THE PMI AIRS / VECTORLINK PROJECT

ZIMBABWE ENTOMOLOGICAL REPORT

OCTOBER 2017 – SEPTEMBER 2018

Recommended Citation: PMI Africa Indoor Residual Spraying (AIRS) and VectorLink. October 2018. *Zimbabwe 2017-2018 Entomological Progress Report.* Rockville, MD. PMI Africa Indoor Residual Spraying, Abt Associates Inc.

Contract: GHN-I-00-09-00013-00
Task Order: AID-OAA-TO-11-00039

Submitted: May 31, 2019

Approved:

Submitted to: President's Malaria Initiative, Washington, DC, and PMI Zimbabwe

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ACRONYMS

AIRS	Africa Indoor Residual Spraying
AU	Africa University
CDC	Centers for Disease Control and Prevention
DDT	Dichlorodiphenyltrichloroethane
ELISA	Enzyme-Linked Immunosorbent Assay
HLC	Human Landing Catch
IRS	Indoor Residual Spraying
LLIN	Long-lasting Insecticidal Net
M	Meter
MOHCC	Ministry of Health and Child Care
NIHR	National Institute of Health Research
NMCP	National Malaria Control Program
OP	Organophosphates
PBO	Piperonyl Butoxide
PCR	Polymerase Chain Reaction
PMD	Provincial Medical Director
PMI	President's Malaria Initiative
PPA	Prokopack Aspirator
PPE	Personal Protective Equipment
PSC	Pyrethrum Spray Collection
USAID	United States Agency for International Development
WHO	World Health Organization
ZAPIM	Zimbabwe Assistance Program in Malaria

EXECUTIVE SUMMARY

Through support from The President's Malaria Initiative (PMI), the African Indoor Residual Spraying (AIRS) Project implemented indoor residual spraying with pirimiphos-methyl during the 2017 campaign in four districts in Manicaland Province (Chimanimani, Mutare, Mutasa, and Nyanga districts). The PMI AIRS Project and the current follow-on project, the PMI VectorLink Project, implemented entomological monitoring for Malaria Vector Control in Zimbabwe in partnership with the National Institute of Health Research (NIHR), the National Malaria Control Program (NMCP), and provincial medical directorates. Monthly longitudinal vector surveillance was conducted at three sites in Manicaland Province, namely, Burma Valley, Chakohwa and Vumba. The residual efficacy of insecticide sprayed at Manicaland Province was also monitored following 2017 spray campaign. Outside of Manicaland, insecticide susceptibility tests were carried out at six sites in four provinces - Makakavhule in Matebeleland South; Ngondo, Manjolo and Simatelele in Matebeleland North; Kamhororo in Midlands; and Beitbridge in Matabeleland South. VectorLink also conducted one-time vector surveillance at four other sites in Manicaland, namely, Chinyamukwakwa, Imbeza, Zindi and Nyamaropa, following at upsurge in malaria cases reported from the province. The project also surveyed Arcturus in Mashonaland East following an outbreak situation in the district that borders with Harare City which the province reported during the introductory meeting involving NMCP and VectorLink. The VectorLink project also supported the Zimbabwe Assistance Program in Malaria (ZAPIM) with entomological surveillance at Angwa Ward; however, this activity is presented as part of a separate report.

Anopheles funestus s.l. was the predominant species at all three longitudinal sites in Manicaland. *Anopheles gambiae* s.l. was found at low densities at the Chakohwa and Vumba (control) sites. The major malaria vectors belong to the two species complexes. Other species found included *An. pretoriensis,* a non-vector, and three potential secondary vectors, *An. coustani*, *An. rufipes*, and *An. maculipalpis*. Mosquito densities at all sentinel sites in Manicaland and using all collection methods were generally low, which did not allow for meaningful conclusions to be made on vector behavior. However, Centers for Disease Control and Prevention (CDC) light trap collections indicated higher numbers of malaria vectors (both *An. funestus* s.l. and *An. gambiae* s.l.) outdoors versus indoors at all sites and might be indicative of the preference to feed outdoors. Wall bioassays conducted monthly following the Indoor Residual Spraying (IRS) campaign in Manicaland showed considerable variation in residual efficacy of pirimiphos-methyl among wall surface types, with greater residual efficacy on mud and brick walls as opposed to cement or painted walls. The residual efficacy was at least five months on cement and painted walls at Burma Valley, and four months on the same wall types at Chakohwa. However, pirimiphos-methyl was effective up to eight months on mud walls at Burma Valley and seven months on mud and brick walls at Chakohwa. The average residual efficacy of pirimiphos-methyl was about six months.

The vectors remain susceptible to most insecticides at most sites but there is resistance to Dichlorodiphenyltrichloroethane (DDT) at Makakavhule and permethrin at Manjolo. Based on the insecticide resistance results from 2017 and 2018, *An. gambiae* s.l. is also susceptible to clothianidin, the active ingredient found in two newly approved next generation insecticides, Sumishield and Fludora Fusion. Therefore, pirimiphos-methyl, Sumishield, and Fludora Fusion should be considered for insecticide rotation strategies for IRS in these districts moving forward. Resistance to DDT at Makakavhule suggest that Sumishield and Fludora Fusion should be considered for the next spray campaign in Matebeleland South. It might also be important to consider next-generation long-lasting insecticidal nets (LLINs) in Matebeleland North, where *An. gambiae* s.l. was found to be resistant to permethrin but susceptible to chlorfenapyr, one of the insecticides used for impregnation of Interceptor G2 LLINs along with a pyrethroid, alpha-cypermethrin.

Outside of Manicaland, in areas sprayed with DDT by the Government of Zimbabwe, *An. funestus* s.l. was collected in both Chinyamukwakwa and Mola, while *An. gambiae* s.l. was present only at Chinyamukwakwa, Chipinge District. *An. pretoriensis*, a non-vector, was also found at Mola.

Although some molecular analysis was completed during this reporting period, results are limited so will be reported as part of an addendum when more results are available to enable more significant conclusions.

1. INTRODUCTION

Malaria is not uniformly distributed in Zimbabwe, with more than 60% of the cases reported from three provinces; Manicaland, Mashonaland Central, and Mashonaland East. Major malaria vectors include *An. arabiensis* and *An. gambiae* s.s., both members of the *An. gambiae* s.l. species complex, and *An. funestus* s.s. More than 98% of malaria cases are due to *Plasmodium falciparum*, with *P. malariae* and *P. ovale* responsible for the remainder of the cases. Malaria incidence was 16.8, 28.8, and 16.3 for 2016, 2017, and 2018, respectively (NMCP, 2018). Malaria deaths were 235, 513, and 191 for 2016, 2017, and 2018, respectively. Twenty-nine districts are considered malaria elimination areas, and 17 are buffer districts between elimination and control districts. Indoor residual spraying (IRS) and long-lasting insecticidal nets (LLINs) are the major strategies for vector control in Zimbabwe. Of the 32 districts targeted for IRS in 2018, 16 were sprayed with pirimiphos-methyl, 15 with (Dichlorodiphenyltrichloroethane) DDT, and one with lambdacyhalothrin, a pyrethroid.

President's Malaria Initiative (PMI) supported IRS and entomological surveillance under the African Indoor Residual Spraying (AIRS) Project from 2013 to February 2018. This support was continued under the follow-on project, PMI VectorLink, starting in March 2018. Prior to 2018, PMI supported IRS in four districts in Manicaland (Chimanimani, Mutare, Mutasa, and Nyanga), but in 2018 transitioned support to two districts in Mashonaland East (Mutoko and Mudzi). This report focuses on activities completed from October 2017 – September 2018 under the PMI AIRS Project (October 2017 to February 2018) and the PMI VectorLink Project (March 2018 to September 2018). The objectives under these two projects include the following:

1. Monitor spray quality and residual efficacy of pirimiphos-methyl following the 2017 IRS campaign in four districts in Manicaland.
2. Perform annual insecticide susceptibility testing at six sites in four provinces to inform vector control decision making.
3. Continue monthly vector bionomics monitoring at three sites in Manicaland province to monitor the impact of IRS (October 2017 – September 2018).
4. Initiate monthly vector bionomics monitoring at four sites in Mashonaland East to collect baseline entomological data prior to the 2018 IRS campaign (August – September 2018). *Results to be reported separately in VectorLink Entomological Monitoring Reports.*
5. Conduct entomological surveillance to support a Zimbabwe Assistance Program in Malaria (ZAPIM)-led assessment in response to increasing malaria incidence in Angwa Ward (Mbire District, Mashonaland Central Province) in May – June 2018. Results reported separately.
6. Conduct one-time entomological surveillance in response to a malaria outbreak in Arcturus (Goromonzi District, Mashonaland East Province) (April 2018).
7. Conduct one-time entomological surveillance at four other sites in Manicaland, namely, Chinyamukwakwa, Imbeza, Zindi and Nyamaropa following at upsurge in malaria cases reported from the province (May-June 2018).

2. MATERIALS AND METHODS

2.1 SITES

Teams consisting of staff from the National Institute of Health Research (NIHR), the provincial medical directors (PMDs), and the PMI AIRS/ VectorLink projects conducted monthly entomological surveillance at three sentinel sites in Manicaland Province - Burma Valley, Chakohwa, and Vumba (control site),starting in October 2017. Surveillance ended in Chakohwa in June 2018 as part of the transition of PMI support from Manicaland to Mashonaland East. No collections were suspended in Vumba or Burma Valley in July and August during the harmonized elections. Collections were also not conducted in Vumba during September as support staff specific to this site attended the Pan-Africa Mosquito Control Association Conference (PAMCA) when collections were done. Collections were still conducted in Burma Valley during September 2018.

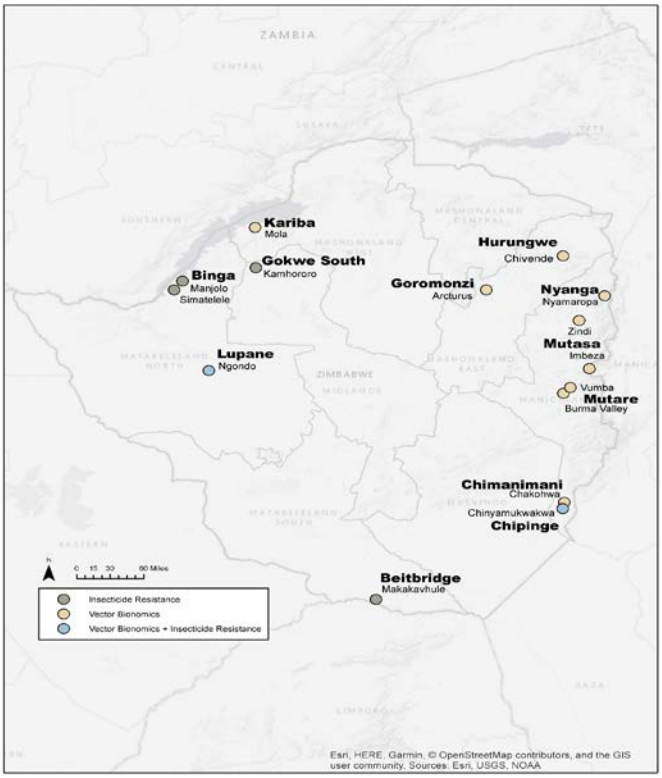
Entomological surveillance was conducted in April 2018 at one site in Mashonaland East (Arcturus) following an outbreak of malaria in the district that borders with Harare City which the province reported during the provincial-level VectorLink introductory meeting. In addition, following an increased number of malaria cases reported by the NMCP, VectorLink conducted one-time entomological surveillance in May and June 2018 at four sites in Manicaland (Chinyamukwakwa, Imbeza, Zindi, and Nyamaropa) and in June 2018 at two sites in Mashonaland West (Mola and Chivende). Insecticide susceptibility tests were carried out at six sites in four provinces, and wall bioassay tests were conducted to monitor residual efficacy of pirimiphos-methyl at two sites sprayed during the 2017 IRS campaign in Manicaland Province. Details of sites and activities accomplished are outlined in Table 1.

TABLE 1. SENTINEL SITES BY GEOGRAPHIC LOCATIONS AND ACTIVITIES.

Province	District	Site	Ento Activity	O	N	D	Ja	F	Mar	A	M	J	Jul	Au	S
Manicaland	Mutare	Burma Valley*	VB	X	X	X	X	X	X	X	X	X			X
			CB	X	X	X	X	X	X	X	X				
		Vumba	VB	X	X	X	X	X	X	X	X	X			
	Chimanimani	Chakowa*	VB	X	X	X	X	X	X	X	X	X			
			CB	X	X	X	X	X	X	X	X				
	Chipinge	Chinyamukwakwa	IR									X			
			VB									X			
	Mutasa	Zindi**	VB									X			
		Imbeza	VB							X					
	Nyanga	Nyamaropa**	VB									X			
Mat South	Beitbridge	Makakavhule**	IR	X	X										
Midlands	Gokwe South	Kamhororo**	IR	X											
Mat North	Binga	Simatelele**	IR												X
		Manjolo**	IR												X
	Lupane	Ngondo**	IR												X
			VB												X
Mash East	Goromonzi	Arcturus	VB							X					
Mash West	Kariba	Mola**	VB									X			
	Hurungwe	Chivende**	VB									X			

**PMI-supported spray districts in 2017 **Non-PMI spray districts*
LEGEND: IR – insecticide resistance testing, VB – vector bionomics, CB – cone bioassays

FIGURE 1. MAP OF ZIMBABWE SENTINEL SITES BY TYPE OF ACTIVITIES ACCOMPLISHED



2.2 ROUTINE VECTOR BIONOMICS MONITORING

Mosquitoes were collected monthly at three vector bionomics monitoring sites in Manicaland (Vumba, Burma Valley, and Chakowa) using pyrethrum spray collections (PSCs), prokopack aspirators (PPA), pit shelters and CDC light traps to assess the following indicators:

1. Vector species composition
2. Indoor and outdoor resting densities
3. Indoor and outdoor human biting rates
4. Sporozoite infection rates

Data for sites in Mashonaland East province will be presented in a separate report.

2.2.1 ESTIMATING INDOOR RESTING DENSITIES USING PYRETHRUM SPRAY COLLECTIONS

Initially, PSCs were used to sample indoor resting mosquitoes from 25 living and 15 non-living rooms monthly at each vector bionomics monitoring per site collection period, as there are fewer non-living rooms than living rooms. Starting in April 2018, collections were no longer done in non-living rooms, only 25 living rooms. PSC was done between 6:00 a.m. and 8:00 a.m. Preparation of the room included removing all people and animals, removing or covering all food and small items of furniture, covering all openings and eaves with cloth or netting, and spreading white calico sheets to completely cover the floor and all flat surfaces of the remaining furniture. The calico sheets were also spread under tables, beds, and other places where mosquitoes may hide. The commercial aerosol Baygon, composed of tetramethrin (0.2%), prallethrin (0.04%), imiprothrin (0.034%), piperonyl butoxide (PBO) (1.0%), and propellant/solvent

(98%), was used to knock down all the resting mosquitoes inside closed rooms. After 10 minutes, mosquitoes knocked down from the sprayed rooms were collected using a pair of forceps or a mouth aspirator.

Mosquitoes collected from the different rooms were transferred to separate, properly-labeled petri dishes. Each petri dish was labeled with method of collection, date, locality, and household name. The abdominal stage of all female *Anopheles* was recorded as unfed, blood-fed, half-gravid, or gravid. In addition, data on the number of people who slept in the house the previous night, the type of house and walls, and the number of treated nets present were recorded.

2.2.2 Estimating Indoor Resting Densities Using the Prokopack Aspirator

Prokopack aspirators were used to sample indoor resting mosquitoes from 25 living and 15 non-living rooms per collection period at each site between 6:00 a.m. and 8:00 a.m. The PPA used a rechargeable, lead, acid-sealed 12-volt battery. One team member entered the room and connected the aspirator to the battery terminals, appropriately using the color codes. Mosquito aspiration was done systematically starting from the door, moving to the walls and furniture, then under the beds and tables, and finishing with the roof or ceiling. Collected (live) mosquitos were released into a Bugdorm cage, counted, and recorded before they were aspirated into a petri dish. Each petri dish was labeled with method of collection, date, locality, and household name. In addition, data on the number of people who slept in the house the previous night, the type of house and walls, and the number of treated nets present were collected. The PPA method was discontinued in June in response to a PMI request to pick either PSC or PPA as a method for estimating indoor resting density. The team chose PSC due to its higher yield.

2.2.3 Estimating Outdoor resting densities using pit shelter collections

Pit shelter collections were set up to estimate outdoor resting density in Burma Valley. Collections were done in June and September 2018. Five pits were established at the site and monitored from 6.00 a.m. and 9:00 a.m. on four consecutive days per month. Overall, pit shelters were two meters (m) deep with a 1.5m x 1.5m opening and at least eight holes (2 holes x 4 sides) for mosquitoes to rest. They were at least 10m away from homes and fenced for animal and human safety. One team member entered the pit with a torch, aspirator tube, and polystyrene cup covered with netting. Mosquitoes were aspirated and released into the polystyrene tube, counted, observed with regard to their abdominal stage, and recorded before being aspirated into a petri dish. Each petri dish was labeled with method of collection, date, geographical coordinate, pit locality, and number. The Environmental Health Technician, who supports both Vumba and Burma Valley, experienced delays in setting up pits in Burma Valley, which led to delays at Vumba. Therefore, no pit shelter data from Vumba is included during this reporting period, but will be included in the next report.

2.2.4 Estimating Indoor And Outdoor Densities Using CDC Light Traps

Battery-operated CDC light traps were used to collect mosquitoes from 6:00 p.m. to 6:00 a.m. for two nights in the control site (Vumba) and three nights in the other two sites. Six sentinel houses were randomly selected, with one trap placed indoors and the other outdoors, for a total of 12 light traps per site. Households selected for PSC and PPA collections were excluded from the sampling pool. Indoor CDC light traps were placed 1m above a person sleeping under a mosquito net. Outdoor CDC light traps were about 10m away from the house and, when possible, in a shaded area and away from wind. 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. A mouth aspirator and a pair of forceps were used to collect all mosquitoes from the traps. Separate petri dishes, appropriately labeled, were used for mosquitoes collected from each trap.

2.2.5 Estimating Biting Behavior Using CDC Light Traps

VectorLink used CDC light traps with human bait as a proxy for human landing catches (HLCs) to evaluate human-vector contact including the place, time, and seasonal distribution of the vectors. Houses used for PSC, PPA, and CDC light trap 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 sleeping under an untreated mosquito net. For outdoor placement, light traps were set about ten meters from the house. The volunteers, acting as mosquito bait, switched from indoor to outdoor positions hourly in order to minimize the bias that could arise from individual attractiveness to mosquitoes. Mosquitoes were collected from each trap hourly from 6:00 p.m. to 6:00 a.m. Mosquito collections were conducted indoors and outdoors simultaneously, using the same set-up, in order to compare vectors' host-seeking activity inside and outside houses. The teams recorded temperature, relative humidity, and precipitation hourly during the night. *Anopheles* mosquitoes were identified morphologically and preserved in silica gel for laboratory analysis.

2.3 Cross-Sectional Vector Bionomics

In the seven sites where one-time collections were conducted in response to a malaria outbreak, cross-sectional vector bionomics data was collected using PSCs as well as indoor and outdoor CDC light traps, following the methods described in Section 2.2.1 and 2.2.4, respectively. The only deviation is that collections were done using CDC light traps over three consecutive nights, rather than two, at each site.

2.4 Measuring Quality of Spray and Insecticide Decay

The standard WHO cone bioassay method was followed to measure the quality of spray and insecticide decay rate of pirimiphos-methyl (Actellic®300CS) in Manicaland following the routine spraying of walls at the Burma Valley and Chakohwa sites in October and November 2017, respectively. To assess the spray quality of the IRS application, bioassays were conducted within 24 hours after spraying and then monthly until average mortality rates fell below 80% to determine residual efficacy. Susceptible *Anopheles arabiensis* (KGB strain) from the NIHR's De Beers Research Laboratory insectary in Chiredzi and from Africa University (AU) in Mutare were used to conduct the cone bioassays. Ten rooms were tested at each site per month; wall types are summarized in Table 2 below.

Table 2: Summary of Wall Types Tested with Cone Bioassays

Wall Type	Number of Houses	
	Burma Valley	Chakohwa
Mud	2	2
Brick	2	3
Cement	3	3
Paint	3	2
Total	**10**	**10**

2.4.1 Wall Cone Bioassay Tests

For the wall cone bioassays, ten unfed, two- to five-day old female mosquitoes were exposed on the treated walls per cone. Three cones were randomly positioned per room at 0.5, 1.0, and 1.5 meters above the floor. Mosquitoes were exposed for 30 minutes, after which they were aspirated to a holding paper cup and provided with 10% sugar solution before mortality was recorded after a 24-hour holding period. Knockdown rates were also recorded at 30 minutes and 60 minutes. Mosquitoes exposed to unsprayed

surfaces were run concurrently as controls. Temperature and relative humidity were recorded during the exposure and the subsequent 24-hour holding period.

2.4.2 Bioassay Tests to Assess Fumigant Effect of Insecticide

To assess the airborne or fumigant effect, bioassays 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 placed on a wire support, designed so it was 10 centimeters from a sprayed wall and one meter above the floor. Mosquitoes were removed after 30 minutes and knockdowns recorded. They were then transferred to holding paper cups using a clean aspirator and provided with 10% sugar solution before mortality was recorded after the 24-hour holding period. Controls for the bioassays were conducted simultaneously using a similar set-up, but in an unsprayed room.

2.5 Insecticide Resistance Monitoring

Insecticide susceptibility and resistance intensity testing was conducted at six sites (Table 2). These sites were selected in coordination with the NMCP to provide geographical representation and were areas identified as malaria "hot spots".

The insecticides tested in 2018 were:

1. deltamethrin (0.05%)
2. permethrin (0.75%)
3. alpha-cypermethrin (0.05%)
4. lambdacyhalothrin (0.05%)
5. pirimiphos-methyl (0.25%)
6. DDT (4%)
7. chlorfenapyr (12.5, 25, 50, 100, 200µg/bottle)
8. clothianidin (13.2mg active ingredient per paper)

Insecticide susceptibility tests were performed using *An. gambiae* s.l. raised from larvae for all sites with the exception of Burma Valley, where adult *An. funestus* s.l. collected from pit shelters were tested, since it is difficult to get the larval stages of this species (Table 2). The team attempted to also conduct insecticide susceptibility testing in Zindi, Imbeza, and Nyamoropa, however due to insufficient mosquitoes, this could not be completed. From the range of insecticides available for testing, priority was given to pirimiphos-methyl and the insecticide under current use for vector control at that site. There was also interest in determining the susceptibility of local vectors to next generation insecticides, clothianidin and chlorfenapyr, for consideration under the national Insecticide Resistance Management Plan. The number of insecticides tested at any given site was determined by the availability of mosquitoes.

Table 2: Summary of Insecticides Tested at Insecticide Resistance Monitoring Sites, 2017-2018

Province	Site	*Anopheles* species	deltamethrin	alpha-cypermethrin	Lambda-cyhalothrin	permethrin	DDT	pirimiphos-methyl	chlorfenapyr	clothianidin
Matebeleland South	Makakavhule		X	X	X		X	X	X	

Matebeleland North	Manjolo	*An. gambiae* s.l.	X		X	X	X	X		X
	Simatelele						X	X		
	Ngondo							X		
Midlands	Kamhororo								X	
Manicaland	Chinyamukwakwa						X	X		

The WHO tube test method was used to test all insecticides except chlorfenapyr, for which the CDC bottle assay method was used. For WHO tests, female adult mosquitoes were exposed for one hour to insecticide-treated filter papers provided by WHO (Universiti Sains Malaysia-Malaysia). Exposure tests were accompanied by negative control tests in which mosquitoes were exposed to filter papers impregnated with oil or solvent. Testing was done according to WHO protocols, with mortality being the primary outcome measure. Four replicates of 25 *An. gambiae* s.l. for most sites were exposed to each concentration. Mortality was recorded every 15 minutes, up to 60 minutes. Clothianidin is a slow acting insecticide, hence mosquito mortalities were monitored up to seven days.

For CDC bottle assays, four replicates of at least 20 *An. gambiae* s.l. were exposed for 60 minutes to chlorfenapyr at 12.5, 25, 50, 100, and 200 ug/bottle. The proportion of mosquitoes knocked down was recorded 60 minutes after the start of the test, while mosquitoes were still in the bottle. After 60 minutes of exposure, mosquitoes were removed from the bottle, transferred to paper cups, and supplied with a sugar solution. Mortality was recorded every 24 hours for three days, as chlorfenapyr is also a slow-acting insecticide, following the 60-minute exposure. The team will continue with the CDC bottle assay method for susceptibility tests, except for clothianidin for which the only method available at the moment is the WHO tube test. However, the team will review the experiences with the CDC bottle bioassay method for future insecticide susceptibility monitoring.

2.6 Laboratory Analyses

All laboratory analyses were conducted in the molecular laboratory of Africa University. The mosquito samples collected from sentinel sites were transported to the laboratory, where processing and analysis were carried out following established protocols.

2.6.1 Identification of Sporozoite Infection Rates

Anopheles mosquitoes from Arcturus (n = 131) and Imbeza (n = 15) were analyzed for circumsporozoite (CS-protein) for *Plasmodium falciparum* at AU laboratory using the enzyme linked immunosorbent assay (ELISA) method developed by Wirtz et al (1987) and described in the MR4 Manual (2010) and modified to prevent false positives as described by Durnez et al, (2011). Only the head and thorax of an individual mosquito specimen were analyzed using ELISA. The number of specimens analyzed represented all the mosquitoes collected at these two sites. The sites were prioritized in order to shed some light on the malaria upsurge reported.

2.6.2 Detection of *KDR* Resistance Gene in *An. gambiae* S.L.

A total of 131 *An. gambiae* s.l. from Arcturus and four Imbeza were analyzed for *kdr* gene East (TCA mutation) and West (TTT mutation) using MR4 protocols. The methods were developed by Martinez-Torres et al (1998) and Ransom et al (2000). The allele-specific PCR protocol was used to detect the L1014S or L1014F alleles for the knockdown resistance (*kdr*) commonly found in East Africa and West Africa respectively Specimens from the two sites were prioritized to provide insights on the malaria upsurges reported.

2.6.3 MOLECULAR IDENTIFICATION OF *ANOPHELES* SPECIES

Due to limited reagents, *Anopheles* mosquitoes collected from the Arcturus and Imbeza sites were prioritized for molecular species identification using ribosomal deoxyribonucleic acid (rDNA) polymerase chain reaction (PCR) methods at the AU laboratory. Briefly, DNA samples were extracted from either single mosquitoes or available parts of mosquitoes using standard extraction protocols and amplified through PCR. Following CDC guidance, all of the extracted DNA was first tested using the *An. gambiae* s.l. PCR method. Unamplified DNA was then assayed on the *An. funestus* s.l. multiplex PCR. This process was continued until all the available rDNA PCR methods were exhausted or all of the samples were amplified. The protocol for *An. gambiae* s.l. is described by Scott et al (1993); while the protocol for *An. funestus* s.l. is described by Koekemoer et al (2002). Details of these methods are also documented in the MR4 Manual (2010). Mosquitoes from other sites will be analyzed during 2019. Results from laboratory analysis will be reported as an addendum to this report after specimens from other sites have been analyzed.

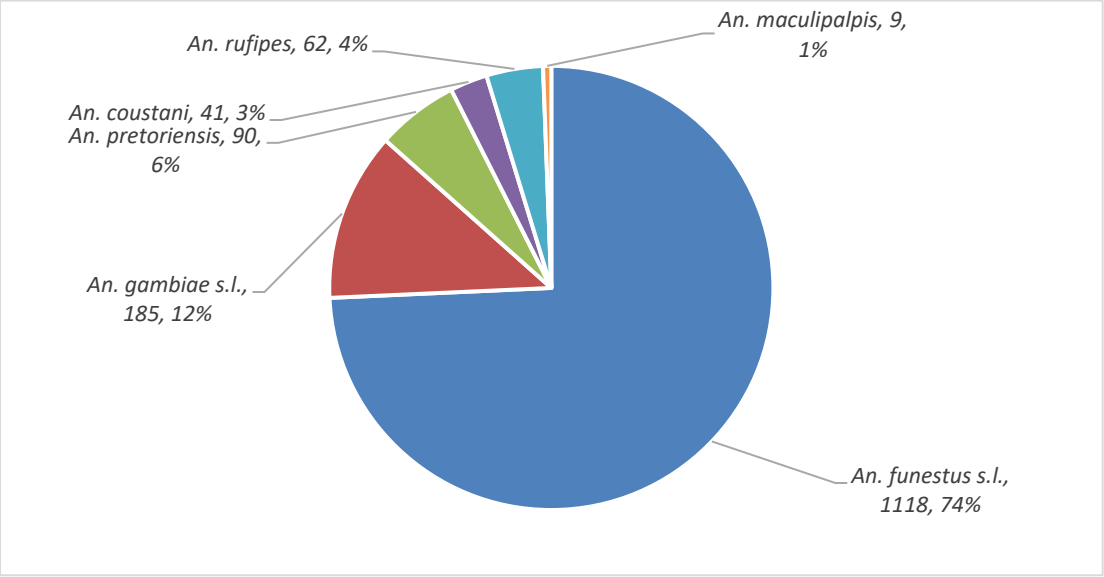
3. RESULTS

3.1 VECTOR SPECIES COMPOSITION

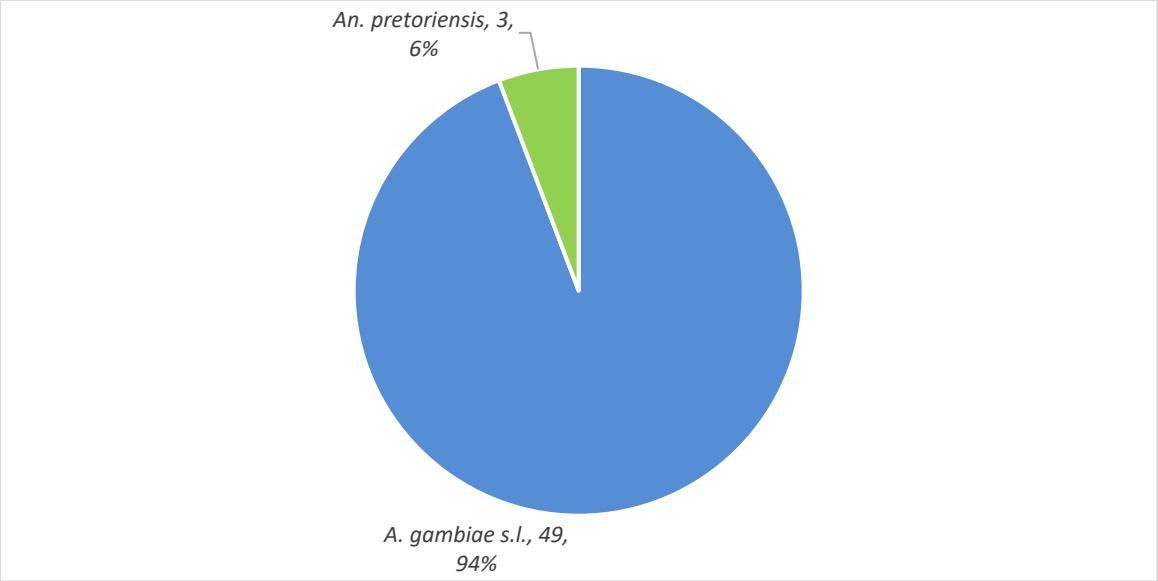
A total of 2020 female *Anopheles* mosquitoes were collected between October 2017 and September 2018 from DDT (two sites) and pirimiphos-methyl (four sites) sprayed sites and three unsprayed sites (Figure 2) using CDC-light traps, pit shelters, PSC and PPA methods. The predominant species in the pirimiphos-methyl sprayed site was *An. funestus* s.l. (N=1118, 74.2%) (Figure 2A), whereas this species was not found in any DDT sprayed areas (Figure 2B). *Anopheles funestus* s.l. was also the dominant species (N= 368, 80.5%) in the unsprayed sites (Figure 2C). *Anopheles gambiae* s.l. represented 11.6% (N=235) of the total collection and was caught mostly in the pirimiphos-methyl sprayed sites (N=185, 78.7%); 49 were caught in the DDT sprayed sites and only one from the unsprayed area. Mosquitoes (N = 1507) for the pirimiphos-methyl sprayed area were collected from four sites in Manicaland: Burma Valley, Chakohwa, Nyamaropa and Zindi. Mosquitoes (N = 56) for DDT sprayed areas were based on three sites, namely, Chinyamukwakwa (Chipinge district in Manicaland province) and from two sites in Mashonaland West province, Mola (Kariba district) and Chivende (Hurungwe district). Mosquitoes (N = 457) for the unsprayed areas were collected from Arcturus in Goromonzi district (Mashonaland East province) and from two sites in Manicaland province; Vumba (Mutare district) and Imbeza (Mutasa district). The figures on the pie charts represent the number of mosquitoes collected and the percentages for each species.

FIGURE 2. *ANOPHELES* SPECIES COMPOSITION IN PIRIMIPHOS-METHYL (A) AND DDT (B) SPRAYED SENTINEL SITES AND UNSPRAYED SENTINEL SITES (C)

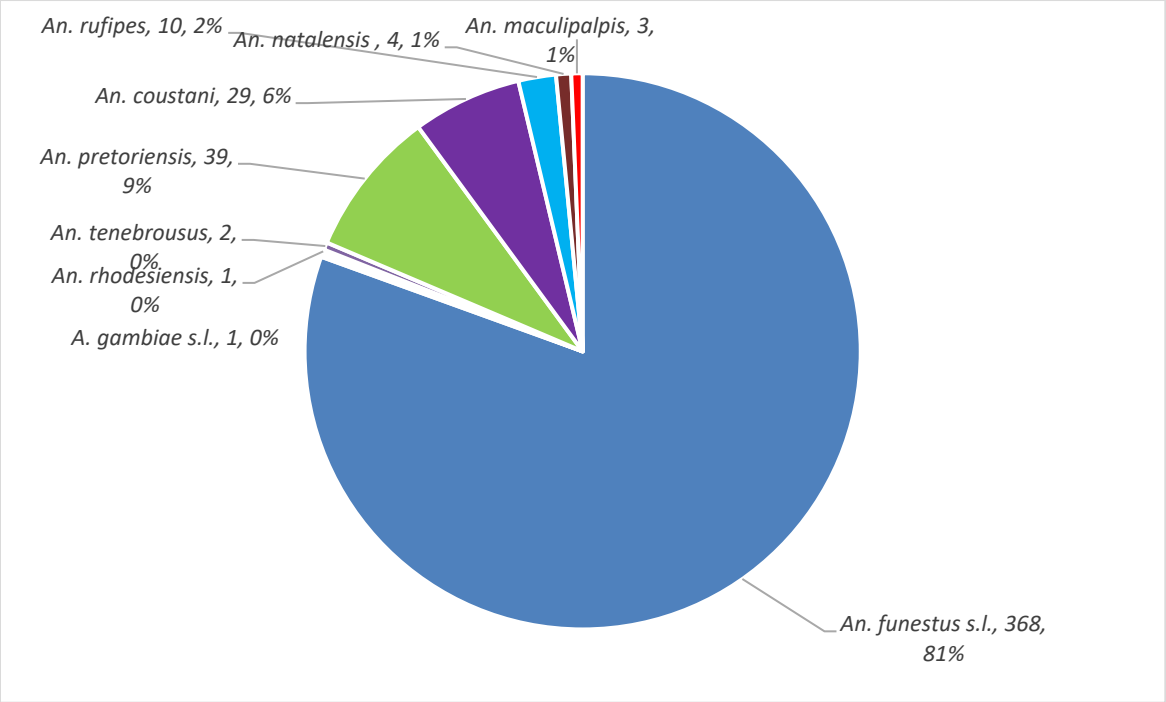
2A- Pirimiphos-methyl sprayed sites (n=1,507)



2B- DDT sprayed sites (n=56)



2C- Unsprayed sites (n=457)



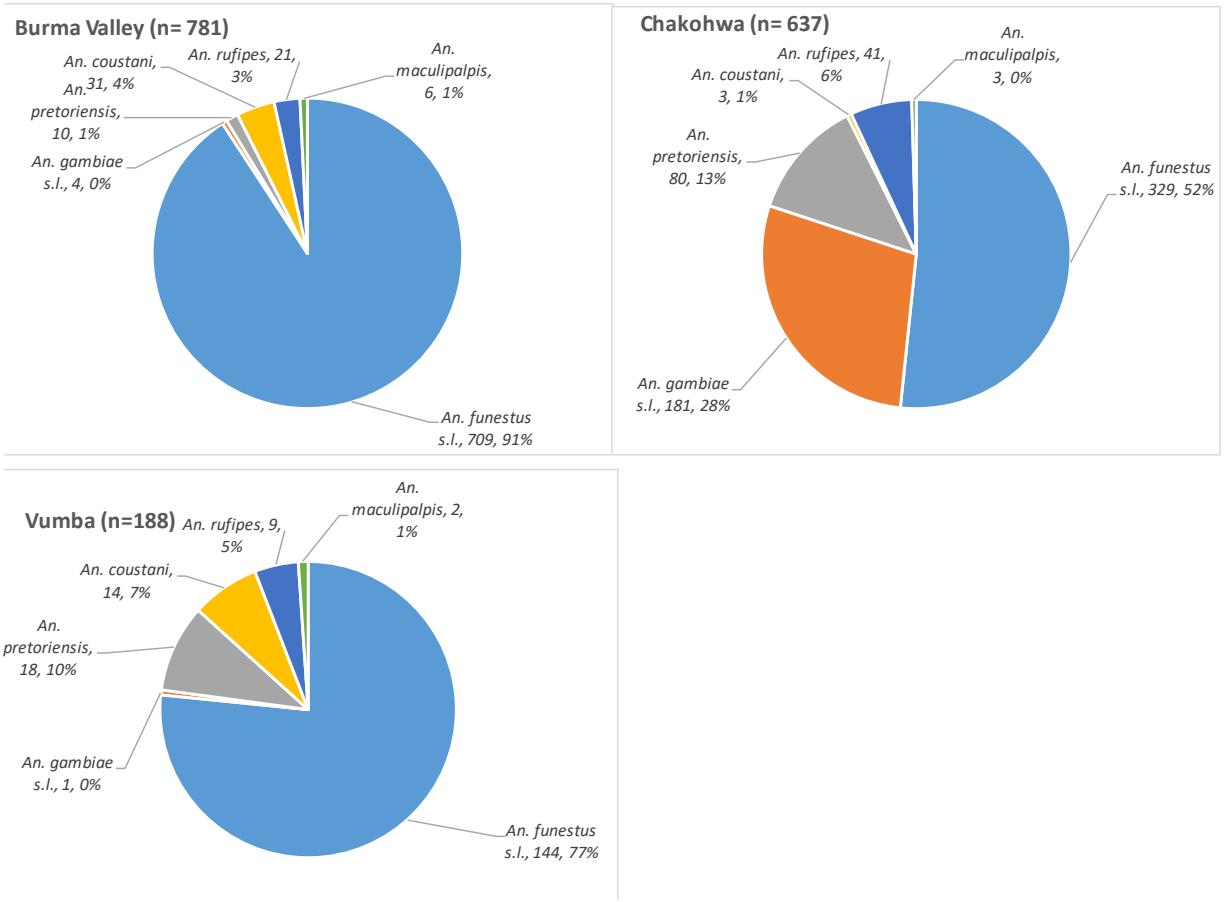
3.2 ROUTINE VECTOR BIONOMICS MONITORING

Below are results from mosquito collections at the sentinel sites in Manicaland Province. The results exclude July and August, when routine surveillance was suspended during the harmonized elections held in July. Chakohwa collections ended in June as planned and Vumba collections ended in June due to the elections and PAMCA conference attendance, held in September. The only site with collections in September was Burma Valley.

3.2.1 VECTOR COMPOSITION

Across the three sites in Manicaland (Burma Valley, Vumba and Chakohwa), the predominant species collected across all methods was *An. funestus* s.l. *An. gambiae* s.l. were collected from all three sites but were a substantial proportion (28%) in Chakohwa (Figure 3). Three other species collected at the sites, *An. coustani, An. rufipes* and *An. maculipoalpis* are considered secondary malaria vectors, whereas, *An. pretoriensis* is not a malaria vector.

FIGURE 3. *ANOPHELES* SPECIES COMPOSITION AT SPRAYED (BURMA VALLEY AND CHAKOHWA)) AND UNSPRAYED (VUMBA) SITES IN MANICALAND PROVINCE.



3.2.2 INDOOR RESTING DENSITIES

PSC and PPA collections indicated very few mosquitoes were resting indoors in both sprayed and unsprayed sites, making it difficult to make meaningful conclusions. Of the 152 mosquitoes collected across all sites and methods, 95.4% were *An. funestus* s.l and 4.6% were *An. gambiae* s.l. At Burma Valley, only *An. funestus* s.l. were collected. Of the 45 *An. funestus* s.l. collected resting indoors at Burma Valley, 66.7% were unfed, 4.4% gravid, 0% half-gravid, and 28.9% blood-fed. Of the 39 *An. funestus* s.l. collected resting indoors at Vumba, 43.6% were unfed, 43.6% blood-fed and12.8% gravid. Nine *An. funestus* s.l. were collected at Chakohwa with the following physiological status: 22.2% unfed, 66.7% fed and 11.1% half-gravid.

3.2.3 Outdoor Resting Densities

Pits shelters used at Burma Valley suggest greater numbers of mosquitoes are resting outdoors than indoors, though collections using this method were only performed in June and September and numbers were still low (n = 129). 23 *An. funestus* s.l. were collected in June, whereas 106 were collected in September, and three *An. pretoriensis* were collected in September. No other anopheline species were collected from the pits at Burma Valley.

3.2.4 Indoor and Outdoor Densities

Overall, *An. funestus* s.l. was the predominant mosquito species collected using CDC lights traps across the three sites in Manicaland, with more being caught indoors than outdoors (Table 3), although it should be noted that the outdoor traps were not baited. *Anopheles gambiae* s.l. was caught only in Chakohwa, and mainly from traps set outdoors.

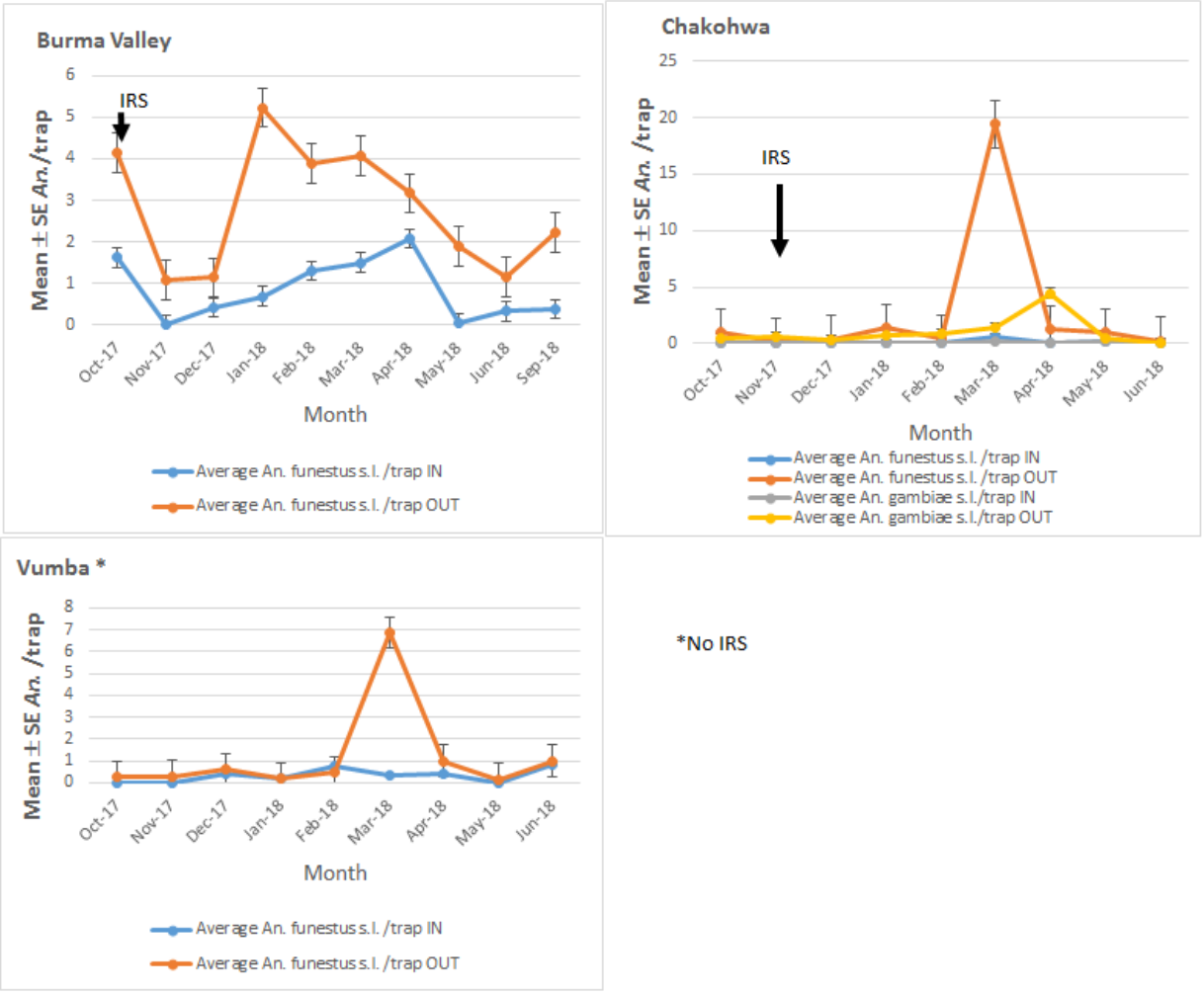
Table 3. Indoor and Outdoor densities of Anopheles mosquito vectors as collected in CDC light traps at three sentinel sites in Manicaland

SITE	VECTOR SPECIES	NUMBER OF CDC LIGHT TRAPS*		NUMBER OF MOSQUITO COLLECTED		DENSITY (NUMBERS/ TRAP/ NIGHT)	
		IN	OUT	IN	OUT	IN	OUT
Burma Valley (sprayed)	*An. gambiae* s.l.	139	147	0	0	0	0
	An. funestus s.l.	139	147	64	240	0.28	1.88
Vumba (unsprayed)	*An. gambiae* s.l.	65	65	0	0	0	0
	An. funestus s.l.	65	65	9	65	0.14	1
Chakohwa (sprayed)	*An. gambiae* s.l.	90	91	3	59	0.01	0.19
	An. funestus s.l.	90	91	9	265	0.1	2.91

**Light trap variances due to differences in the number of collection nights (two for control site and two for intervention sites), collection months, and battery challenges.*

The greatest number of mosquitoes collected outdoors occurred in January 2018 at Burma Valley (average 5.23 *An. funestus* s.l./trap/night), April 2018 at Vumba (6.5 *An. funestus* s.l./trap/night), and March 2018 in Chakohwa (20 *An. funestus* s.l./trap/night) (Figure 4). The overall greatest number of mosquitoes collected indoors occurred in Burma Valley in April, with an average of 2.08 per trap per night.

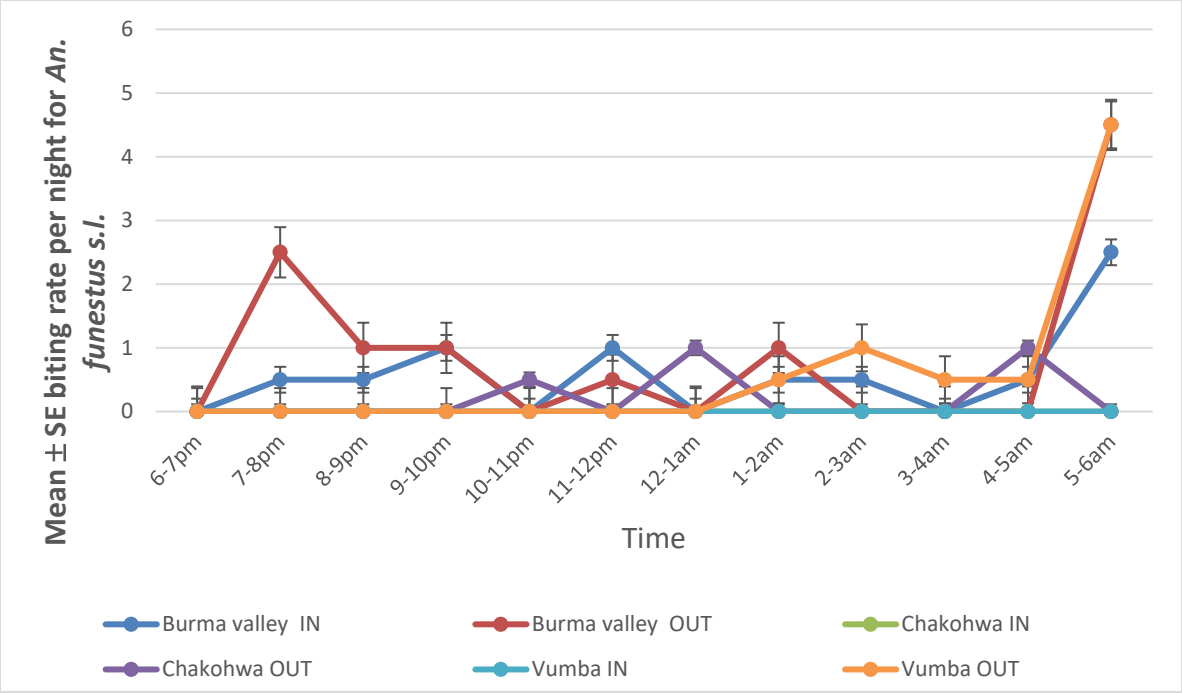
Figure 4. Indoor and Outdoor vector densities (based on CDC light trap collections) in sprayed (Burma Valley and Chakohwa) and unsprayed (Vumba) sites in Manicaland Province.



3.2.5 Indoor and Outdoor Human Biting Rates

Hourly collections using CDC traps revealed peak outdoor biting activity of *An. funestus* s.l. between 7:00 p.m. and 8:00 p.m. and again between 5:00 a.m. and 6:00 a.m. in Burma Valley (Figure 5). In Vumba, *An. funestus* s.l. exhibited peak outdoor biting activity between 5:00 am and 6:00 am. Surprisingly, *An. funestus* s.l. was not found indoors at any of the sites during the collection period. Because biting peaked in the early morning hours (between 5:00 a.m. and 6:00 a.m.) in both Vumba and Burma Valley, collection hours were extended from 6:00 a.m. up to 10:00 a.m., but no mosquitoes were found during this time. Very few mosquitoes were collected in Chakohwa, revealing no trends in hourly biting rates.

Figure 5. Average Indoor and Outdoor Hourly biting Rates of *An. funestus* s.l. as Determined by CDC Light Trap Collections at Three Sentinel Sites in Manicaland



3.3 Cross-Sectional Entomological Surveillance

3.3.1 Manicaland

At three sites in Manicaland, *An. funestus* s.l. was the predominant mosquito collected, comprising 99% (n=86) in Zindi, 100% in Nyamaropa (n=2), 60% (n=15) in Imbeza, whereas *An. gambiae* s.l. was predominant 94% (n=53) in Chinyamukwakwa. *An. natalensis* (4), *An. maculipalpis* (1), and *An. preoriensis* (1) were also collected at Imbeza. A single *An. coustani* was collected in Zindi and three *An. pretoriensis* were collected in Chinyamukwakwa.

3.3.2 Mashonaland East

At Arcturus, of the 131 *Anopheles* collected using multiple methods in April, 43.5% were *An. funestus* s.l., 28.3% were *An. gambiae* s.l., and the remaining proportion (28.2%) was made up of four other species namely, *An. pretoriensis, An. squamosus, An. rufipes* and *An. maculipalpis*. Molecular analysis revealed the presence of four malaria vectors. Detailed results will be presented as an addendum to this report.

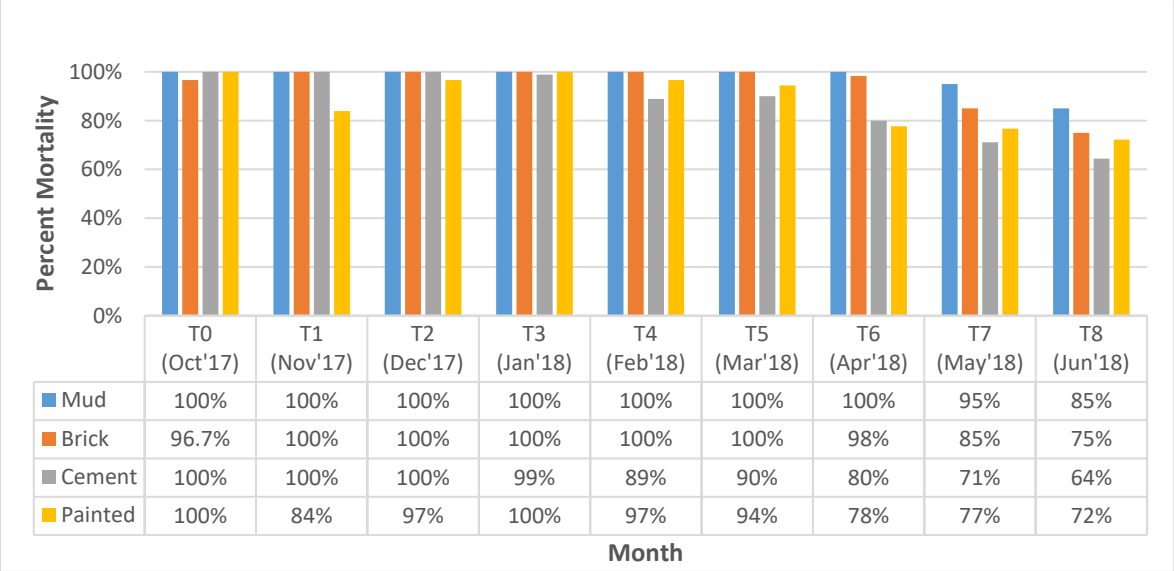
3.3.3 IRS Spray Quality and Residual Efficacy

The team monitored the spray quality and insecticide decay rate of Actellic on sprayed wall surfaces from October 2017 until June 2018, as well as the airborne effect from October 2017 to April 2018.

3.3.4 Cone Bioassay Tests

At Burma Valley, the initial quality of spray was acceptable for all wall surfaces except in one brick room. Residual efficacy of pirimiphos-methyl varied among wall surface types, but was greatest for mud surfaces, for which mosquito mortality remained >80% for eight months (Figure 6). Mosquito mortality remained >80% for up to five, six, and seven months for painted, cement, and brick surfaces, respectively.

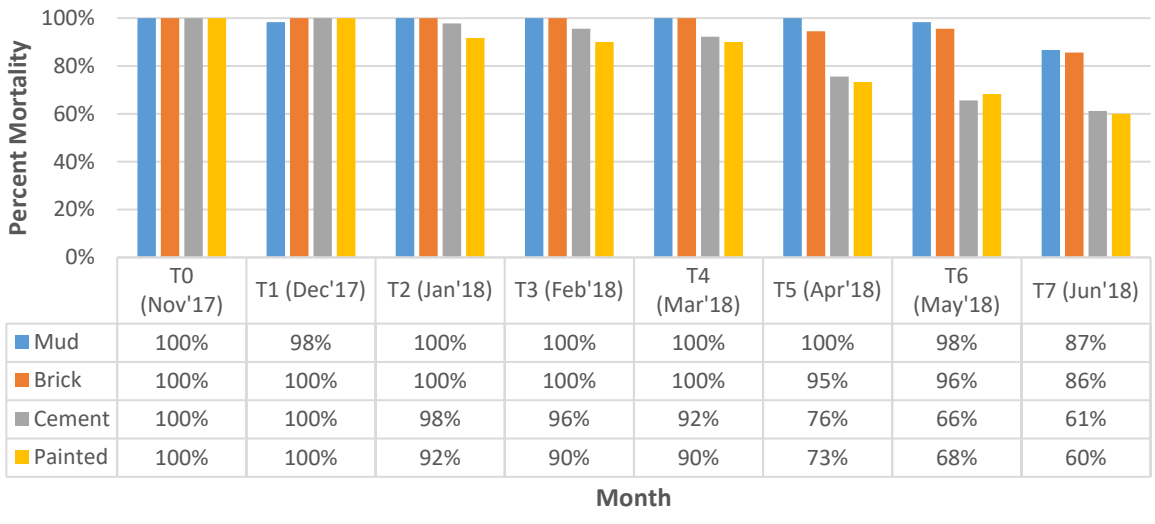
FIGURE 6. RESIDUAL EFFICACY OF PIRIMIPHOS-METHYL IN BURMA VALLEY, MUTARE DISTRICT, REPORTED AS *AN. ARABIENSIS* (KGB STRAIN) MORTALITY AFTER 24-HOUR HOLDING PERIOD IN WHO CONE BIOASSAYS



	T0 (Oct'17)	T1 (Nov'17)	T2 (Dec'17)	T3 (Jan'18)	T4 (Feb'18)	T5 (Mar'18)	T6 (Apr'18)	T7 (May'18)	T8 (Jun'18)
Mud	100%	100%	100%	100%	100%	100%	100%	95%	85%
Brick	96.7%	100%	100%	100%	100%	100%	98%	85%	75%
Cement	100%	100%	100%	99%	89%	90%	80%	71%	64%
Painted	100%	84%	97%	100%	97%	94%	78%	77%	72%

At Chakohwa, a good quality of spray was noted in all 10 rooms recording 100% mosquito mortality after the 24-hour holding period (Figure 7). Residual efficacy of pirimiphos-methyl varied among wall surface types, but was greatest for mud and brick surfaces, for which mosquito mortality remained >80% for seven months. Mosquito mortality remained >80% for up to four months for cement and painted surfaces.

FIGURE 7. RESIDUAL EFFICACY OF PIRIMIPHOS-METHYL IN CHAKOHWA, CHIMANIMANI DISTRICT, REPORTED AS *AN. ARABIENSIS* (KGB STRAIN) MORTALITY AFTER 24-HOUR HOLDING PERIOD IN WHO CONE BIOASSAYS

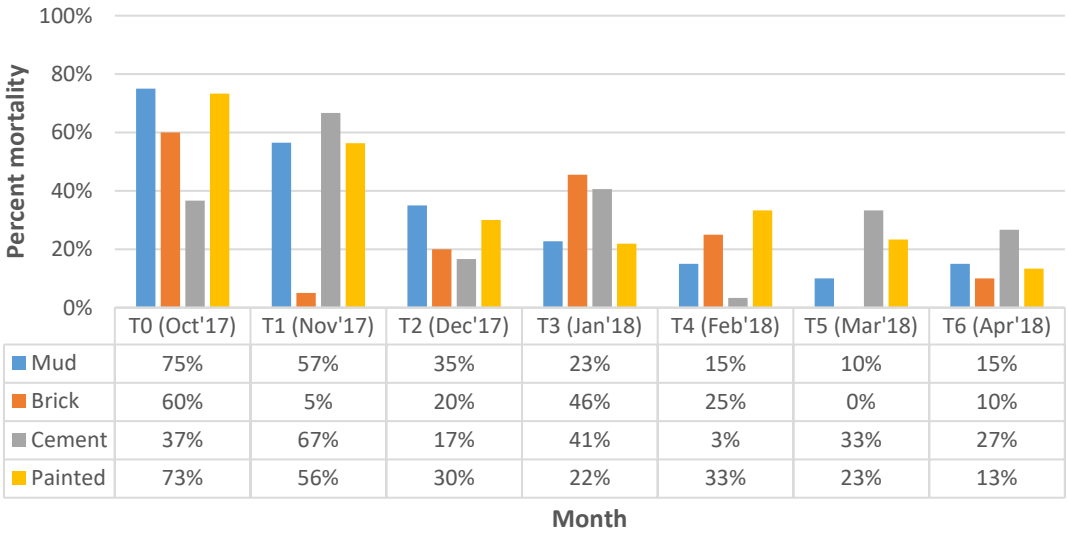


	T0 (Nov'17)	T1 (Dec'17)	T2 (Jan'18)	T3 (Feb'18)	T4 (Mar'18)	T5 (Apr'18)	T6 (May'18)	T7 (Jun'18)
Mud	100%	98%	100%	100%	100%	100%	98%	87%
Brick	100%	100%	100%	100%	100%	95%	96%	86%
Cement	100%	100%	98%	96%	92%	76%	66%	61%
Painted	100%	100%	92%	90%	90%	73%	68%	60%

3.3.5 AIRBORNE EFFECT OF PIRIMIPHOS-METHYL

At Burma Valley, the average mosquito mortality across all four wall types due to the airborne effect of the insecticide 24 hours post-spray was 61.3% in October and declined to a 16.3% in April, six months post-spray (Figure 8). There was greater variability on percentage mortality among houses of the same wall type mainly at the time of spray (October 2017) as compared to March 2018, five months after spraying. The tests for airborne effect were discontinued after April.

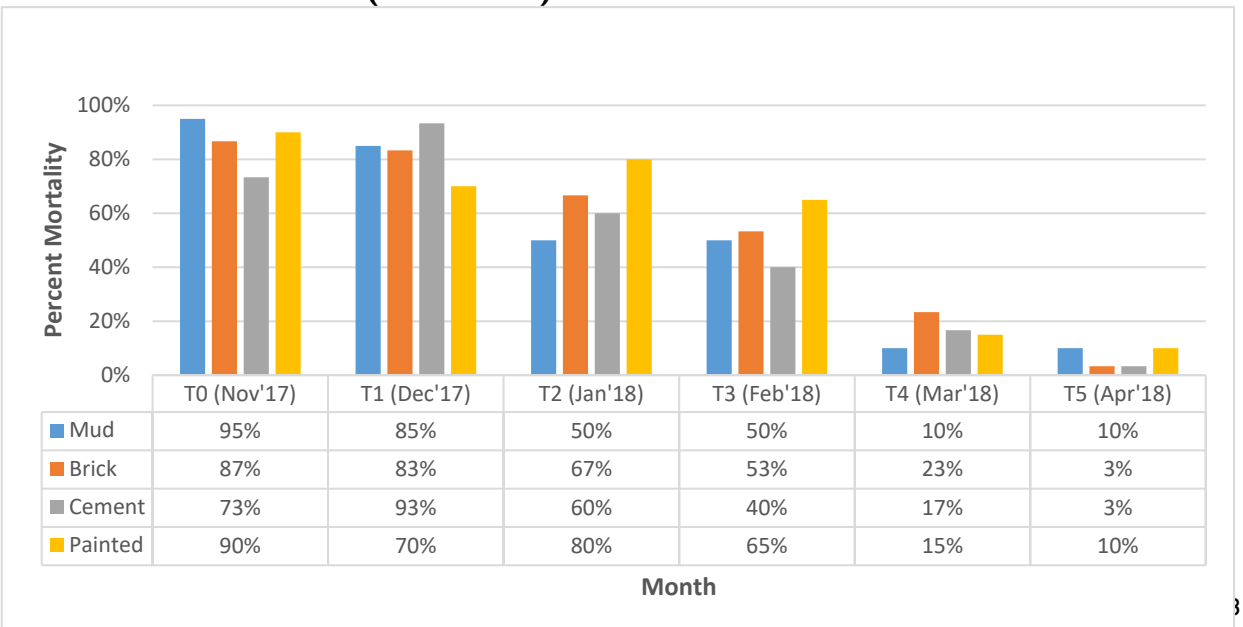
FIGURE 8. AIRBORNE EFFECT OF PIRIMIPHOS-METHYL IN BURMA VALLEY, MUTARE DISTRICT REPORTED AS *AN. ARABIENSIS* (KGB STRAIN) MORTALITY AFTER 24-HOUR HOLDING PERIOD



	T0 (Oct'17)	T1 (Nov'17)	T2 (Dec'17)	T3 (Jan'18)	T4 (Feb'18)	T5 (Mar'18)	T6 (Apr'18)
Mud	75%	57%	35%	23%	15%	10%	15%
Brick	60%	5%	20%	46%	25%	0%	10%
Cement	37%	67%	17%	41%	3%	33%	27%
Painted	73%	56%	30%	22%	33%	23%	13%

At Chakohwa, the average mosquito mortality due to the airborne effect of the insecticide 24 hours post-spray was 86.3% across all four wall types, and this declined to 6.5% in April, five months post-spray (Figure 9). The tests for airborne effect were discontinued after April.

FIGURE 9. AIRBORNE OF PIRIMIPHOS-METHYL IN CHAKOHWA, CHIMANIMANI DISTRICT, REPORTED AS *AN. ARABIENSIS* (KGB STRAIN) MORTALITY AFTER 24-HOUR HOLDING PERIOD

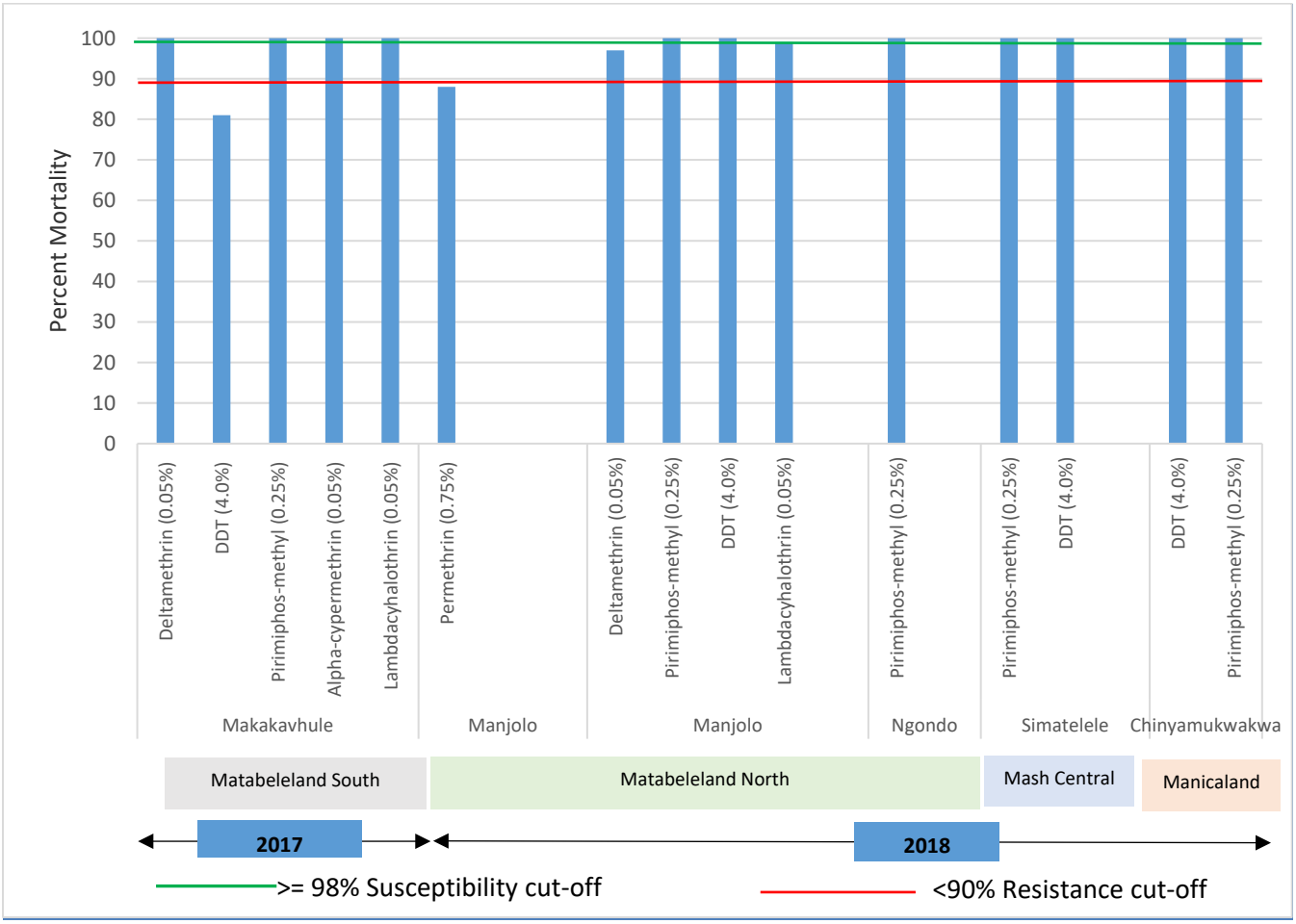


	T0 (Nov'17)	T1 (Dec'17)	T2 (Jan'18)	T3 (Feb'18)	T4 (Mar'18)	T5 (Apr'18)
Mud	95%	85%	50%	50%	10%	10%
Brick	87%	83%	67%	53%	23%	3%
Cement	73%	93%	60%	40%	17%	3%
Painted	90%	70%	80%	65%	15%	10%

3.4 INSECTICIDE RESISTANCE MONITORING

Insecticide susceptibility tests using the WHO method were carried out on *An. gambiae* s.l. mosquitoes collected at various insecticide resistance monitoring sites across the country in 2017 and 2018. *Anopheles gambiae* s.l. was susceptible to most insecticides tested at all sites, with some exceptions (Figure 10). At Manjolo (sprayed with pirimiphos-methyl in 2018), possible resistance to deltamethrin (0.05%) and resistance to permethrin (0.75%) was detected. *Anopheles gambiae* s.l. from Makakavhule (sprayed with DDT in 2018) in Matebeleland South Province were susceptible to pirimiphos-methyl (0.25%) and but resistant to DDT (4%) in tests done in November 2017.

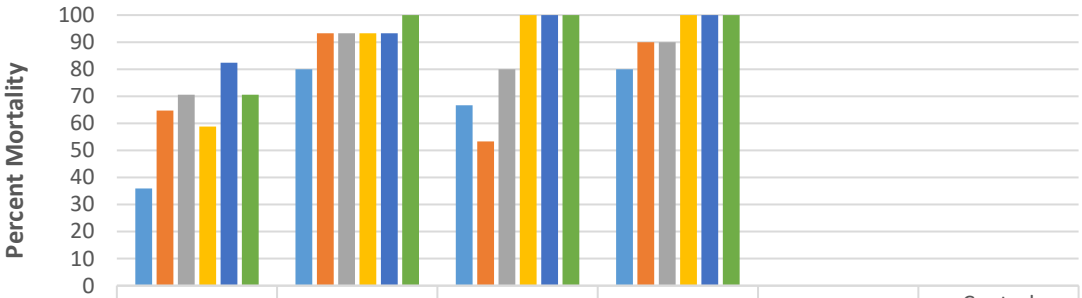
FIGURE 10. INSECTICIDE SUSCEPTIBILITY OF *AN. GAMBIAE* S.L. IN FOUR PROVINCES, AS MEASURED WHO TUBE TEST



An. gambiae s.l. from Makakavhule and Kamhororo were susceptible (100% mortality) to chlorfenapyr 48 hours post-exposure to 12.5, 25, 50, 100, and 200 µg/ml (Figure 11). At Manjolo, *An. gambiae* s.l. was susceptible (100% mortality) 72 hours post-exposure for the 12.5 µg/ml concentration of chlorfenapyr.

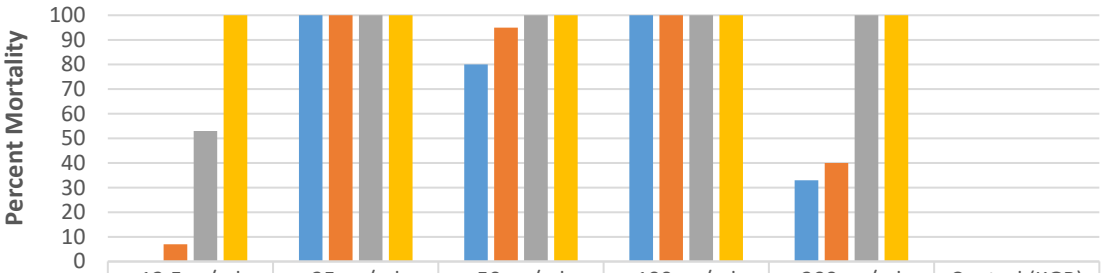
FIGURE 11. SUSCEPTIBILITY OF *AN. GAMBIAE* S.L. COLLECTED FROM MAKAKAVHULE (MATEBELELAND NORTH PROVINCE) AND KAMHORORO (MIDLANDS PROVINCE) TO DISCRIMINATING CONCENTRATIONS OF CHLORFENAPYR USING CDC BOTTLE ASSAYS.

A) Makakavhule



	12.5µg/ml	25 µg/ml	50 µg/ml	100 µg/ml	Control KGB	Control (Field)
15mins	35.9	80	66.7	80	0	0
30mins	64.7	93.3	53.3	90	0	0
35mins	70.6	93.3	80	90	0	0
40mins	58.8	93.3	100	100	0	0
60mins	82.4	93.3	100	100	0	0
24hrs	70.6	100	100	100	0	0

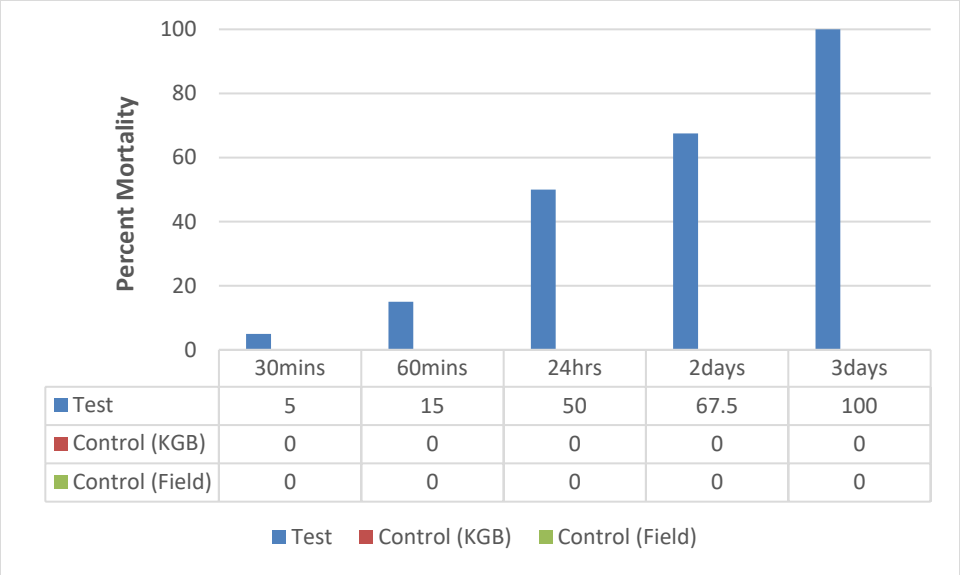
B) Kamhororo



	12.5µg/ml	25 µg/ml	50 µg/ml	100 µg/ml	200 µg/ml	Control (KGB)
30 mins	0	100	80	100	33	0
60 mins	7	100	95	100	40	0
24hrs	53	100	100	100	100	0
48hrs	100	100	100	100	100	0

A small sample size of *An. gambiae* s.l. (n=20) from Manjolo were exposed to a 13.2mg clothianidin-treated paper which resulted in 100% mortality (susceptible) three days post-exposure (Figure 12). The other sites did not collect enough mosquitoes to run this test.

FIGURE 12. SUSCEPTIBILITY OF *AN. GAMBIAE* S.L. COLLECTED FROM MANJOLO (MATEBELELAND NORTH PROVINCE) TO CLOTHIANIDIN USING WHO TUBE TESTS


Percent Mortality
100
80
60
40
20
0
30mins
60mins
24hrs
2days
3days
Test 5 15 50 67.5 100
Control (KGB) 0 0 0 0 0
Control (Field) 0 0 0 0 0
Test
Control (KGB)
Control (Field)

4. DISCUSSION

Entomological monitoring results from October 2017 to September 2018 indicate that *Anopheles funestus* s.l. is the major malaria vector species in all sites in Manicaland, with *An. gambiae* s.l. also found at Chakohwa, though *An. funestus* s.l. was predominant. In this reporting period, *An. funestus* s.l. was the dominant species (74.4%) at Burma Valley compared to 26.6% at the control site, Vumba. Historically, four members of *An. funestus* s.l. have been collected: *An. leesoni, An. parensis*, *An. rivulorum,* and *An. funestus* s.s., with the former two species being the most abundant. Both *An. funestus* s.s. and *An. rivulorum* are known vectors at these sites. The role of the other species within *An. funestus* s.l. is not well documented, but these are considered potential secondary vectors where they occur. For instance, *An. rivulorum*-like mosquito collected from a house at Arcturus was sporozoite positive.

Both *An. funestus* s.l. and *An. gambiae* s.l. were collected at higher densities outdoors than indoors, even though outdoor traps were not baited; 265/274 *An. funestus* s.l. and 59/62 *An. gambiae* s.l. at Chakohwa, while 240/304 *An. funestus* s.l. were collected outdoors at Burma Valley. The peak biting period for *An. funestus* s.l. was between 5 a.m. and 6 a.m. outdoors at all three sites. Insufficient mosquitoes were collected to determine an indoor biting rate. *An. gambiae* s.l. biting rates were too low to establish any clear pattern on peak biting time or location.

The sites where cross-sectional surveillance was conducted reported low densities of the traditional malaria vectors, raising questions about the possible role of other species in disease transmission at these localities. However, these collections were not sampled over time and may not reflect an accurate seasonal picture of species composition.

Entomological monitoring yielded low numbers of mosquitoes overall, limiting the project's ability to identify clear seasonal trends yet highlighting the need for an assessment and potentially a change of mosquito collection methods. Collections of indoor resting *Anopheles* mosquitoes using PSC and PPA methods were generally very low across all the sites monitored during the reporting period; therefore, greater effort should be made to collect outdoor biting and resting *An. funestus* mosquitoes. Inclusion of pit shelters enhanced the average number of mosquitoes collected at most sites. The team will use collections from pit shelters for resistance monitoring at sites with low numbers of mosquitoes for resistance tests by the larval collection method. While pit shelters are have great potential in vector surveillance, there are hazards associated with snakes that occasionally fall into the pits. Pits should be properly fenced to prevent humans and domestic animals from falling in. The pits are also sometimes vandalized or abused by the community. Entomological teams will continue to work with communities and health workers to prevent such occurrences.

Following the 2017 spray campaign in Manicaland, pirimiphos-methyl was effective on cement plastered and painted surfaces up to four months at Chakohwa and five months at Burma Valley. On mud and brick surfaces, its efficacy reached seven to eight months. The most commonly found wall materials used in the IRS districts is not currently known, but these data suggest that this information should be obtained to better inform implementation of IRS in the area. Results of the cone bioassays to assess the fumigant effect of pirimiphos-methyl demonstrate that this phenomenon does occur but is not similar in the two sites.

Insecticide susceptibility tests conducted in September 2017 in Matebeleland South Province initially indicated resistance of *An. gambiae* s.l. to pirimiphos-methyl (74% mortality), which was reported as part of the 2017 annual report; however, 100% mortality was observed when the test was repeated in November 2017. This variance could be the result of collecting larvae at slightly different locations and times. Similarly, *An. gambiae* s.l. was initially found to be resistant to DDT (67% mortality), but mortality

increased to 94% when the test was repeated. Thus, it is recommended that the NMCP consider alternative insecticides from the new generation of IRS (Sumishield or Fludora Fusion) for the next IRS campaign in Matebeleland South Province.

While *An. gambiae* s.l. was fully susceptible to deltamethrin, alpha-cypermethrin, and lambdacyhalothrin in Matebeleland South, resistance to permethrin (88% mortality) was detected in Matebeleland South/North. This is a concern, as LLINs treated with this pyrethroid are planned for distribution in this area. It is recommended that synergist assays be increased to evaluate the potential of PBO to restore susceptibility of local vectors to pyrethroids, and that alternatives to standard pyrethroid treated LLINs (synergist or dual-insecticide next-generation LLINs) be considered for deployment in these areas. Interceptor G2 is a potential addition to the vector control arsenal for insecticide resistance management planning since the vector was susceptible to chlorfenapyr in Matebeleland South (Makakavhule) and Midlands (Kamhororo) provinces. *An. gambiae* s.l. was susceptible to pirimiphos-methyl, DDT, lambdacyhalothrin, and permethrin at several sites, possible *An. gambiae* s.l. possible resistance to deltamethrin was detected at Manjolo. The emergence of *kdr* resistant alleles needs to be monitored regularly. However, the mosquitoes resistant to permethrin from Matebeleland South were fully susceptible to chlorfenapyr, a new insecticide that is one of the active ingredients in next-generation, dual-insecticide LLINs that will become available in the next few years.

5. RECOMMENDATIONS

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

- Resistance to both pirimiphos-methyl and DDT at Makakavhule suggests that next-generation IRS insecticides (Sumishield/Fludora Fusion) which recently received WHO prequalification be considered for IRS in the next campaign in Matebeleland North and South provinces.
- NMCP and partners to consider procuring and distributing alternatives to standard pyrethroid treated LLINs (e.g. synergist or dual-insecticide next-generation LLINs) for deployment in areas where resistance to pyrethroids is observed.
- Evaluate, in collaboration with NIHR, alternative collection methods to potentially increase mosquitoes collected at sentinel sites, including clay pots, pit shelters, resting boxes etc.
- In collaboration with the NMCP, train staff at VL, AU and NIHR laboratories to improve the capacity for morphological identification of *Anopheles* mosquitoes for focused molecular and immunodiagnostic analyses and build similar capacity at sentinel sites.
- Engage the Entomological Task Force in discussions to include spray operation collection of information on wall types in all IRS districts, ideally in the spray operator's data collection tool.
- The NMCP and partners – in collaboration with CDC, AU and NIHR should establish an *An. funestus* s.s. colony.
- The NMCP and partners should continue vector surveillance at established sentinel sites to monitor changes over time apart from ad-hoc surveillance in response to outbreaks and feed into national vector mapping

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ANNEX A: GPS COORDINATES OF SENTINEL SITES

Province	Site (District)	GPS Coordinates
Manicaland	Burma Valley (Mutare)	S19º 06′ 40.032″ E032º 29′ 39.012″ 644m
	Vumba (Mutare)	S19º 06′ 40.032″ E032º 29′ 39.012″ 644m
	Chinyamukwakwa (Chipenge)	S20º 52′ 37.1″ E032º 20′ 51.4” 766m
	Imbeza (Mutasa)	S18º 54′ 24.3″ E32º 41’ 28.96” 1212m
	Zindi (Mutasa)	S18º 54′ 24.3″ E32º 41’ 28.96” 1212m
	Chakowa (Chimanimani)	S20º 52′ 37.1″ E032º 20′ 51.4” 766m
	Nyamaropa (Nyanga)	S17º 52′ 17.7″ E032º 53’ 20.96” 841m
Matabeleland North	Ngondo (Lupane)	S18º 37′ 43.2″ E027º 26′ 57.6″ 917m
	Simatelele (Binga)	S17º 47′ 18.0″ E027º 18′ 56.1” 536m
	Manjolo (Binga)	S17º44'43.3” E027º23'58.2" 500m
Mashonaland East	Arcturus (Goromonzi)	S17º 47′ 0.87″; E031º 20′ 33.4″ 1343m
Matabeleland South	Makakavhule (Beitbridge)	S22º 08′ 21.804″; E029º 55′ 49.404″
Midlands	Kamhororo (Gokwe South)	S17º 28′ 24.24″; E028º 22′ 24.6″
Mashonaland West	Mola (Kariba)	S 16°54’24.1” E 28°22’27.4” 533m
	Chivende (Hurungwe)	S17°19’35” E032°21’09”, 825m