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THE PMI VECTORLINK PROJECT

ZIMBABWE ANNUAL ENTOMOLOGICAL REPORT

MARCH 2022 – DECEMBER 2022

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ACRONYMS

AChE	Acetylcholinesterase
iAChE	Insensitive acetylcholinesterase
AU	Africa University
CDC	U.S. Centers for Disease Control and Prevention
CSP	Circumsporozite protein
DDT	Dichlorodiphenyltrichloroethane
DNA	Deoxyribonucleic acid
ELISA	Enzyme-linked immunosorbent assay
FF	Fludora® Fusion
HLC	Human landing catch
IRS	Indoor residual spraying
ITN	Insecticide-treated nets
kdr	Knockdown resistance
NIHR	National Institute of Health Research
NMCP	National Malaria Control Program
PCR	Polymerase chain reaction
PMI	President's Malaria Initiative
PPA	Prokopack aspirator
PSC	Pyrethrum spray catch
s.l.	*Sensu lato* (in the broad sense)
s.s.	*Sensu stricto* (in the narrow sense)
SOP	Standard operating procedure
UMP	Uzumba Maramba Pfungwe
USAID	United States Agency for International Development
VGSC	Voltage-gated sodium channel
WHO	World Health Organization

EXECUTIVE SUMMARY

With technical support from the U.S. President's Malaria Initiative (PMI), Zimbabwe's National Malaria Control Program (NMCP) implemented indoor residual spraying (IRS) in Uzumba-Maramba-Pfungwe (UMP), Mudzi and Mutoko districts in Mashonaland East Province in 2022.

PMI VectorLink implemented entomological monitoring for malaria vector control in Zimbabwe, in partnership with the National Institute of Health Research (NIHR), NMCP, and Provincial Medical Directorates. Monthly longitudinal vector surveillance was conducted at three sites in Mashonaland East Province, namely Dendera, Kawere, and Makarara. The residual efficacy of Fludora® Fusion and dichlorodiphenyltrichloroethane (DDT) was monitored at the PMI-supported districts in Mashonaland East for the 2021 IRS campaign, and quality of spray was assessed following the 2022 IRS campaign using DDT at the two sites. Insecticide resistance tests were conducted at Dendera, Kawere, and Makarara in Mashonaland East (alpha-cypermethrin, deltamethrin, permethrin, clothianidin and chlorfenapyr), and at Burma Valley in Manicaland (permethrin, deltamethrin and clothianidin). Most susceptibility tests were done on *An. gambiae* s.l., with a few done on *An. demeilloni* at Burma Valley while *An. funestus* s.l. has become scarce following IRS with pirimiphos-methyl and DDT.

Anopheles funestus s.l. and *An. gambiae* s.l. are the main malaria vectors in Zimbabwe. Overall, *An. gambiae* s.l. occurred in slightly greater abundance than *An. funestus* s.l. *An. gambiae* s.l. were more abundant than *An. funestus* s.l. at Kawere, whereas it was the reverse at Makarara. No *An. funestus* s.l. were collected from Dendera during the reporting period. *An. rufipes* was slightly more abundant than *An. pretoriensis* at Dendera and was more abundant than *An. gambiae* s.l. at Kawere. *An. rufipes* is a potential secondary malaria vector whereas *An. pretoriensis* is considered a non-vector. All species were found at low densities, probably due to the vector control interventions in place, in combination with less rains that affect availability and productivity of mosquito breeding sites.

Of the *An. funestus* s.l. species complex, *An. leesoni*, *An. parensis,* and *An. rivulorum* were found at Kawere and Makarara. *An. funestus* s.s. was conspicuous by its absence at all three sites. Two *An. gambiae* s.l. sibling species were recorded at all three longitudinal monitoring sites from adult collections: *An. arabiensis* and *An. quadriannulatus*. Two other sibling species were found from mosquitoes collected as larvae at Kawere (*An. gambiae* s.s. and *An. merus*) and Burma Valley (*An. merus*). Potential secondary vectors found occurring in low numbers included *An. rufipes* and *An. maculipalpis*. However, their propensity to feed on humans was low. *An. pretoriensis* was found at all sites but showed no tendency to feed on humans. Similarly, *An. quadriannulatus* tended to feed on animals except for one specimen from Makarara that had mixed human-goat blood.

Mosquito densities at all sentinel sites in Mashonaland East and Manicaland using all collection methods were generally low, which did not allow for definitive conclusions to be made about vector behavior and seasonal fluctuations, though U.S. Centers for Disease Control and Prevention (CDC) light trap collections and human landing collection (HLC) proxy collected more mosquitoes outdoors than indoors. Moreover, more mosquitoes were collected resting outdoors (from pit shelters by mouth aspiration) than indoors (Prokopack aspirator collections) which suggests a preference to feed and rest outdoors. The low human blood index in both the two main vectors and in other species suggests an opportunistic feeding behavior. None of the specimens were infected with *Plasmodium* parasites. However, there is need to continue monitoring both primary and secondary vectors to understand the ongoing albeit low, disease transmission caused by elusive vectors.

Wall cone bioassays conducted monthly following the 2021 IRS campaign at two sites in Mashonaland East showed a residual efficacy of Fludora® Fusion of at least 11 months at Dendera site and 10 months for DDT at Kawere site. Residual efficacy varied for DDT by wall surface type, with more fluctuations on cement and brick walls than on mud and painted walls. Residual efficacy of Fludora® Fusion was less varied on the four wall types in the observations completed.

Wall cone bioassays done in the week following the 2022 spray campaign with DDT indicated acceptable spray quality at Dendera and Kawere. The team subsequently monitored DDT residual efficacy for only one-month post-spray since project activities ended in December 2022.

The primary vector *An. gambiae* s.l. remains susceptible to alpha-cypermethrin, deltamethrin, permethrin, clothianidin and chlorfenapyr at the sites tested. In Mashonaland East, *An. gambiae* s.l. was susceptible to deltamethrin, permethrin and clothianidin at Dendera; to alpha-cypermethrin, deltamethrin, permethrin, clothianidin, and chlorfenapyr at Kawere. The observations apply to *An. gambiae* s.l. prior to species identification in the laboratory. Molecular species identification, subsequently, indicated that the proportion of the vector *An. arabiensis* was low, ranging from 0 to 15%, while the non-vector *An. quadriannulatus* was dominant. This observation underlines the value of laboratory analysis when monitoring insecticide resistance in local malaria vectors. Laboratory tests indicated absence of knockdown resistance (*kdr*) L1014S alleles, but low frequency (0.3%; n=375) of *kdr* (L1014F) resistant heterozygotes in *An. gambiae* s.l., specifically *An. arabiensis* from Kawere. Insensitive acetylcholinesterase (*Ace*-1) mutation was not analyzed during the reporting period owing to lack of controls. Susceptibility tests should be extended to localities where *An. arabiensis* is predominant, and to *An. rufipes* since this potential vector species is abundant at all three sites.

I. INTRODUCTION

Malaria is heterogeneously distributed in Zimbabwe, with most cases reported from three of the eight rural provinces: Manicaland, Mashonaland Central, and Mashonaland East. In Zimbabwe, malaria is transmitted by *Anopheles arabiensis*, *An. gambiae* s.s., and *An. funestus* s.s. More than 98% of cases are caused by *Plasmodium falciparum*, while *Plasmodium malariae* and *Plasmodium ovale* are responsible for the remainder. Following concerted efforts by stakeholders to prevent and control malaria transmission, the disease burden decreased from 32/1000 population in 2020 to 9/1000 in 2022 (NMCP 2022). Malaria remains one of the most important public health challenges in some parts of the country. To control malaria, Zimbabwe's the National Malaria Control Program (NMCP) relies on two core vector control strategies: deployment of indoor residual house-spraying (IRS) and distribution of insecticide-treated nets (ITNs). Though these two strategies are known to contribute immensely to the prevention, control, and elimination of malaria in most settings, their effectiveness depends greatly on monitoring the behavior and insecticide resistance status of local primary vectors, as well as human behavior. The country's Insecticide Resistance Management Plan guides the program to rotate insecticides for IRS every two years (between classes for which there is no reported cross resistance) although non-adherence with planned use of different insecticides has sometimes been observed due to logistical challenges. For instance, for 2021 IRS, Mudzi used dichlorodiphenyltrichloroethane (DDT), organophosphates (OP) and pyrethroid instead of the DDT that was scheduled. For 2022 IRS, both Mudzi and Mutoko were sprayed with pyrethroid instead of the planned DDT during the start of the 2022 IRS campaign due to logistical challenges in the provision of DDT from national level. Uzumba-Maramba-Pfungwe (UMP) District diverted from an all-pyrethroid 2022 IRS campaign and used DDT after sharing pyrethroid with Mudzi and Mutoko.

Regular entomological monitoring is important for evidenced-based selection and deployment of insecticides for IRS and ITNs because monitoring provides timely, key information on vector species composition and their distribution, resting and feeding behavior, and susceptibility to insecticides. Additionally, these investigations are helpful for monitoring emerging and re-emerging primary and secondary malaria vectors and assessing the role they play in disease transmission. Information collected through entomological monitoring helps program managers and implementers understand the spatial and temporal changes in vector species, quality of IRS application, residual efficacy of insecticides on sprayed surfaces, and effectiveness of vector control interventions deployed to interrupt malaria transmission.

The PMI-supported IRS and entomological surveillance under the Africa Indoor Residual Spraying Project operated from 2013 to February 2018. This support continued as the PMI VectorLink project, which started in March 2018. Prior to 2018, PMI supported IRS in four districts in Manicaland Province (Chimanimani, Mutare, Mutasa, and Nyanga). In 2018, the project transitioned support to two districts in Mashonaland East Province (Mudzi and Mutoko), which then shifted to technical support in five districts (Mudzi, Mutoko, UMP, Goromonzi and Murehwa) initially in 2021 and three districts (Mudzi, Mutoko and UMP) in 2022 as the other two districts transitioned from IRS to ITNs. This report focuses on activities completed from March 2022 to December 2022 under the PMI VectorLink Project. This is the final report on entomological surveillance under VectorLink as the project closes in Zimbabwe in February 2023. The objectives included the following:

1. Monitor spray quality and residual efficacy of Fludora® Fusion (clothianidin and deltamethrin combination for IRS) used in Mashonaland East in the 2021 IRS campaign, and of DDT used in the 2022 IRS campaign.
2. Perform annual insecticide susceptibility testing at four sites in Mashonaland East (three sites) and in Manicaland (one site) to inform vector control decision making.
3. Continue monthly vector bionomics monitoring at three sites in Mashonaland East to monitor the impact of IRS.

The project continued the collaboration with Africa University (AU), which provided support in analyzing mosquitoes to determine species identification, parasite infection (sporozoite rate), host choice, and target site resistance mechanisms. AU also provided all the mosquitoes for bioassays to monitor the residual efficacy of insecticides from the susceptible colony of *An. arabiensis* KGB strain.

2. MATERIALS AND METHODS

2.1 SITES

Entomological surveillance was conducted from March 2022 up to December 2022 in three sites in Mashonaland East Province (IRS sites of Dendera and Kawere, and control site of Makarara).

Insecticide susceptibility tests were conducted in two provinces: Mashonaland East and Manicaland. Wall cone bioassays were conducted to monitor residual efficacy of Fludora® Fusion in Mashonaland East, sprayed in Mudzi during the 2021 IRS campaign, and DDT for the 2022 IRS campaign, and for DDT in Mutoko, following the 2021 and 2022 IRS campaigns. Activities accomplished are outlined in Table 1 at sites shown in the map in Figure 1.

TABLE 1. SENTINEL SITES BY GEOGRAPHIC LOCATIONS AND ACTIVITIES MARCH 2022-DECEMBER 2022*

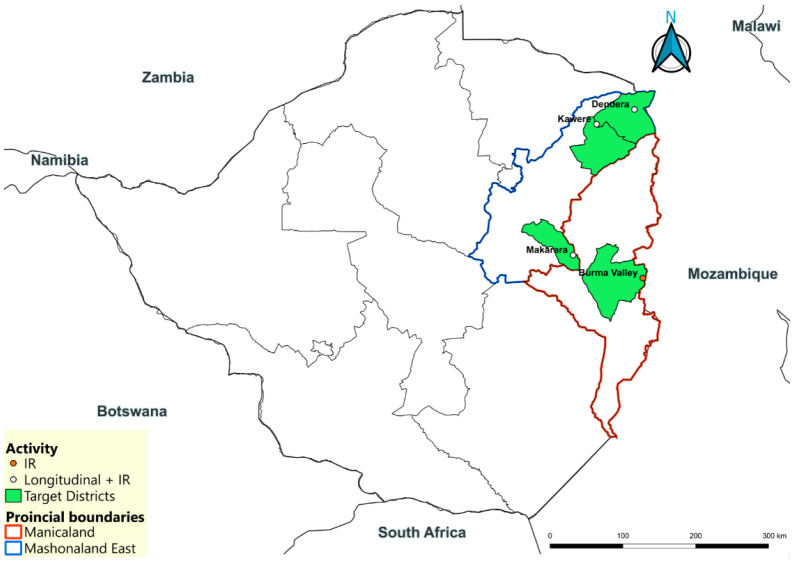
Province	District	Site	VC** intervention	Ento activity	M	A	M	Jn	Ju	A	S	O	N	D
Manicaland	Mutare	Burma Valley	2022: IRS with DDT and Fludora® Fusion	IR		X	X							X
Mashonaland East	Mudzi	Dendera***	2021 & 2022: IRS with Fludora® Fusion and DDT, respectively	VB	X	X	X	X	X	X	X	X	X	X
				CB	X	X	X	X	X	X	X	X	X	X
				IR	X	X								
	Mutoko	Kawere***	2021 IRS with DDT 2022: IRS with DDT	VB	X	X	X	X	X	X	X	X	X	X
				CB	X	X	X	X	X	X	X	X	X	X
				IR	X	X								
	Hwedza	Makarara	2022: ITNs	VB	X	X	X	X	X	X	X	X	X	X
				IR										X

Note:

**VC=vector control, IR=insecticide resistance testing, VB=vector bionomics, CB=cone bioassays

*** PMI IRS technical supported district

FIGURE 1. MAP OF ZIMBABWE SENTINEL SITES IN FOUR DISTRICTS, MARCH 2022-DECEMBER 2022



2.2 ROUTINE VECTOR BIONOMICS MONITORING

Mosquito collections were done to monitor vector bionomics at three sites in Mashonaland East Province (Dendera, Kawere, and Makarara) from March to December 2022. Prokopack aspirator collections (PPA) and pit shelters were used to assess indoor and outdoor resting site densities, respectively. Centers for Disease Control (CDC) light traps were used as a proxy for human landing catches (HLCs) to assess indoor and outdoor human biting rates. Mosquitoes from all collections were used to assess sporozoite infection rates (Table 2).

TABLE 2. SUMMARY OF COLLECTION METHODS

Collection method	Collection time	Frequency	Sample
PPA	6:00 am to 9:00am	Two days per site per month	25 houses per site
CDC light trap as HLC proxy	6:00 pm to 6:00 am, hourly	Two nights per site per month	Two houses per site per night, using four CDC light traps: two indoor, two outdoor
CDC light trap	6:00 pm to 6:00 am collection	Two nights per site per month	Two houses per site (two houses per night), using four CDC light traps: two indoor, two outdoor
Pit shelters	6:00 am to 9:00 am	Two days per site per month	Five pits at each site

All entomological monitoring at each sentinel site was conducted by teams consisting of staff from the National Institute of Health Research (NIHR), the Provincial Medical Directorates, PMI VectorLink, and local youths.

2.21 ESTIMATING INDOOR RESTING DENSITIES USING PPAS

Indoor resting mosquitoes were sampled from 25 houses (one sleeping room per house) per month at each of the vector bionomics monitoring collection sites, following Standard Operating Procedure (SOP) # 11 (PPA).[1] Mosquitoes collected from the different rooms were transferred to separate petri dishes. Each petri dish was labeled with the following information: location, household name, method of collection, and date. The abdominal stage of all female *Anopheles* was recorded as unfed, blood-fed, half-gravid, or gravid. Data on the number of people who slept in the house the previous night, the type of house and walls, and the number of

[1] Complete SOPs can be found here: https://pmivectorlink.org/resources/tools-and-innovations/

ITNs present were recorded on appropriate forms. All *Anopheles* mosquitoes collected were identified morphologically and preserved individually in silica gel for laboratory analysis.

2.2.2 Estimating Outdoor Resting Densities Using Pit Shelter Collections

Outdoor resting mosquitoes were sampled using five pit shelters per site. The pit shelters were dug at least 10m away from nearest household and fenced for human and animal safety. Each pit shelter had a depth of 2m, a 1.5m x 1.5m opening, and at least eight holes (2 holes x 4 sides) cut12-15 cm deep into the sides with a 10 cm wide opening for mosquitoes to enter and rest. Mosquito sampling was conducted following SOP #13.[1] The abdominal stage of all female *Anopheles* mosquitoes was recorded. All *Anopheles* mosquitoes collected were identified morphologically and preserved in silica gel for laboratory analysis.

2.2.3 Estimating Indoor and Outdoor Densities Using CDC Light Traps

A total of four battery-operated CDC light traps per site per month were used to collect mosquitoes from 6:00 p.m. to 6:00 a.m. for two consecutive nights, following SOP #1.[1] Two sentinel houses were randomly selected, with one trap placed indoors and one trap placed outdoors at each house. Households selected for PPA collections were excluded from the sampling pool, and the same houses were used for collections throughout the reporting period. Both indoor and outdoor CDC light traps were suspended 1m above the ground next to a person sleeping under an untreated mosquito net. Outdoor CDC light traps were installed about 10m away from the house and, when possible, in a shaded area. Traps were set at 6:00 p.m. and mosquitoes were collected from each of the traps at around 6:00 a.m. the following morning. The persons did not swap positions, from indoor to outdoor or vice versa, at hourly intervals due to COVID-19 regulations. All *Anopheles* mosquitoes collected were identified morphologically and preserved in silica gel for laboratory analysis.

2.2.4 Estimating Biting Time and Behavior Using CDC Light Trap Proxy

Hourly mosquito collections from CDC light traps with human bait were used as a proxy for HLCs to evaluate human-vector contact, including the place, time, and seasonal activity of the vectors. Traps were set indoor and outdoor on the same nights, alongside a human bait protected by an untreated mosquito net. The procedure is a modification of SOP #1. Houses used for PPAs and CDC light traps for density estimation were excluded from the sample. Collections were done over two consecutive nights at each site. In both indoor and outdoor collections, the light trap was set at the feet of a volunteer. For outdoor placement, light traps were set about 10m from the house. Due to COVID-19 regulations the volunteers did not swap positions, from indoors to outdoors or vice versa, at hourly intervals Mosquitoes were collected from each trap hourly from 6:00 p.m. to 6:00 a.m. The teams recorded temperature, relative humidity, wind status, and precipitation hourly during the night. All *Anopheles* mosquitoes collected were identified morphologically and preserved in silica gel for laboratory analysis.

2.3 Measuring Quality of Spray and Residual Efficacy

The quality of IRS application and insecticide residual efficacy of clothianidin-deltamethrin combination IRS (Fludora® Fusion) in Mudzi and DDT in Mutoko was measured using cone wall bioassays, in accordance with SOP #9[1] and following IRS at the two sites in October and November 2021, respectively. The final months of residual efficacy testing fell within this reporting period, thus the results in full are presented in this report. Bioassays were conducted within a week after spraying to assess the spray quality of the IRS operation and then monthly until the mean mortality rates fell below 80% for two consecutive months. Susceptible *An. arabiensis* (KGB strain), from the insectary at AU in Mutare were used to conduct the cone bioassays. One room in 10 different houses was tested at each site per month. The number of houses from each sentinel site by wall surface types, and insecticide sprayed are summarized in Table 3. The project conducted only two rounds of bioassays following the 2022 spray campaign; the first in November within one week of the IRS application, to assess quality of spray, and the second in December to monitor residual efficacy one month after spraying. As the project is closing out, no further rounds of bioassays are planned.

TABLE 3. SUMMARY OF WALL TYPES TESTED WITH CONE BIOASSAYS, MARCH 2022-SEPTEMBER 2022

Province	District	Sentinel site	Month Year sprayed	Insecticide sprayed	Type of wall	Number of houses
Mashonaland East	Mutoko	Kawere	November 2021	DDT	Mud	4
					Brick	1
					Cement	4
					Painted	1
			November 2022	DDT	Mud	4
					Brick	0
					Cement	2
					Painted	4
	Mudzi	Dendera	October 2021	Fludora® Fusion	Mud	2
					Brick	2
					Cement	4
					Painted	2
			November 2022	DDT	Mud	2
					Brick	3
					Cement	3
					Painted	2

2.3.1 WALL CONE BIOASSAY TESTS

For the wall cone bioassays, 10 unfed, two- to five-day-old female susceptible *An. arabiensis* (KGB strain) mosquitoes were exposed on the treated walls per cone following SOP #9. Three cones were randomly positioned per room at 0.5, 1.0, and 1.5 meters above the floor, all on one wall but within different spray swaths. These positions were marked and used in all subsequent tests. Mosquitoes were exposed for 30 minutes, after which they were transferred to a holding paper cup and provided with 10% sugar solution. Knockdown rates were recorded at 30 minutes (inside the cones) and 60 minutes (30 minutes after removal from the cones). Mortality was recorded at 24 hours after exposure for DDT and up to 120 hours for Fludora® Fusion. Controls were run concurrently using mosquitoes exposed to unsprayed surfaces in an unsprayed room. Temperature and relative humidity were recorded hourly during the exposure and three times per day during the subsequent post-exposure holding periods. Clothianidin (a constituent active ingredient in Fludora® Fusion) is a slow-acting insecticide, hence the extended observation period.

2.3.2 BIOASSAY TESTS TO ASSESS FUMIGANT EFFECT OF INSECTICIDE

Bioassays to assess the fumigant effect of Fludora® Fusion were conducted in each room where wall cone bioassay tests were done. Ten two- to-five-day-old unfed female mosquitoes placed in one paper cup per room were exposed for 30 minutes at the same time as the wall bioassay tests. The paper cup was held by a wire support, designed so it was 10 cm away from a sprayed wall and 1m above the floor. Mosquitoes were removed after 30 minutes, and knockdown recorded. They were then transferred to holding paper cups using a clean aspirator and provided with 10% sugar solution during the holding period. Mortality was recorded up to 120 hours. Controls for the bioassays were conducted simultaneously using a similar set-up, but in an unsprayed room. The fumigant effect was not determined for DDT since the insecticide is not known to have a pronounced airborne effect.

2.4 INSECTICIDE RESISTANCE MONITORING

Insecticide susceptibility testing was conducted at Dendera, Kawere, Makarara, and Burma Valley. Due to low larval availability, testing was extended across several months from March 2022 to December 2022. The insecticides (dosage) tested were:

1. Alpha-cypermethrin (12.5µg/mL)

2. Deltamethrin (12.5µg/mL)
3. Permethrin (21.5 µg/mL)
4. Clothianidin (4µg/mL)
5. Chlorfenapyr (100µg/mL)

Insecticide susceptibility tests were performed using *An. gambiae* s.l. raised from larvae for all sites except for one instance at Burma Valley (Table 4). At the time of conducting the tests, the proportion of the sibling species included in the assays is unknown. Previously the team has tested *An. funestus* s.l. susceptibility but the adult population of this species has since dwindled in Burma Valley following IRS with pirimiphos-methyl. It was planned to test *An. funestus* s.l. from Makarara but insufficient larvae were collected. The team tested the F1 generation of *An. demeilloni* raised from adults collected from pits at Burma Valley. Permethrin was the only insecticide tested on mosquitoes from Makarara. The number of insecticides tested at any given site was determined by the availability of mosquitoes.

TABLE 4. SUMMARY OF INSECTICIDES TESTED IN MONITORING SITES, MARCH 2022-DECEMBER 2022

Province	District	Sentinel site	Anopheles species	Deltamethrin	Clothianidin	Alpha-cypermethrin	Permethin	Chlorfenapyr
Mashonaland East	Mutoko	Kawere	*An. gambiae* s.l.	X	X	X		X
	Mudzi	Dendera		X	X		X	
	Hwedza	Makarara					X	
Manicaland	Mutare	Burma Valley	*An. gambiae* s.l.	X	X		X	
			An. demeilloni				X	

CDC bottle assays (SOP #4) were used to test all insecticides including clothianidin (mixed with acetone and 800ppm Mero surfactant). Four replicates of 25 female *An. gambiae* s.l., 2-5-day old, were exposed. Mortality was recorded at the diagnostic time: 30 minutes for pyrethroids, and 45 minutes for DDT. Chlorfenapyr is a slow-acting insecticides, hence mosquito mortalities were recorded every 24 hours up to three days after exposure.

2.5 LABORATORY ANALYSES

All laboratory analyses of mosquito specimens were conducted following established protocols at the AU molecular laboratory.

2.5.1 MOLECULAR IDENTIFICATION OF *ANOPHELES* SPECIES

Anopheles mosquitoes collected from all four sentinel sites (from all collection methods and from resistance tests) were analyzed for species identification using polymerase chain reaction (PCR) methods. Briefly, DNA was extracted from either single whole mosquitoes or available parts of single mosquitoes using standard extraction protocol which was replaced by the rapid protocol after May 2022 and amplified through PCR. The selection of the PCR protocol was based on morphological identification of the mosquito specimen done initially by the PMI VectorLink team and verified by the AU team. The protocol for *An. gambiae* s.l. is described by Wilkins et al. (2006), while the protocol for *An. funestus* s.l. is described by Koekemoer et al. (2002). For *An. rufipes*, *An. maculipalpis,* and *An. pretoriensis,* AU is using protocols developed by CDC.

2.5.2 Identification of Blood Meals

Mosquitoes collected and recorded as freshly fed or half-gravid from all adult collection methods were tested for the blood meal source using PCR (Kent and Norris 2005).

2.5.3 Sporozoite Rate

Mosquitoes collected from all adult collection methods during the reporting period were tested for sporozoite rate using enzyme-linked immunosorbent assay (ELISA). Specimens positive for circumsporozoite protein (CSP) with ELISA were subsequently processed by the boiling method (Durnez et al. 2011) and further analyzed by PCR to confirm *Plasmodium* infection.

2.5.4 KDR Assays

Target-site mutations encoding the voltage-gated sodium channel (VGSC) cause pyrethroid resistance and confer cross-resistance to the organochlorine DDT. The VGSC mutations are referred to as 'knockdown resistance' (*kdr*). The MR4[2] protocol was used for detecting *kdr* in *An. gambiae* s.l., specifically allele L1014F (*kdr* West) and L1014S (*kdr* East).

2.5.5 Ace-1 Assays

The presence of insensitive acetylcholinesterase (AChE) was determined in *An. gambiae* s.l. mosquitoes. The analysis detects the G119S mutation in the acetylcholinesterase (Ace-1) gene, a target site mutation that is associated with resistance to carbamates and organophosphates. The MR4 protocol was followed for the Ace-1 analysis.

[2] MR4: The Malaria Research and Reference Reagent Resource Center

3. RESULTS

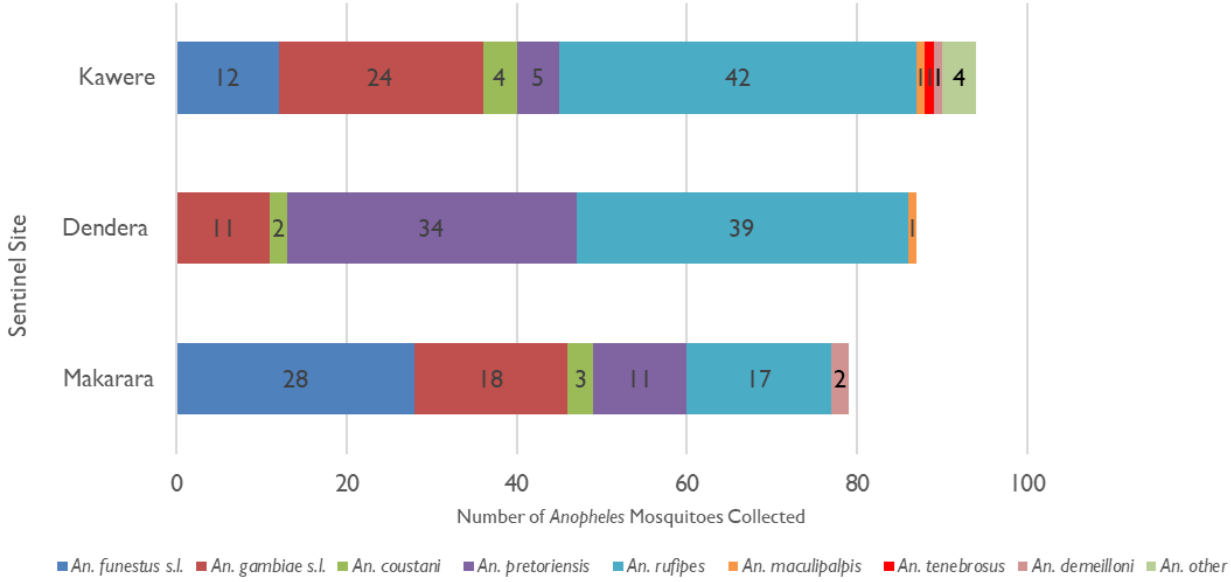
3.1 ROUTINE VECTOR BIONOMICS LONGITUDINAL MONITORING

Sentinel site Dendera (Mudzi District) was sprayed with Fludora® Fusion during the 2021 campaign, and with DDT for the 2022 IRS campaign. Sentinel site Kawere (Mutoko District) was sprayed with DDT consecutively during the 2021 and 2022 IRS campaigns. Makarara (Hwedza District) was not sprayed and served as the control site.

3.1.1 VECTOR COMPOSITION

A total of 260 female *Anopheles* mosquitoes were collected across all methods and sites combined, between March 2022 and December 2022. At all sites, five or more *Anopheles* species were collected, but the species composition varied between sites. *An. rufipes* was the predominant species at Kawere and Dendera, whereas *An. funestus* s.l. was the predominant species at Makarara (Figure 2). The second most common species at Kawere and Makarara was *An. gambiae* s.l., whereas it was *An. pretoriensis* at Dendera. *An. gambiae* s.l. and *An. funestus* s.l. are the major malaria vectors in Zimbabwe. Two species considered secondary malaria vectors, *An. coustani* and *An. rufipes,* and *An. pretoriensis,* a non-vector, were caught at Dendera, Kawere, and Makarara. Other species collected included *An. maculipalpis*, *An. tenebrosus* and *An. demeilloni*.

FIGURE 2. *ANOPHELES* SPECIES MORPHOLOGICAL COMPOSITION AT SENTINEL SITES (KAWERE, DENDERA AND MAKARARA) FROM ALL COLLECTION METHODS COMBINED WHERE N = NUMBER OF *ANOPHELES* MOSQUITOES PER SITE, MARCH 2022-DECEMBER 2022.



3.1.2 INDOOR RESTING DENSITIES

PPA collections caught no *Anopheles* mosquitoes throughout the monitoring period, except at Makarara, where a mean of 0.04 and 0.05 *An. gambiae* s.l. /house/night were caught in May and November respectively.

3.1.3 Outdoor Resting Densities

The mean number of *Anopheles* mosquitoes collected outdoors with the pit shelter collection (Table 5) was more than from indoors with PPA. *An. gambiae* s.l. was collected resting outdoors at all three sites with the highest mean (0.14) at Kawere. Some *An. funestus* s.l. were collected resting outdoors at all sites except Dendera. The species was collected at Kawere and Makarara with a mean of 0.09 and 0.05 mosquitoes/trap/day at these sites, respectively. Fewer *An. funestus* s.l./trap/day were collected than *An. gambiae* s.l. at Dendera, Kawere, and Makarara. The higher outdoor resting densities of both *An. gambiae* s.l. and *An. funestus* s.l. from the pit shelter collections than the indoor resting collections from the PPAs might indicate that these vectors tend to rest outdoors. Other species collected outdoors from pit shelters included *An. rufipes* at Dendera, Kawere and Makarara, *An. pretoriensis* at Dendera and Makarara, and *An. demeilloni* at Kawere and Makarara.

Table 5. Outdoor Vector Mean Densities (based on pit shelter collections) in Sprayed (FF = Dendera, FF /DDT= Kawere,) and Unsprayed (ITNs = Makarara) Sites in Mashonaland East and Manicaland Provinces, March 2022-December 2022

Site	Total number of days sampled/pit	*An. gambiae* s.l.	*An. funestus* s.l.	*An. pretoriensis*	*An. coustani*	*An. demeilloni*	*An. rufipes*	Other species
Dendera*	20	0.04	0	0.09	0	0	0.16	0
Kawere	20	0.14	0.09	0	0	0.01	0.02	0
Makarara	30	0.07	0.05	0.01	0	0.01	0.08	0

Note: FF=Fludora® Fusion

*For Dendera, the team sometimes sampled from more than five pits to increase the yield of mosquitoes. These mosquitoes were analyzed.

3.1.4 Indoor and Outdoor Densities from CDC Light Trap Collections

Overall, CDC light traps set outdoors collected a higher mean number of mosquitoes/trap/night than traps set indoors for all species at Dendera, Kawere, and Makarara, except for more *An. gambiae* s.l. collected indoors at Dendera and Kawere (Table 6). More *An. funestus* s.l. were collected outdoors at Kawere and Makarara, more *An. pretoriensis* outdoors at Dendera and Makarara, and more *An. rufipes* collected outdoors at all three sites, and the only *An. coustani* at Makarara and *An. maculipalpis* at Dendera were collected outdoors. These data indicate that these vectors tend to feed outdoors.

Table 6. Indoor and Outdoor Mean Densities/trap/night of Anopheles Mosquito Vectors as Collected by the CDC Light Traps at Three Sentinel Sites in Mashonaland East and one Sentinel Sites in Manicaland, March 2022-December 2022

Site	In/Out	No. of months sampled	*An. gambiae* s.l.	*An. funestus* s.l.	*An. pretoriensis*	*An. coustani*	*An. maculipalpis*	*An. rufipes*	Other species
Dendera	In	7	0.04	0	0	0	0	0	0
	Out	7	0	0	0.11	0	0.04	0.07	0
Kawere	In	7	0.04	0	0	0	0	0.07	0
	Out	7	0	0.04	0	0	0	0.18	0
Makarara	In	7	0	0.04	0.04	0	0	0	0
	Out	7	0.04	0.11	0.11	0.04	0	0.07	0

3.1.5 Hourly Biting Rates of *An. funestus* s.l. and *An. gambiae* s.l.

Too few mosquitoes were collected by HLC-proxy during the night to determine a clear pattern in hourly biting behavior (Figures 3 – 6 and Table 7). However, there is predominantly more biting outdoors compared with

indoors for both *An. gambiae* s.l. (at Dendera and Kawere) and *An. funestus* s.l. (at Makarara). Crude endophagic indices are generally low – ranging from nil to 0.5 - for both *An. gambiae* s.l. and *An. funestus* s.l. The predominant biting times varied by sentinel site from early night (18-19 pm) indoors for *An. gambiae* s.l. at Makarara and late morning (5-6 am) for *An. gambiae* s.l. outdoors at Kawere (Figures 3 and 4), and earliest from 20-21 pm for *An. funestus* s.l. outdoors and as late as 5 -6 am outdoors at Kawere and Makarara (Figures 5 and 6).

The human biting rate for both *An. gambiae* s.l. and *An. funestus* s.l. was barely detectable indoors at Kawere (Table 7). No *An. gambiae* s.l. were caught indoors from Kawere (Figure 3), and no *An. funestus* s.l. were collected indoors from Dendera and Kawere (Figure 5), and outdoors at Dendera (Figure 6).

Figure 3. Mean daily indoor biting rates, An. gambiae s.l., by site; March – December 2022

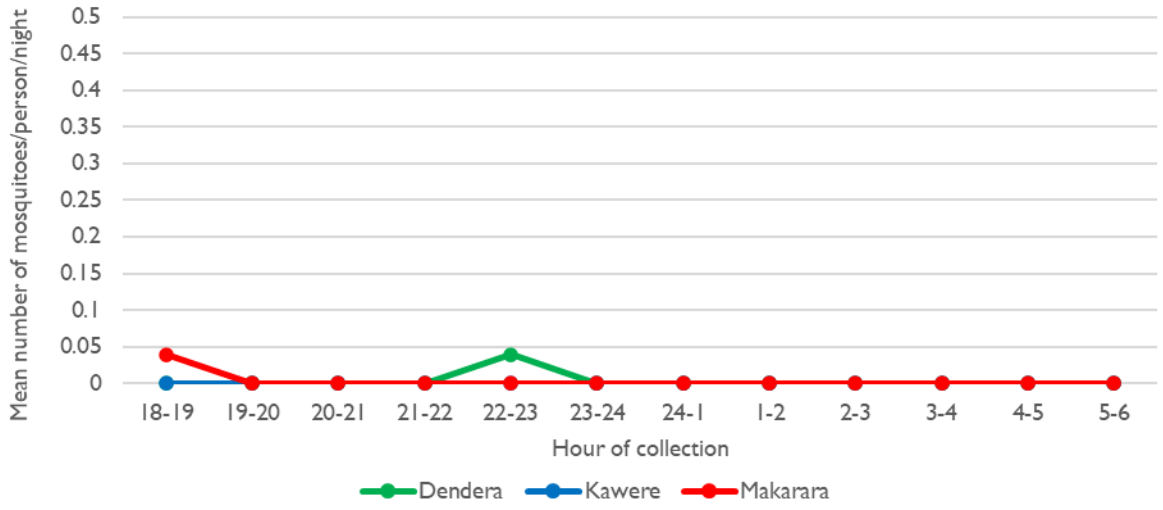


Figure 4. Mean daily outdoor biting rates, An. gambiae s.l., by site; March – December 2022

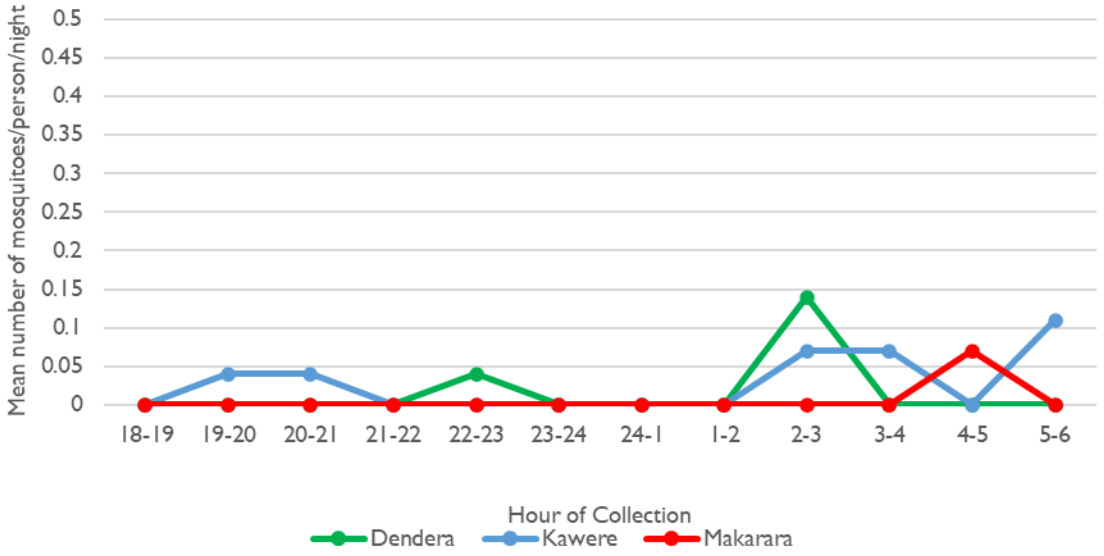


Figure 5. Mean daily indoor biting rates, An. funestus s.l., all sites; March – December 2022

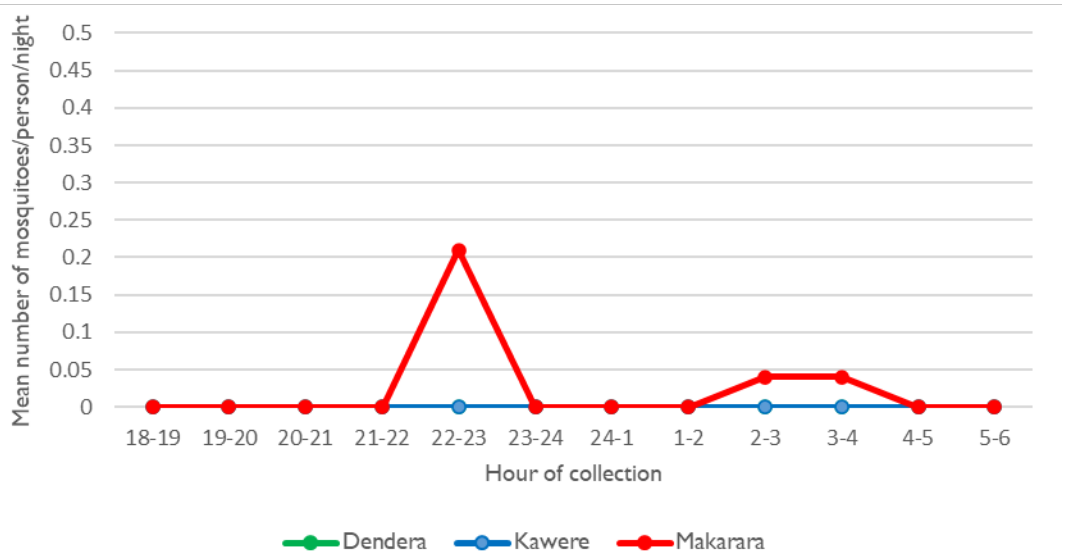


Figure 6. Mean daily outdoor biting rates, An. funestus s.l., all sites; March – December 2022

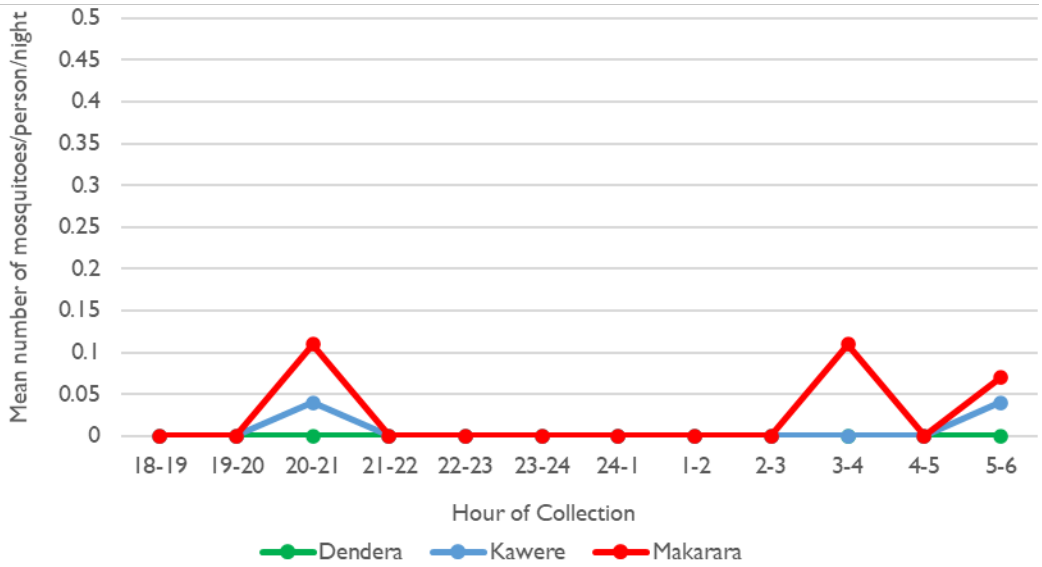


TABLE 7. BITING PATTERN FOR *AN. GAMBIAE* S.L. AND *AN. FUNESTUS* S.L. ACROSS ALL SITES

Site	*An. gambiae* s.l.		*An. funestus* s.l.	
	Indoor	Outdoor	Indoor	Outdoor
Dendera	22-23 pm	22-23 pm, 2-3 am	N/A	N/A
Kawere	N/A	19-20 pm, 20-21 pm, 2-3 am, 3-4 am, 5-6 am	N/A	20-21pm, 5-6am
Makarara	18-19 pm	4-5 am	22-23 pm, 2-3 am, 3-4 am	20-21 pm, 3-4 am, 5-6 am

3.2 IRS SPRAY QUALITY AND RESIDUAL EFFICACY

For the 2021 and 2022 IRS campaigns, the team monitored the spray quality, the residual efficacy, and the fumigant effect of the insecticides sprayed where applicable. In 2021, these were Fludora® Fusion at Dendera (October 2021 until September 2022) and DDT at Kawere (November 2021 until September 2022). For the 2022 IRS campaign, the team monitored DDT at both Dendera and Kawere from November 2022 to December 2022. Cone assays were not performed at Dendera in November 2021 due to unavailability of susceptible colony *An. arabiensis* (KGB strain) mosquitoes from the insectary at AU. Monthly monitoring was stopped after December 2022 as the project is closing in February 2023.

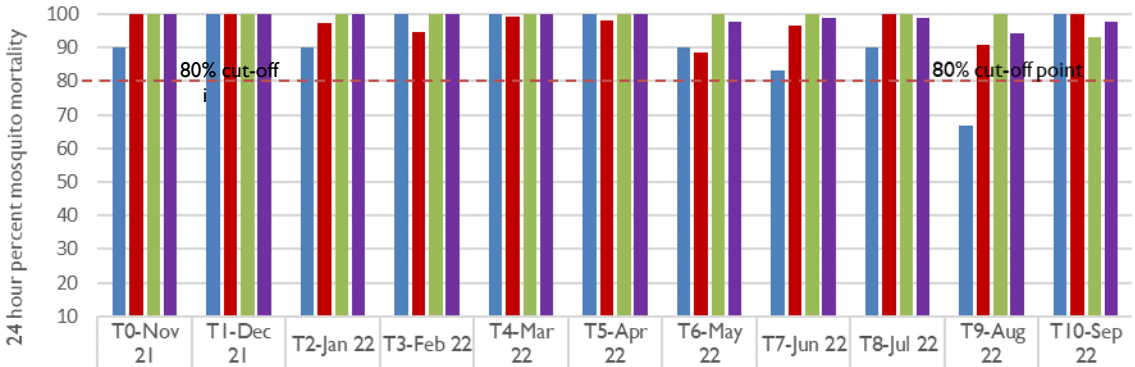
3.2.1 CONE BIOASSAY TESTS AND FUMIGANT EFFECT

2021 IRS Campaign

Quality of spray was acceptable at the two sites sprayed with Fludora® Fusion at Dendera (Zhuwau Village) and with DDT at Kawere (Sispence, Kawere and Botsanzira villages). Residual efficacy of Fludora® Fusion at Dendera and of DDT at Kawere was good with mosquito mortality above 80% on all four wall surface types 11 months after spray at Dendera and 10 months at Kawere (Figures 7 and 8). Bioassays were not done in November 2021 in Dendera because of a lack of colony mosquitoes available from the Africa University (AU) insectary.

Mean mosquito mortality due to fumigant effect of Fludora® Fusion was below 50% six months post-spraying at Dendera so monitoring was discontinued.

Figure 7. Residual Efficacy of Fludora® Fusion in Dendera (ZhuwauVillage), Mudzi District, Reported as *An. arabiensis* (KGB Strain) Mean Mortality After Five-Day Holding Period in WHO Cone Bioassays, October 2021-September 2022

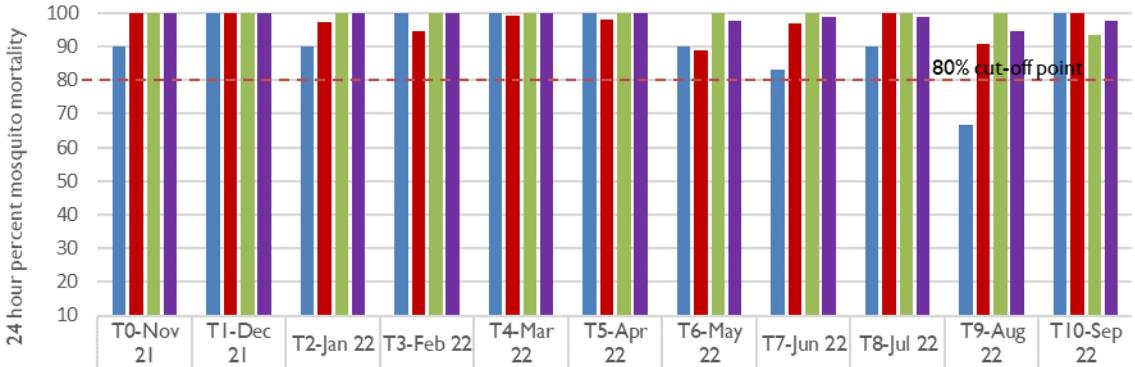


	T0-Nov 21	T1-Dec 21	T2-Jan 22	T3-Feb 22	T4-Mar 22	T5-Apr 22	T6-May 22	T7-Jun 22	T8-Jul 22	T9-Aug 22	T10-Sep 22
Brick	90	100	90	100	100	100	90	83.3	90	66.7	100
Cement	100	100	97.3	94.7	99.3	98	88.7	96.7	100	90.7	100
Mud	100	100	100	100	100	100	100	100	100	100	93.3
Painted Cement	100	100	100	100	100	100	97.8	98.9	98.9	94.4	97.8

Time Points

**Not done due to lack of mosquitoes

Figure 8. Residual Efficacy of DDT in Kawere (Sixpence, Kawere/Botsanzira Villages), Mutoko District, Reported as *An. arabiensis* (KGB Strain) Mean Mortality After 24-Hour Holding Period in WHO Cone Bioassays, November 2021-September 2022



	T0-Nov 21	T1-Dec 21	T2-Jan 22	T3-Feb 22	T4-Mar 22	T5-Apr 22	T6-May 22	T7-Jun 22	T8-Jul 22	T9-Aug 22	T10-Sep 22
Brick	90	100	90	100	100	100	90	83.3	90	66.7	100
Cement	100	100	97.3	94.7	99.3	98	88.7	96.7	100	90.7	100
Mud	100	100	100	100	100	100	100	100	100	100	93.3
Painted Cement	100	100	100	100	100	100	97.8	98.9	98.9	94.4	97.8

Time Points

2022 IRS Campaign

The team monitored the IRS performance in Dendera and Kawere following the 2022 IRS campaign. Quality of spray was acceptable at the two sites sprayed with DDT. DDT at Dendera retained efficacy one month post-spray: mean mosquito mortality is 100% (Figure 9) for all surface types. Likewise, DDT at Kawere retains efficacy after one month post-spray; mean mosquito mortality was 100% for three surfaces (Figure 10). Quality of spray monitoring was ceased in December 2022 as the VectorLink project is closing in February 2023.

FIGURE 9. RESIDUAL EFFICACY OF DDT IN DENDERA (MAPUNDU VILLAGES), MUDZI DISTRICT, REPORTED AS *AN. ARABIENSIS* (KGB STRAIN) MEAN MORTALITY AFTER 24-HOUR HOLDING PERIOD IN WHO CONE BIOASSAYS, NOVEMBER 2022-DECEMBER 2022

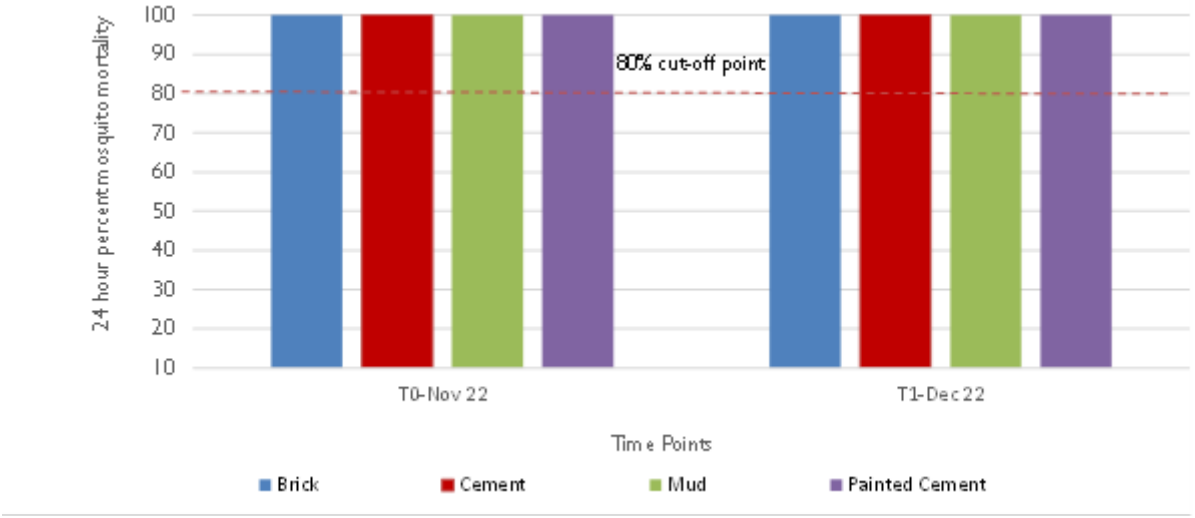
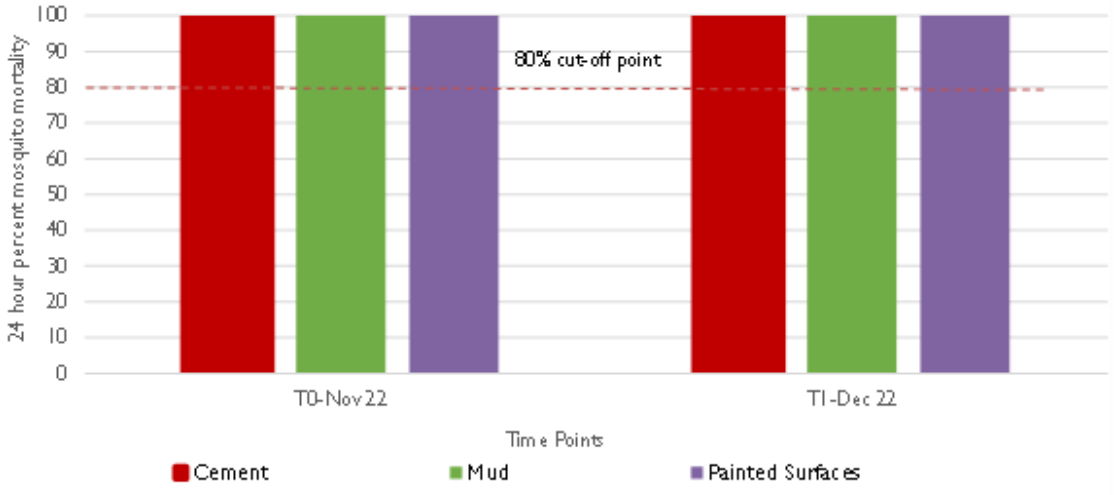


FIGURE 10. RESIDUAL EFFICACY OF DDT AT KAWERE (NDEMERA VILLAGE), MUTOKO DISTRICT, REPORTED AS *AN. ARABIENSIS* (KGB STRAIN) MEAN MORTALITY AFTER 24-HOUR HOLDING PERIOD IN WHO CONE BIOASSAYS, NOVEMBER 2022-DECEMBER 2022



3.3 Insecticide Resistance Monitoring

The insecticide susceptibility of *An. gambiae* s.l. collected from localities in Mashonaland East and Manicaland Provinces was done using the CDC bottle bioassay method for all insecticides tested. The five insecticides tested were alpha-cypermethrin, deltamethrin, permethrin, clothianidin, and chlorfenapyr (Table 8). Few mosquitoes were collected from all sites mostly due to erratic rains, and a drought at Makarara.

An. gambiae s.l. was susceptible (100% mortality) to most of the diagnostic doses of the insecticides tested at the four sites. However, caution should be applied with this interpretation because low number of mosquitoes were tested in the majority of cases. There was limited mosquito breeding due to the dry weather conditions. Sample sizes exceeded 100 for two tests at Dendera for deltamethrin (n = 120) and permethrin (n = 135) whereas the samples in the remaining tests ranged from n = 12 to 78.

An. gambiae s.l. from Burma Valley (collected from Marange area) in Mutare District were susceptible to deltamethrin and clothianidin and to permethrin (collected from Gimboki on the outskirts of Mutare City) but *An. demeilloni* was resistant to deltamethrin. However, these observations are based on fewer than 100 mosquitoes tested.

Table 8. Results of Insecticide Susceptibility Tests on *An. gambiae* s.l. Conducted In Mashonaland East and Manicaland Provinces; 2022

Province	District	Sentinel Site	Larval Collection Site	Month of test	Insecticide Tested (µg/bottle; dose)	Total No. of Mosquitoes Tested	Resistance Status (% Mosquito Mortality)
Mashonaland East	Mudzi	Dendera	Dendera Irrigation	March	Deltamethrin (12.5)	120	S (100%)
			Gatakata	April	Clothianidin (4)	12	S (100%)
			Kotwa Stream	March	Permethrin (21.5)	135	PR (94.1)
	Mutoko	Kawere	Kawere Gardens/Hunda	March	Alpha-cypermethrin (12.5)	75	S (98.7%)
			Kawere Gardens	April	Clothianidin (4)	15	S (100%)
			Hunda	March	Chlorfenapyr (100)	78	S (100%)
			Kawere Gardens	March	Deltamethrin (12.5)	63	S (100%)
	Hwedza	Makarara	Musoko Dam	Dec	Permethrin (21.5)	77	S (100)
Manicaland	Mutare	Burma Valley	Marange/ Mafararikwa	April	Clothianidin (4)	31	S (100%)
			Mafararikwa	April	Deltamethrin (12.5)	56	S (100)
			Brandhill Farm compound	May	*Deltamethrin (12.5)	25	R (72%)
			Gimboki	Dec	Permethrin (21.5)	25	S (100)
					**An. demeilloni*		
			Brandhill Farm Compound	May	Deltamethrin (12.5)	25	R (72%)

***** *An. demeilloni* was tested on deltamethrin using F1 mosquitoes raised from adults collected from pits
*Where S – susceptible; PR – possible resistance; R – resistant

3.4 Results of Laboratory Analysis

3.4.1 Molecular Identification of *Anopheles* Species From Longitudinal Monitoring

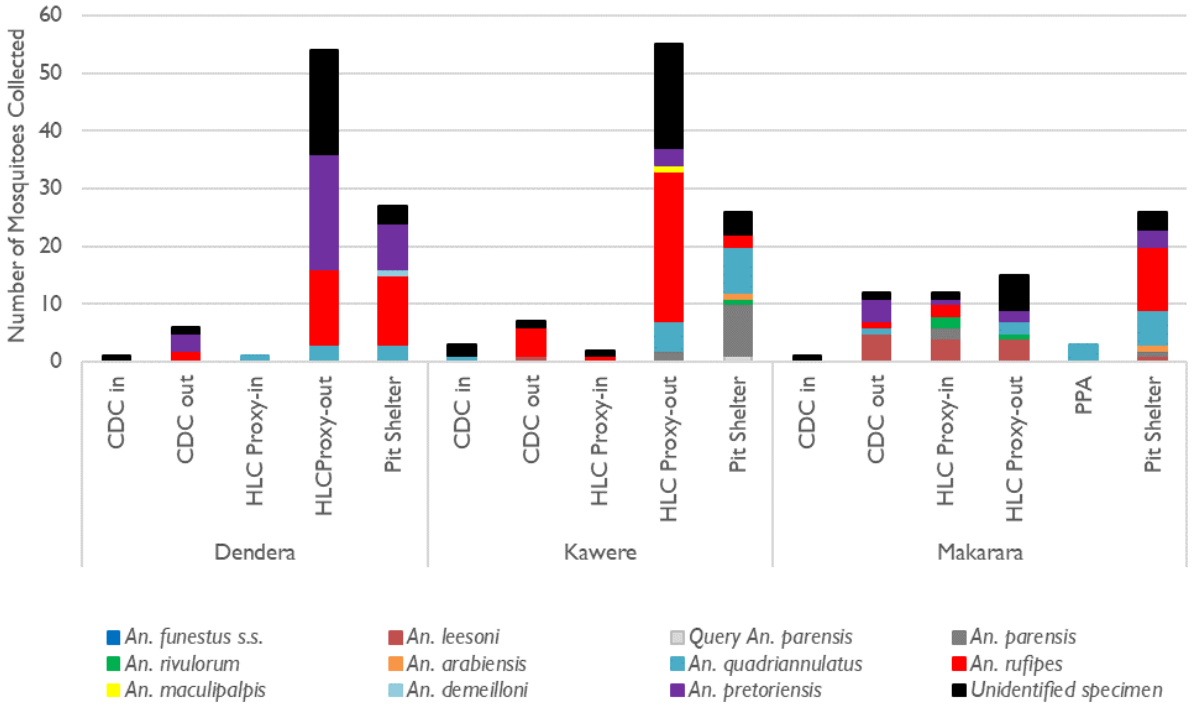
A total of 260 *Anopheles* mosquitoes collected in 2022 were assayed for species identification in the AU laboratory as follows: 92 from Dendera, 93 from Kawere, and 75 from Makarara (Figure 11). HLC-proxy outdoors provided most of the mosquitoes at Dendera (60.7%) and at Kawere (59.1%) for lab analysis whereas pit shelters provided most at the control site Makarara (37.7%).

Most of the species collected at Dendera were *An. pretoriensis* (34.8%; 31/89) and *An. rufipes* (30.3%; 27/89). No *An. funestus* s.l. was collected unlike in the previous year when a sibling species, *An. parensis* was identified. One *An. demeilloni* was collected from pits. All seven *An. gambiae* s.l. collected were identified as *An. quadriannulatus,* and no *An. arabiensis* was recorded. A quarter (25.8%; 23/89) of the specimens collected at Dendera did not amplify.

At Kawere, 15.1% (14/93) of the *Anopheles* collected were *An. funestus* s.l. that were identified as *An. leesoni* (1/14; 71.1%), *An. parensis* (11/14; 78.7%), *An. leesoni* (1/14; 7.1%) and *An. rivulorum* (1/14; 7.1%). Fifteen *An. gambiae* s.l. were collected; most of which were *An. quadriannulatus* (14/15; 93.3%), with *An. arabiensis* constituting a mere 6.7% (1/15). Most of the specimens collected were *An. rufipes* (36.5%; 34/93). A few *An. pretoriensis* (3.2%; 3/93) and one *An. maculipalpis* (1.1%; 1/93) were collected. Close to a third of the specimens could not be identified (28%; 26/93).

Twenty nine percent of the 69 *Anopheles* mosquitoes from Makarara were *An. funestus* s.l., followed by *An. rufipes* at 20.3% and *An. gambiae* s.l at 18.8%.. Most of the *An. funestus* s.l. were identified as *An. leesoni* (70.0%); while the remainder were *An. parensis* 15.0% (3/20), and *An. rivulorum* (15.0%; 3/20). Most of the *An. gambiae* s.l. from Makarara were *An. quadriannulatus* (92.3%; 12/13) with *An. arabiensis* constituting only 7.7% (1/13). Ten were *An. pretoriensis* (14.5%; 10/69). Twelve of the 69 (17.4%) did not amplify.

FIGURE 61. MAIN ANOPHELES MOSQUITOES COLLECTED AT DENDERA, MAKARARA, AND KAWERE IDENTIFIED WITH PCR, 2022, EXCLUDING SPECIMENS NOT IDENTIFIED



Footnote on species: Vectors: *An. funestus* s.s.; *An. leesoni*; *An. parensis*; *An. arabiensis; An. gambiae* s.s.
Potential Vectors: *An. rivulorum; An. rivulorum*-like; *An. rufipes; An. squamosus*
Non Vectors: *An. quadriannulatus; An. maculipalpis; An. pretoriensis*
The Query *An. parensis* refers to the species that amplifies with 2 bands , which is actually *An. longipalpis* C.
CDC: CDC light trap set indoors or outdoors 'IN' or 'OUT'
HLC Proxy: human landing collection set indoors or outdoors 'IN' and 'OUT'
PPA: Prokopack Aspirator

3.4.2 Mosquitoes Collected For Insecticide resistance Monitoring

Laboratory analysis of the *An. gambiae* s.l. specimens tested for insecticide susceptibility showed a low proportion of the malaria vector *An. arabiensis* as its occurrence ranged from 0% for some insecticides tested at Dendera and Kawere to 15% at Dendera (Table 9). Most of the *An. gambiae* s.l. were *An. quadriannulatus*, a species regarded as a non-vector in the preliminary results. A saltwater breeder, *An. merus*, was identified among the *An. gambiae* s.l. specimens collected from Kawere (8%) and from Burma Valley (Gimboki area (7%)). A substantial proportion of the *An. gambiae* s.l. tested could not be identified by the PCR assays currently available. It is difficult to make inferences about the resistance status of known vector species from these sparse data. Getting adequate proportions of known vectors (i.e. *An. arabiensis* and *An. gambiae* s.s.) in wild-caught *An. gambiae* s.l. is an ongoing challenge as the vector population continues to decrease.

Table 9. Siblings Species of *An. gambiae* s.l. following Insecticide Susceptibility Tests

Site	Insecticide	*An. arabiensis*	*An. quadri*annulatus	*An. gambiae s.s.*	*An. merus*	No amplification	Total Tested
Dendera	Deltamethrin	2/140 (1%)	59/140 (42%)	0	0	79/140 (56%)	140
	Clothianidin	0	8/10 (80%)	0	0	2/10 (20%)	10
	Permethrin	6/40 (15%)	29/35 (73%)	0	0	5/40 (13%)	40
Kawere	Alphacypermethrin	5/80 (6%)	75/80 (94%)	0	0	0	80
	Deltamethrin	2/58 (3%)	40/58 (69%)	0	0	16/58 (28%)	58
	Clothianidin	0	20/23 (87 %)	0	0	3/23 (13%)	23
	Permethrin	9/105 (9%)	80/105 (76%)	1/105 (1%)	8/105 (8%)	7/105 (7%)	105
	Chlorfenapyr	1/88 (1%)	43/88 (49%)	0	0	44/88 (50%)	88
Makarara	Permethrin	6/72 (8%)	28/72 (39%)	0	0	38/72 (53%)	72
Burma Valley	Deltamethrin	3/96 (3%)	36/96 (38%)	0	0	57/96 (59%)	96
	Clothianidin	3/36 (8%)	28/36 (78%)	0	0	5/36 (14%)	36
	Permethrin	1/30 (3%)	6/30 (20%)	0	2/30 (7%)	21/30 (70%)	30

One *An. gambiae* s.s. was classified by molecular method as *An. gambiae* (formerly the 'S' molecular form), while *An. coluzzii* (formerly the 'M' molecular form), was absent. The *An. gambiae* s.s. was collected as larvae from Kawere (Hunda locality) in August 2021.

Results of Blood Meal Analysis

A total of 110 blood-fed mosquitoes collected from the three sites were analyzed by PCR to determine the blood meal sources (Table 10). Cows, followed by either goat (Dendera and Kawere) or dog (Makarara) were the most or joint-most common sources of bloodmeals at all three sites.

Only one *An. rufipes* from Dendera and an unidentified species from Makarara had fed solely on humans. Mixed human+animal blood meals for various *Anopheles* species were observed at Kawere and Makarara. At Kawere, there was one human+goat (*An. parensis*), human+dog (*An. rivulorum*), human+goat (*An. arabiensis*) among the major vector species, and human+cow (*An. rufipes*) and human+goat+cow (*An. rufipes*). *An. rivulorum*. At Makarara, there was one specimen with human+goat (*An. quadriannulatus*), human+cow (*An. rufipes*). None of the *An. demeilloni* collected were blood-fed, so there is no indication of the host preference for the species. No clear mosquito feeding behavior is discernable from these data on blood meals.

TABLE 10. RESULTS OF THE BLOOD MEAL ANALYSES OF ANOPHELES SPECIES FROM DENDERA, KAWERE AND MAKARARA

Site	Blood meal source	Species											Total
		An. funestus s.l.				*An. gambiae* s.l.			Other species			Unidentified specimen	
		An. funestus s.s.	*An. leesoni*	*An. parensis*	*An. rivulorum*	*An. arabiensis*	*An. quadriannulatus*	*An. rufipes*	*An. maculipalpis*	*An. demeilloni*	*An. pretoriensis*		
Dendera	Cow	0	0	0	0	0	2	4	0	0	4	2	12
	Goat	0	0	0	0	0	0	4	0	0	3	2	9
	Human	0	0	0	0	0	0	2	0	0	0	0	2
	No amplication	0	0	0	0	0	0	7	0	0	1	5	13
	Total	**0**	**0**	**0**	**0**	**0**	**2**	**17**	**0**	**0**	**8**	**9**	**36**
Kawere	Cow	0	0	2	0	0	4	0	1	0	0	0	7
	Dog	0	0	1	0	0	0	0	0	0	0	0	1
	Goat	0	0	0	0	0	1	0	0	0	0	2	3
	Goat & cow	0	0	1	0	0	0	0	0	0	0	0	1
	Human & cow	0	0	0	0	0	0	1	0	0	0	0	1
	Human & dog	0	0	0	1	0	0	0	0	0	0	0	1
	Human & goat	0	0	1	0	1	0	0	0	0	0	0	2
	Human, goat & cow	0	0	0	0	0	0	2	0	0	0	0	2
	No amplication	0	1	5	0	0	3	3	0	0	0	4	16
	Total	**0**	**1**	**10**	**1**	**1**	**8**	**6**	**1**	**0**	**0**	**6**	**34**
Makarara	Cow	0	0	0	0	0	5	4	0	0	3	1	13
	Dog	0	1	0	1	0	1	1	0	0	0	2	6
	Goat	0	1	1	0	0	2	0	0	0	0	0	4
	Goat & cow	0	1	0	0	0	2	0	0	0	2	0	5
	Human	0	0	0	0	0	0	0	0	0	0	1	1
	Human & cow	0	0	0	0	0	0	1	0	0	0	0	1
	Human & goat	0	0	0	0	0	1	0	0	0	0	0	1
	No amplication	0	0	0	1	0	1	5	0	0	0	2	9
	Total	**0**	**3**	**1**	**2**	**0**	**12**	**11**	**0**	**0**	**5**	**6**	**40**

Footnote on species: Vectors: *An. funestus* s.s.; *An. leesoni*; *An. parensis*; *An. arabiensis; An. gambiae* s.s.
Potential Vectors: *An. rivulorum; An. rivulorum*-like; *An. rufipes; An. squamosus*
Non Vectors: *An. quadriannulatus; An. maculipalpis; An. pretoriensis*

3.4.3 SPOROZOITE INFECTION RATE

A total of 260 *Anopheles* mosquitoes collected from the three sentinel sites by various methods were analyzed by ELISA for *Plasmodium falciparum* CSP. . *funestus* s.l. (n=39), *An. gambiae* s.l. (n=50), and other *Anopheles* (n=171) consisting mainly of *An. rufipes* (n=97), *An. pretoriensis* (n=51), *An. coustani* (n=8), *An. demeilloni* (n=1), *An. maculipalpis* (n=2), and 9 unidentified species. None of these species were positive.

3.4.4 Results of KDR Assays

A total of 732 *An. gambiae* s.l. were tested for the kdr mutation; Leu – Ser (L1014S) and Leu – Phe (L1014F). All specimens were susceptible to homozygous except for a single specimen (*An. arabiensis*) from Kawere which had the resistant heterozygote L1014F gene (Table 11). Most of the *An. gambiae* s.l. analyzed were collected by larval collection for insecticide resistance.

Table 11. Results of *kdr* Assays

Province	District	Site	Number of species tested	L1014S			L1014F		
				SS	RS	RR	SS	RS	RR
Manicaland	Mutare	Burma Valley	107 (LC)	107	0	0	107	0	0
Mashonaland East	Mudzi	Dendera	216 (205 LC+11 LM)	216	0	0	216	0	0
	Mutoko	Kawere	375 (354 LC+21 LM)	375	0	0	374	1	0
	Hwedza	Makarara	34 (LM)	34	0	0	34	0	0
Total			732	732	0	0	731	1	0

Note: SS=Susceptible homozygous, RS=Resistant heterozygous, RR=Resistant homozygous
LC = larval collection; LM = longitudinal monitoring

3.4.5 Results of Ace-1 Assays

No *An. gambiae* s.l. were analyzed for insensitive AChE (acetylcholinesterase) gene by molecular method because AU laboratory did not have the controls necessary for this analysis.

4. DISCUSSION

Entomological monitoring results from March 2022 to December 2022 show variation in the species composition at the three main sites. For the two major malaria vectors, *An. gambiae* s.l. occurred in slightly greater abundance than *An. funestus* s.l. *An. funestus* s.l. was dominant at Makarara, less than *An. gambiae* s.l. at Kawere but was absent at Dendera. Meanwhile, *An. rufipes* was the most abundant species for combined data for the three sites: the predominant species at Kawere and Dendera and third most common at Makarara. *An. pretorienis* was also fairly dominant species at Dendera. The absence of *An. funestus* s.s. – an efficient malaria vector - suggests a positive impact of indoor residual spraying on this endophilic vector. Other sibling species *An. leesoni, An. rivulorum*, *An. parensis* and possibly *An. longipalpis* C are considered secondary vectors although none of the species were sporozoite positive. In contrast to *An. funestus* s.s., the occurrence of *An. arabiensis* suggests the resilience of the species which is partially exophilic and tends to be versatile regarding choice of animals for a blood meal. The predominance of *An. rufipes* in comparison to the major malaria vectors at Dendera and Kawere is noted with interest as this species is considered a secondary vector.

The low mosquito densities are attributed to the erratic rains that affected *An. gambiae* s.l., which typically breeds in temporary rainwater pools, as compared to *An. funestus* s.l., which breeds in semi-permanent water bodies that are available at Kawere and Makarara. The longitudinal monitoring was conducted mostly under drought conditions, especially for Makarara, and hence the low number of mosquitoes collected. Most *Anopheles* mosquitoes were collected by HLC proxy outdoors at Dendera and Kawere, followed by pits. This suggests a vector population that prefers resting outdoors and/or the impact of insecticide from routine IRS on indoor resting mosquitoes. Indoor resting *Anopheles* were collected only at Makarara which further suggests the impact of IRS on vector mosquitoes. However, this observation is based on very low mosquito densities in the areas. Other species, *An. pretoriensis* and *An. rufipes,* were also relatively more abundant outdoors than indoors. CDC light traps set outdoors generally collected more mosquitoes than those set indoors.

Too few mosquitoes were collected to determine indoor and outdoor hourly biting rates. More *An. gambiae* s.l. were collected outdoors than indoors whereas there was no significant difference in biting location for *An. funestus* s.l. Most *An. gambiae* s.l. and *An. funestus* s.l., were observed biting outdoors before early evening (6-7 pm) and after midnight with some biting around sunrise (5-6 am). The outdoor biting behavior presents challenges for vector control using traditional strategies such as IRS and ITNs. However, these data need to be interpreted along with human population behavior (i.e., the time at which people retreat indoors and the time at which they are in bed under an ITN) at each given locality to get a better estimate of exposure risk. Communities at the study sites engage in market gardening, a habit which potentially increases their risk of exposure to vectors outdoors in the evening and early morning.

Laboratory analysis provided insights on species occurrence at all four sites. At Kawere and Makarara, the three sibling species of *An. funestus* s.l. were collected: *An. parensis*, *An. rivulorum*, and *An. leesoni*. These three species are considered potential secondary malaria vectors, with *An. parensis* previously found sporozoite-positive locally. *An. longipalpis* C was also collected in low numbers.

Two sibling species were identified from the *An. gambiae* s.l. collected from longitudinal monitoring: *An. arabiensis* occurring scarcely at Kawere and Makarara and *An. quadriannulatus* occurring in greater abundance at the three sites. There was greater species variety from *An. gambiae* s.l. collected as larvae for resistance testing. *An. gambiae* s.l. was represented by four sibling species at Kawere: *An. arabiensis*, *An. quadriannulatus*, *An. gambiae* s.s. and *An. merus*. Major malaria vectors *An. arabiensis*. s.s and *An. gambiae* s.s. (where applicable) were overshadowed by *An. quadriannulatus* – a non-vector - at Dendera, Kawere, and Makarara. Besides Kawere, *An. merus* was also observed from Burma Valley samples collected at Gimboki area. *An. merus* is generally a saltwater breeder. The water salinity of the breeding site at Kawere and Burma sites was not analyzed.

Mosquitoes that had fed solely on humans were few, consisting of *An. rufipes* from Dendera and one unidentified species from Makarara. The mixed human-animal blood meals that were found at Kawere and Makarara mostly for *An. rufipes* suggest this species is a potential secondary vector. Other species that had fed on humans include *An. arabiensis*, *An. rivulorum*, *An. parensis* at Kawere, and *An. quadriannulatus* at Makarara. This indicates an opportunistic feeding tendency in these species except for *An. arabiensis*.

Of the 260 specimens tested, none of the species were infected with malaria. This is perhaps not surprising given the small numbers tested. Other species found include *An. maculipalpis*, *An. demeilloni,* and *An. pretoriensis*. While *An. rufipes* and *An. demeilloni* are considered potential malaria vectors, none of the other species were CSP-positive. *An. pretoriensis* is probably not a malaria vector despite it sometimes feeding on humans although the species has been reported positive for sporozoites in Eastern Zambia that borders with Mozambique. The low human blood index in most of the other species suggests it is unlikely they feed on humans often enough to transmit malaria. Further evaluation of *An. rufipes* is necessary given this species had the most human blood meals. *An. parensis* as a secondary malaria vector in its geographical range albeit based on a limited sample. The vectorial role of *An. longipalpis* C remains unknown.

Entomological monitoring yielded low numbers of mosquitoes overall, limiting the ability to identify clear seasonal trends yet highlighting the need for an assessment and potential consideration around other mosquito collection methods such as using animal- or human-baited tent traps. The pit shelter has proven to be an affordable but productive outdoor collection method in vector surveillance.

Results of insecticide susceptibility tests on *An. gambiae* s.l. are encouraging since there was generally no resistance. *An. gambiae* s.l. was susceptible to deltamethrin, clothianidin and permethrin at Dendera, to alpha-cypermethrin, deltamethrin, permethrin, clothianidin and chlorfenapyr at Kawere, and to permethrin at Makarara. At Burma Valley, *An. gambiae* s.l. was susceptible to deltamethrin permethrin and clothianidin. However, there is need for caution in making inferences from the susceptibility tests since most mosquitoes tested are non-vector species. While *An. demeilloni* at Burma Valley was resistant to deltamethrin this was also based on a small sample tested, and the significance rests also on the role of this species in disease transmission, which remains unknown.

Laboratory tests for insecticide resistance indicate the presence of heterozygous resistant *kdr* (L1014F) gene in one *An. arabiensis* from Kawere. This represents only 0.3% of the 375 specimens analyzed from Kawere for *kdr* had the heterozygous allele for *kdr* (L1014F). None of the specimens had *kdr* (L1014S) resistance. This augurs well for insecticide use in vector control although monitoring should be done on a wider scope geographically.

For the 2021 IRS campaign, the residual efficacy of Fludora® Fusion was at least 11 months at Dendera while it was at least 10 months for DDT at Kawere. These results indicate the two insecticides can remain effective for the duration of the malaria season provided they are sprayed at the right time. Insecticide build-up on sprayed surfaces after several spray cycles was not assessed. Mean mosquito mortality due to fumigant effect of Fludora® Fusion was below 50% six months post spraying at Dendera. For the 2022 IRS campaign, the residual life of DDT was not monitored sufficiently at Dendera, and Kawere since bioassays were done only one month after spray.

5. RECOMMENDATIONS

Based on the data presented and discussed in this report, the following recommendations and next steps should be considered going forward:

- PMI and NIHR should continue to inform NMCP and sensitize the Vector Control Technical Sub-Committee on insecticide resistance to guide policy and action.
- Because more mosquitoes are caught outdoors than indoors, PMI in collaboration with the NIHR and partners should evaluate alternative collection methods, such as the window trap, to determine mosquito behavior.
- PMI and partners should disseminate the observed outdoor mosquito biting behavior at the sentinel sites and urge NMCP and partners to investigate the trends in other geographical areas to guide targeted intervention.
- PMI and partners in collaboration with the NMCP should determine the role of each sibling species of the now prevalent *An. funestus* s.l. and other Anopheline species (including *An. rufipes*) in malaria transmission and investigate approaches to control residual transmission.

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7. ANNEX

ANNEX 1. DATA ON MAIN ANOPHELES MOSQUITOES COLLECTED AT DENDERA, KAWERE AND MAKARARA IDENTIFIED WITH PCR, 2022 SHOWN IN FIGURE 12

Site	Collection Method	Species												
			An. funestus s.l.				*An. gambiae* s.l.			Other species			Unidentified specimen	Total
		An. funestus s.s.	*An. leesoni*	*An. parensis*	*Query An. parensis*	*An. rivolum*	*An. arabiensis*	*An. quadriannulatus*	*An. rufipes*	*An. maculipalpis*	*An. demeilloni*	*An. pretoriensis*		
Dendera	CDC in	0	0	0	0	0	0	0	0	0	0	0	1	1
	CDC out	0	0	0	0	0	0	0	2	0	0	3	1	6
	HLC Proxy-in	0	0	0	0	0	0	1	0	0	0	0	0	1
	HLC Proxy-out	0	0	0	0	0	0	3	13	0	0	20	18	54
	Pit Shelter	0	0	0	0	0	0	3	15	0	1	8	3	30
	Total	**0**	**0**	**0**	**0**	**0**	**0**	**7**	**30**	**0**	**1**	**31**	**23**	**92**
Kawere	CDC in	0	0	0	0	0	0	1	0	0	0	0	2	3
	CDC out	0	1	0	0	0	0	0	5	0	0	0	1	7
	HLC Proxy-in	0	0	0	0	0	0	0	1	0	0	0	1	2
	HLC Proxy-out	0	0	2	0	0	0	5	26	1	0	3	18	55
	Pit Shelter	0	0	9	1	1	1	8	2	0	0	0	4	26
	Total	**0**	**1**	**11**	**1**	**1**	**1**	**14**	**34**	**1**	**0**	**3**	**26**	**93**
Makarara	CDC in	0	0	0	0	0	0	0	0	0	0	0	1	1
	CDC out	0	5	0	0	0	0	1	1	0	0	4	1	12
	HLC Proxy-in	0	4	2	0	2	0	1	2	0	0	1	1	13
	HLC Proxy-out	0	4	0	0	1	0	2	0	0	0	2	6	15
	PPA	0	0	0	0	0	0	3	0	0	0	0	0	3
	Pit Shelter	0	1	1	0	1	1	10	11	0	0	3	3	31
	Total	**0**	**14**	**3**	**0**	**4**	**1**	**17**	**14**	**0**	**0**	**10**	**12**	**75**