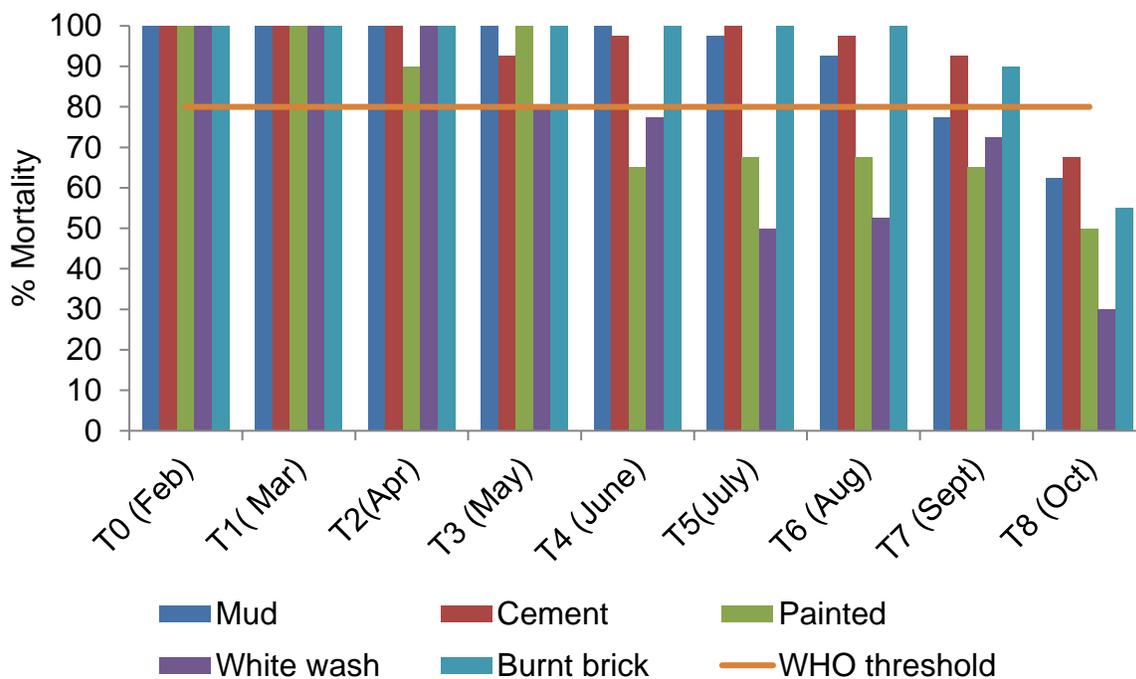
## 3.1 Residual efficacy of IRS with Actellic 300CS against a susceptible strain of *An. gambiae* s.s. in cone bioassay

Cone bioassays of walls sprayed with pirimiphos-methyl (Actellic CS 300) produced mean mortality rates greater than the WHO threshold of 80 percent in all districts six months after spraying. Nevertheless, to note, in Geita Town Council, only four post-spray cone bioassays had been finalized during this reporting period. The following sections discuss results for the decay rate in all 10 sprayed sentinel sites.

### 3.1.1 Ngara district

Mortality was 100 percent for all rooms tested during the baseline in February for the IRS quality spraying assessment (Figure 3). Three months post spraying, mortality rates were  $\geq 90$  percent on all sprayed surfaces except white-wash surfaces (80%). Painted and white-wash surfaces had a residual duration of three months compared to six months for mud and seven for cement and burned brick.

**FIGURE 3: RESIDUAL EFFICACY OF ACTELLIC 300CS IN NGARA**

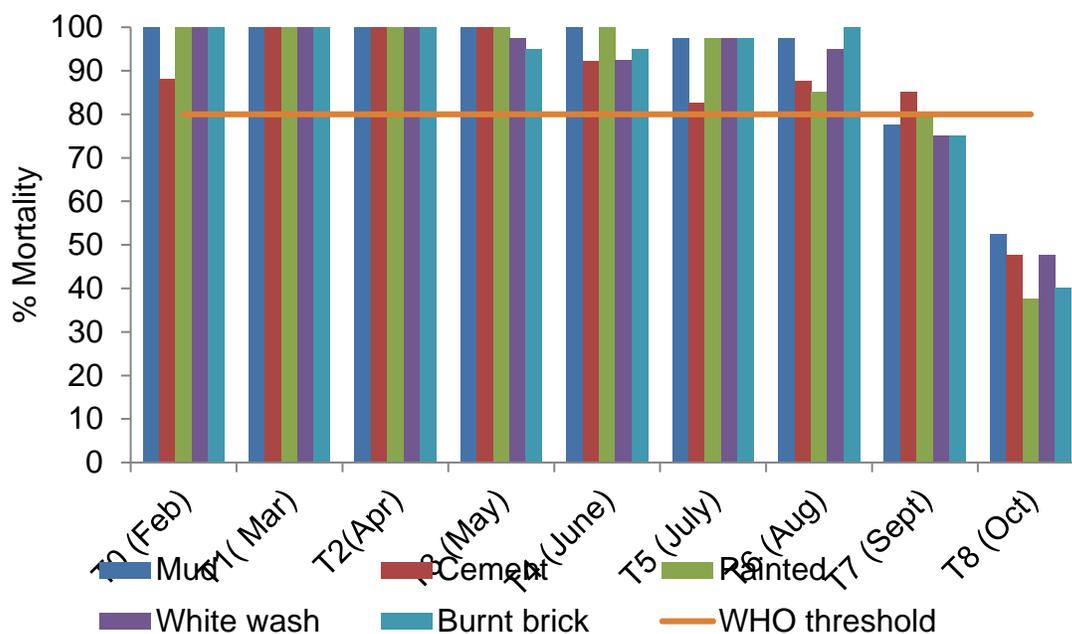


\*WHO cone test results, *An. gambiae* Kisumu mortality 24h after 30-minute exposure to Actellic 300CS

### 3.1.2 Missenyi district

Mortality was 100 percent for all surface types tested except cement (88%) during the baseline in February for the IRS quality spraying assessment (Figure 4). Six months post spraying, the test mortality rates were  $\geq 82.5$  percent in all sprayed surface types. Eight months after spraying, mortality declined substantially on all surfaces to  $< 60$  percent, giving a residual duration of seven months on most substrates.

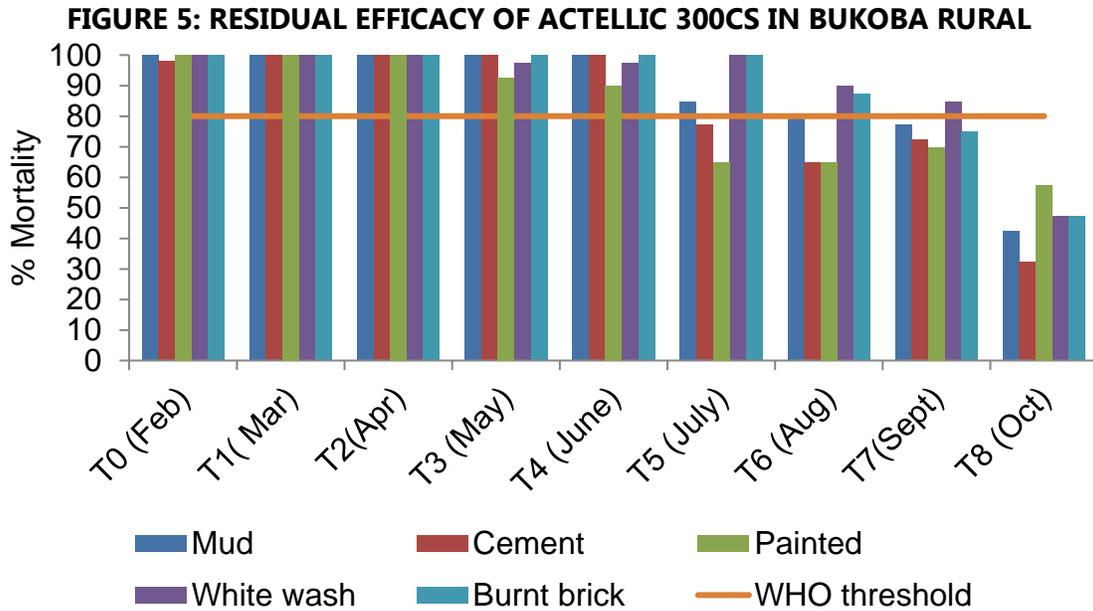
**FIGURE 4: RESIDUAL EFFICACY OF ACTELIC 300CS IN MISSENYI**



\*WHO cone test results, *An. gambiae* Kisumu mortality 24h after 30-minute exposure to Actellic 300CS

### 3.1.3 Bukoba Rural district

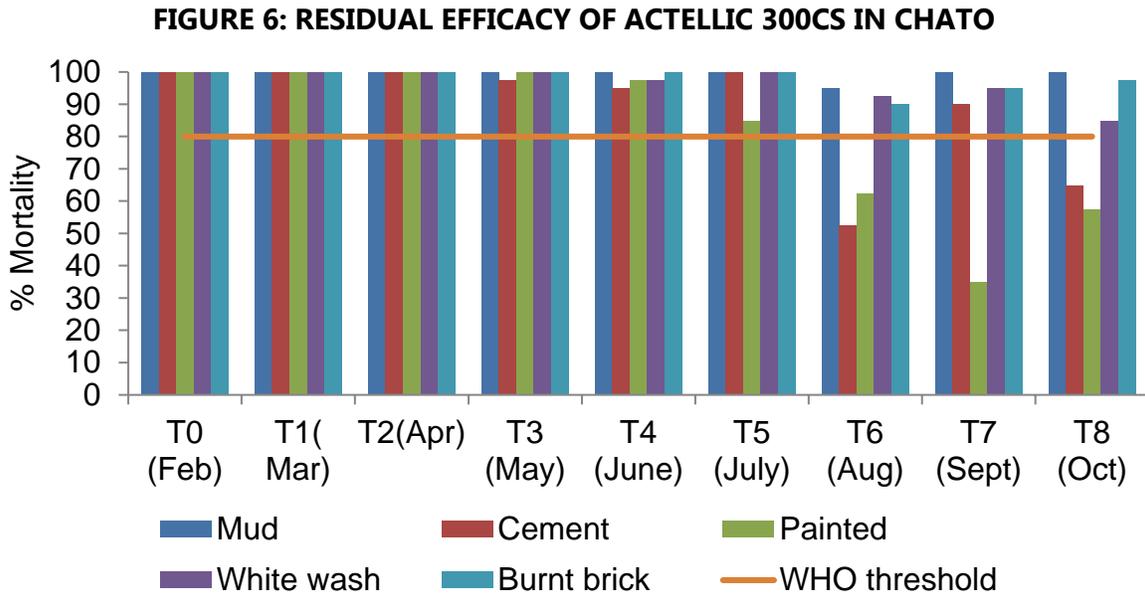
Mortality was 100 percent for all wall surface types tested except cement surface (98.3%) during the baseline in February for the IRS quality spraying assessment (Figure 5). Four months post spraying, the test mortality rates were  $\geq 90$  percent in all sprayed wall surface types. Trends were similar for all substrates with a residual duration of between four and seven months, with duration appearing to be longest for white-wash and burnt brick substrates.



\*WHO cone test results, *An. gambiae* Kisumu mortality 24h after 30-minute exposure to Actellic 300CS

### 3.1.4 Chato district

Mortality was 100 percent for all wall surface types tested during the baseline in February for the IRS quality spraying assessment (Figure 6). Five months post spraying, the test mortality rates were  $\geq 85$  percent on all sprayed wall surfaces. Cement and painted surfaces had a residual duration of five months compared to at least eight months for mud, white wash, and burnt brick.

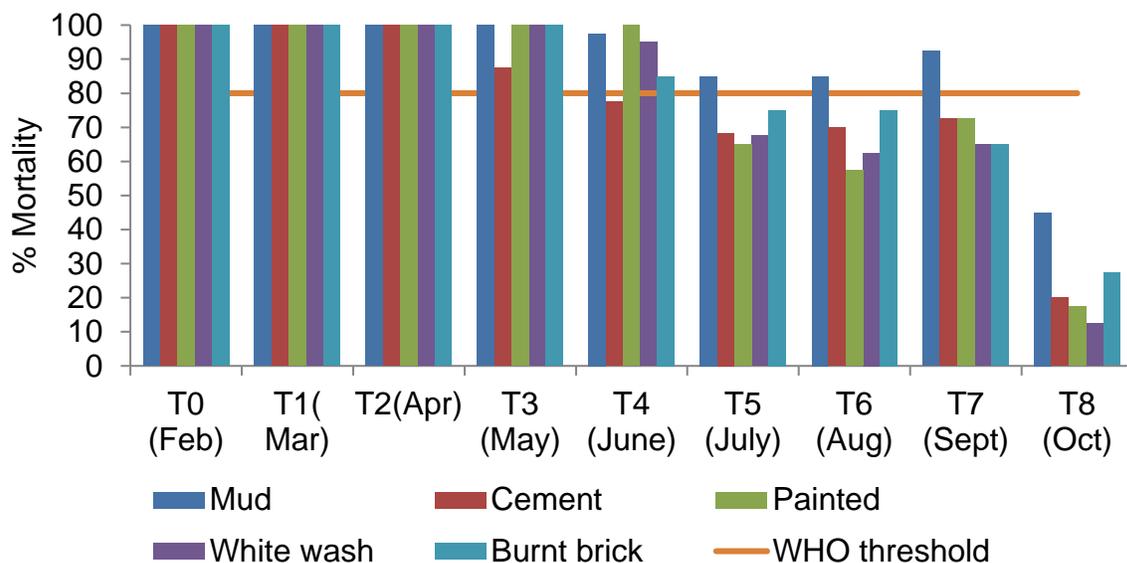


\*WHO cone test results, *An. gambiae* Kisumu mortality 24h after 30-minute exposure to Actellic 300CS

### 3.1.5 Nyang'hwale district

Mortality was 100 percent for all wall surfaces during the baseline in February for the IRS quality spraying assessment (Figure 7). Trends were similar for all substrates, with approximately four months of residual duration with mortality >80 percent.

**FIGURE 7: RESIDUAL EFFICACY OF ACTELIC 300CS IN NYANG'HWALE**

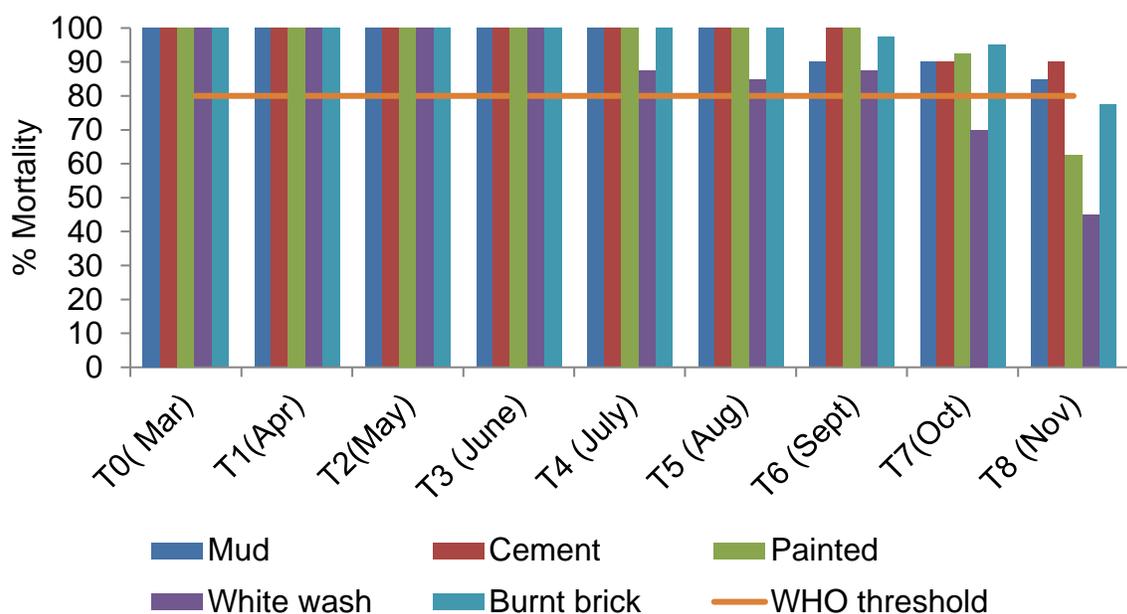


\* WHO cone test results, *An. gambiae* Kisumu mortality 24h after 30-minute exposure to Actellic 300CS

### 3.1.6 Sengerema district

Mortality was 100 percent for all wall surfaces during the baseline in February for the IRS quality spraying assessment (Figure 8). Seven months post spraying, mortality rates were still at ≥80 percent on all sprayed surfaces except white wash.

**FIGURE 8: RESIDUAL EFFICACY OF ACTELIC 300CS IN SENGEREMA**

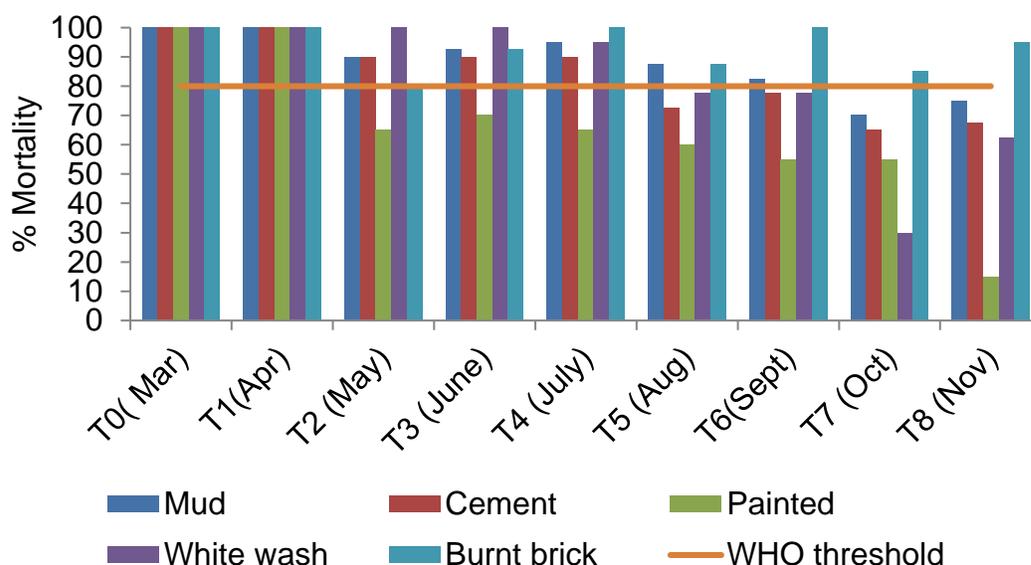


\* WHO cone test results, *An. gambiae* Kisumu mortality 24h after 30-minute exposure to Actellic 300CS

### 3.1.7 Kwimba district

Mortality was 100 percent for all wall surfaces during the baseline in March for the IRS quality spraying assessment (Figure 9). The residual duration of Actellic 300CS was approximately six months on all substrates, except painted walls, which produced mortality <80 percent after only two months.

**FIGURE 9: RESIDUAL EFFICACY OF ACTELLIC 300CS IN KWIMBA**

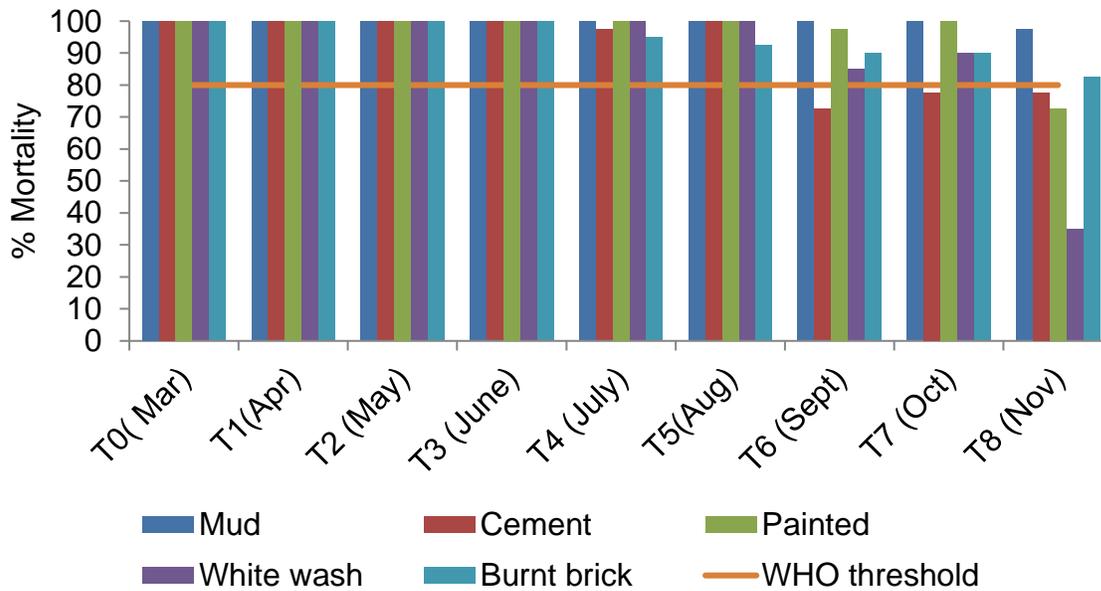


\* WHO cone test results, *An. gambiae* Kisumu mortality 24h after 30-minute exposure to Actellic 300CS

### 3.1.8 Musoma Rural district

Mortality was 100 percent for all wall surfaces during the baseline in March for the IRS quality spraying assessment (Figure 10). Five months post spraying, mortality rates were still at 100 percent on all wall surfaces except burnt brick.

**FIGURE 10: RESIDUAL EFFICACY OF ACTELLIC 300CS IN MUSOMA RURAL**

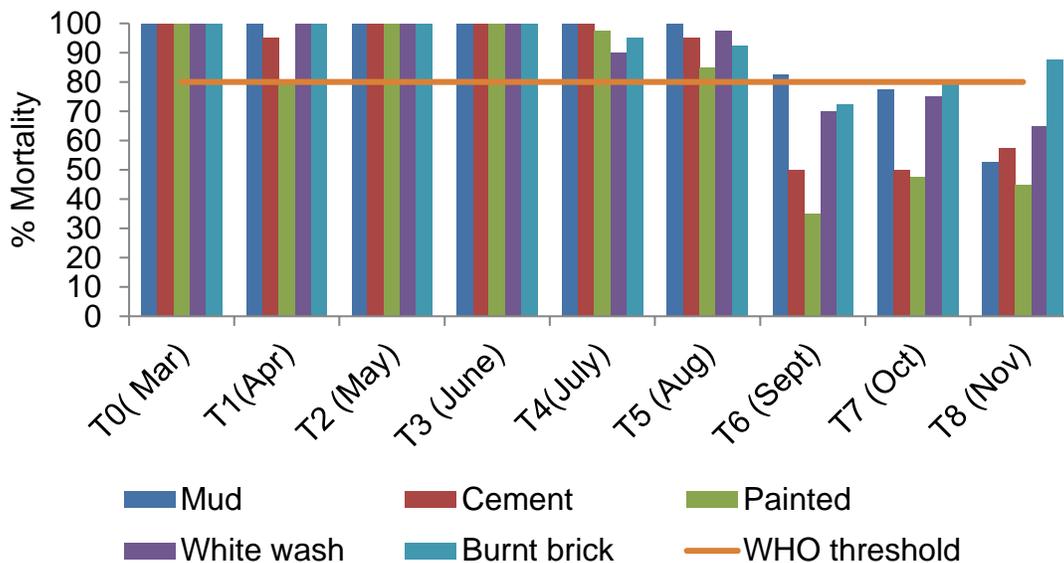


\* WHO cone test results, *An. gambiae* Kisumu mortality 24h after 30-minute exposure to Actellic 300CS

### 3.1.9 Butiama district

Mortality was 100 percent for all wall surfaces during baseline in March for IRS quality spraying assessment (Figure 11). Five months post spraying, the test mortality rates were still at  $\geq 85$  percent on all sprayed wall surfaces.

**FIGURE 11: RESIDUAL EFFICACY OF ACTELLIC 300CS IN BUTIAMA**

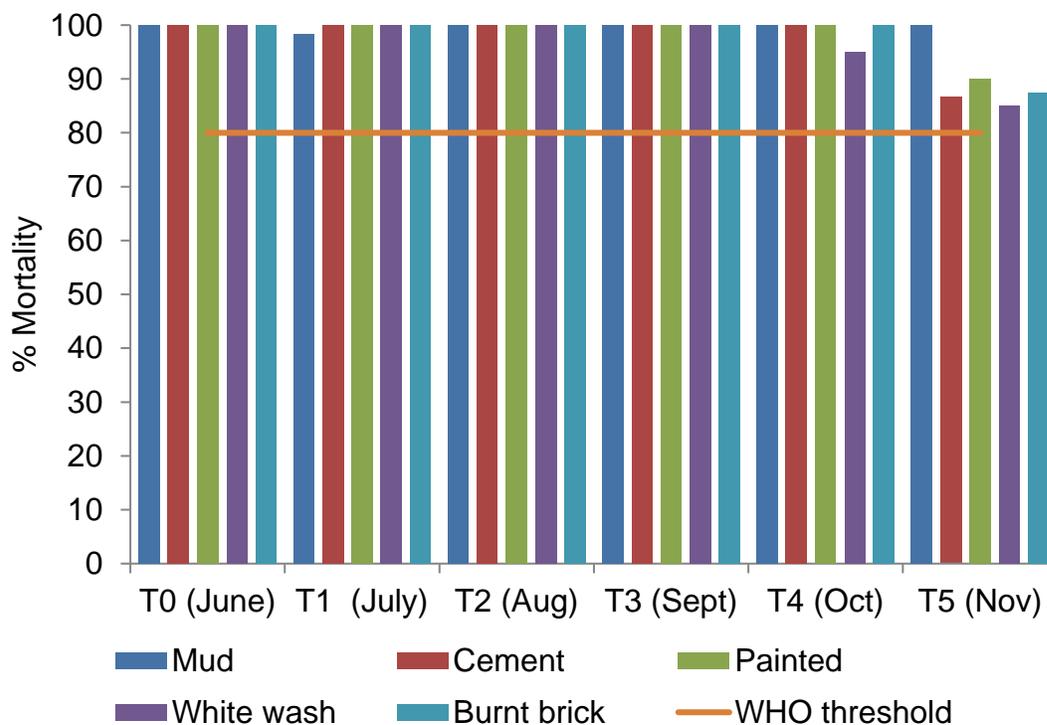


\*WHO cone test results, *An. gambiae* Kisumu mortality 24h after 30-minute exposure to Actellic 300CS

### 3.1.10 Geita Town Council district

Mortality was scored at  $>85$  percent for all wall surface types tested five months after spraying (Figure 12). Monthly cone bioassay is ongoing.

**FIGURE 12: RESIDUAL EFFICACY OF ACTELLIC 300CS IN GEITA TC**



\* WHO cone test results, *An. gambiae* Kisumu mortality 24h after 30-minute exposure to Actellic 300CS

## 3.2 Vector population dynamics in IRS districts

### 3.2.1 Molecular analysis of mosquito species composition and sporozoite rates

A total of 5,845 female *Anopheles* mosquitoes were analyzed by PCR for species identification, while 6,025 samples were analyzed by ELISA for detection of sporozoites. The PCR results confirmed the local vector population to be predominantly *An. arabiensis* (57.6%), *An. funestus* s.s. (12.6%), *An. gambiae* s.s. (5.0%), and *An. parensis* (7.4%). Approximately 17 percent (17.4%) of the samples were non-amplified by *An. gambiae* complex and *An. funestus* PCR (Table 3).

**TABLE 3: OVERALL SPECIES IDENTIFICATION BY PCR AND SPOROZOITE ELISA RESULTS IN SPRAYED AND NON-SPRAYED DISTRICTS (JANUARY–SEPTEMBER 2017)**

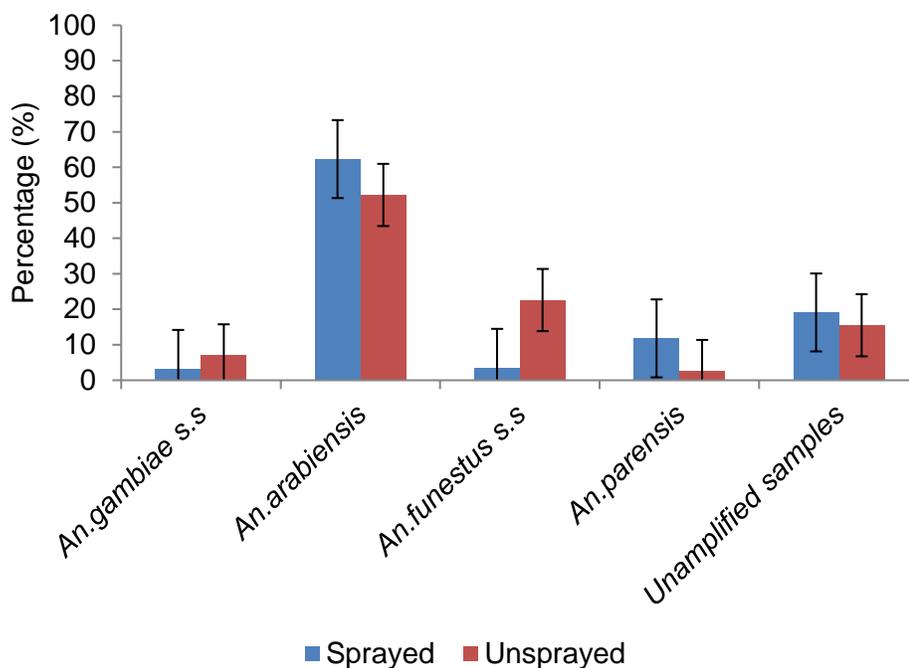
District	Species identification PCR						ELISA results		
	No. tested n	<i>An. gambiae</i> s.s. n (%)	<i>An. arabiensis</i> n (%)	<i>An. funestus</i> s.s. n (%)	<i>An. parensis</i> n (%)	Negative PCR	Total tested n	No. positive n	Sporozoite rates % (95% CI)
<b>Sprayed sites</b>									
Ngara	51	8 (16)	12 (24)	8 (16)	13 (26)	10 (20)	50	0	0
Missenyi	1021	49 (5)	784 (77)	3 (<1)	20 (2)	165 (16)	1066	9	0.8 (0.4-1.6)
Bukoba Rural	211	10 (5)	80 (40)	8 (4)	10 (5)	103 (49)	279	3	1.1 (0.2-3.1)
Chato	1078	23 (2)	471 (44)	52 (5)	311 (29)	231 (21)	1091	12	1.1 (0.6-1.9)
Nyang'hwale*	146	0	124 (85)	10 (7)	0	12 (8)	146	2	1.4 (0.2-4.9)
Geita Town Council*	52	2 (4)	30 (58)	0	3(6)	17 (33)	52	1	1.9 (0.1-10.3)
Sengerema	158	4 (3)	121 (77)	14 (9)	3 (2)	16 (10)	158	3	1.9 (0.4-5.4)
Kwimba	51	1 (2)	25 (49)	5 (10)	2 (4)	18 (35)	52	0	0
Musoma Rural	1	0	1(100)	0	0	0	5	0	0
Butiama	276	1 (<1)	256 (93)	6 (2)	0	13 (5)	276	5	1.8 (0.6-4.2)
Total (sprayed sites)	3045	98 (3.2)	1904 (62.5)	106 (3.5)	362(11.9)	575 (18.9)	3175	35	1.1 (0.8-1.5)
<b>Non-sprayed sites</b>									
Bukombe	698	61 (9)	433 (62)	61 (9)	3 (<1)	140 (20)	712	21	2.9 (1.8-4.5)
Busega	806	1 (<1)	652 (81)	18 (2)	31 (4)	103 (13)	806	9	1.1 (0.5-2.1)
Biharamulo*	540	134 (25)	97 (18)	184 (34)	39 (7)	86 (16)	560	22	3.9 (2.5-5.9)
Tarime*	756	1 (<1)	280 (37)	369 (49)	0	106 (14)	772	17	2.2 (1.3-3.5)
Total (non-sprayed sites)	2800	197 (7.0)	1462 (52.3)	632 (22.6)	73 (2.6)	435(15.5)	2850	69	2.4 (1.9-3.1)
<b>Total (all sites)</b>	<b>5,845</b>	<b>295 (5.0)</b>	<b>3,366 (57.6)</b>	<b>738 (12.6)</b>	<b>435 (7.4)</b>	<b>1,020 (17.4)</b>	<b>6,025</b>	<b>104</b>	<b>1.7 (1.4-2.1)</b>

\* Mosquito collections in the sentinel sites started in March 2017.

There was no significant difference in the proportion of *An. arabiensis* in sprayed and non-sprayed sites ( $p=0.258$ ) whereas six times the proportion of *An. funestus* s.s. was collected in

non-sprayed sites compared to sprayed sites ( $p=0.0088$ ) (Figure 13). Conversely, the proportion of *An. parensis* was higher in sprayed sites than non-sprayed. Sporozoite rates were found to vary across the sentinel districts ranging from 0 percent to 3.9 percent with a mean sporozoite rate of 1.7 percent in the study area (Table 3). The mean sporozoite rate was 1.1 percent (95% CI: 0.8–1.5) (35/3,175) in the sprayed sentinel sites compared with 2.4 percent (95% CI: 1.9–3.1) (69/2,850) in non-sprayed sentinel sites ( $p=0.0001$ ).

**FIGURE 13: HISTOGRAM SHOWING SPECIES COMPOSITION IN SPRAYED AND NON-SPRAYED SITES**



*An. funestus* s.l. had a significantly higher ( $p=0.0006$ ) sporozoite positive rate at 3.0 percent (95% CI: 2.0–4.0) (35/1,173) than *An. gambiae* s.l. at 1.4 percent (95% CI: 1.1–1.8) (53/3,661). Further analysis to species level showed that *An. funestus* s.s. had the highest sporozoite rate (4.1%) of all species analyzed (Table 4). *An. parensis* had a sporozoite rate of 1.1 percent. Sixteen samples that were sporozoite positive could not be identified by PCR to species but were morphologically identified as *An. gambiae* s.l.

**TABLE 4: SPOOROZITE RESULTS BY MOSQUITO SPECIES IDENTIFIED BY PCR**

Mosquito species	No. of samples analyzed	No. of sporozoite positive	Sporozoite rate (%)
<i>An. gambiae</i> s.s.	295	8	2.7
<i>An. arabiensis</i>	3,366	45	1.3

<i>An. funestus</i> s.s.	738	30	4.1
<i>An. parensis</i>	435	5	1.1
Unidentified by PCR	1,020	16	1.6

*An. arabiensis* had a higher sporozoite rate in non-sprayed sites (2.0%; 95% CI 1.4–2.9) than in sprayed sites (0.8%; 95% CI: 0.5–1.3) ( $p=0.003$ ). The *An. funestus* s.s. sporozoite rate was not statistically significant between sprayed and non-sprayed sites ( $p=0.48$ ) (Table 5), although the sample size was small in the sprayed sites.

**TABLE 5: SPECIES SPOROZOITE RESULTS IN SPRAYED AND NON-SPRAYED SITES**

Mosquito species	Spray status	No. of samples analyzed	No. of sporozoite positive	Sporozoite rate (%) (95% CI)	P-value
<i>An. gambiae</i> s.s.	Sprayed	118	3	2.5 (0.5-7.2)	0.8758
	Non-sprayed	177	5	2.8 (0.9-6.5)	
<i>An. arabiensis</i>	Sprayed	1,924	16	0.8 (0.5-1.3)	0.003
	Non-sprayed	1442	29	2.0 (1.3-2.9)	
<i>An. funestus</i> s.s.	Sprayed	108	3	2.8 (0.6-7.9)	0.48
	Non-sprayed	630	27	4.3 (2.8-6.2)	
<i>An. parensis</i>	Sprayed	362	4	1.1 (0.3-2.8)	0.84
	Non-sprayed	73	1	1.4 (0.03-7.4)	
Unidentified by PCR	Sprayed	559	9	1.6 (0.7-3.0)	0.898
	Non-sprayed	461	7	1.5 (0.6-3.1)	

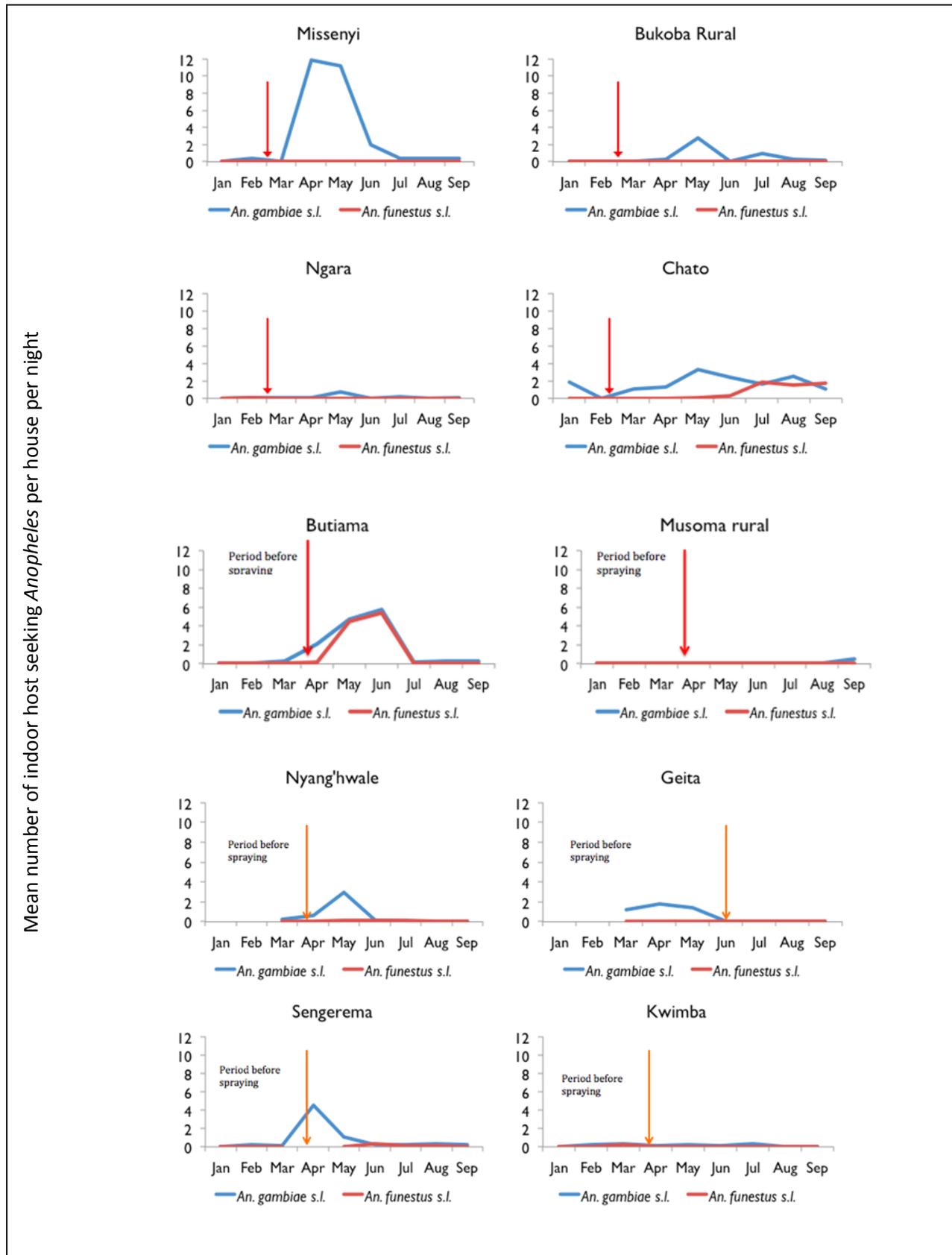
### 3.2.2 Vector seasonality

Indoor biting densities of *An. gambiae* s.l. and *An. funestus* s.l. between January and September 2017 in all sentinel districts are presented in Figure 14 (sprayed sites) and Figure 15 (non-sprayed sites). There were higher densities of *An. gambiae* s.l. than of *An. funestus* s.l. in all study sites. In general, the highest vector densities were observed between March and June in all sites. However, increases in vector densities between March and June were small in most sprayed districts, including in Bukoba Rural, Chato, Nyang'hwale, Sengerema, and Butiama (Figure 14). In Musoma Rural, Kwimba, and Ngara, the densities were nearly zero throughout the year. Biting

rates were particularly high following the rainy season in Missenyi and Butiama. The pattern in Missenyi was particularly concerning because it had the highest biting rate of all sites. This may be attributed to the presence of more breeding sites due to extensive sugarcane plantations and heavy rainfall in this district. Vector densities also increased in the non-sprayed districts of Bukombe, Tarime, Biharamulo, and Busega in May following the long rains (Figure 15).

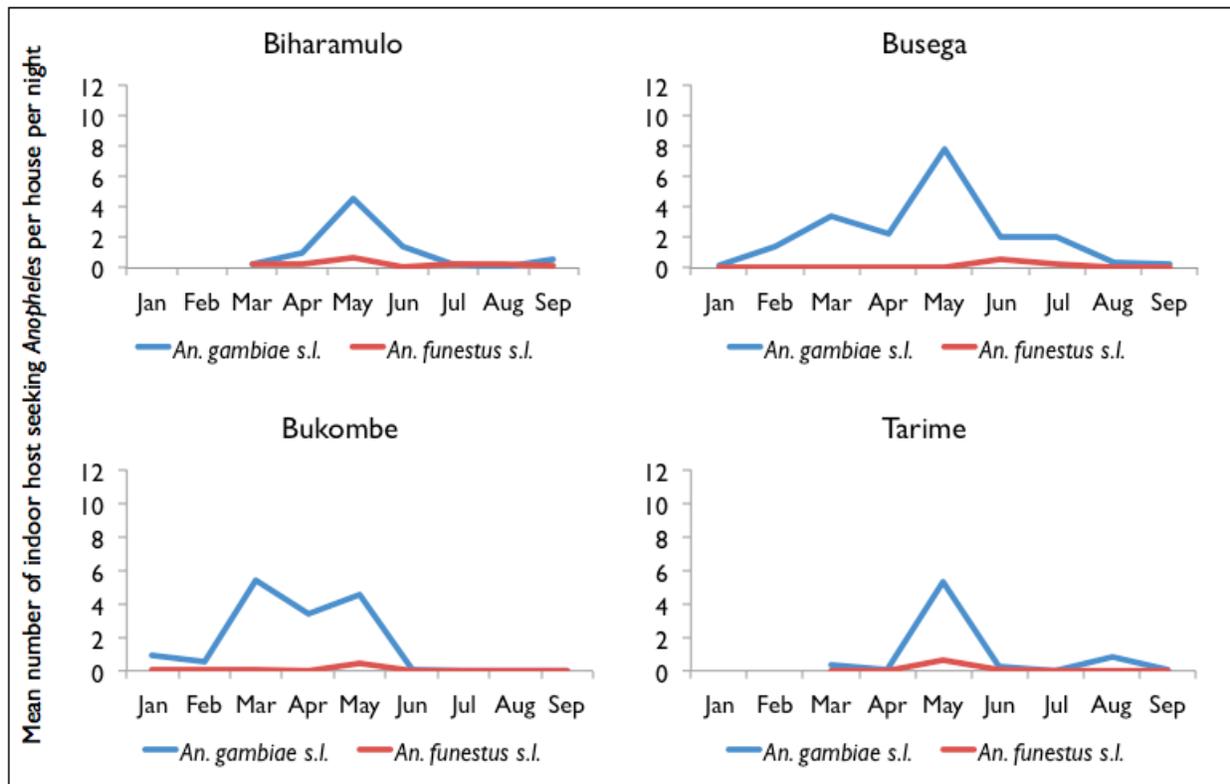
*An. funestus* s.l. was not collected at all in several sites (Bukoba Rural, Musoma Rural, and Geita Town Council districts), and very low densities were recorded in most other sites. A relatively high proportion of *An. funestus* s.l. was observed in Chato during the dry season, June–September. Due to the limited sample size, the biting data are unlikely to be representative of the whole district and comparisons of biting rates between districts should be made with caution.

**FIGURE 14: MONTHLY INDOOR BITING RATES (CDC LIGHT TRAP) OF ANOPHELES MOSQUITOES IN 10 SPRAYED DISTRICTS**



Key: Red and yellow arrows denote spray timing.

**FIGURE 15: MONTHLY INDOOR BITING RATES (CDC LIGHT TRAP) OF ANOPHELES MOSQUITOES IN FOUR NON-SPRAYED DISTRICTS**

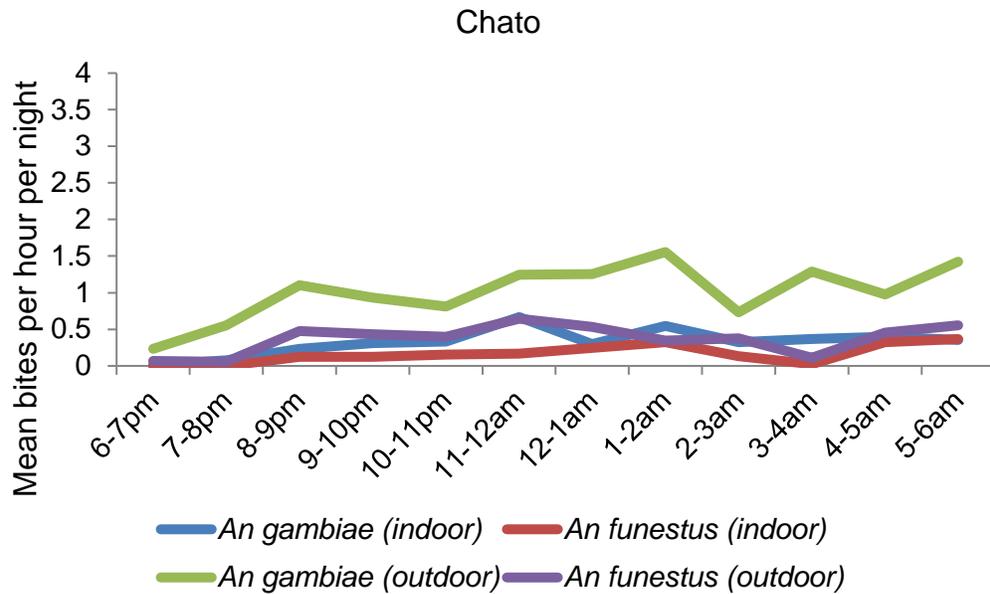


### 3.2.3 Biting times of *An. gambiae s.l.* and *An. funestus s.l.* (indoors and outdoors)

In Chato the *An. gambiae s.l.* biting rate (bites per hour per night) was higher outdoors than indoors, including the hours before people went to bed (18:00–22:00) (Figure 16). In Biharamulo (non-sprayed site), there was more biting risk indoors than outdoors in the early evening (18:00–22:00) (Figure 17).

In Chato and Biharamulo, both indoor and outdoor biting rates were fairly consistent throughout the night except for an early morning indoor biting peak in Biharamulo (Figures 16 and 17).

**FIGURE 16: MEAN INDOOR AND OUTDOOR BITING RATES OF ANOPHELES SPECIES COLLECTED BY CBRS IN CHATO**

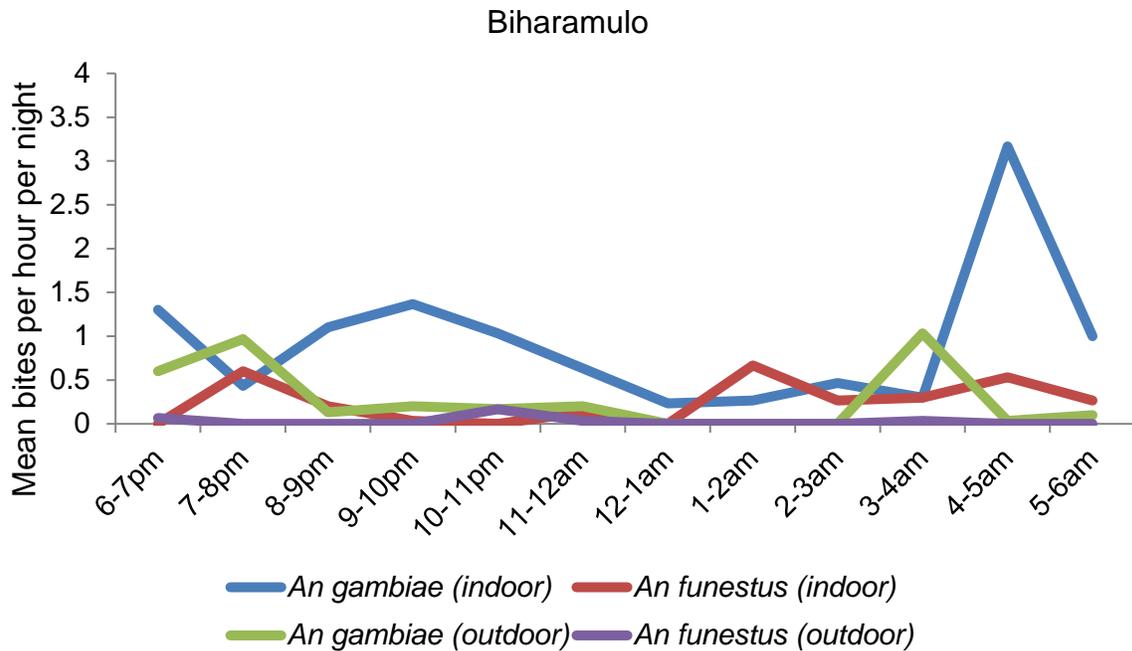


Key: Total number in Chato: *An. gambiae* (indoor) n=353, *An. gambiae* (outdoor) n=1,090, *An. funestus* (indoor) n=178, *An. funestus* (outdoor) n=401. The sampling was for 10 nights every month, from January to September 2017 (90 days in total).

Note: Bites per person per night is estimated as the total number of mosquitoes collected from January to September 2017 divided by the number of trap nights.

Generally, the total number (indoors and outdoors) of *Anopheles* collected was <100 over the trapping period in three sprayed districts: Bukoba Rural (63), Musoma Rural (51), and Sengerema (50); because no clear trends could be observed, the data are not presented in this report.

**FIGURE 17: MEAN INDOOR AND OUTDOOR BITING RATES OF ANOPHELES SPECIES COLLECTED BY CBRS IN BIHARAMULO DISTRICT**



Key: total number in Biharamulo: *An. gambiae* (indoor) n=339, *An. gambiae* (outdoor) n=103, *An. funestus* (indoor), n=90, *An. funestus* (outdoor) n=9; starting June to September 2017 (30 days in total). The sampling was for 10 nights every month.

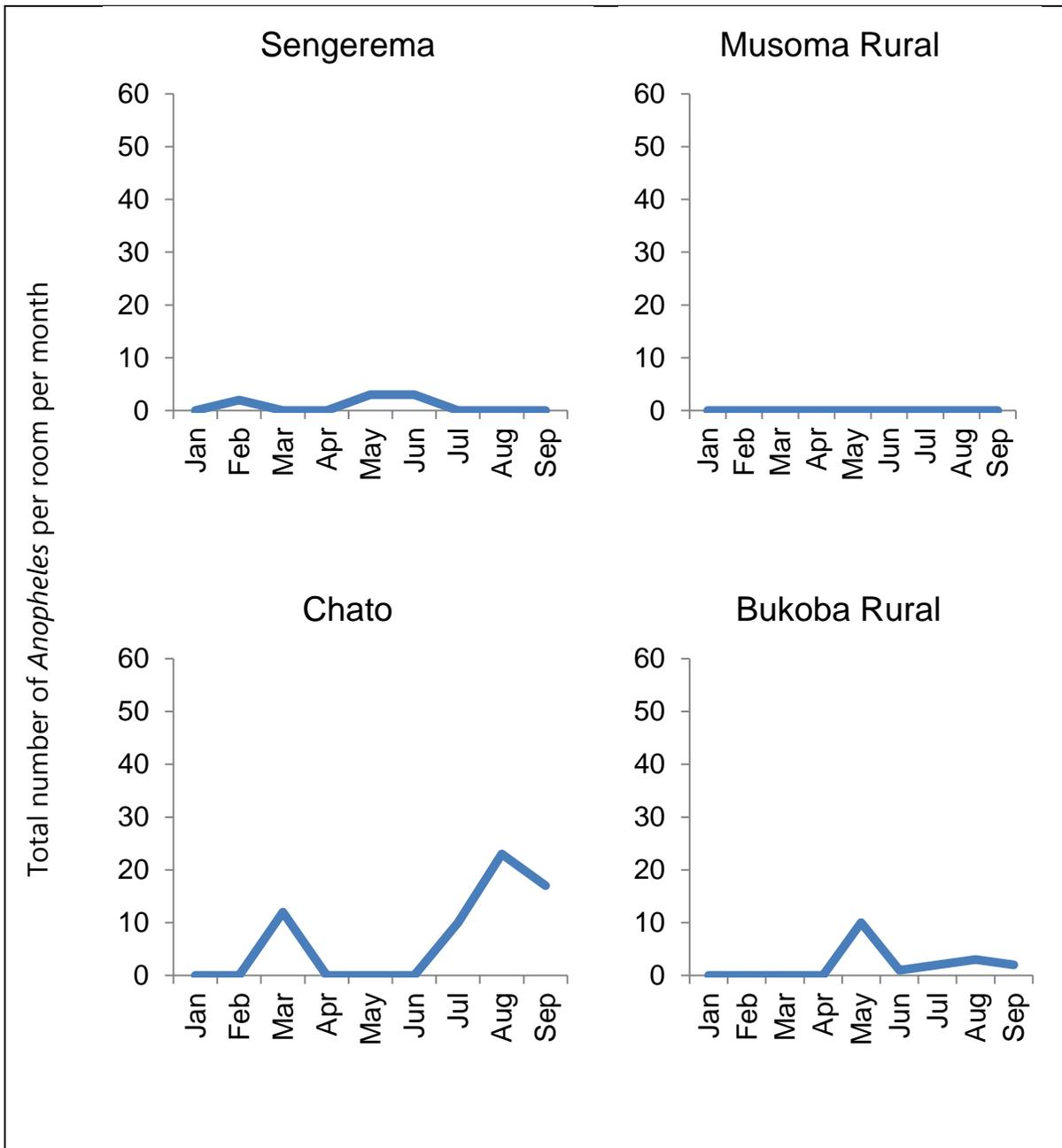
Generally, the total number (indoors and outdoors) of *Anopheles* collected also was <100 over the trapping period in three non-sprayed districts: Bukombe (1), Busega (4), and Tarime (21). Because there were no clear trends, the data are not presented in this report.

### 3.2.4 Indoor resting density

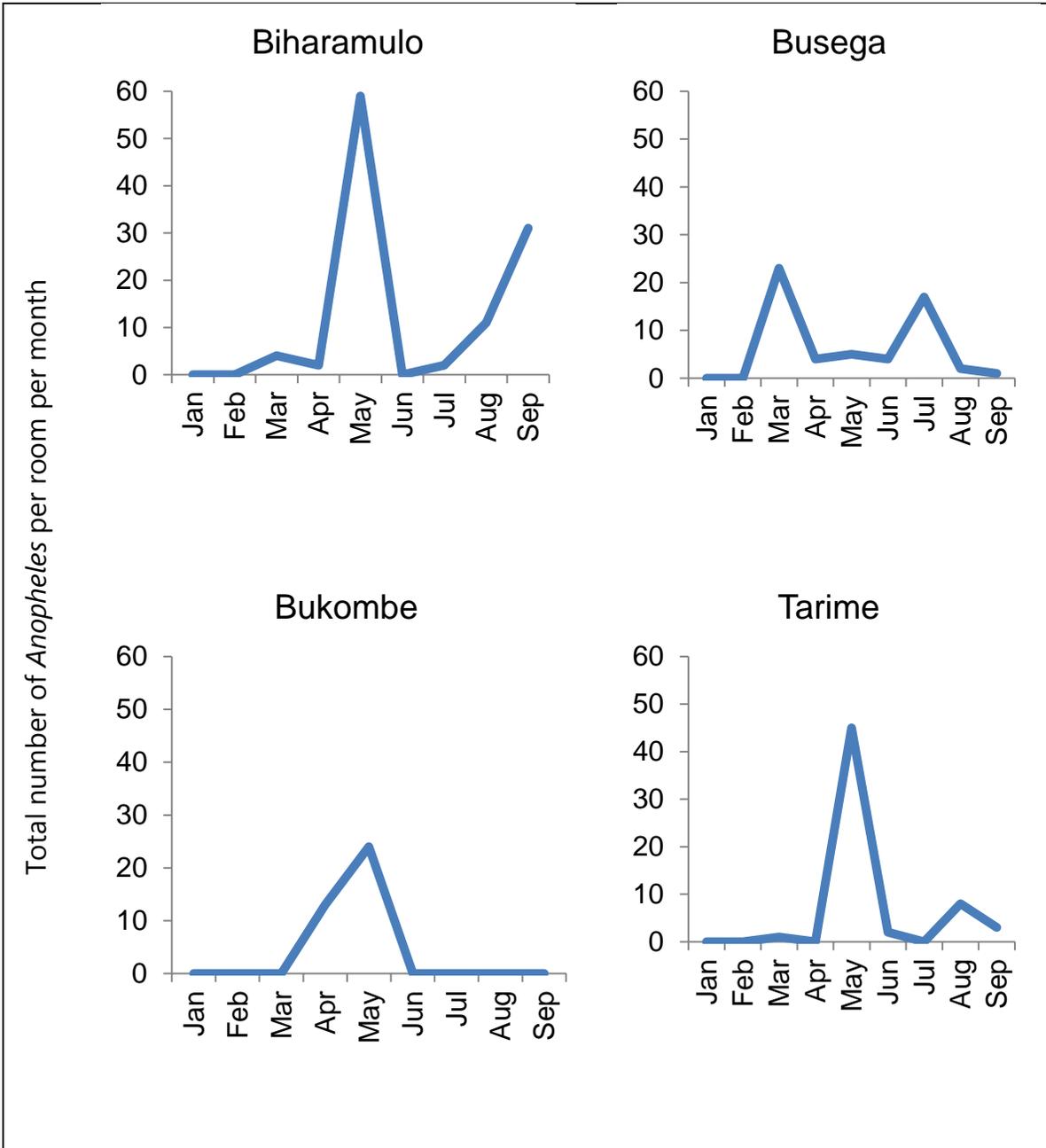
Indoor resting density (IRD) was considerably greater in the non-sprayed sentinel sites of Biharamulo, Bukombe, Busega, and Tarime than in the sprayed sites of Bukoba Rural, Chato, Musoma Rural, and Sengerema (Figures 18 and 19).<sup>1</sup> The highest collections were between March and June. Overall throughout the period of collection, higher IRDs were observed in the non-sprayed districts (Figure 20).

<sup>1</sup> There was no collection throughout the reporting period in Musoma Rural (Figures 18).

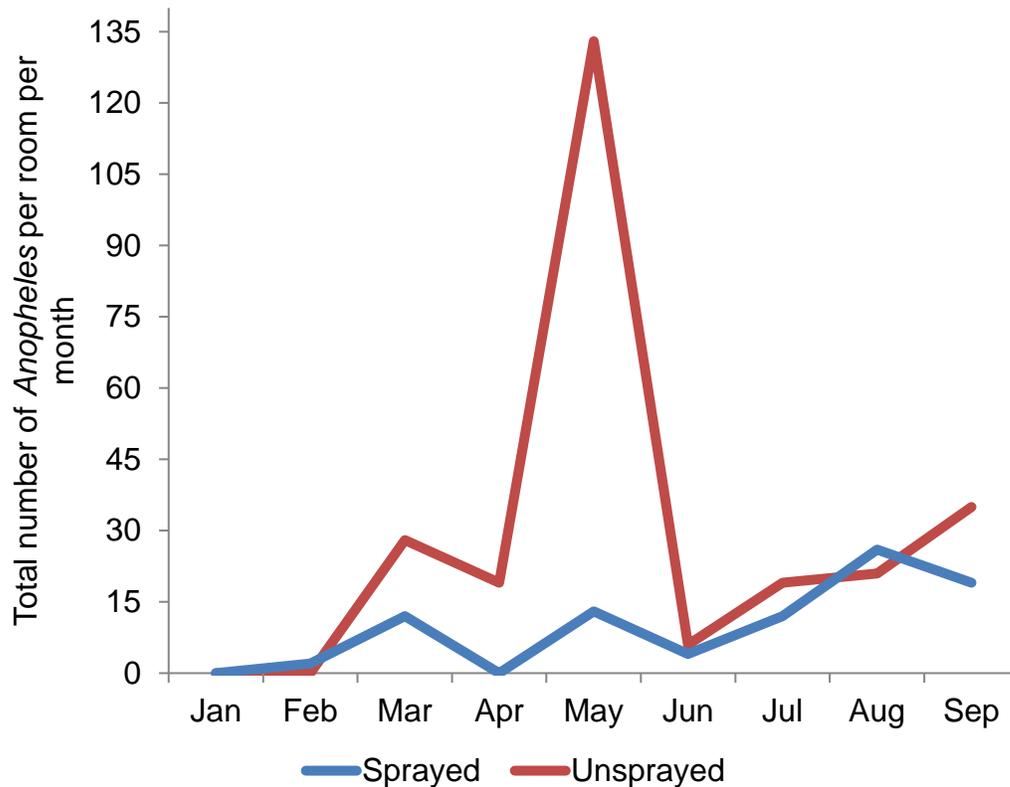
**FIGURE 18: IRD OF AN. GAMBIAE S.L. IN FOUR SPRAYED DISTRICTS**



**FIGURE 19: IRD OF AN. GAMBIAE S.L. IN FOUR NON-SPRAYED DISTRICTS**



**FIGURE 20: IRD OF AN. GAMBIAE S.L. IN SPRAYED AND NON-SPRAYED DISTRICTS**



### 3.2.5 Blood-meal analysis

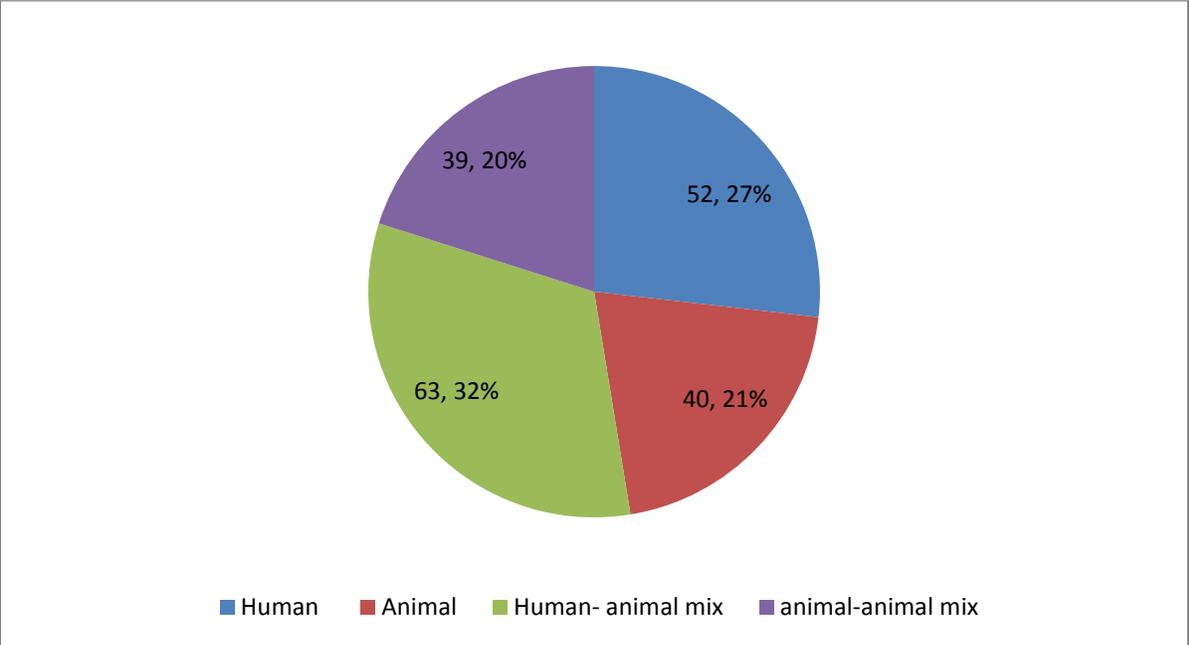
A total of 268 *Anopheles* mosquitoes from sprayed and non-sprayed sites were tested for vertebrate host blood source (human, bovine, goat, and dog). Overall, the proportion of *Anopheles* that fed on humans (including mixed blood meals on both humans and animals) was 66.9 percent (119/178) in sprayed sites and 56.7 percent (51/90) in non-sprayed sites (Table 6).

Molecular species identification indicated that the majority tested for blood-meal host were *An. arabiensis* (Table 6). Thirty-two percent (63/194) of *An. arabiensis* fed on both human and animals, showing opportunistic feeding behavior, while 27 percent (52/194) fed only on humans (Figure 21).

**TABLE 6: SUMMARY OF ALL SAMPLES TESTED AND IDENTIFIED BY ELISA FOR BLOOD MEAL IN THE SENTINEL DISTRICTS ALONG LAKE VICTORIA IN TANZANIA, JANUARY–SEPTEMBER 2017**

District sentinel site	Species	No. tested	Blood-meal sources					
			Human	Cow	Goat or dog	Mixed (human - animal)	Mixed (animal-animal)	Unidentified
Intervention sentinel sites								
Sengerema	<i>An. gambiae</i> s.s.	1	0	0	0	0	1	0
	<i>An. arabiensis</i>	13	3	1	0	3	6	0
	<i>An. funestus</i> s.s.	1	0	0	0	1	0	0
	Unidentified	0	0	0	0	0	0	0
Missenyi	<i>An. gambiae</i> s.s.	2	0	1	0	0	0	1
	<i>An. arabiensis</i>	78	21	18	0	30	9	0
	<i>An. funestus</i> s.s.	0	0	0	0	0	0	0
	Unidentified	24	2	3	0	12	7	0
Bukoba Rural	<i>An. gambiae</i> s.s.	0	0	0	0	0	0	0
	<i>An. arabiensis</i>	17	4	0	4	6	3	0
	<i>An. funestus</i> s.s.	1	1	0	0	0	0	0
	Unidentified	40	3	1	2	32	2	0
Kwimba	<i>An. gambiae</i> s.s.	0	0	0	0	0	0	0
	<i>An. arabiensis</i>	1	1	0	0	0	0	0
	<i>An. funestus</i> s.s.	0	0	0	0	0	0	0
	Unidentified	0	0	0	0	0	0	0
TOTAL	<i>An. gambiae</i> s.s.	<b>3</b>	<b>0</b>	<b>1</b>	<b>0</b>	<b>0</b>	<b>1</b>	<b>1</b>
	<i>An. arabiensis</i>	<b>109</b>	<b>29</b>	<b>19</b>	<b>4</b>	<b>39</b>	<b>18</b>	<b>0</b>
	<i>An. funestus</i> s.s.	<b>2</b>	<b>1</b>	<b>0</b>	<b>0</b>	<b>1</b>	<b>0</b>	<b>0</b>
	Unidentified	<b>64</b>	<b>5</b>	<b>4</b>	<b>2</b>	<b>44</b>	<b>9</b>	<b>0</b>
Control sentinel sites								
Bukombe	<i>An. gambiae</i> s.s.	0	0	0	0	0	0	0
	<i>An. arabiensis</i>	37	6	11	1	2	17	0
	<i>An. funestus</i> s.s.	2	1	1	0	0	0	0
	Unidentified	1	0	0	0	1	0	0
Busega	<i>An. gambiae</i> s.s.	0	0	0	0	0	0	0
	<i>An. arabiensis</i>	48	17	4	0	22	5	0
	<i>An. funestus</i> s.s.	0	0	0	0	0	0	0
	Unidentified	2	1	0	0	1	0	0
TOTAL	<i>An. gambiae</i> s.s.	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>
	<i>An. arabiensis</i>	<b>85</b>	<b>23</b>	<b>15</b>	<b>1</b>	<b>24</b>	<b>22</b>	<b>0</b>
	<i>An. funestus</i> s.s.	<b>2</b>	<b>1</b>	<b>1</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>
	Unidentified	<b>3</b>	<b>1</b>	<b>0</b>	<b>0</b>	<b>2</b>	<b>0</b>	<b>0</b>

**FIGURE 21: BLOOD-MEAL SOURCES OF AN. ARABIENSIS IN THE SENTINEL SITES (N=194)**



# Conclusion

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This report presents results of entomological surveillance following a spray campaign in mainland Tanzania during the period of January–September 2017. With the exception of two sentinel sites (Kwimba and Ngara), IRS using pirimiphos-methyl (Actellic® 300CS) remained efficacious on several types of sprayed wall surface for six to eight months post IRS.

The population density of malaria vectors was low in sprayed districts with the exception of Missenyi district, where relatively high vector densities were observed in April and May, following rainfall. The mean sporozoite rate was lower in sprayed sites at 1.1 percent, compared with 2.4 percent in non-sprayed sentinel sites. *An. gambiae s.l.* remained the predominant vector species complex, with relatively few *An. funestus* caught in sprayed sites. In some sprayed sites, it appeared that *An. parensis* (of the *An. funestus* group) have replaced *An. funestus*, as was reported in coastal Kenya following IRS with DDT in the 1960s (Gillies and Furlong 1964). The finding of *An. parensis* with sporozoites indicates that this species is probably a secondary vector in the area as has been reported in Uganda (Mulamba et al. 2014).

There was considerable outdoor biting risk before people went to bed in the sprayed sentinel site of Chato. We aim to improve monitoring of outdoor biting by determining which trapping method most accurately predicts outdoor human biting rates. Such outdoor monitoring should be included as a routine monitoring tool alongside indoor CDC light traps.

Overall, the highest sporozoite rate was observed in the non-sprayed districts (Biharamulo, Bukombe, Busega, and Tarime). *An. funestus*, which was mostly captured in non-sprayed sites, had the highest sporozoite rate, 4.3 percent, in those non-sprayed sites.

Blood-meal analysis indicated that *An. arabiensis* showed opportunistic feeding behavior, feeding on both human and animal sources. Despite this behavior, the anthropophily index was reasonably high with 59 percent of blood-meals containing human blood (including mixtures with animal blood).

In general, comparison of sprayed and non-sprayed sites shows that IRS is successful in keeping vector densities relatively low and in reducing sporozoite rates.

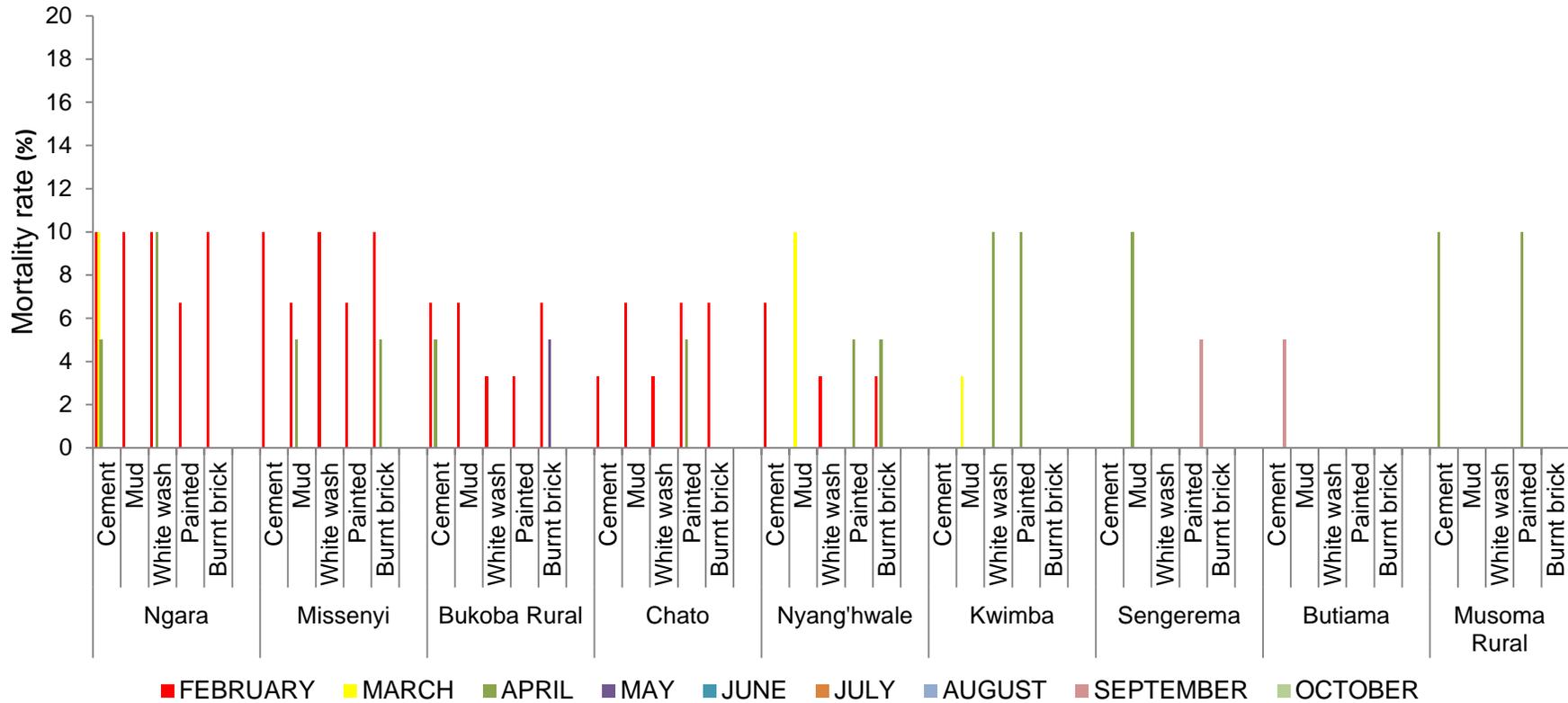
# Annex A: Dates of IRS Campaign and of Cone Bioassays in the IRS Sentinel Sites, 2017

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District	Sentinel site	Date of spray	1 <sup>st</sup> Bioassay	2 <sup>nd</sup> Bioassay	3 <sup>rd</sup> Bioassay	4 <sup>th</sup> Bioassay	5 <sup>th</sup> Bioassay	6 <sup>th</sup> Bioassay	7 <sup>th</sup> Bioassay	8 <sup>th</sup> Bioassay	9 <sup>th</sup> Bioassay
Ngara	Nterungwe	25 - 26 Jan	28 – 29 Jan	24 Mar	11 Apr	10 May	5 Jun	7 July	8 Aug	9 Sep	6 Oct
Missenyi	Gabulanga	26 Jan	30-31 Jan	25 Mar	12 Apr	11 May	6 Jun	8 July	9 Aug	10 Sep	7 Oct
Bukoba Rural	Kangabusharo	27 Jan	1- 2 Feb	26 Mar	13 Apr	12 May	7 Jun	9 July	10 Aug	11 Sep	8 Oct
Chato	Nyamirembe	25 – 26 Jan	3 Feb	27 Mar	14 Apr	13 May	8 Jun	10 July	11 Aug	12 Sep	9 Oct
Nyang'hwale	Izunya	28 - 29 Jan	5 – 6 Feb	23 Mar	10 Apr	9 May	4 Jun	6 July	7 Aug	8 Sep	5 Oct
Geita Town Council	Igence	15 - 18 June	23 July	21 Aug	18 Sept	18 Oct	26 Nov				
Sengerema	Nyamatongo	8 - 10 Mar	13 Mar	17 Apr	15 May	13 June	17 July	18 Aug	15 Sep	17 Oct	28 Nov
Kwimba	Kilyaboya	8 Mar	12 Mar	18 Apr	19 May	14 June	18 July	15 Aug	14 Sep	16 Oct	27 Nov
Butiama	Bisumwa	8 – 10 Mar	15 -16 Mar	20 Apr	21 May	16 June	20 July	17 Aug	17 Sep	15 Oct	25 Nov
Musoma Rural	Etaro	8 Mar	14 Mar	19 Apr	20 May	15 June	19 July	16 Aug	16 Sep	14 Oct	24 Nov



# Annex B: Mortality Rate on Control Surfaces in the Sentinel Sites



# References

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Beier JC, Perkins PV, Wirtz RA, Koros J, Diggs D, Gargan TP, 2nd, Koech DK. 1988. Bloodmeal identification by direct enzyme-linked immunosorbent assay (ELISA), tested on *Anopheles* (Diptera: Culicidae) in Kenya. *J Med Entomol* 25:9-16.

Burkot TR, Williams JL, Schneider I. 1984. Identification of *Plasmodium falciparum*-infected mosquitoes by a double antibody enzyme-linked immunosorbent assay. *Am J Trop Med Hyg* 33:783-788.

Gillies MT, Furlong M. 1964. An investigation into the behaviour of *Anopheles parensis* Gillies at Malindi on the Kenya coast. *Bull Entomol Res* 55.

Mulamba C, Irving H, Riveron JM, Mukwaya LG, Birungi J, Wondji CS. 2014. Contrasting *Plasmodium* infection rates and insecticide susceptibility profiles between the sympatric sibling species *Anopheles parensis* and *Anopheles funestus* s.s: a potential challenge for malaria vector control in Uganda. *Parasit Vectors* 7:71.

Scott JA, Brogdon WG, Collins FH. 1993. Identification of single specimens of the *Anopheles gambiae* complex by the polymerase chain reaction. *Am J Trop Med Hyg* 49:520-529.

Silver JB. 2008. Mosquito ecology: field sampling methods. New York: Springer.

Wirtz RA, Zavala F, Charoenvit Y, Campbell GH, Burkot TR, Schneider I, Esser KM, Beaudoin RL, Andre RG. 1987. Comparative testing of monoclonal antibodies against *Plasmodium falciparum* sporozoites for ELISA development. *Bull World Health Organ* 65:39-45.

Wilkins EE, Howell PI, Benedict MQ. 2006. IMP PCR primers detect single nucleotide polymorphisms for *Anopheles gambiae* species identification, Mopti and Savanna rDNA types, and resistance to dieldrin in *Anopheles arabiensis* *Malar J*, 5:125.

