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ZIMBABWE 2015 ENTOMOLOGICAL ACTIVITIES FINAL REPORT

MAY 2016

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ZIMBABWE
2015 ENTOMOLOGICAL ACTIVITIES
FINAL REPORT

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ACRONYMS

AIRS	Africa Indoor Residual Spraying
CDC	Centers for Disease Control
DDMS	Disease Data Management System
DDT	Dichlorodiphenyltrichloroethane
IRS	Indoor Residual Spraying
LLIN	Long-Lasting Insecticidal Net
NIHR	National Institute of Health Research
NMCP	National Malaria Control Program
OP	Organophosphate
PMI	President's Malaria Initiative
PSC	Pyrethrum Spray Collection
USAID	United States Agency for International Development
WHO	World Health Organization

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Province/ Institution	Name
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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 its target districts and beyond. In 2015, the AIRS Zimbabwe project for the second consecutive year used the insecticide pirimiphos-methyl, an organophosphate, to conduct IRS in the four districts. To monitor impact of PMI-funded IRS on the local vectors, AIRS Zimbabwe conducts monthly entomological monitoring at three sites in Manicaland: Burma Valley and Chakohwa in the project-supported districts of Mutare and Chimanimani and one unsprayed control site (Makoni district). The project also does seasonal entomological monitoring in seven sites in other provinces.

Methods

The project collected baseline entomological data in September 2015, before spraying began in October. This was followed by post-spray data collections. 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, 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 Prokopack methods. The project used the standard World Health Organization protocol to determine resistance in malaria vectors to four insecticides recommended for public health use. The National Institute of Health Research provi
ded results of the analysis completed on specimens submitted in 2013 and 2014. These results will be analyzed and presented separately by end of June 2016.

Results

The project team observed low mosquito densities at sites dominated by either *An. funestus* or *An.gambiae* s.l. In Burma Valley, the density of *An. funestus* increased in unsprayed non-living structures after living structures were sprayed with pirimiphos-methyl. Though the density seems low, the shift in resting behavior is persistent enough to warrant collecting more data to confirm if spraying non-living structures in this area is required. The average residual efficacy of pirimiphos-methyl for all surfaces was four to five months at Burma Valley and Chakohwa. Mud surfaces tended to retain pirimiphos-methyl for longer at both sites.

Resistance was detected to three insecticides: lambdacyhalothrin and bendiocarb at Chakari site, and DDT at Kamhororo and Makakavhule sites. Possible resistance to pirimiphos-methyl was detected at Makakavhule, the first such report for this insecticide. Further follow-up and work need to be done on mechanisms of insecticide resistance. The National Malaria Control Program introduced pirmiphos-methyl for IRS for the first time in Beitbridge district (where Makakavhule is located) during the 2015 IRS campaign to replace DDT after indications of resistance to DDT in the area.

Conclusions

Malaria transmission continues despite the low mosquito densities in the project areas. The residual life of pirimiphos-methyl has been determined to be four months in Burma Valley, but insecticide decay tests are continuing in Chakohwa. Insecticide resistance remains a threat to effective mosquito control and therefore vector surveillance needs to be strengthened.

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 provinces using dichlorodiphenyltrichloroethane (DDT), pyrethroids, and the recently introduced pirimiphos-methyl, an organophosphate (OP) class insecticide. The IRS is done once a year, before peak transmission, and is expected to reduce 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 out of the four target districts in Manicaland province which receive AIRS comprehensive spraying support. AIRS Zimbabwe also assists the NMCP in testing the residual efficacy of insecticides the national program uses in IRS 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 2015 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 (WHOPES) for IRS;
- Identify the vector species, composition, and density;
- Determine vector biting and resting behavior, including vectors resting in non-living structures; and
- Pilot the Prokopack aspirator for sampling vectors resting indoors at three sites in Manicaland and seven sites outside Manicaland.

This report describes collection activities and the results of AIRS Zimbabwe entomological monitoring conducted between March 2015 and February 2016.

2. METHODOLOGY

AIRS Zimbabwe used the following four techniques for entomological surveillance in 2015:

- World Health Organization (WHO) cone bioassay test to determine the quality of spray and residual efficacy of insecticide on sprayed structure walls
- WHO susceptibility test for determination of insecticide susceptibility
- Pyrethrum spray collection (PSC) to determine the vector indoor resting density
- Centers for Disease Control and Prevention (CDC) light traps to determine mosquito density and behavior

In addition, AIRS Zimbabwe continued piloting the Prokopack to test the aspirator's efficacy compared with the PSC method at sentinel sites dominated by *An. gambiae* s.l. The project used the collected mosquitoes to look at vector resting behavior in living versus non-living structures at sentinel sites in Manicaland and at seven sites outside Manicaland.

2.1 STUDY SITES

In the 2015 spray season, AIRS Zimbabwe did entomological monitoring in the 10 sentinel sites shown in Table 1. The project began monitoring in September 2015, to capture baseline information on malaria vector populations during the dry season and prior to IRS. It then continued monitoring the three sites in Manicaland province routinely (monthly) and the seven sites located in the other provinces seasonally (once in the dry (pre-spray) season and another one during the wet (post-spray) season).

In seven sites outside Manicaland, AIRS Zimbabwe conducted insecticide susceptibility tests and collected data on vector density and behavior. In its target districts in Manicaland, it did cone bioassays for spray quality and insecticide decay rate monitoring as well as vector behavior and density data collections on a monthly basis, from October 2015 through February 2016. The project evaluated the Prokopack aspirators by collecting data on vector resting behavior in both living and non-living structures in the Burma Valley site, and at seven sites outside Manicaland.

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

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.
	Chimanimani*	Chakohwa	OP	*An. gambiae* s.l./*An. funestus* s.l.
	Makoni	Mukamba^	Nil (control)	*An. gambiae* s.l.
Mashonaland East	Mutoko	Kawere	Pyrethroids	*An. gambiae* s.l.
Mashonaland West	Sanyati	Chakari	OP	*An. gambiae* s.l.
Masvingo	Chiredzi	Chilonga	DDT	*An. gambiae* s.l.
Matabeleland North	Binga	Manjolo	DDT	*An. gambiae* s.l.
Matabeleland South	Beitbridge	Makakavhule	OP	*An. gambiae* s.l.
Midlands	Gokwe South	Kamhororo	DDT	*An. gambiae* s.l.
Mashonaland Central	Rushinga	Old Mazowe Bridge	OP	*An. gambiae* s.l.

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

^ The site was selected because it borders the IRS intervention areas.

2.2 Species Composition and Vector Seasonality

The project used PSC, Prokopack aspirators, and the CDC light trap techniques at all 10 sites. The light trap was used as a proxy to the human landing catch (HLC) method at the three sites in Manicaland. Mosquitoes collected by the three main methods were identified morphologically to determine species distribution and abundance.

2.3 Pyrethrum Spray Collections for Vector Density

The PSC method was used in all 10 sentinel sites, to sample indoor resting mosquitoes from 25 rooms per site per month. It was carried out in the morning between 06:00 and 11:00. (In the next spray season, the team will move the start of the data collection to 05:00 in the morning.) Before the PSC was performed, verbal consent to do so was secured from the head of the household. 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 were collected.

The room was prepared 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 before insecticide was applied. A commercial aerosol insecticide sprayers Baygon® was used. The active ingredients include the pyrethroids: Tetramethrin, Prallethrin, Imiprothrin and the synergist piperonyl butoxide.

After vigorously shaking the aerosol can, one spray team member sprayed the eaves from 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 cloth was taken outside and spread out carefully on the ground. Knocked down mosquitoes were picked up with forceps and put into a petri dish. The other pieces of cloth were examined in same way. If it was windy or wet, the cloths were examined sequentially inside the room with the aid of a flashlight. One petri dish was used per room and the dish was labeled with the collection method, room code or identity, the locality, and the date of collection. Data on the collection was entered on a form for each room sampled.

The team also investigated mosquito resting behavior in non-living structures (mainly toilets and bathrooms). In an innovation approach, the team used disposable flipchart paper in place of cloth to conduct PSC in these structures (Figure 1). It would be unhygienic to re-use in living rooms calico cloth that had been used in toilets and bathrooms. The team also used disposable gloves in non-living structures.

FIGURE 1: FLIPCHART PAPER SPREAD ON PIT LATRINE FLOOR FOR PSC, BURMA VALLEY

2.4 PROKOPACK ASPIRATOR COLLECTIONS

AIRS Zimbabwe used Prokopack aspirator only at the three sites in Manicaland province. It used Prokopack aspirators to sample indoor resting mosquitoes from 25 rooms per sentinel site per month. While the project targeted the same rooms, it was not always possible to access the same ones because of the availability of the home owners. As it did with PSC, the project carried out the activity in the morning between 06:00 and 11:00. Before the collection was performed, the team secured the household head's verbal consent (Figure 2), asked all occupants to move out of 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 Prokopack aspirator 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 the aspirator would suck and not blow the mosquitoes. After fitting the collection cup, the Prokopack 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 Prokopack collects live mosquitoes, the cup is inserted in a large mosquito cage and the mosquitoes are released into the cage. Then the team removed mosquitoes using a small sucking aspirator, stunned, 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: TECHNICAL TEAM EXPLAINS PROKOPACK TO HEAD OF HOUSEHOLD, BURMA VALLEY

2.5 CDC Light Trap Collections for Vector Density and Behavior

2.5.1 Vector Density

The project used CDC light traps to determine mosquito density inside houses and outdoors at different households. Six traps were set indoor alongside human bait and another six were outdoors without bait. The twelve traps, two per each of the six households, were left overnight and were emptied the following morning. At each household, we set one light trap indoors towards the foot of a bed or sleeping space, after making sure the human bait was protected by a mosquito net. The light traps set outdoors were within 10 to 15 meters of the one set indoors. Thus, because the outdoor traps are not baited, these traps are not comparable to those set indoors. We considered to have 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. Sleeping outdoors alone is not safe as people can be attacked by robbers or wild animals (snakes, crocodiles and scorpions). The households selected for this exercise are located up to four kilometers apart from each other. Traps were hung with the light source about 1.5 meters from the ground. The project operated the traps from sunset (18:00 hours) to sunrise (06:00 hours). We tied the collection sleeve before switching the trap off to ensure no mosquitoes would escape from the collection cup at the bottom. It was also important to ensure that the equipment was secured at the data collection site before traps could be left overnight. The light traps used sealed, lead-acid, rechargeable 6 volt batteries, which we charged during the day to re-use during the next round of data collection.

The project used the CDC light traps at all 10 sentinel sites. The light traps were set over one night per sentinel site per month of data collection: six traps indoors and six outdoors at the same homesteads each month.

2.5.2 Vector Behavior

One CDC light trap was used alongside a human bait as proxy for the HLC to learn where most vector-human contact was occurring (inside and/or outside), vector feeding time, and changes in the feeding behavior of mosquitoes before and after IRS at a selected house per site surveyed monthly from March 2015 to February 2016. The mosquitoes are collected every hour. One person slept indoors while another slept outdoors for these hourly collections from the light traps. A few data collectors stayed near the human bait to help with the collections, but were placed in a different room.

As noted above, AIRS Zimbabwe made collections at hourly intervals from 18:00 hours until 06:00 hours. We assigned two collectors to stay under a net alongside a light trap: one stayed outside and the other inside. The collectors exchanged their positions at midnight. We checked the light trap hourly, and aspirated anopheline mosquitoes in a paper cup labeled with date, locality, and position of the trap. We monitored temperature, relative humidity, and rainfall and recorded them at hourly intervals during the night.

The team conducted a baseline collection in September to assess vector feeding behavior and biting rate before spraying, and subsequent monthly collections after the spray began in October 2015.

We preserved all collected mosquitoes individually in a 1.5 ml Eppendorf tube in Silica gel for species identification and sporozoite rate using ELISA.

We used the CDC light trap collection method for vector behavior analysis only at the three sites in Manicaland Province.

2.6 Insecticide Susceptibility Tests

AIRS Zimbabwe carried out vector susceptibility tests to determine the susceptibility level of the vector population at the sentinel sites. For the tests, we used WHO tubes and non-blood-fed adult female *An. gambiae* s.l. reared from larvae and pupae. We conducted the tests with insecticides from the four classes recommended for public health use in Zimbabwe: OP, organochlorine, carbamate, and pyrethroid.

Historically, *An. funestus* s.l. was the primary vector found in the sentinel sites in the two PMI-sprayed districts (Mutasa and Mutare) in Manicaland. In 2014, its resistance to insecticide was tested at two sentinel sites (Burma Valley, Mutare, district and Honde Valley, in Mutasa district). Since the PMI program began using pirimiphos-methyl for IRS in those sites, *An. funestus* s.l. has become scarce and so there is an insufficient number of larvae to collect. Therefore, AIRS Zimbabwe plans to collect adult *An. funestus* s.l., which will be set to lay eggs to raise F_1 adults for the susceptibility tests. Meanwhile, because of the mosquito scarcity, the project has not yet conducted any susceptibility tests at the three current sites in Manicaland.

At the seven sentinel sites outside Manicaland, we completed insecticide susceptibility tests on local *An. gambiae* s.l. We tested the four insecticides (bendiocarb, DDT, lambdacyhalothrin, and pirimiphos-methyl) at the following sites: Chakohwa (Nyanyadzi area), Old Mazowe Bridge, Chakari (Sanyati area), Kamhororo, Kawere, and Makakavhule. At Manjolo site, we tested only lambdacyhalothrin because of an inadequate number of mosquitoes. In four of the sites, we reared adult mosquitoes at the field insectary from larvae and pupae collected within a 10 km radius of the sentinel sites; in Chakari and Makakavhule, larval collection areas exceeded the 10 km radius. Also, it was impossible to do mosquito collections at Kawere site (Mashonaland East province) because of dry weather and absence of skilled local staff – two key personnel there left their positions, producing a skills gap.

For the tests, we used insecticide-treated papers not more than four times. We used the control papers for the different insecticide classes as follows: olive oil for OP and carbamate, silicone oil for pyrethroids, and risella oil for organochlorine. We followed the WHO standards of two replicates of 25 mosquitoes each for the controls, although on some occasions, we used fewer due to insufficient availability of mosquitoes.

For the test of each insecticide, we first placed 25 1–5-day-old female mosquitoes in a holding tube, where they were observed for 60 minutes to check on their condition. Then we transferred them to an exposure tube lined with insecticide-treated paper. The target was four replicates per insecticide tested and two replicates for the controls. We exposed mosquitoes for a one-hour period, during which we recorded the number of knocked down mosquitoes at regular intervals, and for an additional 20 minutes after exposure. The female mosquitoes were fed on 10 percent sugar solution prior to exposure and during a 24-hour holding period after the exposure. We recorded temperature and relative humidity during exposure and the 24-hour holding period. We recorded final mosquito mortality after the 24-hour holding period as a percentage of the number of mosquitoes exposed per tube. We used Abbott's formula to correct results to take into account any observed mortality in control tubes.

AIRS Zimbabwe used the revised WHO criteria for noting susceptibility to insecticide:

- Susceptibility = Mortality rate of the exposed vector greater than or equal to 98 percent
- Possible Resistance = Mortality rate of the exposed vector equal to or between 90 percent and 97 percent
- Resistance = Mortality rate of the exposed vector is less than 90 percent.

2.7 Cone Bioassays for Spray Quality and Residual Efficacy

We conducted cone bioassay tests to determine the quality of spray 24–48 hours after spray operations at the two sentinel sites in the in AIRS Zimbabwe-supported districts, Burma Valley and Chakohwa. For the tests, we used standard WHO plastic cones. At each site, we 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.

AIRS Zimbabwe used susceptible mosquitoes from two sources for the cone bioassay tests: mosquito collectors employed by the project collected *An. gambiae* s.l. in the field (Midlands Province) and the National Institute of Health Research (NIHR) supplied susceptible *An. arabiensis* (KGB strain).

At the Burma Valley site, we tested in the 10 rooms as follows: in nine of the rooms we used wild-caught *An. gambiae* s.l. and in six rooms we used *An. arabiensis* colony; in five rooms, both wild-caught and colony mosquitoes were used simultaneously. At the Chakohwa site, we tested five rooms using both *An. arabiensis* and *An. gambiae* s.l., while in the five remaining rooms we used only *An. gambiae* s.l.. When using both wild and colony mosquitoes in one room, we used six cones per room. We exposed 10 female *Anopheles gambiae* s.l. mosquitoes to insecticide in the cones and retrieved them after 30 minutes. Upon retrieval, the mosquitoes were transferred to clean cups and provided with 10 percent sucrose solution for the 24-hour observation period. We recorded the number of mosquitoes knocked down at this 30-minute point and again after 60 minutes. We recorded the final mortality at the end of 24-hour observation period.

We 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 the same way as with the cones in sprayed rooms.

We used wild-caught *An. gambiae* s.l. that were reared from larvae collected in Masakadza, in Gokwe South district, one of few areas with breeding grounds to provide large enough numbers of

mosquitoes required for tests. The susceptibility of the wild-caught mosquitoes to pirimiphos-methyl was confirmed prior to their use in cone bioassay tests.

Since the initial bioassay tests to assess quality of spray, we have been conducting subsequent bioassay tests monthly to determine the residual efficacy of insecticide. We will continue the tests until the average mortality falls below 80 percent for two consecutive tests.

3. RESULTS

3.1 VECTOR SPECIES COMPOSITION, DENSITY, SEASONALITY, RESTING BEHAVIOR

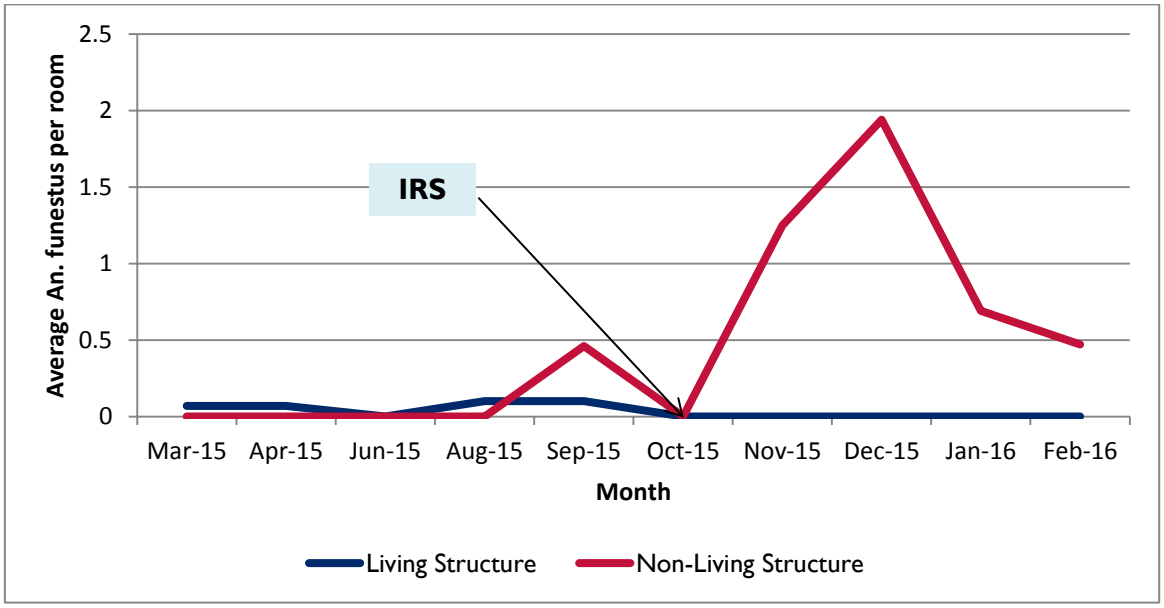
3.1.1 SPECIES COMPOSITION

The primary vector in most sentinel sites was *An. gambiae* s.l., while *An. funestus* s.l. was predominant in the Burma Valley site. Partial data indicate the species diversity is greatest at two representative sites: Burma Valley and Kamhororo. Out of 36 *Anopheles* collected at Burma, 72 percent (n=26) were *An. funestus,* 14 percent (n=5) were *An. coustani,* 8 percent (n=3) were *An. pretoriensis,* and 6 percent (n=2) were *An. maculipalpis*. At Kamhororo, out of 174 *Anopheles*, 77 percent (n=134) were *An. gambiae* s.l., 11.5 percent (n=20) were *An. coustani,* and 11.5 percent (n=20) were *An. pharoensis*.

3.1.2 VECTOR DENSITY

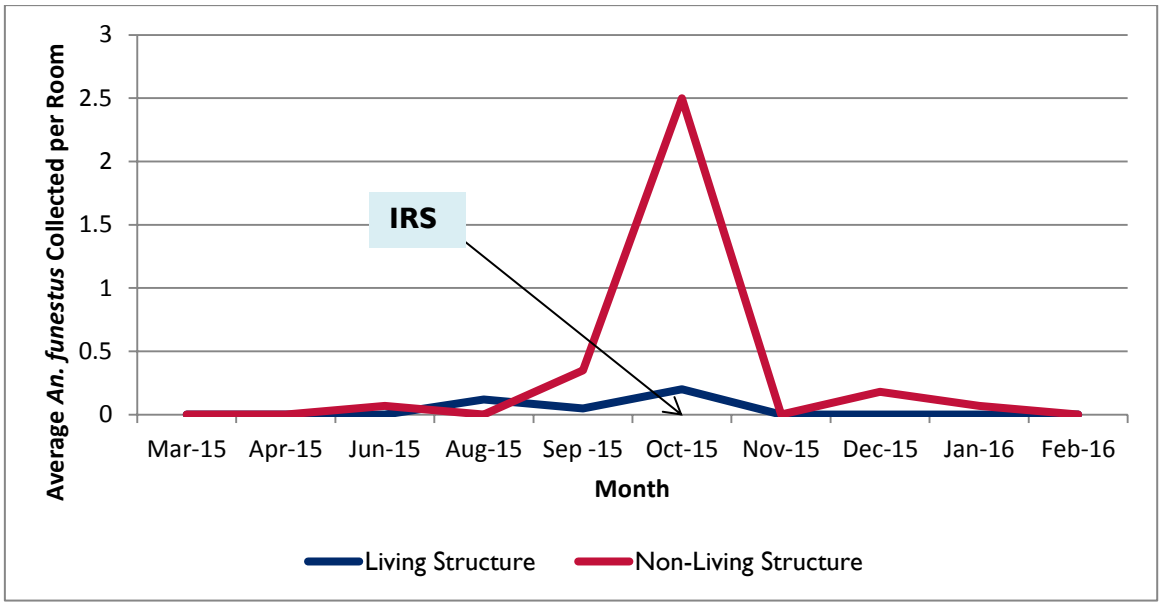
The PSC data on *An. funestus* at Burma Valley show low mosquito densities for all months. While relatively few mosquitoes were found resting in living structures as compared with non-living structures before IRS, no mosquitoes were collected from living structures after spraying. After IRS, almost all *An. funestus* collected were from non-living structures, which are not sprayed during the IRS campaign (Figure 3). The project observed similar results after the 2014 IRS campaign.

FIGURE 3: MEAN INDOOR RESTING DENSITY (PSC) OF *AN. FUNESTUS* S.L., LIVING AND NON-LIVING STRUCTURES, BURMA VALLEY, SEPTEMBER 2015–FEBRUARY 2016



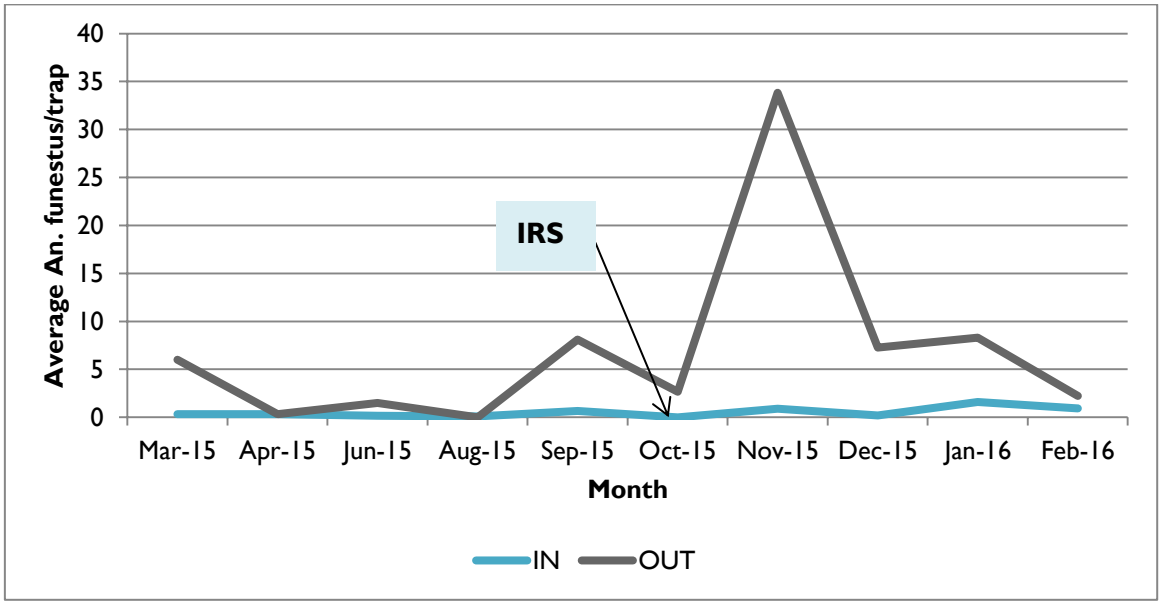
The data from the Prokopack collections also showed more mosquitoes were collected in (unsprayed) non-living structures than in (sprayed) living structures. Indoor resting mosquitoes continued to be detected from non-living structures albeit at lower densities compared with the PSC method above (Figure 4). Collection peaks for Prokopack and PSC occurred after spraying. However, the PSC-collected peak was delayed, whereas the peak for the Prokopack was observed immediately following IRS in October. The PSC and Prokopack collections were done at different localities in Burma Valley.

FIGURE 4: MEAN INDOOR RESTING DENSITY (PROKOPACK) OF *AN. FUNESTUS* S.L., LIVING AND NON-LIVING STRUCTURES, BURMA VALLEY, SEPTEMBER 2015–FEBRUARY 2016



CDC light trap collections at Burma Valley yielded more *An. funestus* than did either PSC or Prokopack aspirators. Results in this section reflect mosquito collections from traps that were set outdoors and were not baited. Even though the traps are not comparable, the team collected more mosquitoes from traps set outdoors than those set indoors alongside human bait (Figure 5). The higher density of mosquitoes from outdoor light traps could partially be due to the deterrent effect of pirimiphos-methyl reported in studies in Cote d'Ivoire.[1]

FIGURE 5: MEAN CDC LIGHT TRAP COLLECTIONS OF *AN. FUNESTUS* S.L., INDOORS AND OUTDOORS, BURMA VALLEY, SEPTEMBER 2015–FEBRUARY 2016

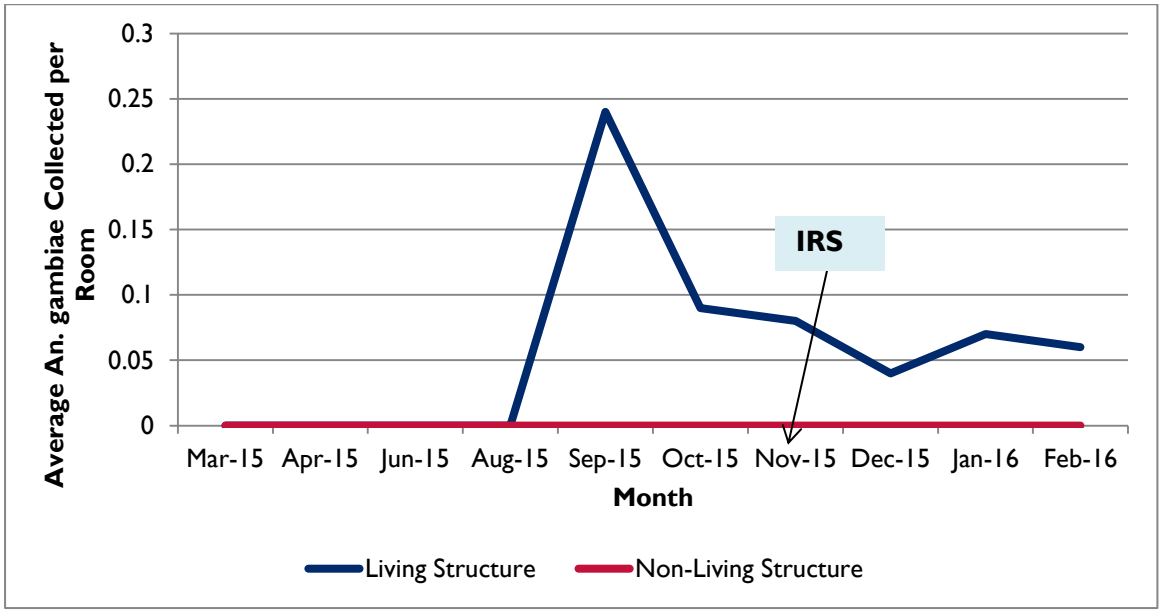


At Chakohwa site, we collected few *An. gambiae* s.l. from living structures using the PSC method, and none from non-living structures (Figure 6). There are very few non-living structures in the area.

[1] Emile S Tchicaya, Christian Nsanzabana, Thomas A Smith et al. 2014. Micro-encapsulated pirimiphos-methyl shows high insecticidal efficacy and long residual activity against pyrethroid-resistant malaria vectors in Central Cote d'Ivoire. *Malaria Journal* 4, 13:332.

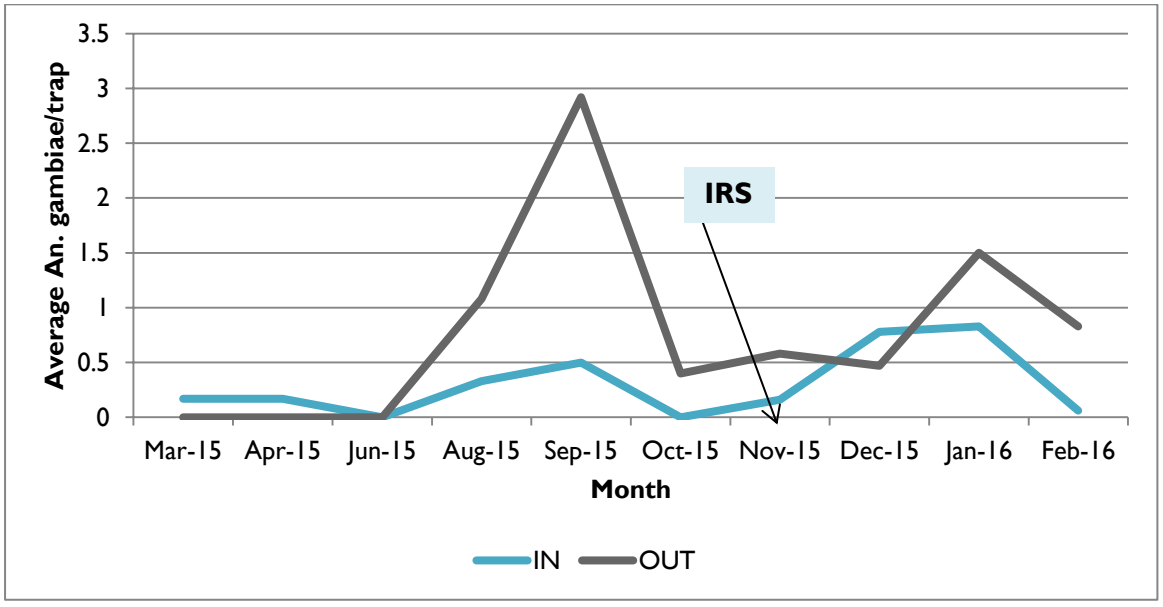
Spraying does not appear to have directly affected vector densities as the decline started well before spraying in November 2015.

FIGURE 6: MEAN INDOOR RESTING DENSITY (PSC) OF *AN. GAMBIAE* S.L., LIVING AND NON-LIVING STRUCTURES, CHAKOHWA, SEPTEMBER 2015–FEBRUARY 2016



CDC light trap collections from Chakohwa yielded more mosquitoes than did the PSC method. Spraying pirimiphos-methyl seems to have caused a decline in mosquitoes collected from both light traps set indoors and outdoors (Figure 7). The Prokopack method did not yield any mosquitoes in the area.

FIGURE 7: MEAN LIGHT TRAP COLLECTIONS OF *AN. FUNESTUS* S.L., INDOORS AND OUTDOORS, CHAKOHWA, SEPTEMBER 2015–FEBRUARY 2016



PSC collections from the control site, Mukamba, yielded few *An. gambiae* s.l.: two from living and five from non-living structures. One mosquito was collected in September, 2015 and the other in January 2016 from living structures, while five mosquitoes were collected in non-living structures in February 2016. The Prokopack aspirators yielded two *An. gambiae* s.l.: one in January 2016 from a living

structure and the other one in February from a non-living structure. CDC light traps collected more *An. gambiae* s.l. at Mukamba than the other methods: 16 mosquitoes from traps set indoors and 60 mosquitoes from outdoor traps. Collections from light traps as HLC proxy did not yield any mosquitoes during the study period.

PSC collections from the seven sites show the scanty *An. gambiae* s.l. densities across the sites. Moderately more mosquitoes were collected from living structures than from non-living structures. The largest collections were made at Makakavhule and Kamhororo sites (Beitbridge and Gokwe South districts, respectively) (Table 2). Most of the data in Table 2 were collected before the routine spraying and therefore the observed mosquitoes may not be directly related to recently applied insecticide. The non-living structures (mostly pit latrines or toilets) were not sprayed.

TABLE 2: *AN. GAMBIAE* S.L. COLLECTED BY PSC, LIVING AND NON-LIVING STRUCTURES, SEVEN PROVINCIAL SITES OUTSIDE MANICALAND, AUGUST 2015–FEBRUARY 2016

Site (Insecticide used for 2014 IRS)	Month of Collection	Type of Structure	No. of Rooms	Total *An. gambiae* s.l. Collected					Average No. of *An. gambiae* s.l. per Room
				UF	F	HG	G	Total	
Kamhororo (DDT)	Aug-15 (pre-IRS)	Living	48	22	32	9	8	71	1.48
		Non-living	10	4	2	1	0	7	0.70
	Mar-16 (post-IRS)	Living	28	5	5	0	0	10	0.36
		Non-living	20	1	0	0	0	1	0.05
Old Mazowe Bridge (DDT)	Aug-15 (pre-IRS)	Living	32	0	2	0	0	2	0.06
		Non-living	3	0	0	0	0	0	0.00
	Feb-16 (post-IRS)	Living	46	0	0	0	0	0	0.00
		Non-living	13	0	0	0	0	0	0.00
Manjolo (DDT)	Sep-15 (pre-IRS)	Living	28	0	2	0	0	2	0.07
		Non-living	6	0	0	0	0	0	0.00
Chilonga (DDT)	Sep-15 (pre-IRS)	Living	41	2	2	0	0	4	0.10
		Non-living	N/A	0	0	0	0	0	0.00
Kawere (Deltamethrin)	Oct-15 (pre-IRS)	Living	50	0	0	1	0	1	0.02
		Non-living	N/A	-	-	-	-	-	0.00
	Mar-16 (post-IRS)	Living	50	0	1	0	0	1	0.02
		Non-living	14	0	0	0	0	0	0.00
Makakavhule (DDT)	Oct-15 (pre-IRS)	Living	22	32	16	3	4	55	2.50
		Non-living	7	0	0	0	0	0	0.00
	Feb-16 (post-IRS)	Living	60	1	5	0	0	6	0.10
		Non-living	16	0	0	0	0	0	0.00
Chakari/Sanyati (Lambdacyhalothrin)	Nov-15 (pre-IRS)	Living	46	2	1	0	0	3	0.07
		Non-living	N/A	-	-	-	-	-	0.00
Total		Living	451	64	66	13	12	155	0.34
		Non-living	89	5	2	1	0	8	0.08

Collections with the CDC light trap method yielded more *An. gambiae* s.l. than did the PSC method. On average, light traps set outdoors attracted more mosquitoes than traps set indoors except at Kamhororo where *An. gambiae* s.l. collected indoors were more than those collected outside (Table 3). CDC light traps collected exceptionally high numbers of un-fed mosquitoes both inside and out. The high yields observed at Kamhororo could be due to the abundance of breeding sites under warm conditions in March. Traps were set around the perennial breeding sites that are associated with the artesian well at Kamhororo. Two *An. pretoriensis* were collected from light traps at Kawere: one inside and one outside. These were also not blood fed.

TABLE 3: *AN. GAMBIAE* S.L. COLLECTED BY CDC LIGHT TRAPS, INDOORS AND OUTDOORS, SEVEN PROVINCIAL SITES OUTSIDE MANICALAND, AUGUST 2015–FEBRUARY 2016

Site	Month of Monitoring	Traps (n x)	Total *An. gambiae* s.l. Collected					Average *An. gambiae* s.l./Trap
			UF	Fed	HG	G	Total	
Kamhororo	Aug-15 (pre-IRS)	IN (18)	47	0	0	0	47	2.62
		OUT (18)	198	0	0	0	198	11.00
	Mar-16 (post-IRS)	IN (24)	887	0	0	0	887	36.95
		OUT (24)	725	0	0	0	725	30.2
Old Mazowe Bridge	Aug-15 (pre-IRS)	IN (23)	2	4	0	0	6	0.26
		OUT (23)	3	1	0	0	4	0.18
	Feb-16 (post-IRS)	IN (30)	1	0	0	0	1	0.03
		OUT (29)	2	0	0	0	2	0.06
Chilonga	Sep-15 (pre-IRS)	IN (30)	12	0	0	0	12	0.40
		OUT (30)	55	0	0	0	55	1.84
Manjolo	Sep-15 (pre-IRS)	IN (25)	11	0	0	0	11	0.44
		OUT (23)	41	0	0	0	41	1.79
Makakavhule	Oct-15 (pre-IRS)	IN (30)	24	0	0	0	24	0.80
		OUT (30)	76	0	0	0	76	2.54
	Feb-16 (post-IRS)	IN (10)	1	0	0	0	1	0.10
		OUT (10)	2	0	0	0	2	0.20
Kawere	Oct-15 (pre-IRS)	IN (22)	0	0	0	0	0	0.00
		OUT (23)	0	0	0	0	0	0.00
	Mar-16 (post-IRS)	IN (22)	1	0	0	0	1	0.04
		OUT (18)	2	0	0	0	2	0.11
Chakari/Sanyati	Nov-15 (pre-IRS)	IN (36)	3	1	0	0	4	0.12
		OUT (12)	1	0	0	0	1	0.09
Total		IN (248)	989	5	0	0	994	4.04
		OUT (216)	1105	1	0	0	1106	5.12

3.1.3 FEEDING TIME

At Burma Valley, the team collected more *An. funestus* s.l. from the light trap placed outdoors than from the one indoors. Even though there was no distinct behavior and the number of mosquitoes

collected was very low, there were indications of increased outdoor vector activity around midnight and from 03:00 to 04:00 hours (Figure 8).

At Chakohwa, the predominant *An. gambiae* s.l. showed peak biting activity between 20:00 and 22:00 hours and between 04:00 and 05:00 hours despite the low mosquito densities (Figure 9). There were no mosquitoes collected by this method at the control site, Mukamba.

FIGURE 8: MEAN BITING RATE OF *AN. FUNESTUS,* BURMA VALLEY (SPRAY SITE), AUGUST 2015 – FEBRUARY 2016

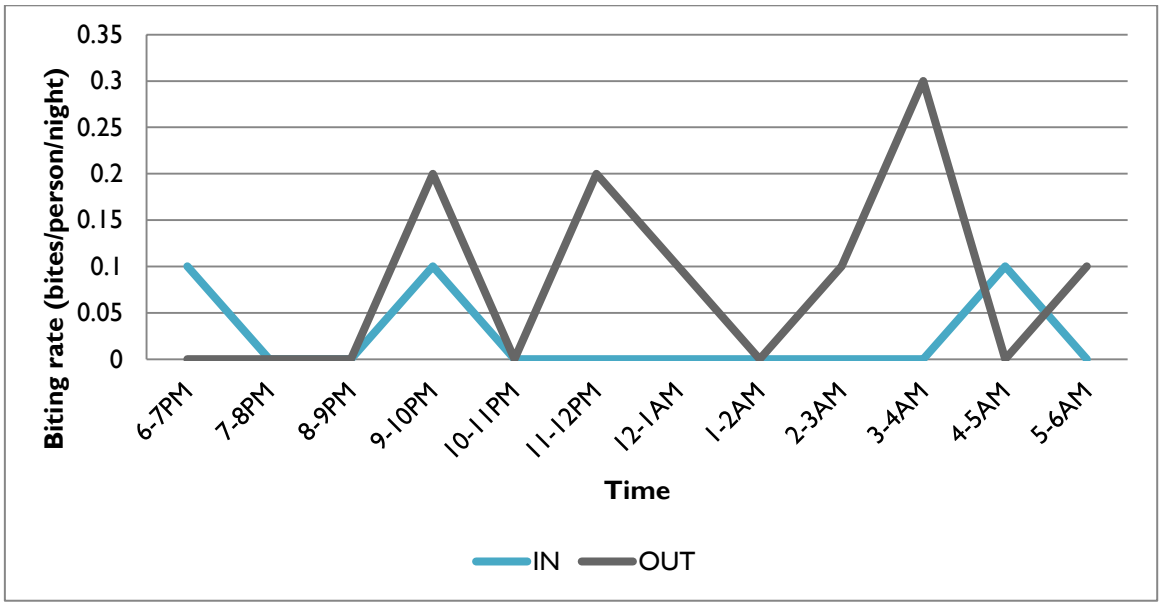
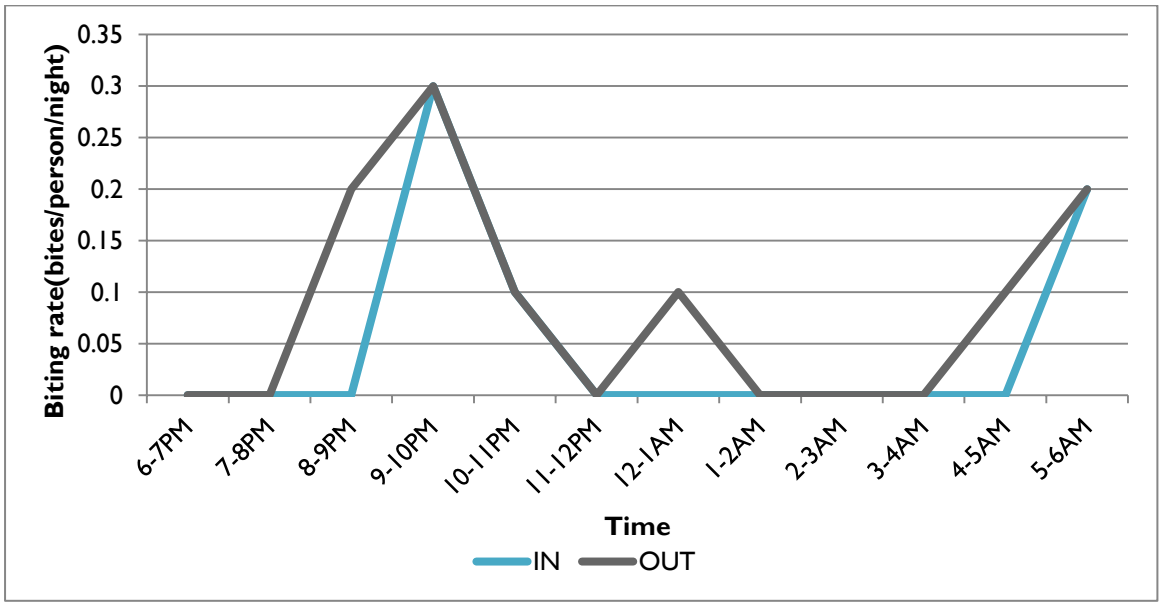


FIGURE 9: MEAN BITING RATE OF *AN. GAMBIAE,* CHAKOHWA (SPRAY SITE), AUGUST 2015 – FEBRUARY 2016



3.1.4 INSECTICIDE SUSCEPTIBILITY

Table 4 and Figure 10 show the results of the insecticide susceptibility tests conducted at seven sentinel sites between April 2015 and February 2016. (Tests could not be completed at the other sites – Burma Valley, Mukamba, and Chilonga – owing to lack of an adequate number of mosquitoes

during the monitoring period.) The results show that the local vector species from Chakohwa, Old Mazowe Bridge, and Kawere were susceptible to all four insecticides (bendiocarb, DDT, lambdacyhalothrin, and pirirmiphos-methyl) tested. At Chakari, the local vector was susceptible to pirimiphos-methyl and DDT but resistant to bendiocarb and lambdacyhalothrin. At Kamhororo, the local vector was resistant to DDT, possibly resistant to lambdacyhalothrin, but susceptible to pirimiphos-methyl and bendiocarb. At Manjolo, possible resistance to lambdacyhalothrin was detected; susceptibility to three other insecticides could not be ascertained due to inadequate number of mosquitoes available for the tests in 2015. At Makakavhule, the local vector was susceptible to bendiocarb and lambdacyhalothrin, but resistant to DDT and possibly resistant to pirimiphos-methyl. This is the first indication of possible vector resistance to pirimiphos-methyl in Zimbabwe, and it will be important to verify it.

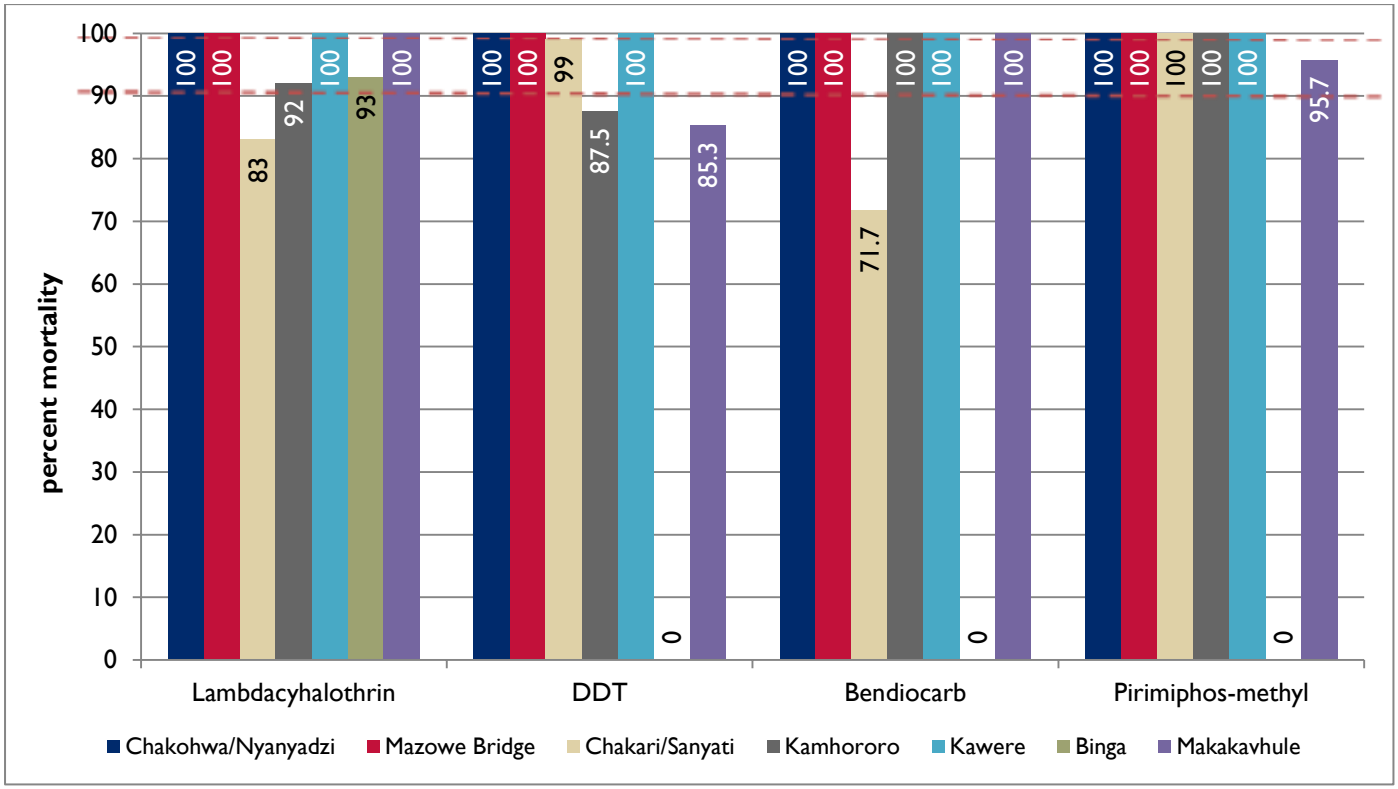
As a trial of skills, we requested the Insectary Manager at Jotsholo site in Lupane district (Matabeleland North province) to conduct the susceptibility tests as well. Jotsholo is one of the nine NMCP sites that AIRS Zimbabwe supplied equipment to and will support when the seconded entomologist is recruited. The susceptibility tests could not be done because the new Insectary Manager collected only *An. pretoriensis* instead of *An. gambiae* s.l. This shortcoming highlights the need for entomological training for both new and experienced staff (outside of Manicaland) who are involved in routine surveillance in 2016. Refresher training for Insectary Managers and Field Officers will be conducted May 16-19, 2016 in Binga district in Matabeleland North Province.

TABLE 4: WHO SUSCEPTIBILITY TEST RESULTS WITH *AN. GAMBIAE* S.L., 2015-2016

Province (District)	Site	Lambdacyhalothrin		DDT		Bendiocarb		Pirimiphos methyl	
		% mort.	# tested	% mort.	# tested	% mort.	# tested	% mort.	# tested
Manicaland (Chimanimani)	Chakohwa	100S	50	100S	25	100S	25	100S	50
Mashonaland Central (Rushinga)	Old Mazowe Bridge	100S	100	100S	100	100S	100	100S	100
Mashonaland West (Sanyati)	Chakari	83R	100	99S	100	71.7R	100	100S	100
Midlands (Gokwe South)	Kamhororo	92PR	100	87.5R	100	100S	100	100S	100
Mashonaland East (Mutoko)	Kawere	100S	100	100S	100	100S	100	100S	100
Matabeleland North (Binga)	Manjolo	93PR	100	-	-	-	-	-	-
Matabeleland South (Beitbridge)	Makakavhule	100S	100	85.3R	100	100S	100	95.7PR	100

Note: S – susceptible; PR - possibly resistant; R - resistant

FIGURE 10: WHO SUSCEPTIBILITY ASSAYS WITH *AN. GAMBIAE* S.L., SEVEN SITES



3.2 IRS RESIDUAL EFFICACY/ QUALITY OF SPRAYING AND INSECTICIDE DECAY RATE

3.2.1 QUALITY OF SPRAY

As described in the Methodology section, cone bioassay tests were done on four types of insecticide-sprayed walls 24-48 hours after spraying at the Burma Valley and Chakohwa sentinel sites. The team recorded complete (100 percent) mosquito mortality after the 24-hour holding period (T0) as shown in Figures 11, 12, and 13. This indicated that the spraying was of good quality. The test mortality rates of both susceptible colony and wild mosquitoes on mud, cement, brick, and painted surfaces were 100 percent (Figure 11, 12). We did not observe a knockdown effect 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 CS.

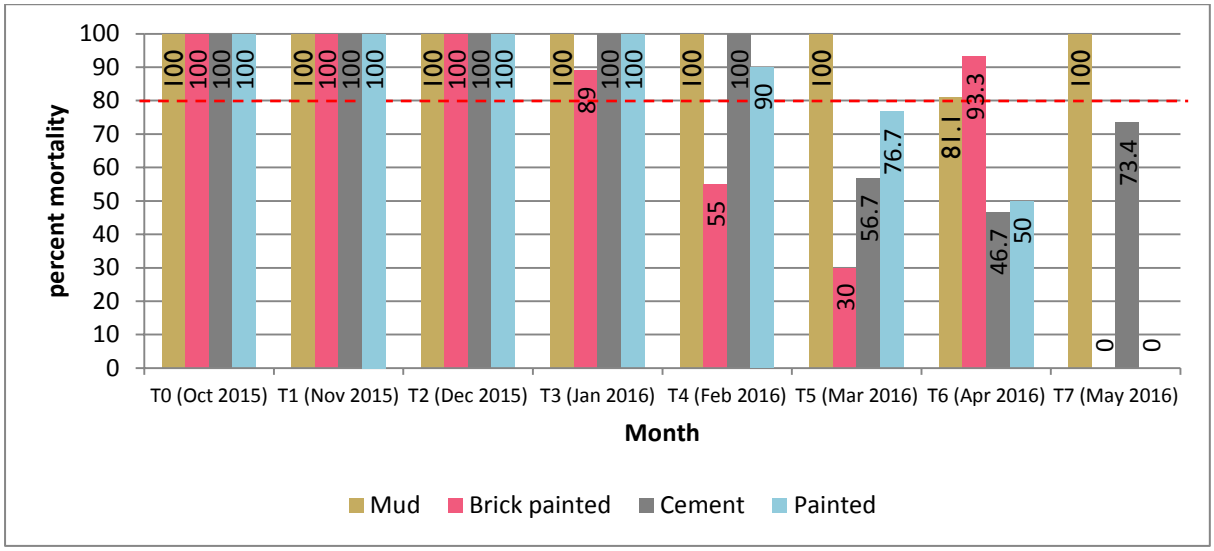
3.2.2 INSECTICIDE DECAY RATE

a) Burma Valley

Mortalities of wild mosquito (*An. gambiae* s.l.) continued at 100 percent at three weeks and eight weeks post-spray on all four types of wall surfaces at Burma Valley (Figure 11). At 13 weeks post-spray, the brick surface showed the first decline (89 percent), while the mud, cement, and painted surface maintained 100 percent mortality. After 16 weeks of spray, the mortality on the brick surface declined to 55 percent, the painted wall to 90 percent, while the mud and cement surfaces

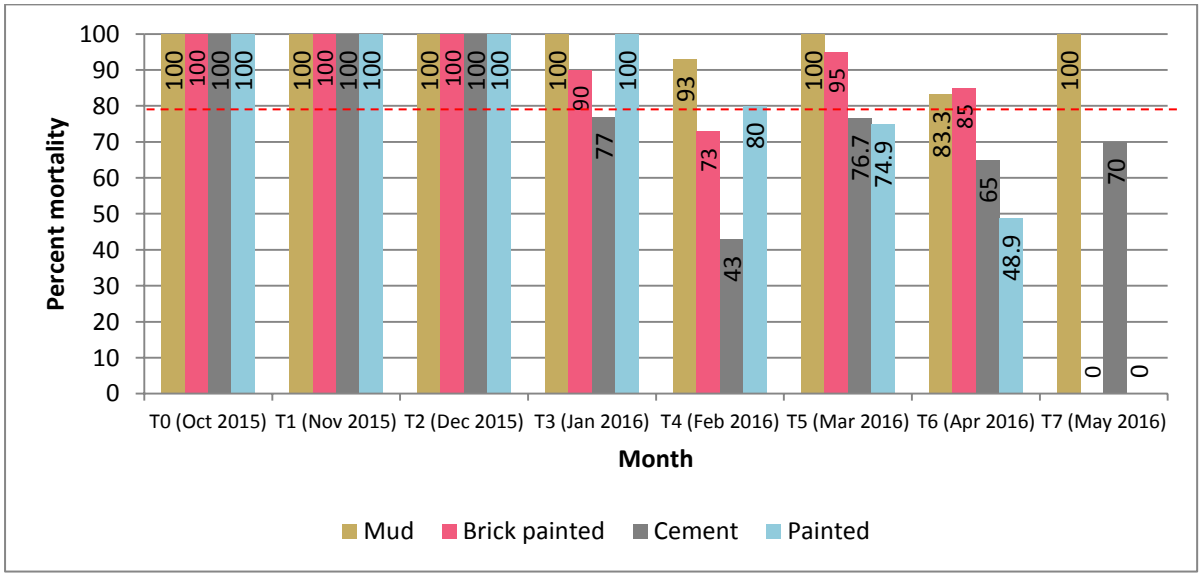
maintained 100 percent. Mud surfaces produced 100 percent mortality 7 months after spraying in May 2016 despite having declined to 81 percent in April.

FIGURE 11: WHO CONE TEST RESULTS, *AN. GAMBIAE* S.L., MORTALITY AFTER 30 MINUTES EXPOSURE TO PIRIMIPHOS-METHYL, BURMA VALLEY, MUTARE DISTRICT.



Using susceptible *An. arabiensis* (KGB strain) at Burma Valley (Figure 12), at 16 weeks post-spray, the mortality had fallen to 43 percent, 73 percent, 80 percent, and 93 percent for the cement, brick, painted, and mud surfaces, respectively. At Burma Valley, mortalities of *An. arabiensis* on mud surfaces were 100% 29 weeks post-spray despite declining to 93% and 83.3% at 16 and 25 weeks post-spray, respectively. These results suggest insecticide bio-efficacy is retained best on mud surfaces. The results indicated the residual efficacy of pirimiphos-methyl at four months although there were differences on different types of walls.

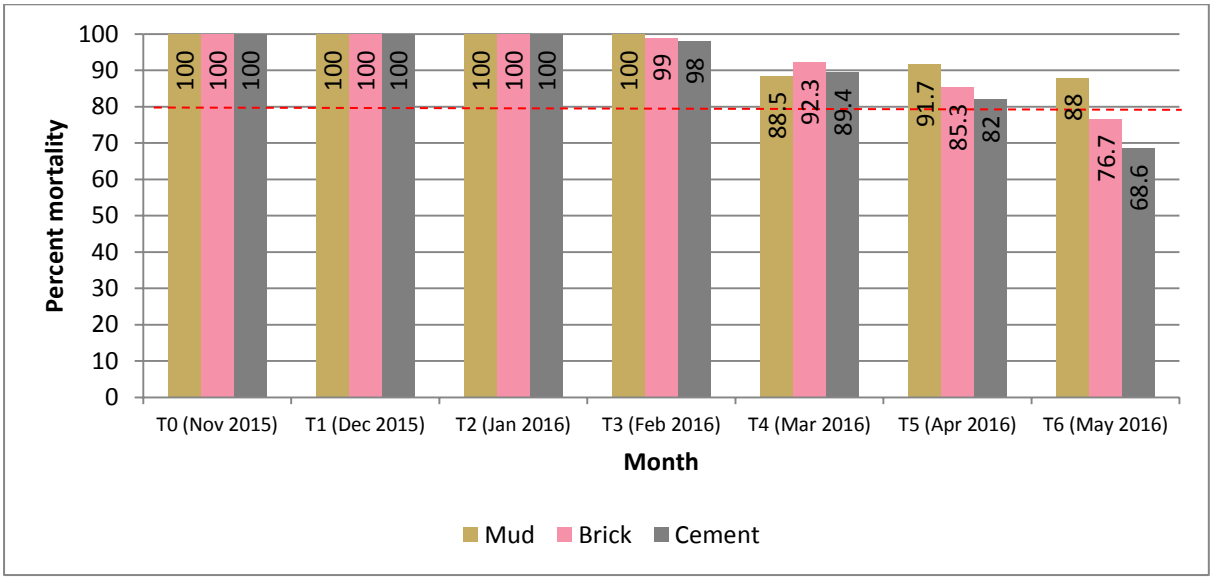
FIGURE 12: WHO CONE TEST RESULTS, *AN. ARABIENSIS* (KGB STRAIN), MORTALITY AFTER 30 MINUTES EXPOSURE TO PIRIMIPHOS-METHYL, BURMA VALLEY, MUTARE DISTRICT



b) Chakohwa

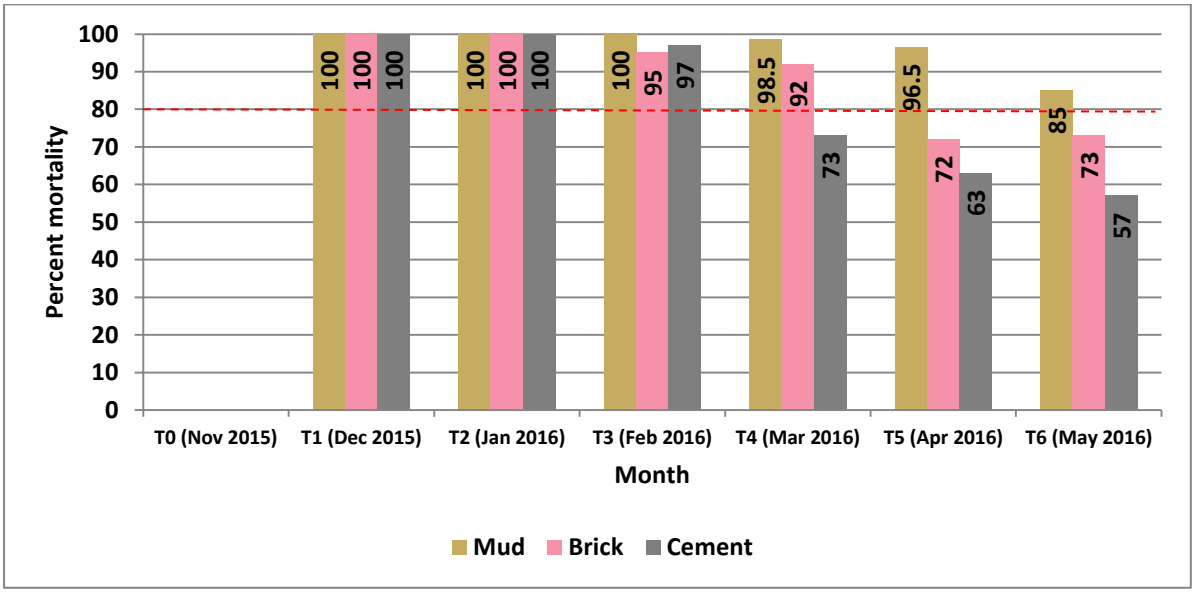
Mosquito mortalities on the three types of wall surfaces in Chakohwa were 100 percent eight weeks after spray, but started to decline after 12 weeks (Figure 13). There was no major difference between the mud, brick and cement surfaces using *An. gambiae* s.l.

FIGURE 13: WHO CONE TEST RESULTS, *AN. GAMBIAE* S.L., MORTALITY AFTER 30 MINUTES EXPOSURE TO PIRIMIPHOS-METHYL, CHAKOHWA, CHIMANIMANI DISTRICT



Mortalities observed on *An. arabiensis* (KGB strain) showed similar decline rates on the mud, brick, and cement surfaces (Figure 14). Further tests will determine the residual span of pirimiphos-methyl at Chakohwa.

FIGURE 14: WHO CONE TEST RESULTS, *AN. ARABIENSIS* (KGB STRAIN), MORTALITY AFTER 30 MINUTES EXPOSURE TO PIRIMIPHOS-METHYL, CHAKOHWA, CHIMANIMANI DISTRICT[2]



[2] While cone bioassay tests were done in November, *An. arabiensis* (KGB strain) from the insectary at NIHR were not adequate for tests at Chakohwa. Only *An. gambiae* s.l. was used at Chakohwa.

4. Discussion, Limitations, and Recommendations

4.1 Discussion

Collections of resting mosquitoes provide useful information on resting preferences, vector population density, and host preferences (human or animal) that can be derived from blood-fed adults. Similar to previous observations, in 2015 the project recorded low mosquito densities at all three sentinel sites monitored in Manicaland and at the seven sites outside of Manicaland. Despite low numbers, *An. funestus* continues to be the main malaria vector in Burma Valley. The scarcity of mosquitoes at Manicaland sites continues to affect the prospects to test insecticide susceptibility. No susceptibility tests have been done since pirimiphos-methyl was introduced for IRS during the 2014 IRS campaign. Data from CDC light traps continues to show greater densities outdoors than indoors for *An. funestus*. Results from laboratory analyses will indicate whether the *An.* funestus s.l. collected from traps set indoor and outdoor are the same species.

Despite its advantages, the Prokopack collected similar data, compared to PSC in sampling densities of indoor resting mosquitoes whether *An. funestus* or *An. gambiae* s.l. during this collection season.

Bioassay data showed good quality of spraying at Burma Valley and Chakohwa. The drastic decline in bioefficacy of insecticide experienced during the 2014 IRS campaign did not repeat after the 2015 campaign, suggesting improved spraying techniques and supervision.

The average mosquito mortalities suggest the residual life of pirimiphos-methyl at Burma Valley is four months (using wild *An. gambiae* s.l.) and five months (using susceptible *An. arabiensis* KGB strain) months, although the insecticide can persist longer on some wall surfaces especially mud. This is consistent with the observations made last year. In contrast, at Chakohwa, the residual efficacy is four months based on susceptible *An. arabiensis* (KGB strain) and five months using *An. gambiae* s.l. from the wild. Since the bioefficacy of pirimiphos-methyl declines towards the peak of malaria transmission, it may be necessary to delay spraying slightly.

Comparative data suggest *An. funestus* rest predominantly in living structures, which are more numerous than non-living structures. However, once IRS is done, the vector appears to shift its preference to resting in unsprayed non-living structures. Though the numbers of mosquitoes collected are few, this shift in behavior could affect the impact of insecticide on the survival of the vector population. These observations reveal the need to collect more data to determine if spraying non-living structures is justifiable when planning IRS programs for maximum impact on the vectorpopulation. If the molecular species identification confirms the mosquitoes collected in these structures are indeed *An. funestus* s.s., PMI and in country stakeholders can discuss whether including non-living structures for spraying into the IRS protocol will impact the vector population.

Insecticide resistance was detected in four localities: at Sanyati/Chakari to lambdacyhalothrin, at Kamhororo and Makakavhule to DDT, and at Chakari/Sanyati to bendiocarb. Possible resistance to pirimiphos-methyl has been detected at Makakavhule. Further surveillance is needed to determine the mechanisms of resistance in the affected localities.

The scarcity of malaria vector mosquitoes is persistent on the malaria vector landscape in Zimbabwe. Low numbers of both *An. gambiae* s.l. and *An. funestus* in most areas under IRS indicate the impact that decades of spraying and the mass distribution of LLINs have had on the vector populations. We realize the limitations of generalizing conclusions based on the data from a sentinel site. Therefore, there is a need to continue entomological monitoring and spraying to prevent the resurgence of the vector. Last year's introduction to the AIRS Zimbabwe project of the Disease

Data Management System (DDMS) as an entomological database could provide a rich repository of longitudinal data that should guide decision making for effective vector control.

4.2 Positive Developments

- In February, NIHR released partial results of the laboratory analysis they completed with the project's 2013-2014 samples. They submitted additional data in late April. We will present analysis of the NHIR data in a forthcoming report in June 2016.
- The insectary at NIHR has reliably improved its supply of susceptible colony mosquitoes for cone bioassay tests. However, the supply cannot meet the numbers required for the two sites. We still need to collect mosquitoes from field. If the NIHR insectaries (Harare and Chiredzi) manage to maintain current productivity then the next series of cone bioassay tests could rely solely on susceptible colony mosquitoes.
- The establishment of the DDMS should improve the management of entomological data.
- The hiring of an Entomological Officer seconded to NMCP should enhance national malaria vector support and ability to provide better quality surveillance.

4.3 Limitations

- Hopefully the insectary at De Beers, in Chiredzi district, can supply sufficient mosquitoes for cone bioassay tests without affecting the colony. AIRS Zimbabwe will continue to provide technical support to the NIHR laboratory. It also will lead an assessment of the laboratory at De Beers to provide a lasting solution to the problem. More mosquitoes will be required for the cone bioassay tests at sentinel sites and for net durability studies.
- Unavailability of test mosquitoes at some sentinel sites resulted in insecticide susceptibility tests not being carried out during the period under review.
- Recent laboratory results suggest that *An. quadriannulatus*, the non-vector sibling species of *An. gambiae* s.l., dominates the intended collection of *An. gambiae* s.l. as larvae. This underlines the need for prompt laboratory analysis of specimens to guide both cone bioassay and susceptibility tests.
- Insectary Managers continue to misidentify mosquitoes, and the refresher training will potentially help overcome the problem.
- The infrastructure at most sentinel sites is inadequate for the management of both mosquitoes and equipment.

4.4 Recommendations

- NIHR should release regular, timely feedback of completed sets of laboratory results on vector species identification, infection rates, host preferences, and resistance mechanisms.
- Establish regular dialog between AIRS Zimbabwe and NHIR to ensure the NHIR plays an active role in producing data for decisions and also shares challenges that need to be addressed collectively and in a timely manner.
- CDC supplied NIHR with primers for resistance mechanisms. PMI AIRS will request NIHR to process specimens accordingly: identify species first and then do the other analyses including resistance mechanisms.
- There is need to improve infrastructure at sentinel sites through concerted partner support to NMCP.
- Regular refresher trainings for Insectary Managers and their supervisors should be considered to continue strengthening their skills in vector surveillance.