

USAID Okoa Maisha Dhibiti Malaria (OMDM)

Entomological Surveillance in Lake Zone Regions of Mainland Tanzania: Year 4 Annual Report

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ENTOMOLOGICAL SURVEILLANCE IN LAKE ZONE REGIONS OF MAINLAND TANZANIA: YEAR 4 ANNUAL REPORT

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TABLE OF CONTENTS

LIST OF FIGURES

[Table 1. Entomological surveillance and insecticide residual efficacy monitoring sites](#page-9-0) 3 [Table 2. Mosquito collection methods used for entomological surveillance](#page-14-0) 8 [Table 3. Number of Anopheles collected by collection method and location](#page-25-0) 19 [Table 4.Table showing other anopheline species collected](#page-26-0) 20

ABBREVIATIONS AND ACRONYMS

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EXECUTIVE SUMMARY

To define the entomological impact of indoor residual spraying (IRS) of households (HHs) with insecticide with clothianidin (SumiShield® 50WG) and Fludora® Fusion, the U.S. President's Malaria Initiative (PMI) supported the USAID Okoa Maisha Dhibiti Malaria (OMDM) activity to carry out entomological surveillance and insecticide residual efficacy testing in IRS and non-IRS districts of the northwestern Lake Zone Regions.

Data included in this report were collected from 10 field sites; 6 of these sites were in 6 districts where IRS operations had been conducted in October and November 2021, and the remaining 4 sites were control sites where IRS operations had not been conducted.

For insecticide residual efficacy testing, cone and fumigant bioassays were carried out as per standard World Health Organization (WHO) protocol in randomly selected HHs that had been sprayed with clothianidin or Fludora® Fusion during programmatic IRS operations. For entomological surveillance activities, mosquito-collection techniques incorporated U.S. Centers for Disease Control and Prevention (CDC) light traps, clay pots, Prokopack aspirators, and CDC light traps with collection bottle rotators (CBRs) with monthly mosquito collections conducted in all study sites. The National Institute for Medical Research (NIMR) Mwanza Centre conducted all entomological activities.

Insecticide residual efficacy. Fludora® Fusion was retained effectively: all wall surfaces showed ≥80% mortality nine months after IRS. Clothianidin was also retained well: its sprayed wall surfaces showed ≥80% mortality eight months after IRS.

A total of 15,593 female *Anopheles* mosquitoes were collected by all collection methods from October 2021 through September 2022 in all districts, both those with IRS and those without. Of those, 4,604 (29.5%) were morphologically identified as *An. gambiae s.l.*; 10,204 (65.4%) as *An. funestus s.l.*; 411 (2.6%) as *An. coustani*; 323 (2.1%) as *An. pharoensis*; and 51 (0.3%) as *An. rufipes*. *An. gambiae* and *An. funestus* complex mosquitoes were the vast majority (14,808 or 95%) of those captured, with 7,550 (51%) of them captured by CDC light traps; 3,305 (22.3%) by CBR; 2,577 (17.4%) by Prokopack aspirator; and 1,376 (9.3%) by clay pots.

An. gambiae s.l. was the most abundant vector species sampled by all collection methods in the sprayed sites, with the exception of the Biharamulo, Kibondo, and Kasulu District Councils (DCs). Whereas in all the unsprayed sites, *An. funestus s.l.* was the main vector species collected. The percentage of parity was lower in sprayed sites compared to the unsprayed sites.

Identification of species by polymerase chain reaction (PCR)–based methods showed the vector population across sites to be predominantly *An. funestus s.s.* (8,505; 64.6%), *An. arabiensis* (3,013; 22.9%), *An. gambiae s.s.* (1,014; 7.7%), and *An. parensis* (48; 0.4%). Of the processed samples, 591 (4.5%) were not amplified by *An. gambiae s.l.* and *An. funestus s.l.* PCR. In sprayed sites the collected mosquito species varied. *An. gambiae s.s.* was the abundant collected species in Kakonko and Ukerewe sites while *An. arabiensis* was the most common species in Bukombe DC. *An. funestus s.s.* was found in most abundance in the Kibondo DC, Kasulu DC, and Biharamulo sentinel sites. A limited number of *An. parensis* was found in all the sentinel sites except Ukerewe. Moreover, in unsprayed sites *An. funestus s.s.* was the most dominant species.

Overall, sporozoite rates varied across the sites, ranging 0%–1.2 % in sprayed sites and 0.5%–2.6% in unsprayed sites. Sporozoite rate was higher in unsprayed sites (1%) compared to sprayed sites (0.3 %), and the observed difference was statistically significant with p < 0.00001. The highest of the sporozoites were found in *An. funestus s.s.* (1.1%) among the *Anopheles* species. The highest proportion of *An. gambiae s.l.* was observed indoors in sprayed sentinel sites, and the indoor biting rate (IBR) lowered post-IRS operations throughout the year except in Ukerewe, Kibondo, and Bukombe DCs. Among unsprayed sentinel sites *An. funestus s.l.* dominated in IBR.

In sprayed sites after IRS application, the level of *An. funestus s.l.* mean bites per person per night both indoors and outdoors was quite low compared with *An. gambiae s.l.*, whose peak biting hours after IRS were 11:00 p.m.–12:00 a.m. indoors and 12:00 a.m.–1:00 a.m. outdoors. Generally, there was a higher number of indoors and outdoors *An. funestus s.l.* in unsprayed sites compared with sprayed sites. Mean bites per person per hour were below 0.2 before and after deployment of IRS in contrast to the unsprayed sites.

Data showed a decrease in indoor resting density (IRD) nine to ten months after IRS operations were conducted in all districts, except for Bukombe, Kibondo, Kakonko, and Ukerewe DCs, which subsequently had the highest number of mosquitoes (mostly *An. gambiae s.l.*) resting indoors over the measured months. By contrast, the data showed higher observed trends of IRD in non-IRS districts, especially in Geita and Muleba DCs, throughout the entire monitoring period.

The majority of the tested blood-meal hosts indicated that *An. funestus s.s.* was the major vector whose blood-meal source was found to be human. In unsprayed sites the highest 12 month EIR was 58.1, unlike the sprayed sites where the highest EIR was 3.0.

The study indicates that continuous rounds of IRS using various insecticides has been fruitful in reducing the *An. gambiae s.l.* and *An. funestus s.l.* abundance and sporozoite rate. IRS contributed to reducing the sporozoite rate and significantly decreased the EIR.

1. INTRODUCTION

The Indoor Residual Spraying (IRS) program in Tanzania is a joint U.S. Government and Government of Tanzania initiative and is part of the U.S. President's Malaria Initiative (PMI) to reduce the impact of malaria in sub-Saharan African countries. RTI, through the PMIfunded Okoa Maisha Dhibiti Malaria (OMDM) activity, is in its fourth year of implementation, including entomological monitoring in IRS-targeted districts in the Lake Zone. OMDM 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.

In this report are the results of the assessment of the quality of IRS operations conducted in study sites (Table 1) and entomological monitoring. National Institute for Medical Research (NIMR) Mwanza staff conducted entomological monitoring, a vital component of any malaria prevention and control program, to evaluate the efficacy of the IRS operations, justify the selection of insecticides and target spray areas, and monitor the behavioral and ecological response of vector species to IRS operations.

During the period of October 2021–September 2022, NIMR Mwanza carried out the following entomological monitoring activities as per the fiscal year (FY) 2021–2022 work plan:

- 1. Identification of malaria vector species in IRS intervention and control districts
- 2. Assessment of vector ecology: density, distribution, and seasonality in intervention and control sentinel sites
- 3. Monitoring of vector feeding and resting behavior in designated sites across the IRS intervention and control districts
- 4. Assessment of insecticide residual efficacy after IRS using cone wall bioassays
- 5. Rearing and maintaining a colony of susceptible *An. gambiae* s.s. (Kisumu strain) in NIMR Mwanza's insectary.

In this report are the results of IRS-operations-related entomological monitoring activities carried out from October 2021–September 2022.

2. METHODS

2.1 Study Sites

Entomological data were collected October 2021–September 2022 from 10 villages in sentinel districts (Table 1 presents a summary of activities, and Figure 1 shows geographical locations).

Table 1. Entomological surveillance and insecticide residual efficacy monitoring sites

Figure 1. Map showing the distribution of 10 districts used in entomological surveillance and insecticide residual efficacy monitoring

2.2 Rearing of Susceptible *An. gambiae s.s.* **(Kisumu Strain)**

Adult *An. gambiae s.s.* of the susceptible Kisumu strain had their numbers increased to meet the demand of field activities involving monthly cone wall bioassays and were reared according to standard protocol at the insectary of NIMR Mwanza.[1] The adult mosquito room was maintained at $27 \pm 1^{\circ}$ C and 60%–80% relative humidity; the larval room environment was maintained at 30 \pm 1°C and 60%–80% relative humidity.

2.3 Insecticide Residual Efficacy Monitoring

Cone bioassays were carried out as per the standard WHO protocol.[2] The tests were carried out using two to five-day-old, sucrose-fed, and laboratory-reared, known susceptible *An. gambiae s.s.* Kisumu strain mosquitoes. Batches of 10 female mosquitoes were exposed for 30 minutes inside a WHO plastic cone on sprayed wall surfaces in each of the rooms and houses sampled (Figure 2). Generally, clothianidin is regarded as a slow-acting insecticide; Fludora® Fusion contains a mixture of clothianidin and deltamethrin. The usual WHO protocol for cone bioassays was modified so that mortality was recorded every 24 hours for 6 consecutive days after insecticide exposure; exposure time remained at 30 minutes. For the initial IRS quality assessment, three locations on walls were sampled: low (0.5 m above the floor), middle (1.0 m above the floor), and upper level (1.5 m above the floor). After the initial IRS quality assessment, measuring insecticide residual efficacy was limited to one sampled room, and two locations on the walls were sampled: low (0.5 m above the floor) and upper level (1.5 m above the floor).

A control cone bioassay was done for every house tested by exposing mosquitoes to an unsprayed surface of a similar wall material. Some insecticide formulations used for IRS, such as clothianidin and pirimiphos-methyl, have a fumigant airborne effect that can last for several months after spraying. A strong fumigant effect in sprayed rooms can result in cone bioassays on unsprayed walls showing 100% mosquito mortality. To avoid the possibility of control mortality increasing because of clothianidin's fumigant effect, bioassays on the unsprayed portable surface were conducted outside sprayed houses in the shade to avoid mosquito mortality from direct sunlight's heat (Figure 2E). To test the airborne efficacy of clothianidin and Fludora® Fusion, fumigant assays using nets suspended in a wire cylinder were carried out in all villages inside and outside of sampled houses (Figures 2F and 2G).

At the end of each bioassay, mosquitoes were transferred using an aspirator to paper cups and supplied with a 10% glucose solution. Cups were placed in a cool box that was covered with a wet towel. Knockdown was assessed 60 minutes after the end of exposure. A mosquito was considered alive if it could fly. When control mortality was 5%–20%, experimental mortality was corrected using Abbott's formula.[3]

Figure 2. Different wall surfaces during testing

2.4 Vector Ecology

Entomological surveillance was carried out to determine vector ecology in IRS and non-IRS sites.

The entomological sampling methods used in all sites comprised Centers for Disease Control and Prevention (CDC) light traps, clay pots, Prokopack aspirators, and CDC light traps with collection bottle rotators (CBRs). These traps were utilized to collect adult mosquitoes to determine basic entomological indicators, including species distribution and abundance, resting behavior, feeding and biting behavior, and seasonality (Table 2).

Table 2. Mosquito collection methods used for entomological surveillance

Identification of the collected specimens was done in the field, and mosquitoes were sorted by species according to standard morphological keys.[4] Subsamples of host-seeking females were dissected for determination of the parity rate. Blood-fed females were independently preserved in filter paper wraps to determine the blood-meal source, while unfed females were preserved for further laboratory analysis, including species-specific identification and detection of malaria infection.

2.4.1 CDC light trap

This collection method is used for indoor biting (endophagic) mosquitoes and can be seen in the top left image of Figure 3. Two houses per night were randomly selected in each village of a study district, with CDC light traps in place on 28 consecutive nights of each month. The traps were installed about 1.5 meters above the floor next to the head of a sleeping person in the room. Each person was requested to sleep under an untreated mosquito net overnight. The CDC light traps were set to trap mosquitoes overnight, from 6:00 p.m. through 6:00 a.m. Captured mosquitoes were transferred separately into mosquito-holding cups.

Figure 3. Entomological sampling methods

(Clockwise from top left) CDC light trap, clay pot, CBR, and Prokopack aspirator.

2.4.2 Clay pot method

This collection method is used for outdoor resting (exophilic) mosquitoes. Local clay pots (molded from clay soil, diameter of \sim 0.5 m, an opening of 20 cm, and a 2-cm hole at the bottom allowing rainwater to drain) were used to collect outdoor resting mosquitoes. Four clay pots were positioned outdoors overnight from 6:00 p.m. to 6:00 a.m.; houses were the same ones that were sampled using CDC light traps. The pots were positioned at an inclined angle to let mosquitoes enter and rest inside the dark inner wall surface of the pot. At 6:00 a.m., community mosquito collectors covered the openings with netting that had a small hole for inserting an aspirator to suck out mosquitoes and transfer them into a mosquito-holding cup.

2.4.3 Prokopack aspirator

This collection method is used to sample indoor resting (endophilic) mosquitoes. The Improved Prokopack Aspirator Model 1419 was used. Mosquitoes were collected from five selected houses (two of the houses were selected for the CDC light tap collection; three more were randomly selected houses) for 10 days a month. Aspiration was carried out between 6:00 a.m. and 8:00 a.m. 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. The number of people who slept in the house the previous night was recorded on the data sheet.

The mosquitoes were put in clearly labeled moist Petri dishes and taken to the field office where they were sorted morphologically by species. The abdominal status of all female anophelines collected was noted, and mosquitoes were sorted into one of the following categories: gravid, semi-gravid, unfed, and blood-fed. The collected mosquitoes were preserved for later molecular assay analysis to identify the sibling species and determine malaria infection rates. The preserved mosquitoes were also subjected to enzyme-linked immunosorbent assays (ELISAs) to identify the source of the blood meal.

2.4.4 CBRs

This collection method is used to monitor indoor and outdoor mosquito biting times. Use of CBRs has replaced human landing catches because of the ethical concerns with using human subjects. One CBR was placed indoors and outdoors, respectively, in a randomly selected house.

CBR sampling was conducted over 10 nights each month, 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. Moonlight can affect the biting behavior of mosquitoes and therefore the collections.[5] An estimate of the presence and period of moonlight was calculated using a lunar calendar.[6] It was assumed that the mosquitoes entering a trap were those actively seeking hosts and who in most cases would have bitten human hosts in the same hour and house if the bed-net trap had been absent. The indoor and outdoor human biting rates and bite timing of the *Anopheles* mosquitoes were determined and recorded throughout the whole sampling period.

CBRs were set indoors with a person sleeping under an untreated net and outdoors from 6:00 p.m. to 6:00 a.m.; the collection of mosquitoes occurred in an interval of one hour. Samples of anophelines were preserved in a 1.5-ml Eppendorf tube in silica gel for further ELISA and molecular analysis.

2.5 Laboratory Analyses

In the field, mosquitoes were morphologically identified, labeled, and transported to the NIMR Mwanza Centre laboratory for further analyses. An ELISA circumsporozoite assay was used to determine the sporozoite index in 13,171 samples of collected *An. gambiae s.l.* and *An. funestus s.l.*[7,8] A PCR-based assay was used to differentiate sibling species of *An. gambiae s.l.*[9] and *An. funestus s.l.*[10] mosquitoes.

Furthermore, blood-meal analysis to determine host preferences of collected mosquitoes was performed using a direct ELISA.[11] Mosquitoes for blood-meal analysis were obtained from clay pots, Prokopack aspirators, and CBRs.

2.6 Rainfall Data

Rainfall data to align with mosquito monthly abundance were accessed via an online database.[12]

2.7 Data Analysis

Vector density was calculated as the number of adult female vectors collected per sampling method and unit time. Indoor biting rate (IBR) was determined as a proportion of adult female vectors that attempted to feed or were freshly blood-fed per person per unit time. Biting time was calculated as the number of adult female vectors that attempted to feed or

were freshly blood-fed per person per night. Indoor resting density (IRD) was calculated as the proportion of adult female vectors collected resting indoors by Prokopack aspirators.

3. RESULTS

3.1 Insecticide Residual Efficacy Monitoring

3.1.1 Residual efficacy of Fludora® Fusion and clothianidin against susceptible An. gambiae s.s. in cone wall bioassays

Figures 4–9 show the results of the cone wall bioassays by site and month postexposure to Fludora® Fusion–treated walls (Kakonko District Council [DC], Kibondo DC, and Kasulu DC) or clothianidin-treated walls (Biharamulo DC, Bukombe DC, and Ukerewe DC). Day 6 on the x-axes refers to when total mortality was determined with measurements being taken every 24 hours for 6 consecutive days after insecticide exposure.

Fludora® Fusion was retained effectively, with all wall surfaces showing ≥80% mortality on Day 6 for 9 months after IRS. Clothianidin was also retained effectively, with all wall surfaces showing ≥80% mortality on Day 6 for 8 months after IRS. The detailed results of cone wall bioassays for each district are shown in **Annex 1**.

Figure 5. Residual efficacy of Fludora® Fusion for An. gambiae s.s. for different wall surfaces in Kibondo DC

Figure 6. Residual efficacy of Fludora® Fusion for An. gambiae s.s. for different wall surfaces in Kasulu DC

■Mud ■Cement ■Painted ■White wash ■Burnt brick

Figure 7. Residual efficacy of clothianidin for An. gambiae s.s. for different wall surfaces in Biharamulo DC

Figure 8. Residual efficacy of clothianidin for An. gambiae s.s. for different wall surfaces in Ukerewe DC

3.1.2 Residual efficacy of Fludora® Fusion and clothianidin against susceptible An. gambiae s.s. in fumigant bioassays

Figures 10–15 show the results of the fumigant bioassays by site and month postexposure to Fludora® Fusion and clothianidin-treated walls. Day 6 on the x-axes refers to when total mortality was determined, with measurements taken every 24 hours for 6 consecutive days after insecticide exposure.

The insecticide airborne effect in all treated wall surfaces eight months post-IRS with Fludora® Fusion was retained to 100% mortality on Day 6 at all sites. Similarly, seven months post-IRS with clothianidin the insecticide airborne effect in all treated surfaces was 100% mortality on Day 6 at all sites. The details of results of cone fumigant bioassays for each district are shown in Annex 2.

Figure 10. Residual efficacy of Fludora® Fusion for An. gambiae s.s. exposed on fumigant assay in Kakonko DC

Figure 11. Residual efficacy of Fludora® Fusion for An. gambiae s.s. exposed on fumigant assay in Kibondo DC

Figure 12. Residual efficacy of Fludora® Fusion for An. gambiae s.s. exposed on fumigant assay in Kasulu DC

Figure 13. Residual efficacy of clothianidin for An. gambiae s.s. exposed on fumigant assay in Biharamulo DC

Figure 14. Residual efficacy of clothianidin for An. gambiae s.s. exposed on fumigant assay in Ukerewe DC

Figure 15. Residual efficacy of clothianidin for An. gambiae s.s. exposed on fumigant assay in Bukombe DC

3.2 Vector Ecology

3.2.1 Abundance, distribution, and species composition

A total of 15,593 female *Anopheles* mosquitoes were collected by all collection methods from October 2021 to September 2022 in all sprayed and unsprayed districts. A sum of 4,604 (29.5%) were morphologically identified as *An. gambiae s.l.*; 10,204 (65.4%) as *An. funestus s.l.*; 411 (2.6%) as *An. coustani*; 323 (2.1%) as *An. pharoensis*; and 51 (0.3%) as *An. rufipes*.

CDC light traps collected 7,550 (51% of total) female *An. gambiae* and *An. funestus* complex mosquitoes, CBRs collected 3,305 (22.3%), Prokopack aspirators collected 2,577 (17.4%), and clay pots collected 1,376 (9.3%) (Table 3).

An. gambiae s.l. was the most abundant vector species complex sampled by all collection methods in all sprayed sites except Biharamulo, Kibondo, and Kasulu DCs. Whereas in all the unsprayed sites, *An. funestus s.l.* was the main vector species complex collected.

A summary of other anopheline species collected during the period in both sprayed and unsprayed areas is indicated in Table 4.

Abbreviations: *N,* Number collected; (*n*), mean number per trap night

Table 4.Table showing other anopheline species collected

3.2.2 Parity

Ten percent of unfed mosquitoes collected from all traps from October 2021 to September 2022 were dissected to determine whether they were parous or nulliparous before and after IRS intervention. Dissection results show that Biharamulo had the highest percentage of parity pre-IRS among other intervention sites while Geita DC had the highest percentage of parity against all other unsprayed sites. Results indicate that after deployment of IRS the percentage of parity significantly decreased in Biharamulo (Table 5).

Table 5. Parity results in both sprayed and unsprayed sentinel sites pre- and post-IRS

3.2.3 Molecular analysis of mosquito species composition and sporozoite rate

Molecular analysis of mosquito species

A total of 13,171 mosquito samples were analyzed by PCR for speciation and ELISA for detection of sporozoites (Table 6).

Mosquito species composition

PCR showed the local vector population across sites to be *An. funestus s.s.* with 8,505 (64.6%); *An. arabiensis* 3,013 (22.9%); *An. gambiae s.s.* 1,014 (7.7%); and *An. parensis* 48 (0.4%). Of the processed samples, 591 (4.5%) were not amplified by *An. gambiae s.l.* and *An. funestus s.l.* PCR (Table 6).

Furthermore, the species collected in sprayed sites varied. *An. gambiae s.s.* was the main species in Kakonko and Ukerewe sites, while *An. arabiensis* was the most abundant species in Bukombe DC. *An. funestus s.s.* was found in most abundance in the Kibondo DC, Kasulu DC, and Biharamulo sentinel sites. A limited number of *An. parensis* was found in all the sentinel sites except Ukerewe. However, *An. funestus s.s.* was the most dominant species in all the unsprayed sites (Table 6).

The different mosquito species and the sporozoite rate collected by CBR traps to assess the human biting rate are indicated in Table 7. All mosquitoes collected in sprayed sites had no sporozoite unlike to unsprayed sites in which the sporozoite rate ranged 0.7% to 5% in Bunda and Kasulu TC sentinel sites, respectively.

Table 6. Results on species identification by PCR and sporozoite ELISA results in sprayed and unsprayed districts

Mosquito sporozoite rate

Overall, sporozoite rates were found to vary across the sites, ranging 0%–1.2 % in sprayed sites and 0.5%–2.6% in unsprayed sites. Sporozoite rate was higher in unsprayed sites (1%) compared to sprayed sites (0.3 %), and the observed difference was statistically significant with p < 0.00001(Table 6). Further analysis to the species level showed that the highest of the sporozoites were found in *An. funestus s.s.* (1.1%) among the *Anopheles* species (Table 8).

Mosquito species	Number of samples analyzed	Number of sporozoite- positive samples	Sporozoite rate (%)
An. gambiae s.s.	1014		0.4
An. arabiensis	3013		0.1
An. funestus s.s.	8505	94	1.1
An. parensis	48		0.0
Unidentified by PCR	591		0.0

Table 8. Sporozoite results by PCR-identified mosquito species

As shown in Table 9, preliminary results indicate that the sporozoite rates for *An. gambiae s.s.* were significantly higher in unsprayed (1.8%) than in sprayed sites (0.0%) (P = 0.0002). Although, the sporozoite rate of *An. funestus* was higher in unsprayed sites (1.1%) than in sprayed sites (0.9%), the difference was not statistically significant ($P = 0.5395$).

Mosquito species	Spray status	Number of samples analyzed	Number of sporozoite- positive samples	Sporozoite rate $(\%)$	P-value
An. Gambiae s.s.	Sprayed	793	0	0.0	0.0002
	Unsprayed	221	$\overline{4}$	1.8	
An. Arabiensis	Sprayed	1,813	Ω	0.0	
	Unsprayed	1,200	3	0.3	
An. funestus s.s.	Sprayed	1,156	10	0.9	0.5395
	Unsprayed	7,349	84	1.1	
An. parensis	Sprayed	13	Ω	0.0	
	Unsprayed	35	0	0.0	
Unidentified by PCR	Sprayed	160	0	0.0	
	Unsprayed	431	0	0.0	

Table 9. Species sporozoite results in sprayed and unsprayed sites

Blood-Meal Analysis

A total of 29 samples were analyzed to determine their blood-meal source. Out of those, 20 were from sprayed sites and nine from unsprayed sites. The samples were from six sites, four from sprayed sites (Bukombe, Ukerewe, Kasulu, and Kibondo DCs) and two from unsprayed sites (Geita and Bunda DCs). There were no samples from Kasulu TC, Muleba, Kakonko, and Biharamulo DCs. Human, cow, goat, and dog antibodies were used for bloodmeal analysis. Table 10 shows the species composition for mosquitoes analyzed for their blood meal, and Table 11 shows host preference of the analyzed mosquitoes through ELISA to determine the blood meal, hence the use of antibodies and not primers. Overall, the proportion of *Anopheles* that fed exclusively on humans was 34.5% (10/29). Molecular species identification indicated that the majority tested for blood-meal host were *An. funestus s.s.* (65.5%), followed by *An. arabiensis* (17.2%). All *An. arabiensis* analyzed fed on both humans and animals, showing opportunistic feeding behavior (Table 11).

Table 11. Table showing blood-meal source, species, and host preference

3.2.4 Biting and resting behavior

IBR of An. gambiae s.l. and An. funestus s.l.

IBR lowered in all sprayed sentinel sites except for Ukerewe, Kibondo, and Bukombe DCs where the highest proportion of *An. gambiae s.l.* was observed. Bunda DC had the highest IBR among unsprayed sentinel sites, with *An. funestus s.l.* exhibiting the highest IBR in unsprayed sites (Figure 16).

Figure 16. Monthly IBR (using CDC light traps) of Anopheles mosquitoes in sprayed (n = 6), and unsprayed (n = 4)

Arrows indicate when IRS operations were carried out.

Biting times of An. gambiae s.l. and An. funestus s.l.

In sprayed sites post-IRS, the level of *An. funestus s.l.* mean bites per person per night both indoors and outdoors was quite low compared with *An. gambiae s.l.* Generally, there was a higher number of indoor and outdoor *An. funestus s.l.* in unsprayed sites compared to sprayed sites (Figure 17). The biting rate of *An. funestus* complex indoors peaked at 12:00 a.m.–1:00 a.m. and 2:00 a.m.–3:00 a.m., while outdoors peaked at 11:00 p.m.–12:00 a.m. and 2:00 a.m.–3:00 a.m. in control sites. Moreover the biting rate of *An. gambiae* complex indoors peaked at 11:00 p.m.–12:00 a.m. while outdoors peaked at 10:00 p.m.–11:00 p.m.

After IRS the biting rate of *An. gambiae* complex indoors peaked at 11:00 p.m.–12:00 a.m., while outdoors peaked at 12:00 a.m.–1:00 a.m. Biting rate of *An. funestus* complex outdoors peaked at 1:00 a.m.–2:00 a.m. but indoors 7:00 p.m.–8:00 p.m. and 10:00 p.m.–11:00 p.m. (Figure 17). Mean indoor and outdoor biting rates of Anopheles collected by CBR in sprayed and unsprayed districts

IBR for both *An. gambiae s.l.* and *An. funestus s.l.* was higher in unsprayed sites compared to sprayed sites except for Bukombe DC, which had a slight increase of IBR, especially in *An. gambiae s.l.*, which were confirmed to be mostly *An. arabiensis* post-PCR (Table12). This confirms the zoophilic and anthropophilic feeding tendencies of *An. arabiensis*.

District	INDOOR CBR [N(n)]		OUTDOOR CBR[N(n)]	
	NUMBER OF Anopheles gambiae s.l.	NUMBER OF Anopheles funestus s.l.	NUMBER OF Anopheles gambiae s.l.	NUMBER OF Anopheles funestus s.l.
Biharamulo	20(0.2)	23(0.2)	14(0.1)	33(0.3)
Ukerewe	28(0.2)	2(0.0)	5(0.0)	1(0.0)
Bukombe	129(1.1)	11(0.1)	192 (1.6)	7(0.1)
Kasulu DC	3(0.0)	31(0.3)	0(0.0)	6(0.1)
Kibondo	53 (0.4)	45(0.4)	42(0.4)	54(0.5)
Kakonko	54(0.5)	0(0.0)	6(0.1)	1(0.0)
Bunda*	66 (0.6)	286 (2.4)	33(0.3)	118(1.0)
Muleba*	43(0.4)	527(4.4)	9(0.1)	304(2.5)
Geita DC*	65(0.5)	256(2.1)	54(0.5)	150(1.3)

Table 12. Table showing biting rates for mosquitoes collected by CBR

* Control sites)

Abbreviations: *N,* Number collected; (*n*), mean number per trap night

IRD of An. gambiae s.l. and An. funestus s.l.

In most sprayed sites, with the exception of Biharamulo and Kibondo DCs, *An. gambiae s.l.* was dominant to *An. funestus s.l.* The sentinel sites of Bukombe, Kibondo, and Ukerewe DCs had the highest number of *An. gambiae s.l.* mosquitoes resting indoors (Figure 18). There were more *An. funestus s.l.* than *An. gambiae s.l.* in all unsprayed sites.

Figure 17. Monthly IRD (using Prokopack aspirators) of Anopheles mosquitoes in sprayed (n = 6), and unsprayed (n = 4) districts

Arrows indicate when IRS operations were carried out.

Entomological Inoculation Rate

Entomological inoculation rate is a measure of exposure to infectious mosquitoes. It is used to assess the impact of vector control on malaria parasite transmission and elimination from data collected both indoors and outdoors. Here CBR was used to collect mosquitoes to estimate EIR.

Overall, the entomological inoculation rate (EIR) was lower in sprayed sites when compared to the unsprayed from October 2021 to September 2022, with exception of Kibondo (Table 13).

Study site	IRS status	Sporozoite rate (%), (number of CBR positive/ number of CBR tested)	Human biting rate * (Total Anopheles collected/sampling duration)	EIR (Sporozoite rate X HBR X 365)
Biharamulo	Sprayed	(0/98)	0.8(98/120)	$\mathbf{0}$
Kasulu DC	Sprayed	(0/36)	0.3(36/120)	0
Kibondo	Sprayed	(1/183)	1.5 (183/120)	3.0
Kakonko	Sprayed	(0/64)	0.5(64/120)	0
Bukombe	Sprayed	(0/329)	2.7 (329/120)	0
Ukerewe	Sprayed	(0/35)	0.3(35/120)	0
Bunda	Unsprayed	(2/467)	3.9 (467/120)	6.1
Muleba	Unsprayed	(3/707)	5.9 (707/120)	9.1
Geita DC	Unsprayed	(4/498)	4.2 (498/120)	12.3
Kasulu TC	Unsprayed	(19/561) .	4.7 (561/120)	58.1

Table 13. Entomological inoculation rate for sprayed and unsprayed sites

*Note: The human biting rate was obtained from mosquitoes collected by CBR both indoor and outdoor

4. DISCUSSION AND CONCLUSION

The use of clothianidin and Fludora® Fusion as insecticides of choice during IRS operations in the six sprayed sites has shown to be efficacious. Fludora® Fusion was retained effectively, with all wall surfaces showing ≥80% mortality for nine months after IRS. Clothianidin was also retained effectively, with all wall surfaces showing ≥80% mortality for eight months after IRS.

Among the six sprayed sites, sporozoites were only found in Biharamulo DC (0.2%), Kibondo DC (0.6%), and Kasulu DC (1.2%). Overall, unsprayed sites had greater sporozoite rates when compared with the sprayed sites. Further analysis to the species level showed that within PCR-identified species the sporozoites were found only in *An. funestus s.s.*, *An. Arabiensis*, and *An. gambiae s.s.*, with *An. funestus s.s.* showing 0.9% sporozoite rate in sprayed sites and 1.1% in unsprayed sites. *An. arabiensis* exhibited no sporozoites in sprayed sites and a 0.3% sporozoite rate in unsprayed sites, while *An. gambiae s.s.* displayed no sporozoites in sprayed sites and a 1.8% sporozoite rate in unsprayed sites. Despite Bunda DC having the highest number of mosquitoes collected, the sporozoite rate was the lowest (0.5%) compared to other unsprayed sites, of which Kasulu TC had the highest (2.6%). All unsprayed sites had *An. funestus s.s.* as the dominant species. Meanwhile, 65.5% tested blood-meal host was *An. funestus s.s.* whose blood-meal source was found to be approximately 53% human.

An. gambiae s.l. was the most common mosquito type resting indoors in Ukerewe, Kibondo, Kakonko, and Bukombe DCs. The most *An. gambiae s.l.* biting indoors was found in Ukerewe, Kakonko, and Bukombe DCs. In Bukombe DC, *An. gambiae s.l.* had the highest IBR after IRS during the reporting period, unlike the rest of the sprayed sites. However, unsprayed sites had higher IBR and IRD when compared to sprayed sites. *An. gambiae s.l.* was observed to be dominant in sprayed sites compared to unsprayed sites, where *An. funestus s.l.* exhibited the highest IBR and IRD. Also, the mean bites per person per hour were below 0.2 before and after deployment of IRS in contrast to the unsprayed sites.

The impact on how IRS has affected the parity of mosquitoes post-IRS could have been much better if there were more than just one-month surveillance pre-IRS. The percentage of

parity pre-IRS was higher compared to post-IRS in all sprayed sites. The percentage of parity in unsprayed sites was higher with Geita DC taking the lead compared to the sprayed sites pre-IRS and post-IRS. Moreover, the EIR was lower in sprayed sites unlike the unsprayed sites with exception to Kibondo. In unsprayed sites the highest 12-month EIR was 58.1 (Kasulu TC), whereas in sprayed sites the highest EIR was 3.0 (Kibondo DC). These data indicate that IRS has reduced the level of human exposure to infective mosquito bites in sprayed sites.

Annex 1: Cone wall bioassay tests. Percentage mortality obtained for female An. gambiae s.s. Kisumu exposed on sprayed surfaces

Annex 2: Fumigant bioassay tests. Percentage mortality obtained for female An. gambiae s.s. Kisumu exposed on sprayed surfaces

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