PMI | Africa IRS (AIRS) Project

Indoor Residual Spraying (IRS 2) Task Order Six

ZIMBABWE 2016 ENTOMOLOGICAL ACTIVITIES ANNUAL REPORT

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ZIMBABWE
2016 ENTOMOLOGICAL ACTIVITIES ANNUAL REPORT

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ACRONYMS

AIRS	Africa Indoor Residual Spraying
CDC	Centers for Disease Control
DDMS	Disease Data Management System
DDT	Dichlorodiphenyltrichloroethane
ELISA	Enzyme-Linked Immune-Sorbent Assay
HLC	Human Landing Catch
IRS	Indoor Residual Spraying
LLIN	Long-Lasting Insecticidal Net
NIHR	National Institute of Health Research
NMCP	National Malaria Control Program
OP	Organophosphate
PCR	Polymerase Chain Reaction
PMI	President's Malaria Initiative
PPA	Prokopack Aspirator
PSC	Pyrethrum Spray Collection
USAID	United States Agency for International Development
WHO	World Health Organization

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EXECUTIVE SUMMARY

Background

The Africa Indoor Residual Spraying (AIRS) Zimbabwe project, funded by the United States Agency for International Development (USAID) through the President's Malaria Initiative (PMI), does indoor residual spraying (IRS) in four districts of Manicaland province and implements entomological monitoring in two of those IRS target districts as well as in other districts. In 2016, the AIRS Zimbabwe project for the third consecutive year used the insecticide pirimiphos-methyl, an organophosphate, to conduct IRS in the four districts. To monitor the impact of PMI-funded IRS on the local vectors, AIRS Zimbabwe conducts monthly entomological monitoring at three sites in Manicaland province, Burma Valley and Chakohwa in the project-sprayed districts of Mutare and Chimanimani, and at one unsprayed control site, Vumba, in Mutare. It does seasonal entomological monitoring in 16 sites in other provinces.

Methods

The project collected baseline entomological data at all 19 sites starting in August 2016, before spraying began in October. Post-spray data were collected monthly at all four sites in Manicaland province and at seven sites outside of Manicaland in January and March 2017. The project used cone bioassay tests to determine quality of spraying and longevity of insecticide in sprayed rooms. To determine entomological indicators, the AIRS Zimbabwe team used three mosquito collection methods: pyrethrum spray collection (PSC), Prokopack aspirator (PPA), and Centers for Disease Control and Prevention (CDC) light traps. The project compared resting behavior of malaria vectors in living and non-living structures using the PSC and PPA methods. The two methods were alternated at the households monthly. The project used the standard World Health Organization (WHO) protocol to determine resistance in malaria vectors to four insecticides recommended for public health use. The National Institute of Health Research (NIHR) completed analyzing specimens from non-living structures and this report incorporates these results. The project team used the standard WHO method to assess vector susceptibility to insecticides.

Results

The project team observed low mosquito densities at sites dominated by either *An. funestus* s.l. or *An. gambiae* s.l. In Burma Valley, the density of *An. funestus* s.l. was greater in unsprayed non-living structures than in living structures sprayed with pirimiphos-methyl. The 24-hour percent mortality after five months was over the 80 percent threshold for all surface types, except painted surfaces in Burma Valley. For Chakohwa, where spraying was done a month later than in Burma Valley, the mortality was about 100 percent for almost all surface types except cement which was four months after spraying. Mud surfaces tended to retain pirimiphos-methyl for longer durations at both sites.

Results from NIHR indicate that malaria vectors rest in non-living structures.

The project team did not test insecticide resistance in 2016 due to unavailability of breeding sites as a result of the severe drought. However, susceptibility tests were conducted on DDT (4%) for *An. gambiae* s.l. from Chakari site in March 2017.

Conclusions

Malaria transmission continues despite the low mosquito densities in the project areas. The residual life of pirimiphos-methyl is being monitored at Burma Valley and Chakohwa. Insecticide resistance remains a threat to effective mosquito control and therefore vector surveillance needs to be strengthened.

A wide variety of *Anopheles* mosquitoes, which transmit malaria, were collected from non-living structures. The project team therefore recommends that non-living structures be sprayed.

1. Introduction

1.1 Background

In Zimbabwe, malaria vector control relies to a great extent on the use of indoor residual spraying (IRS) and long-lasting insecticidal nets (LLINs). The National Malaria Control Program (NMCP) coordinates IRS in eight malaria-endemic rural provinces using dichlorodiphenyltrichloroethane (DDT), pyrethroid, and pirimiphos-methyl, an organophosphate (OP) class insecticide. The IRS is done once a year, before peak transmission, and is expected to reduce the vector population during the transmission period and for an extended time after that.

Entomological surveillance is a component of the NMCP's IRS monitoring. The Africa Indoor Residual Spraying (AIRS) Zimbabwe project, funded by the United States Agency for International Development (USAID) through the President's Malaria Initiative (PMI), performs entomological monitoring in two of the four target districts in Manicaland province that receive AIRS comprehensive spraying support. AIRS Zimbabwe also assists the NMCP in testing the residual efficacy of insecticides the national IRS program uses in other provinces and in collecting data on insecticide resistance and vector behavior nationwide. By evaluating the past performance of IRS, this entomological monitoring provides the NMCP with information to use in planning future IRS campaigns.

1.2 Objectives of Entomological Monitoring Activities

The objectives of the AIRS Zimbabwe entomological monitoring activities for 2016 were the following:

- Determine the quality of spraying and insecticide decay rate following spray operations;
- Determine vector susceptibility to the four classes of insecticides approved by the World Health Organization Pesticide Evaluation Scheme for IRS;
- Identify the vector species, composition, and density; and
- Determine vector biting and resting behavior, including vectors resting in non-living structures.

This report describes the findings of the entomological monitoring done based on those objectives, conducted between March 2016 and February 2017. It includes results of cone bioassay tests done in March 2017.

1.3 Challenges

Weather conditions prevented the project team from conducting insecticide resistance tests in 2016. A severe drought affected the country throughout most of the year, preventing the formation of rainwater pools from which the AIRS Zimbabwe team normally would have collected larvae of the vector *An. arabiensis* for insecticide resistance tests. Instead the project team used the limited results of tests that the National Institute of Health Research (NIHR) did in early 2016 and included in the 2015 Annual Entomological Report. They indicated the dominance of the non-vector *An. quadriannulatus* in larval collections on which its resistance tests were based. The weather pattern changed late in the year, with excessive rains from December 2016 to March 2017 that washed away potential mosquito breeding sites. Once the rains stop, the project team will resume insecticide resistance testing for the remainder of 2017.

2. METHODOLOGY

AIRS Zimbabwe used three techniques for entomological surveillance in 2016:

- WHO cone bioassay test to determine the quality of spray and residual efficacy of insecticide on sprayed structure walls
- PSC and the PPA to determine the vector indoor resting density
- CDC light traps to determine mosquito density and behavior

The project team used the PSC and the PPA techniques to collect mosquitoes in order to compare vector resting behavior in living versus non-living structures[1] at three sentinel sites in Manicaland province and at 16 sentinel sites outside Manicaland.

2.1 STUDY SITES

In the 2016 spray season, AIRS Zimbabwe did entomological monitoring in 19 sentinel sites, located in 18 districts and eight provinces (Table 1). The project team began monitoring in August 2016, to capture baseline information on malaria vector populations during the dry season and prior to IRS. It continued monitoring monthly at the three sites in Manicaland province, for five days at the IRS sites of Burma Valley and Chakohwa and for three days at the control site of Vumba. It did seasonal monitoring at the other 16 sites, for five days in the dry (pre-spray) season and another five in the wet (post-spray) season.

AIRS Zimbabwe did cone bioassay tests as well as vector density and behavior data collections on a monthly basis in its target districts in Manicaland.

For all collections and tests, the project team received verbal consent from the heads of households to allow access into rooms and the household perimeter.

[1] 'Living structure' refers to bedrooms, living rooms, and kitchens that constitute sprayable structures. In contrast 'non-living' refers to other structures like latrines, bathrooms, and animal shelters in which people do not sleep.

TABLE 1: LOCATION OF SENTINEL SITES USED FOR ENTOMOLOGICAL MONITORING

Province	District	Sentinel Site	Insecticide Sprayed	Primary Vector
Manicaland	Mutare*	Burma Valley	OP	*An. funestus* s.l.
		Vumba	Nil (control)	*An. funestus* s.l.
	Chimanimani*	Chakohwa	OP	*An. gambiae* s.l./*An. funestus* s.l.
	Makoni	Mukamba^	Nil (control)	*An. gambiae* s.l.
Mashonaland East	Mutoko	Kawere	DDT	*An. gambiae* s.l.
	Mudzi	Kotwa	OP & DDT	*An. gambiae* s.l.
	UMP	Mtawatawa	OP & DDT	*An.gambiae* s.l.
Mashonaland West	Sanyati	Chakari	DDT	*An. gambiae* s.l.
	Hurungwe	Kasimure	OP	*An. gambiae* s.l.
Masvingo	Chiredzi	Chilonga	OP & DDT	*An. gambiae* s.l.
	Bikita	Mashoko	OP	*An. gambiae* s.l.
Matabeleland North	Binga	Manjolo	DDT	*An. gambiae* s.l.
	Lupane	Jotsholo	DDT	*An. gambiae* s.l.
Matabeleland South	Beitbridge	Makakavhule	Pyrethroid	*An. gambiae* s.l.
	Matobo	Tshelanyemba	Nil	*An. gambiae* s.l.
Midlands	Gokwe South	Kamhororo	DDT	*An. gambiae* s.l.
	Kwekwe	Sidhakeni	DDT	*An. gambiae* s.l.
Mashonaland Central	Rushinga	Old Mazowe Bridge	OP	*An. gambiae* s.l.
	Centenary	Muzarabani	OP	*An. gambiae* s.l.

*Districts supported by AIRS Zimbabwe, sprayed with OP. Other districts were supported by the government's NMCP.

^ The Mukamba site was last monitored in June 2016, and replaced by Vumba in Mutare district in August 2016.

2.2 Species Composition and Vector Seasonality

AIRS Zimbabwe used PSC, PPAs, and the CDC light trap techniques to collect mosquitoes at all 19 sites. The light trap was used as a proxy to the human landing catch (HLC) method at the three sites in Manicaland. The team conducted morphological identifications of collected mosquitoes to determine species distribution and abundance.

2.3 PSC Collections for Vector Density

The AIRS Zimbabwe team used the PSC method in all 19 sentinel sites, to sample indoor resting mosquitoes from 25 living structures and 15 non-living structures per collection period. The team carried out the collection in the morning between 05:00 and 08:00 in both living and non-living structures. Before entering the structures, the project team secured verbal consent to do so from the head of the household. The project team collected data on the number of people and domestic animals who had slept in the house the previous night, and the type of the house and walls for living structures.

Before insecticide was applied, the project team prepared the room by removing all occupants (people and, occasionally, animals), removing or covering all food, and covering all openings and eaves with cloth. Two people laid out white calico cloth to cover the floor and all other flat surfaces of furniture. Sheets were also spread under tables and beds. The project team used the commercial aerosol insecticide sprayer Baygon®, whose active ingredients include the pyrethroids tetramethrin, prallethrin, and imiprothrin and the synergist piperonyl butoxide.

After vigorously shaking the aerosol can, one spray team member sprayed the eaves from the outside while another sprayed inside after closing the door. After completing the spraying, the room was left undisturbed for 10 minutes. After the 10 minutes, the team moved into the room and, starting from the doorway, picked up one piece of cloth at a time by the corners. The project team took each cloth outside, carefully spread it out on the ground, and using forceps picked up the knocked down mosquitoes and put them into a petri dish. This was repeated for each piece of cloth. If it was windy or wet, the team examined each cloth sequentially inside with the aid of a flashlight. The project team used one petri dish per room and labeled it with the collection method, room code or identity, locality, and date of collection. The project team also recorded the collection data on a form for each room sampled.

The team also investigated mosquito resting behavior in non-living structures (mainly latrines and bathrooms). In an innovative approach, the team used sheets of disposable flipchart paper in place of white calico cloth to conduct PSC in these structures, because it would be unhygienic to re-use in living rooms the cloth that had been used in toilets and bathrooms. The team also used disposable gloves in non-living structures. In almost all areas, there were no doors on non-living structures and so a white or green cloth was used to close the entrance of these structures (Figure 1).

FIGURE 1: WHITE CLOTH COVERING THE ENTRANCE OF A PIT LATRINE FOR PSC, BURMA VALLEY

2.4 PPA Collections for Vector Resting Behavior

AIRS Zimbabwe used PPA to sample indoor resting mosquitoes from both living and non-living structures at sites in all 19 sites in eight provinces. The target was to collect mosquitoes from 25 rooms for living structures and 15 non-living structures per collection period at all the sentinel sites. While the project tried to do collections from the same rooms, it was not always possible to access the same rooms because of the availability of the home owners. As it did with PSC, the project carried out the activity in the morning between 05:00 and 08:00. Before the collection was performed, the team secured the household head's verbal consent, asked all occupants to leave the

house, and collected data on the number of people and domestic animals who had slept in the house the previous night and the type of house and walls.

The PPA used a sealed, lead acid, rechargeable 12-volt battery. One team member entered the room and connected the aspirator to the battery terminals. The wires were color-coded to ensure correct polarity so that the aspirator would suck and not blow the mosquitoes. After fitting the collection cup, the PPA handler worked systematically, starting from the door, moving on to the walls and furniture and then under beds and tables, and finishing with the roof or ceiling. Because PPA collects live mosquitoes, the cup is inserted into a large mosquito cage and the mosquitoes are released into the cage. The team removed the mosquitoes from the cage using a small sucking aspirator, counted, and recorded their physiological status on the form, and placed them in a petri dish. Then the team labeled the petri dish with method of collection, date, locality, and household name.

FIGURE 2: TECHNICIAN USING PPA TO COLLECT MOSQUITOES FROM UNDER A BED, MANJOLO SITE, BINGA DISTRICT

2.5 CDC Light Trap Collections for Vector Density and Behavior

The project team used CDC light traps in two different ways to collect mosquitoes for vector density and behavior data. They are described below.

2.5.1 Vector Density

The project team used CDC light traps to determine mosquito density indoors and outdoors. The households selected for this exercise were located up to 4 kilometers from each other. For each of six houses in a sentinel site, the collectors hung two light traps (12 traps in total per site) with the light source about 1.5 meters from the floor/ground. They ensured that the equipment was secured at the site before traps were left overnight.

One trap was set indoors toward the foot of a bed or sleeping space, alongside human bait, after making sure the person was protected by a mosquito net. Another was set outdoors, within 10–15 meters of the one set indoors, without human bait. The project team considered having persons sleeping outdoors alongside light traps but decided not to do so. Unlike the data collection for vector behavior described in Section 2.5.2, the CDC light traps set for vector density data are not monitored throughout the night, thus any person outside would be on his/her own, and sleeping outdoors alone is not safe as people can be attacked by robbers or wild animals (snakes, crocodiles, and scorpions). Because the outdoor traps are not baited, they are not comparable to those set indoors. All traps were left overnight from sunset (6 pm) to sunrise (6 am hours) and emptied the following morning. The project team tied the collection sleeve before switching the trap off to ensure no mosquitoes would escape from the collection cup at the bottom.

The project team used the CDC light traps at all 19 sentinel sites. The project team set traps over one night per sentinel site per month of data collection: six traps indoors and six outdoors at the same households each month. The light traps used sealed, lead-acid, rechargeable 6-volt batteries, which the project team charged during the day to re-use during the next round of data collection.

2.5.2 Vector Behavior

The project team used CDC light traps as a proxy for HLC to learn where most vector-human contact was occurring (indoors and/or outdoors), vector feeding time, and changes in the feeding behavior of mosquitoes before and after IRS. The test was carried out only at the three sites in Manicaland, at a selected house in each site; the test was performed monthly from March 2016 to February 2017. In Manicaland, the team collected baseline data in September, before the start of 2016 IRS campaign, and then monthly collections after the spray began in October 2016.

One person slept indoors under a net alongside a light trap while another slept outdoors, also under a net and alongside a light trap. They exchanged their positions hourly. A few data collectors stayed near the human bait to help with the collections, but were placed in a different room. They did collections at hourly intervals from 18:00 until 06:00. In the hourly check, the data collectors aspirated *Anopheline* mosquitoes into a paper cup labeled with date, locality, and position of the trap. They also monitored temperature, relative humidity, and rainfall and recorded them at hourly intervals during the night. The project team preserved all collected mosquitoes individually in a 1.5 ml Eppendorf tube in Silica gel for species identification by PCR and sporozoite rate using enzyme-linked immune-sorbent assay (ELISA).

2.6 Insecticide Susceptibility Tests

The project team tested the susceptibility of *An. gambiae* s.l. from Chakari site to DDT (4%) in March 2017. The team tested 2-5 day-old female *An. gambiae* s.l. as follows: 60 mosquitoes exposed to DDT (4%) treated papers and 25 mosquitoes for the untreated control paper, 85 mosquitoes in total. The tests consisted of three replicates: two replicates with 25 mosquitoes each and one tube with ten mosquitoes. The project team tested susceptibility according the standard WHO protocol.

2.7 Cone Bioassays for Spray Quality and Residual Efficacy

The project team conducted cone bioassay tests to determine the quality of spray 24–48 hours after spray operations at the two sentinel sites in the AIRS Zimbabwe-supported districts, Burma Valley and Chakohwa. For the tests, the project team used standard WHO plastic cones. At each site, the project team completed tests in 10 rooms per site, with three cones per room placed diagonally on the sprayed wall at 0.5, 1.0, and 1.5 m from the floor.

The project team used susceptible female *An. arabiensis* (KGB strain) mosquitoes from NIHR for the cone bioassay tests.

The project team exposed 10 mosquitoes to the insecticide in the cones and retrieved them after 30 minutes. Upon retrieval, the project team transferred the mosquitoes to clean cups and provided them with 10 percent sucrose solution for the 24-hour observation period. The project team recorded the number of mosquitoes knocked down at the 30-minute point and again after 60 minutes, and recorded the final mortality at the end of 24-hour observation period.

The project team set control cones with 10 mosquitoes on clean (free of insecticide) white paper, placed in a Bugdorm® cage to avoid any fumigant (airborne) effect of insecticides, and recorded knockdown and 24-hour mortality in the same way as they did with the cones in sprayed rooms.

Since the initial bioassay tests to assess quality of spray, the project team has conducted the bioassay tests monthly to determine the residual efficacy of insecticide. The project team will continue the tests until the average mortality falls below 80 percent for two consecutive tests.

2.8 Laboratory Analysis of Specimens From Non-Living Structures

To help determine if resources should be put to spraying non-living structures, in November 2016 AIRS Zimbabwe submitted 64 specimens collected from non-living structures in eight sentinel sites to NIHR for analysis. First, NIHR identified the specimens morphologically. Then, it analyzed them by molecular and immunological techniques to identify the species and determine *Plasmodium* sporozoites, respectively. NIHR used the polymerase chain reaction (PCR) method for species identification and the ELISA for sporozoites. The laboratory used three primers for *An. gambiae* s.l. and seven primers for the *An. funestus* s.l. PCR. NIHR released the results on March 13, 2017.

3. RESULTS

3.1 VECTOR SPECIES COMPOSITION, DENSITY, SEASONALITY, RESTING BEHAVIOR

3.1.1 SPECIES COMPOSITION

The primary vector in most sentinel sites outside Manicaland was *An. gambiae* s.l., although *An. funestus* s.l. was predominant in the Burma Valley and Vumba sites. Available data combined for all collection methods indicate the species diversity is greatest at three sites monitored monthly in Manicaland: Burma Valley, Chakohwa, and Vumba (Table 2 and Figure 3). At Burma Valley, out of 484 *Anopheles* mosquitoes collected, 80 percent were *An. funestus,* s.l. 13.6 percent were *An. coustani,* 3.3 percent were *An. gambiae* s.l., and 3.1 percent were *An. pretoriensis.* At Chakohwa, out of 361 *Anopheles*, 60.9 percent were *An. gambiae* s.l., 22.2 were *An. pretoriensis,* 10.8 percent were *An. funestus* s.l., 0.3 percent *An. coustani,* and 5.8 percent were other, unidentified *Anopheles*. Out of 308 *Anopheles* collected at Vumba, 48.7 percent were *An. funestus* s.l., 24.7 percent *An. natalensis,* 12.3 percent *An. coustani,* 7.8 percent *An. pretoriensis* and 6.5 percent *An. gambiae* s.l.

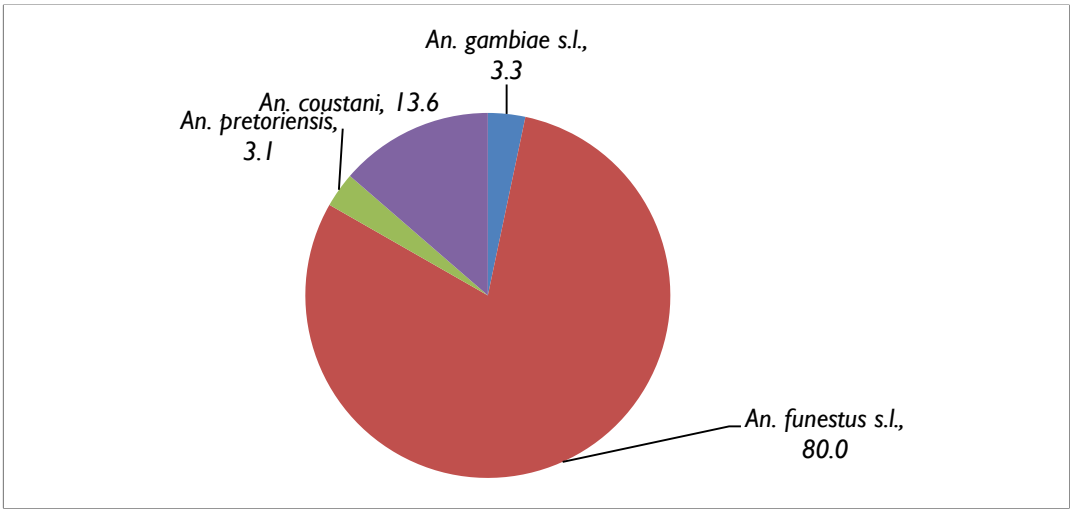
TABLE 2: *ANOPHELES* SPECIES COMPOSITION, ALL COLLECTION METHODS, FOUR SENTINEL SITES, MANICALAND

Species	Burma Valley	Chakohwa	Vumba*	Mukamba*
An. gambiae s.l.**	16 (3.3%)	220 (60.9%)	20 (6.5%)	0
An. funestus s.l.**	387 (80%)	39 (10.8%)	150 (48.7%)	0
An. pretoriensis	15 (3.1%)	80 (22.2%)	24 (7.8%)	27 (100%)
An. coustani	66 (13.6%)	1 (0.3%)	38 (12.3%)	0
An. natalensis	0	0	76 (24.7%)	0
Other *Anopheles*	0	21 (5.8%)	0	0
Total	484	361	308	27

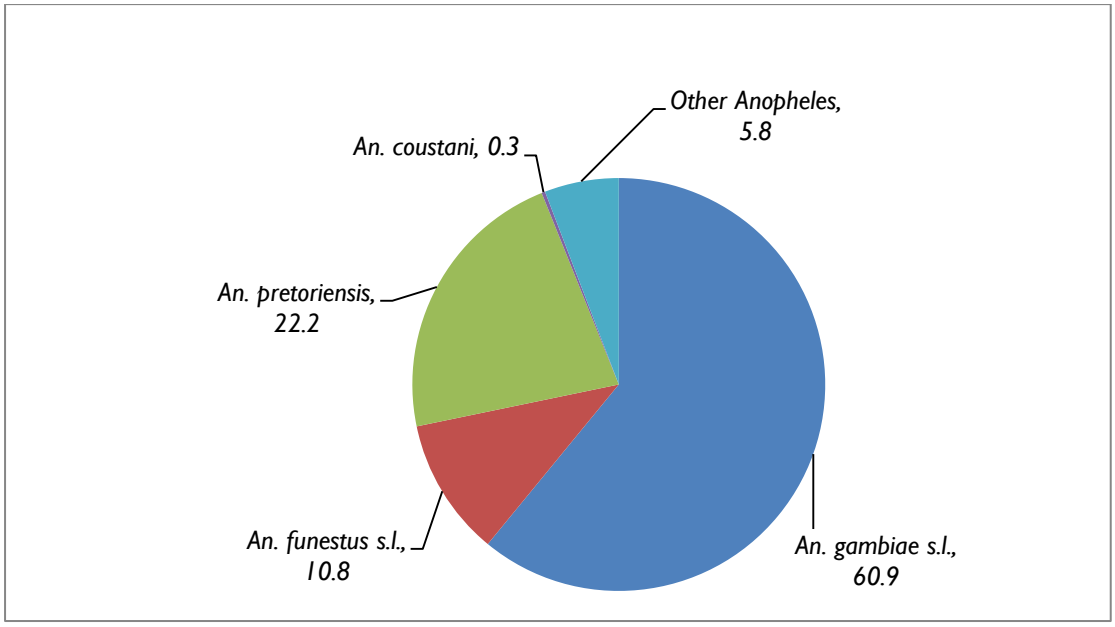
*Vumba replaced Mukamba as a control site in August 2016. ** Malaria vectors are members of these two species complexes

FIGURE 3: *ANOPHELES* SPECIES COMPOSITION (%), THREE SITES IN MANICALAND

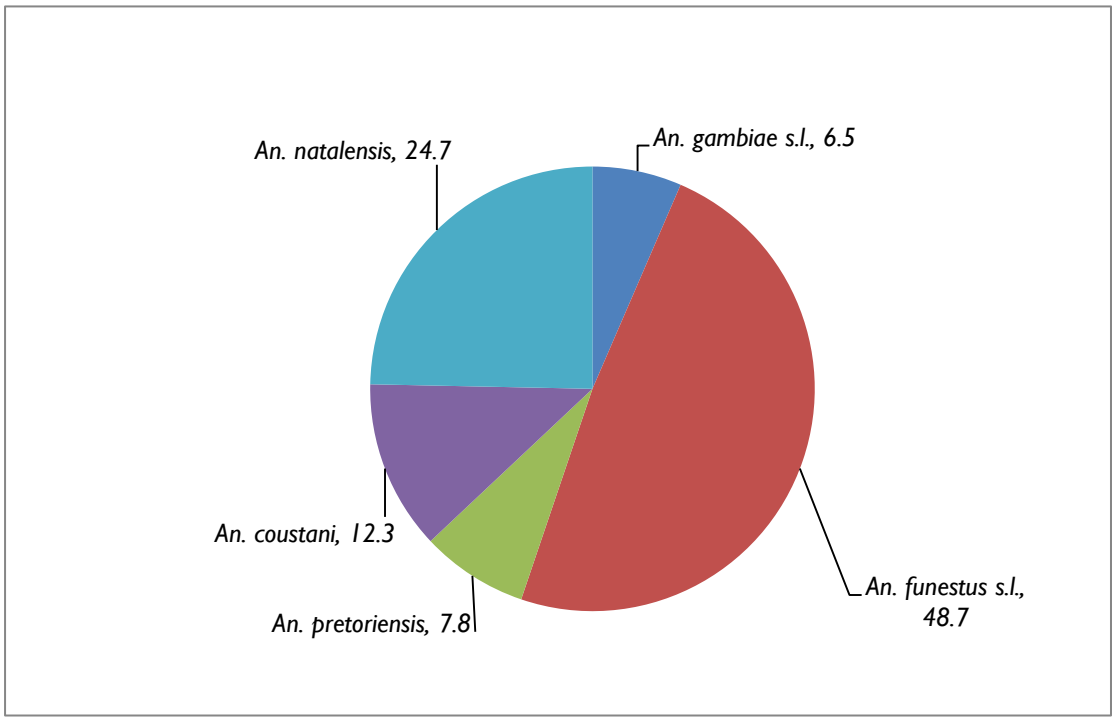
A. Burma Valley (N = 484)



B. Chakohwa (N = 361)



C. Vumba (N = 308)



3.1.2 Vector Density

The PSC data on *An. funestus* s.l. at Burma Valley show low mosquito densities for all months. More mosquitoes were found resting in non-living structures than in living structures (Table 3). The project team sampled 15 non-living structures and 25 living structures each month.

TABLE 3: RESULTS FOR MONTHLY INDOOR RESTING *AN. FUNESTUS* S.L. COLLECTED BY PSC, BURMA VALLEY

Month of collection	*An. funestus* s.l. collected					
	Living structures			**Non-living structures**		
	No. of Rooms	**Total**	**Average No. of *An. funestus* s.l. per Room**	**No. of Rooms**	**Total**	**Average No. of *An. funestus* s.l. per Room**
Mar 2016	46	0	0	23	3	0.13
Apr 2016	27	2	0.07	11	0	0
May 2016	25	0	0	16	4	0.25
Jun 2016	25	0	0	16	2	0.13
Aug 2016	30	0	0	15	0	0
Sep 2016	25	1	0.04	20	22	1.1
Oct 2016	25	0	0	14	0	0
Nov 2016	33	0	0	17	0	0
Dec 2016	27	2	0.07	15	0	0
Jan 1017	26	0	0	13	0	0
Feb 2017	29	1	0.03	9	4	0.44
Total	318	6	0.02	169	35	0.21

The data from the PPA collections also showed more mosquitoes were collected in (unsprayed) non-living structures than in (sprayed) living structures. Table 4 shows the project team collected more *An. funestus* s.l. than *An. gambiae* s.l. at the Burma Valley. Collection peaks for PSC and for PPA occurred in September and October, respectively. The province sprayed Burma Valley on October 24, 2016, and the AIRS/NIHR team collected mosquitoes within a week after spraying. The project team sampled 15 non-living structures and 25 living structures each month.

TABLE 4: RESULTS FOR INDOOR RESTING MONTHLY *AN. FUNESTUS* S.L. COLLECTED BY PPA, BURMA VALLEY

Month of collection	No. of Rooms		*An. funestus* s.l.				*An. gambiae* s.l.			
	Living	**Non-Living**	**Living**		**Non-living**		**Living**		**Non-living**	
			Total	**Average No. of *An. funestus* s.l. per Room**	**Total**	**Average No. of *An. funestus* s.l. per Room**	**Total**	**Average No. of *An. gambiae* s.l. per Room**	**Total**	**Average No. of *An. gambiae* s.l. per Room**
Mar 2016	13	3	0	0	0	0	0	0	0	0
Apr 2016	28	26	0	0	0	0	0	0	0	0
May 2016	34	25	0	0	4	0.16	0	0	0	0

Jun 2016	31	20	0	0	1	0.05	0	0	0	0
Aug 2016	26	17	0	0	4	0.24	0	0	0	0
Sep 2016	12	11	0	0	5	0.45	0	0	0	0
Oct 2016	21	20	0	0	15	0.75	0	0	0	0
Nov 2016	30	22	3	0.1	5	0.23	0	0	0	0
Dec 2016	32	24	0	0	10	0.42	1	0.03	0	0
Jan 1017	29	45	0	0	1	0.02	0	0	0	0
Feb 2017	25	16	1	0.04	0	0	0	0	0	0
Total	281	229	4	0.01	45	0.20	1	0	0	0

CDC light trap collections at Burma Valley yielded more *An. funestus* s.l. than *An. gambiae* s.l. Results from CDC light trap collections were greater than from either PSC or PPA collections. The project team collected more *An. gambiae* s.l. indoors than outdoors. As was discussed in the Methodology section, traps set outdoors were not baited, so the indoor and outdoor traps are not comparable; nevertheless, the traps set outdoors collected more *An. funestus* s.l. mosquitoes than those set indoors alongside human bait (Table 5). In contrast, the project team collected more *An. gambiae* s.l. from traps set indoors than those outdoors. The project team used six light traps indoors and six light traps outdoors each month.

TABLE 5: RESULTS FOR MONTHLY *AN. FUNESTUS* S.L. AND *AN. GAMBIAE* S.L. COLLECTED INDOORS AND OUTDOORS, CDC LIGHT TRAPS, BURMA VALLEY

Month of collection	No of traps per month		*An. funestus* s.l.				*An. gambiae* s.l.			
	IN	**OUT**	**IN**		**OUT**		**IN**		**OUT**	
			Total	**Average No. of *An. funestus* s.l. per Trap**	**Total**	**Average No. of *An. funestus* s.l. per Trap**	**Total**	**Average No. of *An. gambiae* s.l. per Trap**	**Total**	**Average No. of *An. gambiae* s.l. per Trap**
Mar 2016	18	18	5	0.28	31	1.72	0	0	0	0
Apr 2016	17	17	12	0.71	17	1	0	0	0	0
May 2016	18	18	9	0.5	26	1.44	0	0	1	0.05
Jun 2016	12	12	4	0.33	8	0.67	0	0	0	0
Aug 2016	18	18	5	0.28	16	0.89	0	0	0	0
Sep 2016	12	12	18	1.5	8	0.67	0	0	0	0
Oct 2016	18	18	13	0.72	21	1.17	9	0.5	2	0.11

Nov 2016	18	18	33	1.83	21	1.17	1	0.05	0	0
Dec 2016	18	18	8	0.44	5	0.28	0	0	0	0
Jan 1017	18	18	12	0.67	9	0.5	2	0.11	0	0
Feb 2017	16	16	10	0.63	6	0.38	0	0	0	0
Total	183	183	129	0.70	168	0.92	12	0.06	3	0.02

The few *An. gambiae* s.l. the team collected at the Chakohwa site using the PSC method were mostly from non-living structures given that less of these structures were sampled (Table 6). No mosquitoes were collected during the dry period from August to December. After spraying, *An. gambiae* s.l. was only found in unsprayed non-living structures (Table 6).

TABLE 6: RESULTS FOR MONTHLY INDOOR *AN. FUNESTUS* S.L. COLLECTED BY PSC, CHAKOHWA

Month of collection	No. of Rooms		*An. funestus* s.l.				*An. gambiae* s.l.			
	Living	Non-Living	Living		Non-living		Living		Non-living	
			Total	Average No. of *An. funestus* s.l. per Room	Total	Average No. of *An. funestus* s.l. per Room	Total	Average No. of *An. gambiae* s.l. per Room	Total	Average No. of *An. gambiae* s.l. per Room
Mar 2016	34	19	0	0	0	0	0	0	0	0
Apr 2016	24	8	0	0	0	0	0	0	0	0
May 2016	25	11	1	0.04	1	0.09	0	0	0	0
Jun 2016	27	1	0	20	0	0	3	0.11	2	2
Aug 2016	34	22	0	0	0	0	0	0	0	0
Sep 2016	24	10	0	0	0	0	0	0	0	0
Oct 2016	41	6	0	0	0	0	0	0	0	0
Nov 2016	36	6	0	0	0	0	0	0	0	0
Dec 2016	27	4	0	0	0	0	0	0	0	0
Jan 1017	26	6	0	0	0	0	0	0	1	0.17
Feb 2017	33	3	0	0	0	0	0	0	0	0
Total	331	96	1	0	1	0.01	3	0.01	3	0.03

Using PPA, at Chakohwa, the team collected a few *An. funestus* s.l. and *An. gambiae* s.l. from living structures in April and in May, respectively, and a few *An. gambiae* s.l. from non-living structures in January 2017 (Table 7).

TABLE 7: TOTAL *AN. FUNESTUS* S.L. AND *AN. GAMBIAE* S.L. COLLECTED INDOORS BY PPA, CHAKOHWA

Month of collection	No. of Rooms		*An. funestus* s.l.				*An. gambiae* s.l.			
	Living	Non-Living	Living		Non-living		Living		Non-living	
			Total	Average No. of *An. funestus* s.l. per Room	Total	Average No. of *An. funestus* s.l. per Room	Total	Average No. of *An. gambiae* s.l. per Room	Total	Average No. of *An. gambiae* s.l. per Room
Mar 2016	25	7	0	0	0	0	0	0	0	0
Apr 2016	29	17	0	0	0	0	2	0.07	0	0
May 2016	28	14	3	0.11	0	0	0	0	0	0
Jun 2016	48	6	0	0	0	0	0	0	0	0
Aug 2016	35	14	0	0	0	0	0	0	0	0
Sep 2016	29	14	0	0	0	0	0	0	0	0
Oct 2016	20	7	0	0	0	0	0	0	0	0
Nov 2016	30	12	0	0	0	0	0	0	0	0
Dec 2016	25	13	0	0	0	0	0	0	0	0
Jan 1017	24	12	0	0	0	0	0	0	1	0.08
Feb 2017	32	17	0	0	0	0	0	0	0	0
Total	325	133	3	0.01	0	0	2	0.01	1	0.01

CDC light trap collections in Chakohwa yielded more mosquitoes than did the PSC and PPA methods (Table 8). CDC light traps set outdoors collected more *An. gambiae* s.l. (n=170) than the traps set indoors (n=27). Most of the *An. gambiae* s.l. were collected between April and May 2016; hardly any mosquitoes were collected from August to January. The project team also collected other *Anopheles* from Chakohwa that could not be identified because they did not have sufficient body features necessary for morphological identification.

TABLE 8: RESULTS FOR MONTHLY *AN. FUNESTUS* S.L. AND *AN. GAMBIAE* S.L. COLLECTED BY CDC LIGHT TRAPS, CHAKOHWA

Month of collection	No of traps per month		*An. funestus* s.l.				*An. gambiae* s.l.			
	IN	OUT	IN		OUT		IN		OUT	
			Total	Average No. of *An. funestus* s.l. per Trap	Total	Average No. of *An. funestus* s.l. per Trap	Total	Average No. of *An. gambiae* s.l. per Trap	Total	Average No. of *An. gambiae* s.l. per Trap
Mar 2016	16	16	4	0.25	5	0.31	5	0.31	13	0.81
Apr 2016	12	13	0	0	0	0	7	0.58	61	4.70
May 2016	18	18	1	0.05	3	0.17	0	0	42	2.33
Jun 2016	18	18	3	0.17	2	0.11	10	0.56	25	1.39
Aug 2016	18	18	1	0.05	0	0	1	0.05	2	0.11
Sep 2016	18	18	0	0	9	0.5	0	0	4	0.22
Oct 2016	18	18	1	0.05	3	0.17	0	0	1	0.05
Nov 2016	17	17	0	0	0	0	1	0.06	0	0
Dec 2016	17	17	0	0	0	0	1	0.06	3	0.18
Jan 1017	17	17	0	0	0	0	0	0	2	0.12
Feb 2017	17	17	0	0	2	0.12	2	0.12	17	1
Total	186	187	10	0.05	24	0.13	27	0.15	170	0.91

The project team collected non-vector mosquitoes but did not collect any malaria vector mosquitoes in the original control site, Mukamba, in March–June 2016; therefore the site was dropped from further observations. Among non-vectors, the team collected 28 *An. pretoriensis* using PSC, PPA, and CDC light traps. In April, 12 were collected from living structures using the PPA method; eight were collected from living structures and one was collected from a non-living structure using the PSC method; and seven were collected, two from indoors and five from outdoors, using CDC light traps. Collections from light traps as a proxy for the HLC method did not yield any mosquitoes.

Because the team did not collect any malaria vector mosquitoes in Mukamba, it replaced that site with Vumba in August 2016. In Vumba, only *An. funestus* s.l. was found and only in living structures, regardless of the collection method (Table 9.1 and 9.2).

TABLE 9.1: RESULTS FOR MONTHLY *AN. FUNESTUS* S.L. COLLECTED BY PSC FROM LIVING AND NON-LIVING STRUCTURES, VUMBA

Month of collection	Living			Non Living		
	No. of Rooms	**Total**	**Average No. of *An. funestus* s.l. per Room**	**No. of Rooms**	**Total**	**Average No. of *An. funestus* s.l. per Room**
Aug 2016	13	1	0.08	0	0	0
Sep 2016	9	0	0	0	0	0
Oct 2016	12	0	0	2	0	0
Nov 2016	15	3	0.20	1	0	0
Dec 2016	11	0	0	1	0	0
Jan 1017	16	6	0.38	0	0	0
Feb 2017	16	4	0.25	0	0	0
Total	92	14	0.15	4	0	0

TABLE 9.2: RESULTS FOR MONTHLY *AN. FUNESTUS* S.L. COLLECTED BY PPA FROM LIVING AND NON-LIVING STRUCTURES, VUMBA

Month of collection	Living			Non Living		
	No. of Rooms	**Total**	**Average No. of *An. funestus* s.l. per Room**	**No. of Rooms**	**Total**	**Average No. of *An. funestus* s.l. per Room**
Aug 2016	2	0	0	1	0	0
Sep 2016	17	1	0.05	17	0	0
Oct 2016	16	3	0.19	4	0	0
Nov 2016	12	4	0.33	4	0	0
Dec 2016	12	0	0	4	0	0
Jan 1017	11	1	0.10	0	0	0
Feb 2017	15	1	0.07	2	0	0
Total	85	10	0.12	32	0	0

The project team collected more *An. funestus* s.l. than *An. gambiae* s.l. in Vumba. The largest collections were made from CDC light traps set outdoors (Table 10). Collection of *An. gambiae* s.l. peaked (albeit at a low level) in January for traps set indoors and in February for traps set outdoors. More *An. funestus* s.l. were collected outdoors (102) than indoors (24).

TABLE 10: RESULTS FOR *AN. FUNESTUS* S.L. AND *AN. GAMBIAE* S.L. COLLECTED INDOORS AND OUTDOORS BY CDC LIGHT TRAPS, VUMBA

Month of collection	No of traps per month		*An. funestus* s.l.				*An. gambiae* s.l.			
	IN	**OUT**	**IN**		**OUT**		**IN**		**OUT**	
			Total	**Average No. of *An. funestus* s.l. per Trap**	**Total**	**Average No. of *An. funestus* s.l. per Trap**	**Total**	**Average No. of *An. gambiae* s.l. per Trap**	**Total**	**Average No. of *An. gambiae* s.l. per Trap**
Aug 2016	0	36	0	0	4	0.11	0	0	0	0
Sep 2016	12	12	0	0	1	0.08	0	0	0	0
Oct 2016	14	14	2	0.14	38	2.71	0	0	0	0
Nov 2016	18	18	0	0	0	0	0	0	0	0
Dec 2016	12	12	0	0	2	0.17	0	0	0	0
Jan 1017	17	17	10	0.60	10	0.60	7	0.41	0	0
Feb 2017	16	16	12	0.75	47	2.94	1	0.06	8	0.5
Total	89	125	24	0.27	102	0.82	8	0.09	8	0.06

3.1.3 ANOPHELES COLLECTED FROM OTHER SENTINEL SITES

PSC collections from the 16 sites that the AIRS/Zimbabwe team monitors seasonally show low *An. gambiae* s.l. densities across the sites. Moderately more mosquitoes were collected from living structures than from non-living structures, which are routinely sprayed outside Manicaland. The project team made the largest collections at Makakavhule (Beitbridge district) and Kamhororo (Gokwe South district) (Table 11), which is consistent with findings in the 2015 Annual Entomological Report. Most of the data in Table 11 were collected before routine spraying took place. The non-living structures (mostly pit latrines or toilets) were routinely sprayed except at Zindi (Mutasa district). In Zindi in February 2017 (three months after the 2016 IRS campaign), the project team collected seven *An. funestus* s.l. from living (sprayed) structures, using PSC. The project team monitored the sites in Kawere, Kotwa, and Chilonga districts during or soon after the 2016 spray campaign.

TABLE 11: *AN. GAMBIAE* S.L. COLLECTED SEASONALLY BY PSC, 16 PROVINCIAL SITES , AUGUST 2016–FEBRUARY 2017

Site	Month of Collection	Type of Structure	No. of Rooms	Total *An. gambiae* s.l. Collected					Average No. of *An. gambiae* s.l. per Room
				Un Fed	Fed	Half Gravid	Gravid	Total	
Kamhororo	Nov-16 (pre-IRS)	Living	25	38	0	0	0	38	1.52
		Non living	16	6	0	0	0	6	0.38
	Jan-17 (post-IRS)	Living	26	0	2	0	0	2	0.08
		Non living	6	0	0	0	0	0	0.00
Sidhakeni	Nov-16 (pre-IRS)	Living	25	0	0	0	0	0	0.00
		Non living	9	2	0	0	0	2	0.22
	Jan-17 (post-IRS)	Living	29	0	0	0	0	0	0.00
		Non living	3	0	0	0	0	0	0.00
Old Mazowe Bridge	Sep-16 (pre-IRS)	Living	29	7	0	0	0	7	0.24
		Non living	6	0	0	0	0	0	0.00
	Jan-17 (post-IRS)	Living	28	1	0	0	0	1	0.04
		Non living	0	0	0	0	0	0	0.00
Muzarabani	Sep-16 (pre-IRS)	Living	13	1	0	0	0	1	0.08
		Non living	16	1	0	0	0	1	0.06
Chakari	Oct-16 (pre-IRS)	Living	35	0	0	0	0	0	0.00
		Non living	0	0	0	0	0	0	0.00
Kasimure	Aug-16 (pre-IRS)	Living	45	0	1	0	0	1	0.02
		Non living	0	0	0	0	0	0	0.00
Makakavhule	Oct-16 (pre-IRS)	Living	25	134	40	0	0	174	6.96
		Non living	8	3	0	0	0	3	0.38
	Jan-17 (post-IRS)	Living	31	3	0	0	0	3	0.10
		Non living	2	0	0	0	0	0	0.00
Tshelanyemba	Dec-16 (no IRS)	Living	29	1	0	0	0	1	0.03
		Non living	16	0	0	0	0	0	0.00
Kawere	Dec-16 (post-IRS)	Living	13	0	0	0	0	0	0.00
		Non living	11	0	0	0	0	0	0.00
	Feb-17 (post-IRS)	Living	17	1	0	0	0	1	0.06
		Non living	8	0	0	0	0	0	0.00
Kotwa	Dec-16 (pre-IRS)	Living	11	0	0	0	0	0	0.00
		Non living	7	0	0	0	0	0	0.00
	Feb-17 (post-IRS)	Living	28	0	0	0	0	0	0.00
		Non living	0	0	0	0	0	0	0.00
Maramba	Aug-16 (pre-IRS)	Living	16	0	0	0	0	0	0.00
		Non living	10	0	0	0	0	0	0.00
Chilonga	Nov-16 (pre-IRS)	Living	6	0	0	0	0	0	0.00
		Non living	16	0	2	0	0	2	0.13
Mashoko	Nov-16 (pre-IRS)	Living	21	0	0	0	0	0	0.00
		Non living	7	0	0	0	0	0	0.00
Jotsholo	Oct-16	Living	13	0	0	0	0	0	0.00

Site	Month of Collection	Type of Structure	No. of Rooms	Total *An. gambiae* s.l. Collected					Average No. of *An. gambiae* s.l. per Room
				Un Fed	Fed	Half Gravid	Gravid	Total	
	(pre-IRS)	Non living	4	0	0	0	0	0	0.00
Manjolo	Oct-16 (pre-IRS)	Living	25	4	0	0	0	4	0.16
		Non living	12	0	0	0	0	0	0.00
Zindi	Sept-16 (pre-IRS)	Living	9	0	0	0	0	0	0.00
		Non living	14	0	0	0	0	0	0.00
	Feb-17 (post-IRS)	Living	44	0	0	0	0	0	0.00
		Non living	6	0	0	0	0	0	0.00

Most sentinel sites did not yield any mosquitoes using the PPA method (Table 12). More mosquitoes were collected during the pre-IRS period. The highest collections were at Makakavhule, Kamhororo, and Chakari. More mosquitoes were collected from living structures than from non-living ones, but this is partly because there are fewer non-living structures at most sentinel sites. The project team collected fewer indoor resting *An. gambiae* s.l. by PPA than by PSC. The PPA method produced two *An. funestus* s.l. from Zindi, one each from living and non-living structures, in September.

TABLE 12: *AN. GAMBIAE* S.L. COLLECTED SEASONALLY BY PPA, LIVING AND NON-LIVING STRUCTURES, 16 SITES, , AUGUST 2016–FEBRUARY 2017

Site	Month of Collection	Type of Structure	No. of Rooms	Total *An. gambiae* s.l. Collected					Average No. of *An. gambiae* s.l. per Room
				Un Fed	**Fed**	**Half Gravid**	**Gravid**	**Total**	
Kamhororo	Nov-16 (pre-IRS)	Living	25	1	0	0	0	1	0.04
		Non living	16	4	0	0	0	4	0.25
	Jan-17 (post-IRS)	Living	26	0	0	0	0	0	0.00
		Non living	6	0	0	0	0	0	0.00
Sidhakeni	Nov-16 (pre-IRS)	Living	31	0	0	0	0	0	0.00
		Non living	21	0	0	0	0	0	0.00
	Jan-17 (post-IRS)	Living	26	0	0	0	0	0	0.00
		Non living	14	0	0	0	0	0	0.00
Old Mazowe Bridge	Sep-16 (pre-IRS)	Living	27	0	0	0	0	0	0.00
		Non living	10	0	0	0	0	0	0.00
	Jan-17 (post-IRS)	Living	29	1	0	0	0	1	0.03
		Non living	11	0	0	0	0	0	0.00
Muzarabani	Sep-16 (pre-IRS)	Living	25	0	0	0	0	0	0.00
		Non living	7	1	0	0	0	1	0.14
Chakari	Oct-16 (post-IRS)	Living	26	1	1	4	0	6	0.23
		Non living	6	0	0	0	0	0	0.00
Kasimure	Aug-16 (pre-IRS)	Living	39	0	0	0	0	0	0.00
		Non living	32	0	1	0	0	1	0.03

Site	Month of Collection	Type of Structure	No. of Rooms	Total *An. gambiae* s.l. Collected					Average No. of *An. gambiae* s.l. per Room
				Un Fed	Fed	Half Gravid	Gravid	Total	
Makakavhule	Oct-16 (post-IRS)	Living	34	28	6	0	0	34	1.00
		Non living	4	7	0	0	0	7	1.75
	Jan-17 (post-IRS)	Living	26	1	0	0	0	1	0.04
		Non living	11	0	0	0	0	0	0.00
Tshelanyemba	Dec-16 (No IRS)	Living	30	0	0	0	0	0	0.00
		Non living	16	0	0	0	0	0	0.00
Kawere	Dec-16 (pre-IRS)	Living	13	0	0	0	0	0	0.00
		Non living	11	0	0	0	0	0	0.00
	Feb-17 (post-IRS)	Living	20	0	0	0	0	0	0.00
		Non living	15	0	1	0	0	1	0.07
Kotwa	Dec-16 (pre-IRS)	Living	13	0	0	0	0	0	0.00
		Non living	11	0	0	0	0	0	0.00
	Feb-17 (post-IRS)	Living	28	0	0	0	0	0	0.00
		Non living	15	0	0	0	0	0	0.00
Maramba	Aug-16 (pre-IRS)	Living	24	0	0	0	0	0	0.00
		Non living	13	0	0	0	0	0	0.00
Chilonga	Nov-16 (pre-IRS)	Living	27	0	0	0	0	0	0.00
		Non living	9	0	0	0	0	0	0.00
Mashoko	Nov-16 (pre-IRS)	Living	23	0	0	0	0	0	0.00
		Non living	9	0	0	0	0	0	0.00
Jotsholo	Oct-16 (pre-IRS)	Living	21	0	0	0	0	0	0.00
		Non living	11	0	0	0	0	0	0.00
Manjolo	Oct-16 (pre-IRS)	Living	25	0	0	0	0	0	0.00
		Non living	15	1	0	0	0	1	0.07
Zindi	Sept-16 (pre-IRS)	Living	13	0	0	0	0	0	0.00
		Non living	17	0	0	0	0	0	0.00
	Feb-17	Living	21	0	1	0	0	1	0.05
		Non living	13	0	0	0	0	0	0.00

The project team collected an exceptionally high number of *An. gambiae* s.l. from Kamhororo in January from CDC light traps set indoors and outdoors (Table 13). Other productive sentinel sites were Makakavhule, Old Mazowe Bridge, Mashoko, and Muzarabani. On average, light traps set outdoors attracted more *An. gambiae* s.l. than traps set indoors, except at the sites where vector abundance was relatively high. Many of the mosquitoes collected both indoors and outdoors were un-fed. The high density of mosquitoes observed at Kamhororo could be due to the abundance of breeding sites, warm temperatures in March, and the fact that the traps were set around perennial breeding sites that are associated with the artesian well at Kamhororo. No *Anopheles* mosquitoes were collected from Tshelanyemba, Kotwa, Jotsholo, and Maramba sites using CDC light traps.

TABLE 13: *AN. GAMBIAE* S.L. COLLECTED SEASONALLY BY CDC LIGHT TRAPS, INDOORS AND OUTDOORS, 16 PROVINCIAL SITES , AUGUST 2016–FEBRUARY 2017

Site	Month of Monitoring	Location	No of traps	Total *An. gambiae* s.l. Collected					Average *An. gambiae* s.l./Trap
				Un Fed	Fed	Half Gravid	Gravid	Total	
Kamhororo	Nov-16 (pre-IRS)	IN	14	33	0	0	0	33	2.36
		OUT	12	20	0	0	0	20	1.67
	Jan-17 (post-IRS)	IN	18	295	38	0	0	333	18.50
		OUT	18	178	16	0	0	194	10.78
Sidhakeni	Nov-16 (pre-IRS)	IN	18	0	0	0	0	0	0.00
		OUT	15	1	0	0	0	1	0.07
	Jan-17 (post-IRS)	IN	26	0	0	0	0	0	0.00
		OUT	26	3	0	0	0	3	0.12
Old Mazowe Bridge	Sep-16 (pre-IRS)	IN	12	9	0	0	0	9	0.75
		OUT	18	13	0	0	0	13	0.72
	Jan-17 (post-IRS)	IN	18	1	0	0	0	1	0.06
		OUT	18	10	0	0	0	10	0.56
Muzarabani	Sep-16 (pre-IRS)	IN	19	5	0	0	0	5	0.26
		OUT	17	5	1	0	0	6	0.35
Chakari	Oct-16 (post-IRS)	IN	22	8	0	0	0	8	0.36
		OUT	12	6	0	0	0	6	0.50
Kasimure	Aug-16 (pre-IRS)	IN	18	0	0	0	0	0	0.00
		OUT	18	0	0	0	0	0	0.00
Makakavhule	Oct-16 (pre-IRS)	IN	18	28	0	0	0	28	1.56
		OUT	15	26	0	0	0	26	1.73
	Jan-17 (post-IRS)	IN	17	1	0	0	0	1	0.06
		OUT	16	2	0	0	0	2	0.13
Tshelanyemba	Dec-16 (no IRS)	IN	15	0	0	0	0	0	0.00
		OUT	15	0	0	0	0	0	0.00
Kawere	Dec-16 (pre-IRS)	IN	18	0	0	0	0	0	0.00
		OUT	18	0	0	0	0	0	0.00
	Feb-17 (post-IRS)	IN	30	0	0	0	0	0	0.00
		OUT	30	4	0	0	0	4	0.13
Kotwa	Dec-16 (pre-IRS)	IN	18	0	0	0	0	0	0.00
		OUT	18	0	0	0	0	0	0.00
	Feb-17 (post-IRS)	IN	18	0	0	0	0	0	0.00
		OUT	18	0	0	0	0	0	0.00
Maramba	Aug-16 (pre-IRS)	IN	10	0	0	0	0	0	0.00
		OUT	10	0	0	0	0	0	0.00
Chilonga	Nov-16 (pre-IRS)	IN	19	1	0	0	0	1	0.05
		OUT	20	3	0	0	0	3	0.15
Mashoko	Nov-16 (pre-IRS)	IN	24	6	0	0	0	2	0.08
		OUT	24	14	0	0	0	14	0.58
Jotsholo	Oct-16	IN	17	0	0	0	0	0	0.00

Site	Month of Monitoring	Location	No of traps	Total *An. gambiae* s.l. Collected					Average *An. gambiae* s.l./Trap
				Un Fed	Fed	Half Gravid	Gravid	Total	
	(pre-IRS)	OUT	16	0	0	0	0	0	0.00
Manjolo	Oct-16 (pre-IRS)	IN	8	0	0	0	0	0	0.00
		OUT	8	0	0	0	0	0	0.00
Zindi	Sept-16 (pre-IRS)	IN	24	0	0	0	0	0	0.00
		OUT	24	0	0	0	0	0	0.00
	Feb-17 (post-IRS)	IN	24	0	0	0	0	0	0.00
		OUT	24	0	0	0	0	0	0.00

[#] Zindi is included in this table because it is monitored seasonally unlike its counterpart sites in Manicaland that we monitor monthly. Zindi in Manicaland is an exception.

3.1.4 Feeding Time

At Burma Valley, the team sampled one household over two consecutive nights per month and collected more *An. funestus* s.l. (n=23) than *An. gambiae* s.l. (n=14) (Table 14). The outdoor peak for *An. funestus* s.l. was between 8 and 9 pm; indoors, there were two peaks, around midnight and between 3-4 am. This is consistent with observations reported previously for *An. funestus* s.l. in the area. For *An. gambiae* s.l., the indoor peak was at midnight; there was almost no collection outdoors, except for one mosquito collected between 3 and 4 am.

TABLE 14: NUMBER OF *AN. FUNESTUS* S.L. AND *AN. GAMBIAE* S.L. COLLECTED HOURLY INDOORS AND OUTDOORS, BURMA VALLEY

Time of collection	*An. funestus* s.l.		*An. gambiae* s.l.	
	IN	**OUT**	**IN**	**OUT**
6-7 pm	0	0	0	0
7-8 pm	0	1	0	0
8-9 pm	0	3	1	0
9-10 pm	1	2	0	0
10-11 pm	0	0	1	0
11-12	2	1	2	0
12-1 am	4	0	2	0
1-2 am	1	0	0	0
2-3 am	0	0	1	0
3-4 am	4	0	0	0
4-5 am	0	0	0	0
5-6 am	3	1	6	1
Total	15	8	13	1

At Chakohwa one household was sampled over two consecutive nights per month, and the predominant *An. gambiae* s.l. showed peak biting activity indoors between 7 and 9 pm and between 3 and 4 am, which is similar to observations for 2015 albeit based on low mosquito densities (Table 15). Outdoors, peak biting was between 6pm and 7pm, which represents a shift in peak hours to

earlier in the evening as compared with the 9 to 10 pm peak biting observed in 2015. Again, because the biting rate was very low in 2015, it is difficult to discern a pattern. It may be important to closely monitor the biting pattern by extending collection to the early hours of the evening to determine if there has been any significant shift in *An. gambiae* s.l. biting behavior.

TABLE 15: RESULTS FOR *AN. GAMBIAE* S.L. COLLECTED HOURLY INDOORS AND OUTDOORS, CHAKOHWA

Time of collection	*An. gambiae* s.l. collected at hourly intervals	
	IN	**OUT**
6-7 pm	0	5
7-8 pm	1	0
8-9 pm	1	2
9-10 pm	0	3
10-11 pm	0	0
11-12	0	1
12-1 am	0	0
1-2 am	1	0
2-3 am	0	0
3-4 am	2	0
4-5 am	0	1
5-6 am	0	0
Total	5	12

The team did not collect any mosquitoes by HLC proxy method at the former control site, Mukamba. At the new control site, Vumba, *An. funestus* s.l. activity was twice as much (n=12) outdoors than indoors (n=5). The peak activity indoors was between 3 and 4 am , whereas outdoors the biting activity was sporadic with most collections made between 6 and 7 pm, and between 8 to 10 pm (Table 16).

TABLE 16: RESULTS FOR *AN. FUNESTUS* S.L. COLLECTED HOURLY INDOORS AND OUTDOORS, VUMBA

Time of collection	*An. gambiae* s.l. collected at hourly intervals	
	IN	**OUT**
6-7 pm	0	5
7-8 pm	1	0
8-9 pm	1	2
9-10 pm	0	3
10-11 pm	0	0
11-12	0	1
12-1 am	0	0
1-2 am	1	0
2-3 am	0	0
3-4 am	2	0

4-5 am	0	1
5-6 am	0	0
Total	5	12

3.2 INSECTICIDE SUSCEPTIBILITY

All 60 *An. gambiae* s.l. tested were susceptible to DDT (4%). Table 17 shows the results of the tests on DDT (4%) for three replicates and a control for *An. gambiae* s.l. from Chakari site, Mashonaland West province. That few *An. gambiae* s.l. were tested was because of the incessant rains that hampered mosquito breeding. WHO recommends a minimum of 100 mosquitoes should be tested for each insecticide.

TABLE 17: WHO SUSCEPTIBILITY TEST RESULTS, *AN. GAMBIAE* S.L., DDT (4%), CHAKARI SITE, 2017

Replicate	No. tested	& Mortality after 24-hours observation period	Susceptibility status
1	25	100	Susceptible
2	25	100	Susceptible
3	10	100	Susceptible
Control	25	0	N/A

3.3 IRS RESIDUAL EFFICACY/QUALITY OF SPRAYING AND

3.3.1 QUALITY OF SPRAY

As described in the Methodology section, the team completed cone bioassay tests on four types of insecticide-sprayed walls at the Burma Valley sentinel site and on three types at Chakohwa site 24–48 hours after spraying. The team recorded complete (100 percent) mosquito mortality after the 24-hour holding period (T0) as shown in Figures 4 and 5 in the next section. These results are based on *An. arabiensis* (KGB strain) that were used in the cone bioassay tests. This indicated that the spraying was of good quality. The test mortality rates of susceptible colony mosquitoes on mud, cement, brick, and painted surfaces were 100 percent. The team did not observe mortality after exposure of mosquitoes to control (paper) surfaces. Therefore, it was not necessary to use Abbott's formula to correct the observed mortalities on sprayed surfaces.

There were no differences in test mortality rates of mosquitoes exposed to the sprayed walls at three different heights at baseline. This indicates that the spraying was relatively homogeneous along the walls since mosquito mortalities persisted beyond the period of the airborne effect of Actellic 300CS.

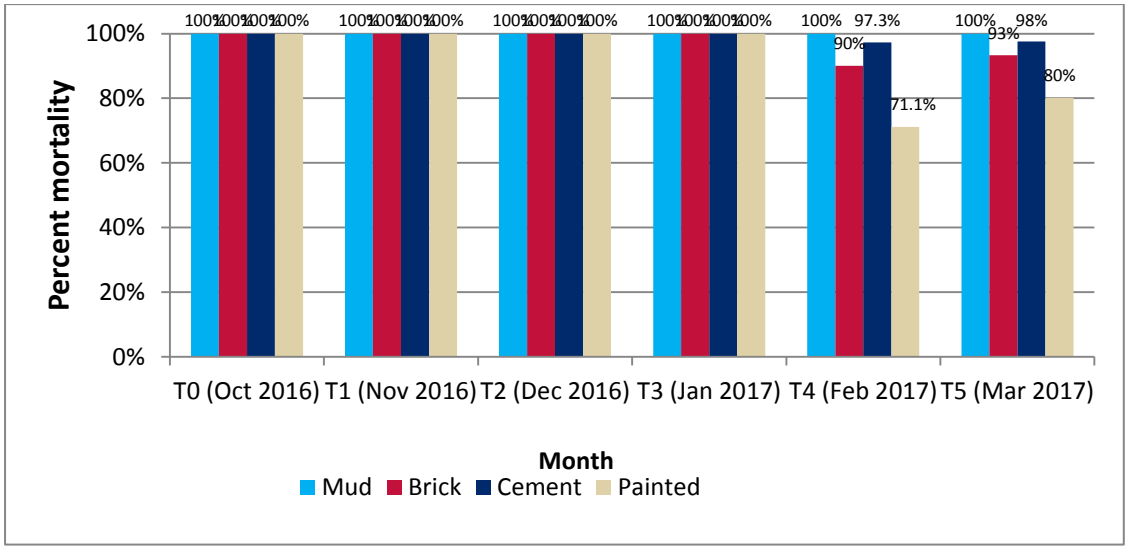
3.3.2 INSECTICIDE DECAY RATE

a) Burma Valley

Mortalities for laboratory colony mosquitoes A*n. arabiensis* (KGB strain) continued at 100 percent for three months post-spray on mud, brick, cement, and painted surfaces (Figure 4) at Burma Valley. Mortality remained at 100 percent for the mud surface but decreased to 90.0 percent, 97.3 percent, and 71.1 percent for brick, cement, and painted surfaces, respectively, in the fourth month, and to 93, 98, and 80 percent in the fifth month, suggesting that insecticide bio-efficacy is retained best on

mud and cement surfaces. Further tests will determine the residual span of the insecticide at Burma Valley.

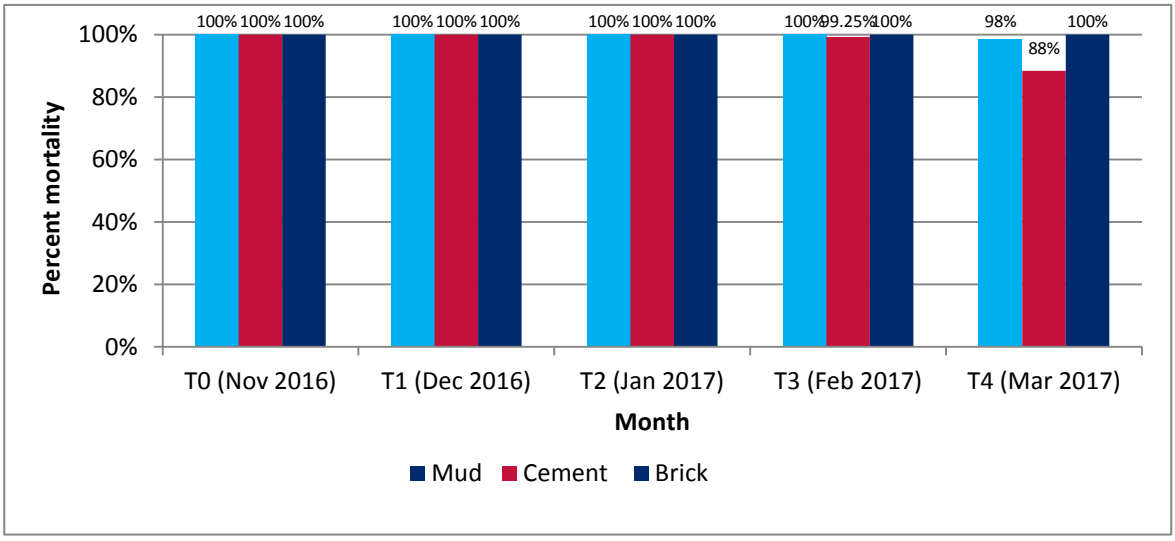
FIGURE 4: WHO CONE BIOASSAY TEST RESULTS, *AN. ARABIENSIS* (KGB STRAIN), MORTALITY AFTER 24-HOUR HOLDING PERIOD BURMA VALLEY, MUTARE DISTRICT



b) Chakohwa

In Chakohwa, the team observed *An. arabiensis* (KGB strain) mortalities of 100 percent on mud, brick, and cement surfaces two months after spraying but this declined in the third month to 99.25 percent on cement and in the fourth month to 98.0 and 88.0 percent for mud and cement, respectively (Figure 5). Further tests will determine the residual span of pirimiphos-methyl at Chakohwa.

FIGURE 5: WHO CONE TEST RESULTS, *AN. ARABIENSIS* (KGB STRAIN), MORTALITY AFTER 24-HOUR HOLDING PERIOD, CHAKOHWA, CHIMANIMANI DISTRICT



3.4 *ANOPHELES* SPECIES COLLECTED FROM NON-LIVING STRUCTURES

The project team collected only *An. gambiae* s.l. and *An. funestus* s.l. from non-living structures. NIHR morphological identifications agreed with AIRS identifications for all but two specimens from

Makakavhule. NIHR used three primers for *An. gambiae* s.l. and seven primers for *An. funestus* s.l. separately, based on the morphological identifications of the specimens. For the 33 *An. funestus* s.l. collected in 135 non-living structures from Burma Valley, NIHR identified *An. funestus* s.s. (6.06%), *An. leesoni* (27.28%), and *An. parensis* (33.34). Eleven (33.34%) of the samples which did not amplify with *An. funestus* s.l. primers were re-analyzed with *An. gambiae* s.l. primers and two (18.18%) of these were subsequently identified as *An. gambiae* s.s.; the remainder (81.81%) did not amplify (Table 18). NIHR identified two sibling species from *An. gambiae* s.l. from Burma Valley: *An. gambiae* s.s. *and An. quadriannulatus.* Overall, NIHR identified three *An. gambiae* s.s. from the sample collected from non-living structures in Burma Valley. The project team collected nine specimens from Chakohwa: eight *An. gambiae* s.l. and one *An. funestus* s.l. For the eight *An. gambiae* s.l. collected in 45 non-living structures from Chakohwa, NIHR identified two as *An. quadriannulatus*; the remaining six did not amplify but were subsequently identified as *An. funestus* s.s. (1/6) and *An. leesoni* (5/6) using the *An. funestus* s.l. protocol. NIHR identified the two *An. funestus* s.l. collected from 15 non-living structures from Kasimure as *An. leesoni* and *An. parensis*. The three *An. gambiae* s.l. collected from Makakavhule were identified as *An. gambiae* s.s. (n = 1) and *An. quadriannulatus* (n =2). Out of the 11 samples from Mukamba, four were identified as *An. quadriannulatus* while the seven that did not amplify were subsequently identified as *An. leesoni* using the *An. funestus* s.l. protocol. The single *An. funestus* s.l. specimen from Vumba was identified as *An. parensis*. Thirteen samples did not amplify: nine from Burma Valley , two from Chabwino , one each from Chakohwa and Zindi . None of the 64 tested specimens were sporozoite positive. Both AIRS and NIHR incorrectly identified eight specimens morphologically. Two *An. gambiae* s.l. were misidentified as *An. funestus* s.l., while six *An. funestus* s.l. were mis-identified as *An. gambiae* s.l.

TABLE 18: PCR IDENTIFICATION *AN. FUNESTUS* S.L. AND *AN. GAMBIAE* S.L. COLLECTED FROM NON-LIVING STRUCTURES, EIGHT SITES

Site	Total Tested	Method of Collection	PCR ID	Total per Species	% Vector Species	
					Known vector[#]	Possible vector[#]
Burma Valley	35	PSC	*An. funestus* s.s.	2	6.06	
		PSC	*An. leesoni*	9	27.27	
		PSC/PPA	*An. parensis*	11		33.33
		PSC/PPA	*An. gambiae* s.s.	3	75.00	
		PSC	*An. quadriannulatus*	1		
		PSC/PPA	Unidentified	9		
Chabwino	2	PSC	Unidentified	2		
Chakohwa	9	PSC/PPA	*An. funestus s.s*	1	11,1	
		PSC/PPA	*An. leesoni*	5	55,5	
		PSC/PPA	*An. quadriannulatus*	2		
		PSC	Unidentified	1		
Kasimure	2	PPA	*An. leesoni*	1	50	
		PPA	*An. parensis*	1		50
Makakavhule	3	PSC	*An. gambiae* s.s.	1	33,3	
			An. quadriannulatus	2		
Mukamba	11	PSC/PPA	*An. leesoni*	7	63,6	
		PSC/PPA	*An. quadriannulatus*	4		
Vumba	1	PPA	*An. parensis*	1		100
Zindi	1	PPA	Unidentified	1		

. The denominator is number of specimens morphologically identified as either *An. funestus* s.l. or *An. gambiae* s.l. at each sentinel site.

4. Discussion and Recommendations

4.1 Discussion

In Manicaland, the *An. funestus* s.l. was the predominant vector species at Burma Valley and Vumba in contrast to *An. gambiae* s.l. at Chakohwa. Other common species found were *An. coustani* and *An. gambiae* s.l. at Burma Valley, *An. pretoriensis* and *An. funestus* s.l. at Chakohwa, and *An. natalensis* and *An. coustani* at Vumba. *An. gambiae* s.l. is re-emerging at Burma Valley after apparently disappearing after the introduction of OP in 2014. Most *An. funestus* s.l. was found in Burma Valley, an area of routine spraying.

There is less species diversity at sentinel sites outside Manicaland, where *An. gambiae* s.l. is predominant. The scarcity of mosquitoes at Manicaland sites continues to affect the prospects of testing insecticide susceptibility. No susceptibility tests have been done since pirimiphos-methyl was introduced for IRS during the 2014 IRS campaign. The low mosquito densities can be attributed to the drought, which affected the availability of mosquito breeding sites. Indoor resting densities were low regardless of collection method. More mosquitoes were collected from non-living compared to living structures using both PSC and PPA. This was observed for *An. funestus* s.l. and *An. gambiae* s.l. at Burma Valley where non-living structures were not sprayed. More *An. funestus* s.l. were collected from living than non-living structures at Chakohwa (by PPA method) where non-living structures were not sprayed and at Vumba by both PSC and PPA methods. It must be noted that non-living structures are scarce at most sentinel sites. The team discusses the laboratory results of species collected from non-living structures in this report.

CDC light traps collected more *Anopheles* than did either PSC or PPA methods, and outdoor traps collected more *Anopheles* than did indoor traps.

The HLC proxy yielded more *Anopheles* mosquitoes in 2016 than in 2015, which suggests a growing number of vector species. The biting peaks for *An. funestus* s.l. and *An. gambiae* s.l. at Burma Valley imply the population is exposed both before midnight, at midnight, and during the early hours of the morning. At Chakohwa, indoor biting peaked at 20:00–21:00 and 03:00–04:00 for *An. funestus* s.l. and outdoor biting peaked at 18:00–19:00 for *An. gambiae* s.l.

PSC performed slightly better than PPA as observed from collections from the sites with high vector abundance, Kamhororo, Mashoko, Makakavhule, and Muzarabani, irrespective of species (*An. funestus* s.l. or *An. gambiae* s.l.) in 2016.

Susceptibility of *An. gambiae* s.l. from Chakari to DDT (4%) is good for insecticide resistance management planning for Zimbabwe despite that the results are based on a limited sample size. These results remain preliminary until the laboratory identifies the mosquito species in order to distinguish the proportion of vectors in the *An. gambiae* s.l. tested. The project team will submit the sample from the tests to the laboratory for species identification. The project will conduct further tests with DDT and other insecticides for a fuller picture of the susceptibility status of the *An. gambiae* s.l. from Chakari and other sentinel sites when mosquito breeding sites are abundant. The heavy rains have continued to affect the availability of mosquito larvae for insecticide resistance testing.

Bioassay data showed good quality of spraying at Burma Valley and Chakohwa for T0 and T1 collection periods. A large decline in the bio-efficacy of pirimiphos-methyl was observed after the 2014 IRS campaign and was attributed to inadequate spray performance; more rigorous training and supervision of spray operators improved bio-efficacy after the 2015 campaign, and in the three

months since the 2016 campaign. The residual efficacy of pirimiphos-methyl after the 2015 IRS campaign ranged from four to five months at both Burma Valley and Chakohwa. Although it is too early to make a final pronouncement on the residual efficacy of the insecticide sprayed in 2016, observations show residual efficacy is at least five months and four months at the Burma Valley and Chakohwa sites, respectively. The cone bioassays will continue until the mortality drops to less than 80 percent mortality for two consecutive months.

Malaria vector mosquitoes continue to be scarce at most sites in Zimbabwe. Atypical rainfall in 2016 prevented the AIRS Zimbabwe team from monitoring insecticide resistance at the sentinel sites in 2016. Most of the year was extremely dry, which prevented mosquitoes from finding pools in which to breed. In contrast, in December and through the time this report was written in March 2017, the pattern has been one of heavy rains, which are washing away normal breeding sites.

There is also longer-term scarcity of mosquitoes. Low numbers of both *An. gambiae* s.l. and *An. funestus* s.l. in most areas under IRS indicate the impact that decades of spraying and mass distribution of LLINs have had on the vector populations. The project team realizes the limitations of generalizing conclusions based on the data that come only from sentinel sites. The NMCP can collect more data by increasing the number of sentinel sites and/or by conducting vector surveillance in the areas not covered by the sentinel sites.

The species identified from specimens collected from non-living structures show a surprising mixture of *An. gambiae* s.l.; *An. gambiae* s.s. and *An. quadriannulatus* were found in such disparate sites as Burma Valley and Makakavhule in the absence of the expected *An. arabiensis.* The vectorial efficiency of *An. gambiae* s.s. is comparable to that of *An. funestus* s.s. The combination of these two efficient malaria vectors could explain the high disease burden in Burma Valley and Beitbridge if these species are widely distributed within these localities. For *An. funestus* s.l., the three sibling species found also reflect a wide diversity for most sites. While *An. funestus* s.s. is a known malaria vector, previous work has incriminated *An. leesoni,* but is not conclusive for *An. parensis.* These results provide ample evidence that malaria vectors rest in non-living structures.

The specimens that failed to amplify could indicate the need to expand the range of primers for both *An. funestus* s.l. and *An. gambiae* s.l. or even other species during analysis. NIHR used three primers for the *An. gambiae* s.l. (GA for *An. gambiae* s.s., AR for *An. arabiensis,* and QD for *An. quadriannulatus*) and excluded the primer for *An. merus* (MR) and this could account for some of the sample that did not amplify. *An. merus* has a patchy distribution in Zimbabwe. For *An. funestus* s.l., NIHR excluded primers for four sibling species, *An. fuscivenosus* whose known distribution is limited to Zimbabwe, *An. confusus, An. brucei* (described from one locality in Nigeria), and *An. aruni* (described from Zanzibar). It is also interesting to note that *An. quadriannulatus* was found indoors, as it normally is regarded as a non-vector because of its largely zoophilic and exophilic behavior.

The analysis of samples from non-living structures also revealed the weakness in morphological identification. This does not necessarily point to incompetence on the part of entomologists at AIRS and NIHR. The accuracy of morphological identification relies on intact specimens, but the labs sometimes receive incomplete material from the field – mosquito legs, wings, or palps are missing or distorted. This can be the result of careless handling – in future, the project team will handle all specimens with greater care during collection, transportation, storage, and handling – but also of equipment failure. For example, CDC light traps sometimes damage the mosquitoes when the fan dismembers the specimens during suction.

Manicaland province considered spraying Vumba following an upsurge of malaria cases in December and January. A new control site will be needed should the province spray Vumba as part of the 2017 IRS campaign, which starts in October 2017.

4.2 Positive Developments

- AIRS Zimbabwe's hiring of the Entomological Officer seconded to the NMCP has enhanced national malaria vector support and ability to provide better surveillance. The project and NMCP managed to increase the number of sentinel sites covered during the 2016/17

entomological monitoring period from 11 to 19 (excluding Mutare City). In 2015, the project team supplied equipment at 17 sites, and in 2016 the team formally trained all staff from all the sentinel sites. In 2016, the team further provided on-the-job training for the same officers during the first-ever episode data collection conducted during the dry season prior to the 2016 IRS campaign. The NIHR insectaries at Harare and Chiredzi have reliably improved their supply of susceptible colony mosquitoes for cone bioassay tests. AIRS Zimbabwe currently relies solely on susceptible colony mosquitoes for cone bioassay tests.

- The management of entomological data improved with the establishment of the Disease Data Management System (DDMS). Data from 2015 and 2016 have been entered in the DDMS. The database is updated monthly, soon after entomology teams complete data collection at sentinel sites.
- The establishment and equipping of a molecular laboratory at Africa University is expected to speed up the processing of mosquito specimens.
- Having an insectary at Africa University will alleviate pressure on NIHR to supply susceptible colony mosquitoes. It will ensure the constant availability of susceptible colony mosquitoes for cone bioassay tests in Manicaland and adjacent provinces. Cone bioassay tests can be expanded to more districts both in and outside Manicaland. Currently, cone bioassay data collection is limited to two sites in Manicaland (Burma Valley and Chakohwa), and the province wants to expand this to the other five districts. The project will provide technical assistance to initiate cone bioassay tests in the other districts once the Africa University insectary can regularly provide adequate numbers of colony mosquitoes. The team anticipates this can happen gradually in 2018.
- NIHR has completed analyzing mosquito specimens collected from non-living structures from March to October 2016. The results indicate the presence of malaria vectors in non-living structures.
- Refresher training held in May 2016 helped insectary managers and field officers with critical mosquito identification skills.
- AIRS Zimbabwe successfully hosted the PMI Regional Training in Entomology in Harare June 26–July 3, 2016. National-level, junior entomologists from 11 African countries were trained.

4.3 RECOMMENDATIONS

- Refresher entomology training for district supervisors (district environmental health officers) and environmental health technicians is required to continue strengthening their skills in vector surveillance.
- There is need to improve infrastructure (shelves, cabinets, and work stations) at sentinel sites through concerted partner support to NMCP.
- Insecticide susceptibility tests should be conducted at all sentinel sites once weather conditions permit.
- There is need to expand the range of primers for both *An. funestus* s.l. and *An. gambiae* s.l. or even other species to cater for the samples that fail to amplify with the existing primers.
- There is need for careful handling all mosquito specimens with greater care during collection, transportation, storage, and handling to ensure that sample arrive intact for easy morphological identification.
- The entomology teams will continue to monitor vector resting behavior in non-living structures for another year in order to decide whether to spray them or not.