The PMI Africa IRS (AIRS) Project

Indoor Residual Spraying (IRS 2) Task Order Six

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AIRS MADAGASCAR ENTOMOLOGICAL MONITORING FINAL REPORT

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ACRONYMS

AIRS	Africa Indoor Residual Spraying
CDC	Centers for Disease Control
CHL	Central Highlands
DDT	dichlorodiphenyltrichloroethane
HLC	Human Landing Collections
IRS	Indoor Residual Spraying
KD	Knockdown
LLIN	Long-Lasting Insecticidal Net
MBR	Man Biting Rate
ODC	Outdoor Collection
PMI	President's Malaria Initiative
PSC	Pyrethrum Spray Collections
RBM	Roll Back Malaria
USAID	United States Agency for International Development
WHO	World Health Organization
WHOPES	World Health Organization Pesticide Evaluation Scheme

EXECUTIVE SUMMARY

Background

In Madagascar, indoor residual spraying (IRS) is an important component of the malaria control strategy, as noted in the current National Strategic Plan. Madagascar currently receives donor support for implementing IRS from the President's Malaria Initiative (PMI) and the Global Fund.

During the 2016 spray round, the PMI Africa Indoor Residual Spraying (AIRS) Madagascar Project covered 36 communes in the East Coast and 54 communes in the South East with blanket IRS. Pirimiphos-methyl CS, an organophosphate insecticide, was used for the campaign that happened from July 25 to August 22, 2016 in the South East and from September 5 to October 1, 2016 in the East coast. Entomological monitoring is an integral component of the PMI AIRS Madagascar Project. The 2016/2017 entomological monitoring activities included collection of comprehensive entomological data on vector density, species composition, seasonal patterns, biting behavior, insecticide resistance and parity of anopheline mosquitoes from seven sentinel sites, five intervention (IRS) and two control sites (non-IRS). Data on vector species composition, density and behavior was collected using various mosquito-sampling methods that included pyrethrum spray catches (PSCs), human landing catches (HLC) and sucking tube (aspirator) collections. One month's data was collected prior to the spray campaign to serve as a baseline from both the intervention and control sites, and subsequent monthly data were collected post spray to help understand if there was any change in the species composition, density and behavior following IRS. Wall bioassay tests were done to assess the quality of spray within 24 hours of the spray, and monthly thereafter to monitor the decay rate of the insecticide sprayed. Insecticide susceptibility data was also collected from eleven sentinel sites, including the seven sentinel sites used to collect comprehensive entomological data to inform insecticide based malaria vector control programming (IRS and LLINs).

Results

Vector density and seasonality: A total of 4,763 female anophelines and 5,544 culicine mosquitoes were collected during the monitoring period. The most abundant vector species was *An. gambiae* s.l. that constituted 35.6% (n=1,694) of the total anopheline mosquitoes collected. The two other anophelines that are vectors of malaria in Madagascar, *An. funestus* and *An. mascarensis,* accounted only for 6.4% (n=307) and 4.5% (n=215) of the collection respectively. Eighty-six (86) *An. gambiae* s.l., eighteen (18) *An. funestus,* and eleven *An. mascarensis* were collected resting indoors with PSC. At the same time 224, 19 and 34 *An. gambiae* s.l., *An. funestus,* and *An. mascarensis*, respectively, were collected from artificial pit shelters resting outdoors with aspirators. 9,361 (90.8%) mosquitoes were collected while seeking human blood through the HLC method. It is apparent that the number of vector mosquitoes collected resting both indoors and outdoors was too low to make conclusions about the resting habits of the vectors or to assess the impact of IRS on indoor resting density.

Feeding time and location: At the baseline before IRS, *An. gambiae* s.l. human biting rates ranged from 0.0 bites per person per night in Lopary to 2.7 bites per person per night in Lanivo (Vohipeno district) and Manambotra (Farafangana district) indoors, and from 0.0 bites per person per night in Mahambo (Fenerive Est district) to 4 per person per night in Lanivo (Vohipeno district) outdoors. In all sentinel sites but Ambodifaho, *An. gambiae* s.l. exhibited exophagic tendencies pre-IRS. No change in the feeding habit of *An. gambiae* s.l. was noted after IRS when compared to pre-IRS. It is apparent that the vector prefers feeding outdoors as compared to indoors in both the intervention and control sites (Table 1).

The low mean biting rates noted at baseline as compared to after spray could be explained by the limited availability of breeding sites before the rainy season when the baseline data was collected.

Table 1. Comparison of indoor and outdoor man biting rates before and after IRS

Intervention status	Spray Status	Mean MBR		P=value
		Indoor	Outdoor	
Intervention	Pre-IRS	1.3	1.3	1
Control	Pre-IRS	0.3	1.9	0.280
Intervention	Post-IRS	0.9	2.3	0.433
Control	Post-IRS	1.2	2.3	0.556

An. gambiae s.l. engaged in biting throughout the night but peak biting was variable between sites. Peak biting time was observed, both indoors and outdoors between 9:00 and 10:00 pm in two sites (Ambodifaho and Manambotra Sud), and between 9:00 pm and 1:00 am in Mahambo. In Vohitrambato, biting was observed between 8:00 pm and 3:00 am both outdoors and indoors. In Vavatenina, peak biting was recorded between 10:00 pm and 12:00 am both outdoors and indoors. In Lanivo, peak time was between 01:00 to 02:00 am both outdoors and indoors. In Lopary, indoor peak biting was between 7:00 and 9:00 pm and 11:00 pm and 2:00 am. The outdoor biting rates, however, peaked between 10:00 pm to 12:00 am in Lopary. Except Lopary, in all other sentinel sites there was an overlap in outdoor and indoor peak bites times. In Lopary and Vohitrambato (Toamasina II) significant mosquito biting started as early as 20:00 before people go to bed and are sleeping under ITNs, which might affect the impact of ITNs on vector-human contact.

Quality of spraying and residual life: The results of wall bioassays indicated that the spray quality, both in the East Coast and in the South East, was good; mortality was 100% for all the structures sampled at T0 (24 hours after spraying) and T1 (one month after spray). In the South East and in the East Coast pirimiphos-methyl CS killed > 80% test mosquitoes and proved effective for 7 months.

Susceptibility tests: The results of the vector susceptibility tests indicated full susceptibility of *An. gambiae* s.l. to bendiocarb and pirimiphos-methyl in all areas where the tests were conducted. The test results also showed that *Anopheles gambiae* s.l. had developed resistance to DDT in Imerina Imady, Vohimarina, and Ankafina Tsarafidy; to permethrin in Mahambo, Vavatenina, Bekily and Ankafina Tsarafidy; and to lambda-cyhalothrin in Bekily. Suspected resistance was noted for DDT in Mahambo and Ambodifaho; for deltamethrin in Vohitrambato and Vavatenina; for permethrin in Ankafina Tsarafidy, Ambodifaho and Vohitrambato; for lambda-cyhalothrin in Imerina Imady; and for alphacypermethrin in Vohitrambato, Mahambo and Imerina Imady.

1. INTRODUCTION

In Madagascar, malaria is endemic across 90% of the country; however, the entire population is considered to be at risk for the disease. In 2013, it was the second leading cause of death among children under five as reported by district hospitals.

Madagascar's national malaria strategy from 2008 - 2012 recommended blanket IRS. In 2012, there was a change in the vector control strategy to include focal IRS and epidemic alert reporting in addition to blanket IRS. Villages in the Central Highlands were selected for the focal spraying based on health facility malaria cases and rapid diagnostic test positivity rates. PMI supported spraying in the Central Highlands (CHL) and the Fringe areas in 2008 and 2009. In 2010, the South was added for spraying. Blanket IRS was conducted in the CHL, Fringe areas, and the South in 2011. However, in 2012 and 2013, spraying in the CHL and Fringe areas were switched from blanket spraying to focal spraying. In 2014, the CHL received focal spray but the Fringe districts were moved back to blanket spray with three districts in the East Coast (Brickaville, Toamasina II and Fenerive Est) also receiving blanket coverage.

In 2015, the annual IRS campaign was performed between August 3rd – August 26th, in the South East and August 31st - September 26th, in the East Coast with pirimiphos-methyl CS. The National Malaria Control Programme (NMCP) implemented IRS in 16 districts of the Central High Lands (CHL) in December, using pyrethroids.

In 2016, the annual IRS campaign was performed with pirimiphos-methyl between July 25 and August 22 in the South East, and September 5 – October 1 in the East Coast. The National Malaria Control Program (NMCP) implemented IRS as a response to some malaria outbreaks in the Central High Lands, using pyrethroids.

This report presents the results of the entomological monitoring activities completed by the PMI AIRS Madagascar project between June 2016 and May 2017, including data on the residual efficacy of insecticides, insecticide susceptibility, mosquito density, and mosquito behavior.

2. OBJECTIVES

The objectives of the entomological surveillance were:

- To identify the vector species, composition, and density;
- To determine vector biting and resting behavior;
- To determine the quality of spraying and insecticide decay rate following spray operations; and
- To ascertain vector susceptibility to the four classes of insecticides approved by the World Health Organization Pesticide Evaluation Scheme (WHOPES) for IRS.

Entomological surveillance will continue to play a critical role in informing vector control programs, including the impact of IRS on vector density, resting and feeding behavior, and it will identify insecticides that are effective against local vectors to guide vector control programming.

3. MATERIALS AND METHODS

3.1. STUDY SITES

All sentinel sites, where entomological surveillance was performed during the 2016 IRS campaign are listed in Table 2. They are the same as those for the 2015 IRS round, except Lanivo (Vohipeno), a new IRS area in the South East that replaced Bekily, a non-IRS area located in the South sub-desert that was serving as an insecticide resistance testing site.

Table 2. List of Sentinel Sites

Region	District	Sentinel Site Location	Notes
Antsinanana (East Coast)	Toamasina II	Vohitrambato	Used as a sentinel site since 2014-2015 IRS campaign
Antsinanana (East Coast)	Brickaville	Ambodifaho	Used as a sentinel site since 2014-2015 IRS campaign
Analanjirofo (East Coast)	Fenerive Est	Mahambo	Used as a sentinel site since 2014-2015 IRS campaign
Analanjirofo (East Coast)	Vavatenina	Vavatenina (control site)	Used as a control site since 2014-2015 IRS campaign for the East Coast
Atsimo Antsinanana (South East)	Farafangana	Manambotra Sud	Used as a sentinel site since 2015-2016 IRS campaign
Atsimo Antsinanana (South East)	Vangaindrano	Lopary (control site)	Used as a control site since 2015-2016 IRS campaign for the South East
Vatovavy Fito Vinany	Vohipeno	Lanivo	New sentinel site in a new IRS district
Amoron'I Mania (CHL)	Fandriana	Milamaina	Non IRS area for susceptibility test (old site for 2014/2015 round)
Amoron'I Mania (CHL)	Ambositra	Imerina Imady	Non IRS area for susceptibility test (old site for 2014/2015 round)
Haute Matsiatra (CHL)	Ambohimahasoa	Ankasina Tsarafidy	Non IRS area for susceptibility test (old site for 2014/2015 round)
Haute Matsiatra (CHL)	Fianarantsoa II	Vohimarina	Non IRS area for susceptibility test (old site for 2014/2015 round)

3.2. ADULT MOSQUITO COLLECTIONS

Baseline entomological data was collected one month before the start of the IRS campaign in two spray zones (in June 2016 in the South East and in August 2016 in the East Coast). The East Coast has three entomological sentinel sites that were used for comprehensive entomological data collection, Ambodifaho (Brickaville district), Vohitrambato (Toamasina II district), and Mahambo (Fenerive Est, district) as well as one control site, Vavatenina. The South East had three sites, one control and two intervention sites, used for entomological monitoring: Manambotra Sud (intervention site in Farafangana district), Lanivo (intervention site in Vohipenio district), and Lopary (control site in Vangaindrano district).

Data on species composition, vector densities, and vector behavior were gained via collecting adult mosquitoes using human landing collections (HLCs), pyrethrum spray collections (PSCs), and outdoor resting collections (ODCs) using sucking tube.

Human Landing Catches (HLCs)

HLCs were intended to determine vector biting location and time. HLCs were conducted indoors and outdoors in three houses per sentinel site, for two nights per month. Collections were made over a period of 12 hours (18:00 – 6:00) indoors and outdoors. One mosquito collector was seated indoors and another seated outdoors from 6 p.m. to 6 a.m. to collect blood-seeking mosquitoes. Outdoor and indoor collectors switched sites every hour. Collectors adjusted their clothing so that the legs were exposed up to the knees. When a mosquito was felt, collectors quickly turned on the torch, collected the mosquito with the sucking tube and transferred it to a paper cup. One cup was used for each hour of collection. Hourly temperature and humidity were recorded. At the end of the collection, mosquitoes were transported to the field lab and were identified using taxonomic keys (Gilles and Coetzee, 1987).

Pyrethrum Spray Collection (PSC)

PSC was used to estimate the room resting density, and indirectly measure the MBR. PSC activity was completed in the morning between 06:00am and 09:00am, once a month. AIRS Madagascar entomology staff conducted PSC at ten houses per sentinel site per month. Before the PSC was performed, all occupants were cordially asked to move out of the house. AIRS Madagascar entomology staff then covered the floor of a room in the house with white sheets and closed all other openings that would allow the mosquito to escape from the house. The walls and roof space inside the houses were then sprayed with insecticide that knocks down the mosquitoes. Knocked-down mosquitoes were collected using forceps and kept separately in pill-boxes until species identification could be performed along with the determination of blood digestion stage. Identification of all mosquitoes was done using morphological keys.

Outdoor Resting Collection (ODC)

Mosquitoes resting outdoors were collected from natural resting places and pit shelters using aspirators. Four pit shelters per sentinel site and natural resting places were used to collect outdoor resting mosquitoes. Mosquitoes collected from outdoors were kept in paper cups separately labeled for each collection site and were morphologically identified to species.

For all mosquito collections, after species identification, malaria vector mosquitoes were preserved individually in Eppendorf tubes with silica gel for ELISA tests, and molecular identification to be completed by Institut Pasteur-Madagascar or other collaborating labs.

3.3. INSECTICIDE SUSCEPTIBILITY TESTS

Vector susceptibility was tested for all four classes of insecticides recommended for public health (carbamates, pyrethroids, organochlorines, and organophosphates). The team performed the tests using World Health Organization (WHO) tube assays with insecticide-impregnated papers on two- to four-day-old adult, non-blood-fed female mosquitoes (reared from field collected larvae). Mortality was recorded after a 24-hour holding period. The 2013 WHO resistance classification criteria was used to interpret susceptibility test results.

Definitions:

- Vector species were characterized as fully susceptible when the test mortality rate of the vector was greater than or equal to 98%.
- The susceptibility of the vector was classified as suspected resistance that needs confirmation when the test mortality rate of the exposed vector was equal to or between 90% and 97%.
- The vector was classified as resistant when the mortality rate of the exposed vector was less than 90%.

3.4. RESIDUAL EFFICACY METHODOLOGY

WHO cone bioassay tests were used to determine the residual efficacy of an insecticide on sprayed surfaces. Since AIRS Madagascar does not have access to a susceptible colony in Madagascar, wild-caught mosquitoes reared from larvae at sentinel sites were used to determine the quality of spraying and subsequently to monitor the residual efficacy of insecticides sprayed. The susceptibility of the local vector to the insecticide sprayed in the area was determined before mosquitoes from the same population were used for the cone bioassay testing. Bioassays were used to evaluate the quality of spraying by spray operators during the first two weeks of the start of IRS campaigns. The residual bio-efficacy of the insecticides was then monitored on monthly intervals. Two common surface types were selected from each of the different sites: thatch (Falafa) and wood or bamboo, were used for the cone bioassay data collection.

The mosquitoes were exposed to the sprayed surfaces for 30 minutes and the "knock-down" rate was recorded at 30 minutes and 60 minutes post exposure. The vector mortality was observed after a 24-hour recovery period.

4. RESULTS AND DISCUSSION

4.1. SPECIES COMPOSITION, VECTOR DENSITIES, AND VECTOR BEHAVIOR OBSERVED DURING THE SURVEILLANCE PERIOD

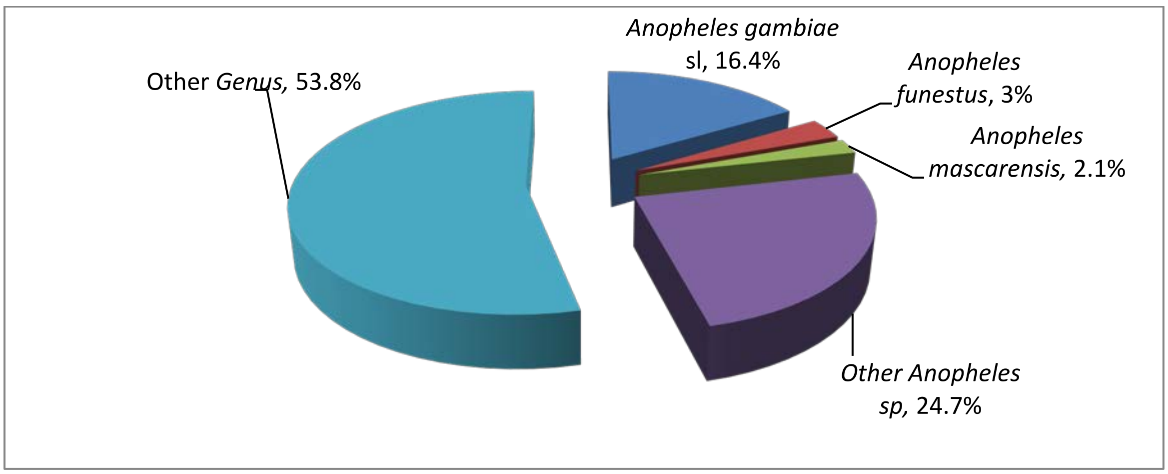
AIRS Madagascar entomologists and vector control officers collected 10,307 mosquitoes in total from all the sentinel sites between June 2016 and March 2017 in the South East and August 2016 and May 2017 in the East, using HLC, PSC, and outdoor collection (ODC) with aspirators. Listed below are the number and proportion of mosquitoes collected via each mosquito sampling method:

- HLC: 9,361(90.8%)
- PSC: 211 (2%)
- ODC: 735 (7.2%)

The results clearly indicate that HLC is the most productive sampling method in the collection of mosquitoes in Madagascar.

Species composition of the mosquitoes collected during the investigation period is noted in Figure 1, below, 46.2% of the mosquitoes collected were anopheline species and 24.1% represented *Anopheles gambiae* s.l., *Anopheles funestus* group, and *Anopheles mascarensis*, species.

Figure 1: Vector Species as a Percentage of Total Mosquitoes Collected



Vector species, *An. gambiae* s.l., *An. funestus* group, and *An. mascarensis*, distribution varied by sentinel site.

Overall, *An. gambiae* s.l. was collected from all sentinel sites and was noted as the primary and predominant vector species in the PMI-supported spray areas. *An. funestus* was collected from Ambodifaho (Brickaville), Vohitrambato (Toamasina II), Vavatenina, Manambotra Sud (Farafangana), Lanivo (Vohipeno), and Lopary (Vangaindrano). *An. mascarensis* was collected from Vohitrambato (Toamasina II), Vavatenina, Mahambo (Fenerive Est), Manambotra Sud (Farafangana), Lanivo (Vohipeno), and Lopary (Vangaindrano) sentinel sites (Table 3).

An. gambiae s.l., *An. funestus* and *An. mascarensis*, the three vectors of malaria in Madagascar were found co-existing in five districts: Toamasina II (Vohitrambato), Vavatenina (Vavatenina, control site of the East), Farafangana (Manambotra Sud), Vohipeno (Lanivo) and Vangaindrano (Lopary, control site of the South East). The highest vector density was recorded in Vohitrambato during the surveillance period for all the three vectors (n=661). *An. gambiae* s.l. was the most prevalent anopheline species found and accounted for 76.4% (n=1694) of the three vectors, followed by *An. funestus* (13.9%) (n=307) (Table 3). *An. mascarensis* constituted 9.7% (n=215). At baseline the proportion of An*. gambiae* s.l., *An. mascarensis, and An. funestus* was 42.8% (n=140), 27.5 %(n=90), and 29.7 %(n=97), respectively. At the baseline, non-anopheline mosquitoes accounted for 66.3% (597/900) of all the mosquitoes collected in the East Coast and 63.7% (408/641) in the South East.

Two of the three vector mosquitoes were found in Ambodifaho (Brickaville) and Mahambo (Fenerive Est): *An. gambiae* s.l. and *An.* funestus in Ambodifaho *and An. gambiae* s.l. and *An. mascarensis* in Mahambo. During this entomological monitoring work, *An. mascarensis* was absent in Ambodifaho (Brickaville) and *An. funestus* was not detected in Mahambo (Fenerive Est) (Table 3). All vector mosquito samples were preserved for further laboratory analysis that included identification of species by PCR and detection of sporozoites by ELISA.

Table 3. Number of mosquitoes collected at each sentinel site, disaggregated by species.

	Ambodifaho (Brickaville)	**Vohitra mbato (Toamasi na II)**	**Mahambo (Fenerive Est)**	**(Vavateni na)**	**Manambo tra Sud (Farafanga na)**	**Lanivo (Vohipe no)**	**Lopary (Vangain drano)**	**Total**
An. gambiae s.l.	233	494	288	395	94	113	77	1694
Anopheles funestus	1	80	0	76	8	51	91	307
An. mascarensis	0	87	30	72	17	8	1	215
Other *Anopheles* sp.	0	1219	131	313	556	21	307	2547
Other *Genus*	857	787	840	477	521	1297	765	5544
Total	1091	2667	1289	1333	1196	1490	1241	10307

4.2. RESULTS OF HUMAN LANDING COLLECTION

During the surveillance period, 1,384 female *An. gambiae* s.l. were collected from seven sentinel sites using human landing catches. Among these, 415 *An. gambiae* s.l. (30%) were collected indoors and 969 (70%) outdoors.

Regarding the other malaria vectors, 106 *An. funestus* were collected indoors and 164 outdoors, while 33 *An. mascarensis* were collected indoors and 137 outdoors

The vectors showed an exophagic tendency in all sites. When HLC data from all the villages were combined, the proportion of vectors caught while seeking human blood outdoors was significantly higher than indoors:

Table 4. Indoor vs Outdoor Landing Mosquitoes

Vector	# indoor	# outdoor	P value
An. gambiae s.l.	415	969	<0.0001
An. funestus	106	164	0.002
An. mascarensis	33	137	<0.0001

Before spraying, *An. gambiae* s.l. human biting rates were very low (<1), except in Lanivo (2.7 b/p/n indoor; 4 b/p/n outdoor), Vavatenina (3 b/p/n outdoor) and Ambodifaho (1.8 b/p/n outdoor), most likely related to environmental factors (low or no rain fall and hence few breeding sites before IRS during the dry season). In most areas, the vector biting rates inside houses were low at the baseline and remained low post IRS. The dry season before IRS and possibly the impact of IRS after spray might partly explain the low biting rates observed (Table 5). *An. gambiae* s.l. appeared to have exophagic tendency both in the East and in the South East. Post IRS, the overall proportion of *An. gambiae* s.l. caught while seeking a blood meal indoors was lower than those caught outdoors. The results were statistically significant (p<0.011). Owing to the small number of mosquitoes collected, the village-by-village comparison in feeding location did not result in statistically significant differences between outdoor and indoor feeding.

Table 5. Number of Mosquitoes Collected by HLC and Man Biting Rates (bites/person/night = b/p/n) between June 2016 and March 2017 in the South East and between August 2016 and May 2017 in the East Coast.

Sites	Month	*Anopheles gambiae s.l.*				*Anopheles funestus*				*Anopheles mascariensis*				Other *Anopheles*			
		Indoor	Indoor (b/p/n)**	Out door	Outdoor (b/p/n)**	Indoor	Indoor (b/p/n)**	Out door	Outdoor (b/p/n)**	Indoor	Indoor (b/p/n)**	Outdoor	Outdoor (b/p/n)**	Indoor	Indoor (b/p/n)**	Outdoor	Outdoor (b/p/n)**
Ambodifaho, Brickaville	August*	4	0.7	11	1.8	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	September	1	0.2	7	1.2	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	October[1]	2	0.3	9	1.5	0	0.0	1	0.2	0	0.0	0	0.0	0	0.0	0	0.0
	November	1	0.2	11	1.8	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	December	4	0.7	3	0.5	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	January	8	1.1	15	2.1	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	February	4	0.6	2	0.3	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	March	3	0.4	7	1	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	April	15	2.1	25	3.6	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	May	11	1.6	22	3.1	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Vohitrambato, Toamasina II	August*	3	0.5	4	0.7	14	2.3	24	4.0	10	1.7	47	7.8	35	5.8	181	30.2
	September	7	1.2	35	5.8	0	0.0	4	0.7	2	0.3	11	1.8	12	2.0	67	11.2
	October	1	0.2	50	8.3	1	0.2	10	1.7	0	0.0	1	0.2	3	0.5	128	21.3
	November	29	4.8	86	14.3	0	0.0	1	0.2	0	0.0	0	0.0	39	6.5	251	41.8
	December	19	3.2	54	9.0	1	0.2	11	1.8	0	0.0	2	0.3	4	0.7	41	6.8
	January	20	3.3	47	7.8	0	0.0	0	0.0	0	0.0	0	0.0	28	4.7	63	10.5
	February	3	0.5	35	5.8	1	0.2	3	0.5	0	0.0	2	0.3	10	1.7	160	26.7
	March	7	1.2	12	2.0	1	0.2	4	0.7	0	0.0	1	0.2	8	1.3	58	9.7
	April	5	0.8	15	2.5	0	0.0	1	0.2	0	0.0	3	0.5	6	1.0	56	9.3
	May	6	1.0	16	2.3	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	38	6.3
Mahambo,	August*	2	0.3	0	0.0	0	0.0	0	0.0	3	0.5	6	1.0	3	0.5	2	0.3

Fenerive Est	September	0	0.0	2	0.3	0	0.0	0	0.0	0	0.0	5	0.8	0	0.0	2	0.3
	October	0	0.0	6	1.0	0	0.0	0	0.0	0	0.0	3	0.5	0	0.0	5	0.8
	November	2	0.3	11	1.8	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	December	4	0.7	11	1.8	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	5	0.8
	January	6	1.0	14	2.3	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	6	1.0
	February	10	1.7	18	3.0	0	0.0	0	0.0	0	0.0	0	0.0	2	0.3	6	1.0
	March	14	2.3	22	3.7	0	0.0	0	0.0	1	0.2	1	0.2	4	0.7	7	1.2
	April	10	1.7	20	3.3	0	0.0	0	0.0	0	0.0	6	1.0	7	1.2	8	1.3
	May	15	2.5	23	3.8	0	0.0	0	0.0	0	0.0	0	0.0	10	1.7	11	1.8
Vavatenina, control East (control site for East)	August*	3	0.5	18	3.0	8	1.3	9	1.5	2	0.3	6	1.0	0	0.0	26	4.3
	September	1	0.2	7	1.2	1	0.2	5	0.8	3	0.5	4	0.7	1	0.2	6	1.0
	October	20	3.3	84	14.0	5	0.8	12	2.0	4	0.7	6	1.0	3	0.5	14	2.3
	November	2	0.3	30	5.0	0	0.0	1	0.2	0	0.0	1	0.2	7	1.2	15	2.5
	December	27	4.5	34	5.7	2	0.3	1	0.2	0	0.0	1	0.2	8	1.3	29	4.8
	January	17	2.8	18	3.0	5	0.8	10	1.7	2	0.3	0	0.0	25	4.2	33	5.5
	February	8	1.3	8	1.3	1	0.2	1	0.2	3	0.5	7	1.2	12	2.0	18	3.0
	March	9	1.5	9	1.5	0	0.0	0	0.0	1	0.2	5	0.8	10	1.7	15	2.5
	April	7	1.2	17	2.8	3	0.5	2	0.3	0	0.0	5	0.8	18	3.0	33	5.5
	May	8	1.3	16	2.7	0	0.0	0	0.0	0	0.0	0	0.0	8	1.3	16	2.7
Manambotra Sud, Farafangana	June*	16	2.6	1	0.2	0	0.0	0	0.0	2	0.5	2	0.0	0	0.0	0	0.0
	July	0	0.0	0	0.0	1	0.2	1	0.2	0	0.0	2	0.3	2	0.3	33	5.5
	August	0	0.0	0	0.0	1	0.2	1	0.2	0	0.0	0	0.0	6	1.0	44	7.3
	September	0	0.0	9	1.5	0	0.0	0	0.0	0	0.0	0	0.0	2	0.3	49	8.2
	October	1	0.2	16	2.7	1	0.2	3	0.5	0	0.0	0	0.0	3	0.5	38	6.3
	November	0	0.0	7	1.2	0	0.0	0	0.0	0	0.0	5	0.8	14	2.3	49	8.2
	December	2	0.3	3	0.5	0	0.0	0	0.0	0	0.0	0	0.0	18	3.0	32	5.3
	January	4	0.7	3	0.5	0	0.0	0	0.0	0	0.0	0	0.0	13	2.2	19	3.2
	February	2	0.3	5	0.8	0	0.0	0	0.0	0	0.0	0	0.0	9	1.5	14	2.3
	March	5	0.8	9	1.5	0	0.0	0	0.0	0	0.0	0	0.0	36	6.0	42	7.0

Lanivo, Vohipeno	June*	16	2.7	24	4.0	5	0.8	9	1.5	0	0.0	0	0.0	0	0.0	5	0.8
	July	11	1.8	9	1.5	3	0.5	12	2.0	0	0.0	0	0.0	0	0.0	8	1.3
	August	1	0.2	2	0.3	1	0.2	2	0.3	0	0.0	0	0.0	0	0.0	7	1.2
	September	0	0.0	3	0.5	0	0.0	3	0.5	0	0.0	2	0.3	0	0.0	1	0.2
	October	0	0.0	1	0.2	0	0.0	1	0.2	0	0.0	0	0.0	0	0.0	0	0.0
	November	1	0.2	1	0.2	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	December	1	0.2	4	0.7	2	0.3	3	0.5	0	0.0	0	0.0	0	0.0	0	0.0
	January	1	0.2	2	0.3	0	0.0	0	0.0	0	0.0	1	0.2	0	0.0	0	0.0
	February	3	0.5	3	0.5	0	0.0	0	0.0	0	0.0	1	0.2	0	0.0	0	0.0
	March	3	0.5	6	1.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Lopary, Vangaindrano, control South East (control site for South East)	June*	0	0.0	4	0.7	4	0.7	7	1.2	0	0.0	0	0.0	15	2.5	22	3.7
	July	6	1.0	7	1.2	9	1.5	2	0.3	0	0.0	1	0.2	37	6.2	87	14.5
	August	0	0.0	1	0.2	14	2.3	9	1.5	0	0.0	0	0.0	5	0.8	13	2.2
	September	0	0.0	1	0.2	21	3.5	4	0.7	0	0.0	0	0.0	6	1.0	16	2.7
	October	3	0.5	1	0.2	1	0.2	7	1.2	0	0.0	0	0.0	13	2.2	26	4.3
	November	4	0.7	7	1.2	0	0.0	0	0.0	0	0.0	0	0.0	11	1.8	13	2.2
	December	4	0.7	1	0.2	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	3	0.5
	January	5	0.8	1	0.2	0	0.0	0	0.0	0	0.0	0	0.0	2	0.3	5	0.8
	February	5	0.8	2	0.3	0	0.0	0	0.0	0	0.0	0	0.0	3	0.5	2	0.3
	March	3	0.5	2	0.3	0	0.0	0	0.0	0	0.0	0	0.0	1	0.2	1	0.2

Figure 2. Monthly Distribution of Indoor Man Biting Rates (bites/person/night: b/p/n) for *An. gambiae* s.l. at Sentinel Sites

Note: June was baseline for the South East districts: Farafangana, Vangaindrano and August was baseline for Ambodifaho, Vohitrambato, Mahambo, and Vavatenina

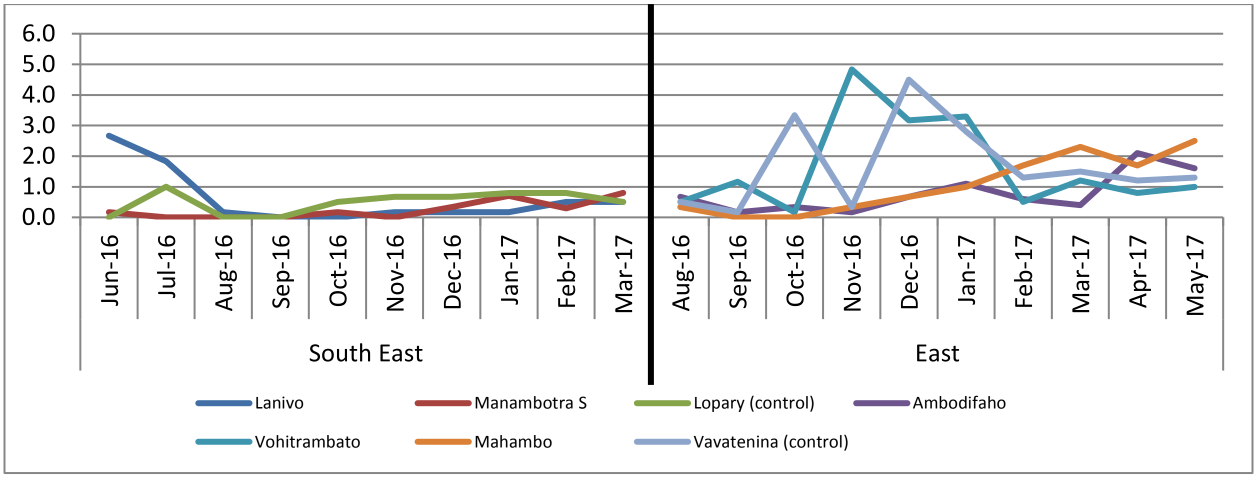
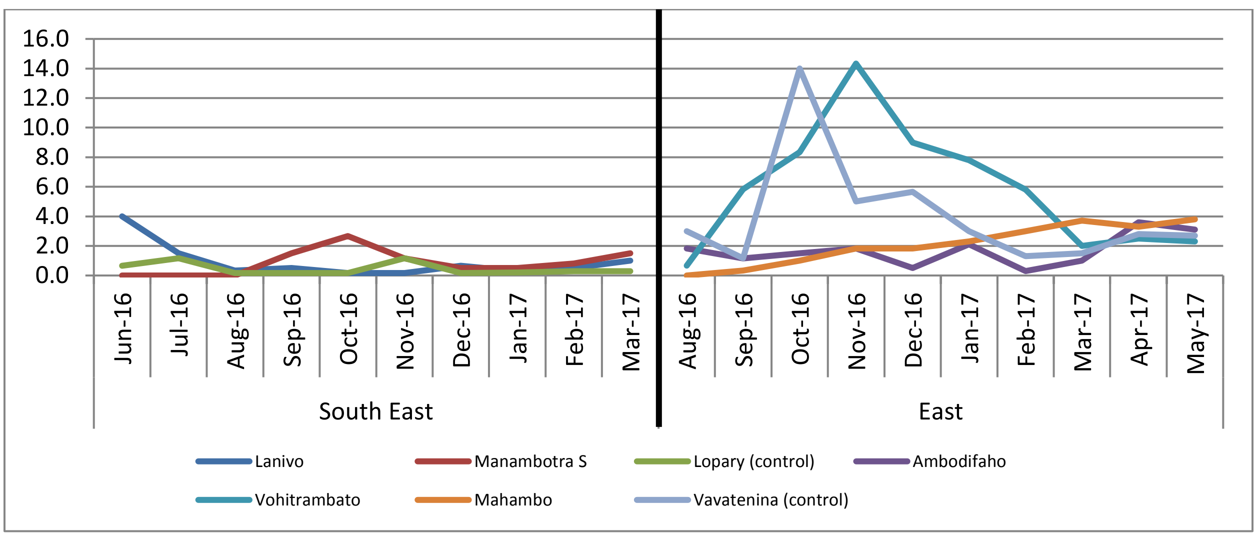


Figure 3. Monthly Distribution of Outdoor Man Biting Rate (bites/person/night: b/p/n) for *An. gambiae* s.l. at Sentinel Sites



4.3. PEAK BITING TIME

From the entomological monitoring data collected during the period that this report covers, it was noted that the peak biting time for mosquitoes seemed to vary by sentinel site (Figure 4 and Figure 5). Peak biting time was observed, both indoors and outdoors, between 9:00 and 10:00 pm in two sites (Ambodifaho and Manambotra Sud), and between 9:00 pm and 1:00 am in Mahambo. In Vohitrambato peak biting was observed between 8:00 pm and 3:00 am both outdoors and indoors. In Vavatenina peak biting was recorded between 10:00 pm and 12:00 am both outdoors and indoors. In Lanivo peak biting was between 01:00 and 02:00 am both outdoors and indoors. In Lopary indoor peak biting rates were between 7:00 and 9:00 pm and 11:00 pm and 2:00 am. The outdoor biting rates, however, peaked between 10:00 pm and 12:00 am. Except Lopary, in all other sentinel sites there was an overlap in outdoor and indoor peak bites time. In Lopary and Vohitrambato (Toamasina II) significant mosquito biting started as early as 20:00 before people go to bed and are sleeping under ITNs, which might affect the impact of ITNs on vector-human contact. Except in Lanivo, in all other sentinel sites most *An*. *gambiae* s.l. mosquitoes seem to bite in the first half of the night. It was difficult to draw a conclusion on the feeding habit of the vector based on the current data for the other two vectors (i.e., *Anopheles* funestus and Anopheles mascariensis), either due to the absence of a consistent biting pattern or the small number of mosquitoes collected.

FIGURE 4: *Anopheles gambiae* s.l. Biting Hours At Eastern Sentinel Sites And Control

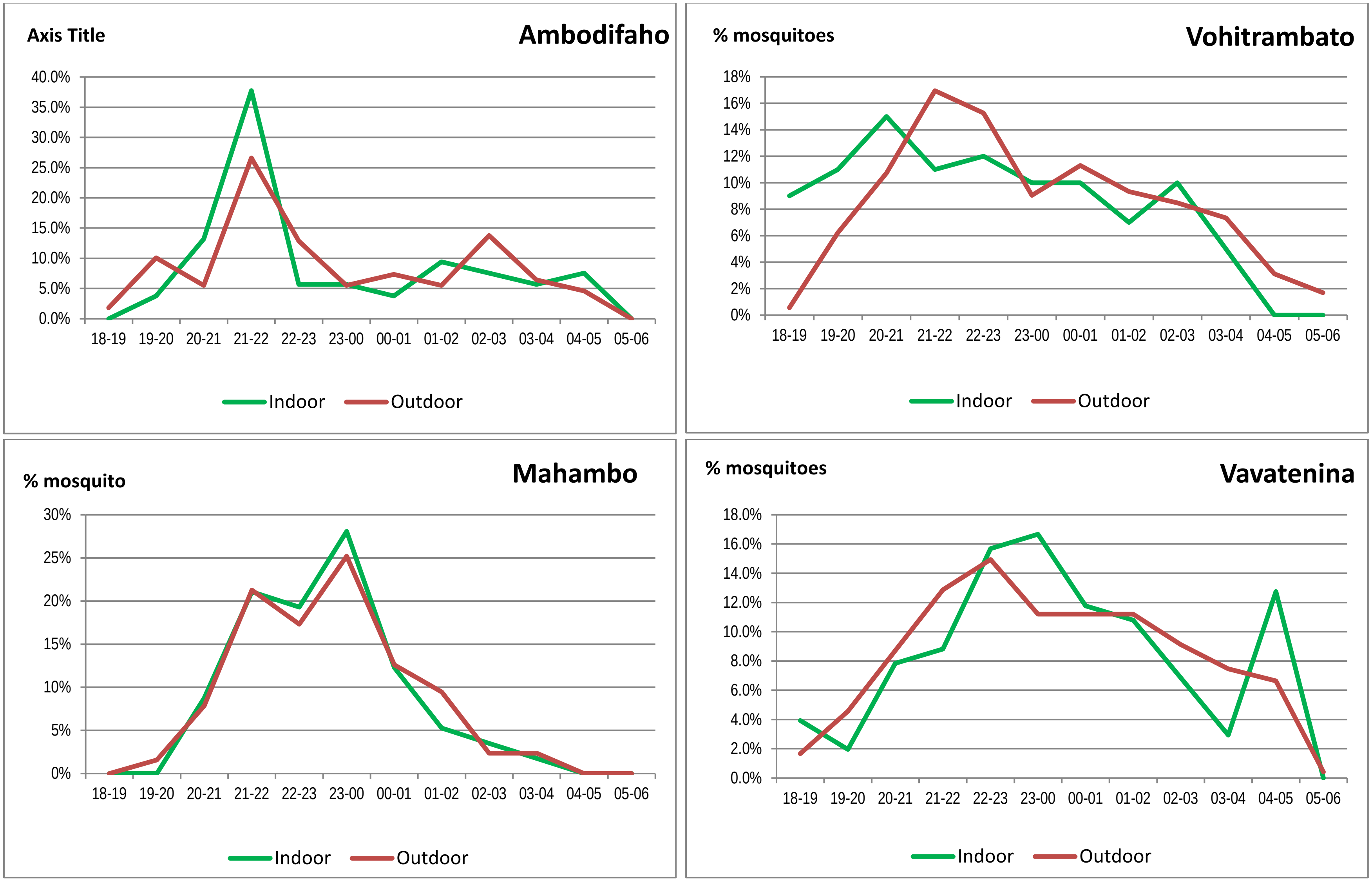
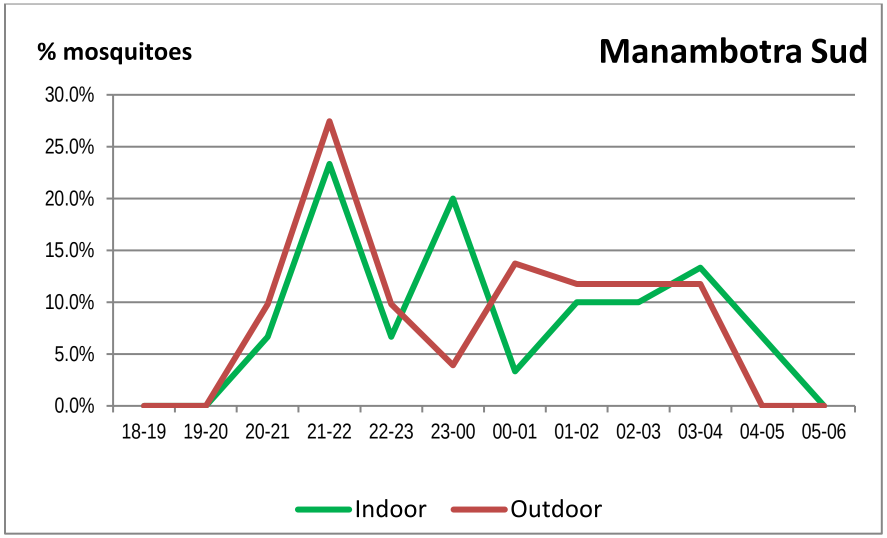
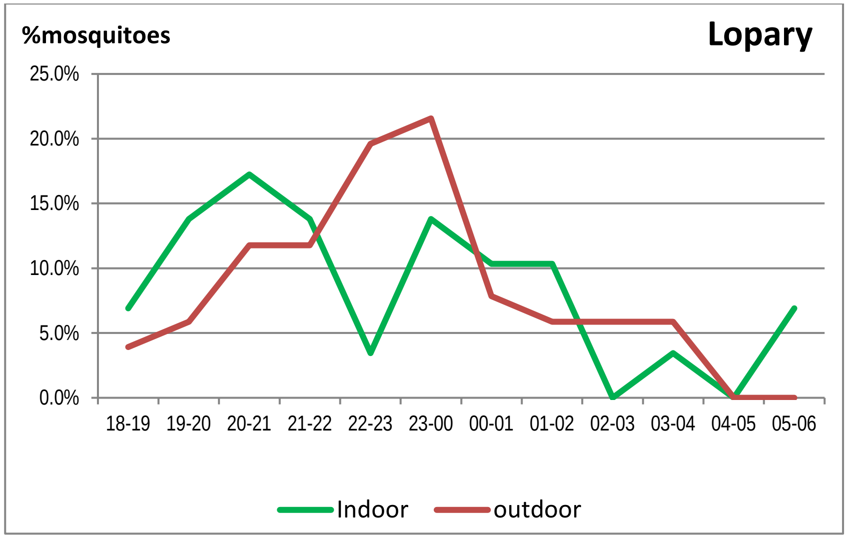
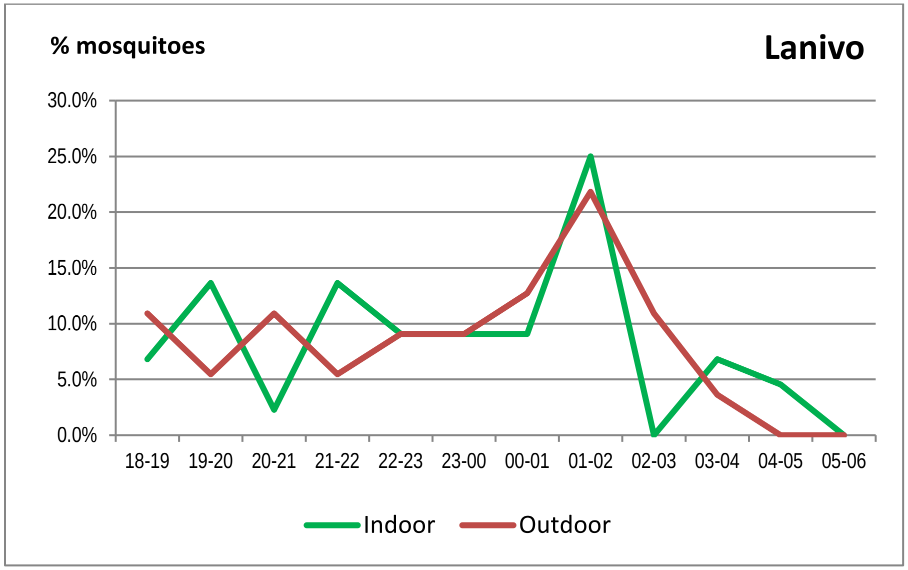


FIGURE 5: *Anopheles gambiae* s.l. Biting Hours At South Eastern Sentinel Sites And Control







4.4. RESULTS OF OUTDOOR COLLECTION

A total of 224 *Anopheles gambiae* s.l. were collected resting outdoors in natural and pit shelters using aspirators from all seven sites in the South East and East Coast of Madagascar. Only nineteen *Anopheles funestus* was collected via outdoor collection at four sites: Vohitrambato, Vavatenina, Lopary and Lanivo. Thirty-four *Anopheles mascarensis* were collected from five sites (Vohitrambato, Mahambo and Vavatenina, Manambotra Sud and Lanivo) via outdoor collection (Table 6 and Annex-16).

Table 6. Total Number of Mosquitoes Collected by Outdoor Collection with Aspirator (ODC) Method between June 2016 and March 2017 in the South East and August 2016 and May 2017 in the East.

Species	East				South East			
	Ambodifaho (Brickaville)	Vohitrambato (Toamasina II)	Mahambo (Fenerive Est)	Vavatenina	Manambotra Sud (Farafangana)	Lopary (Vangaindrano)	Lanivo (Vohipeno)	Total
An. gambiae s.l.	43	32	94	31	5	8	11	224
An. funestus	0	3	0	6	0	5	5	19
An. mascariensis	0	5	5	17	3	0	4	34
Other *Anopheles* sp.	0	26	51	11	53	17	0	158
Other Genus	0	26	157	29	48	40	0	300
Total	43	92	307	94	109	70	20	735

4.5. INDOOR RESTING ANOPHELINE DENSITIES

Indoor resting density was determined using PSC in seven sentinel sites. Vector density was estimated by dividing the number of mosquitoes collected by the number of houses sampled (ten houses per site). The indoor vector density was low (0 to 0.8 vector per room per day) at the baseline.

Indoor resting density for *An. gambiae* s.l. and the other vectors collected during this entomological monitoring period was very low and it is difficult to make a sound inference of the resting habit and impact of IRS on indoor resting density. In most sites, the indoor resting density was zero or close to zero. Table 7 provides more information about the indoor resting density at each sentinel site.

Table 7: Number of Malaria Vector Mosquitoes Collected By Psc and Indoor Resting Densities In Study Sites

Species	Month	Ambodifaho		Vohitrambato		Mahambo		Vavatenina		Manambotra Sud		Lopary		Lanivo	
		#	Vector Density	#	Vector Density	#	Vector Density	#	Vector Density	#	Vector Density	#	Vector Density	#	Vector Density

An.gambiae s.l.	June									1	0.1	0	0	4	0.4
	July									0	0	0	0	0	0
	August	8	0.8	0	0	4	0.4	0	0	0	0	0	0	0	0
	September	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	October	0	0	0	0	0	0	1	0.1	0	0	5	0.5	0	0
	November	2	0	0	0	0	0	3	0.3	0	0	2	0.2	0	0
	December	2	0	0	0	0	0	1	0.1	0	0	1	0.1	0	0
	January	3	0.3	0	0	0	0	2	0.2	0	0	1	0.1	0	0
	February	2	0.2	0	0	0	0	0	0	0	0	1	0.1	2	0.2
	March	2	0.2	7	0.7	0	0	11	1.1	5	0.5	2	0.2	4	0.4
	April	2	0.2	0	0	0	0	2	0.2						
	May	4	0.4	1	0.1	0	0	1	0.1						
An. funestus	June									0	0	0	0	5	0.5
	July									0	0	4	0.4	0	0
	August	0	0	0	0	0	0	2	0.2	0	0	2	0.2	0	0
	September	0	0	0	0	0	0	1	0.1	0	0	2	0.2	0	0
	October	0	0	0	0	0	0	1	0.1	0	0	0	0	0	0
	November	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	December	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	January	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	February	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	March	0	0	1	0.1	0	0	0	0	0	0	0	0	0	0
	April	0	0	0	0	0	0	0	0						
	May	0	0	0	0	0	0	0	0						
An. mascariensis.	June									3	0.3	0	0	0	0
	July									0	0	0	0	0	0
	August	0	0	2	0.2	0	0	0	0	0	0	0	0	0	0
	September	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	October	0	0	0	0	0	0	1	0.1	0	0	0	0	0	0
	November	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	December	0	0	0	0	0	0	1	0.1	0	0	0	0	0	0
	January	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	February	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	March	0	0	1	0.1	0	0	3	0.3	0	0	0	0	0	0
	April	0	0	0	0	0	0	0	0						
	May	0	0	0	0	0	0	0	0						

Note: In June and July, collections happened only in Manambotra Sud (Farafangana district), Lanivo (Vohipeno district) and Lopary (control site in Vangaindrano district)

4.6. PARITY RATES

At the baseline, parity rate of *An.gambiae* s.l. was high in Ambodifaho 100% (n=25), Vavatenina 87.5% (n=32) and Lanivo 84.2% (n=19). The number of mosquitoes collected and dissected from Vohitrambato, Mahambo, Manambbotra Sud and Lopary was small with a parous rate of 100% (n=8), 100% (n=9), 88.9% (n=9), 88.9 (n=9), respectively. After IRS, parity rates reduced to 58.3% (n=79), 42.3% (n=208), 52 %(n=475), 47.9 %(n=282), 44 %(n=100), 29.9% (n=71), and 30.6% (n=352) in Manambbotra Sud, Ambodifaho, Vohitrambato, Mohambo, Lanivo, Lopary and Vavatenina, respectively. Two-sided McNemar's chi-square test of two paired samples was used to assess if the observed parity rate reduction was statistically significant when pre-spray data was compared with post spray. In all the five spray areas where vector surveillance was conducted and the two control sites, the reduction was statistically significant (Table 7). Whilst we observed reduction in parity rates in the control sites, in absolute terms the reduction noted was low when compared with intervention villages, but was statistically significant (Table 8).

The most conservative two-sided Fisher's exact test was used to conduct significance testing to compare parity rate data between the intervention and control sites. No statistically significant difference was observed between the intervention sites in the South East (Manambotra Sud and Lanivo) and Lopary (control) when pre-spray data was compared (p=0.99). However, post spray parity rate in intervention sites was significantly lower than Lopary, the control (P<0.0001). This reduction in parity might at least partially be attributed to the impact of IRS. Similarly, we used data obtained from the East Coast (intervention) and Vavatenina (control) to compare the parity rate between the intervention and control sites. At the baseline, before spray, parity rate was higher in the intervention villages as compared to the control sites (p<0.037). However, post IRS parity rate in the intervention villages were reduced, the gap between the two arms was narrowed. Hence, no statistically significant difference was observed (Table 8).

At the baseline, *An. funestus* was collected from three out of the six sentinel sites, namely Lopary, Toamasina II and Vavatenina. The proportions of parous mosquitoes recorded in these three sites were 33.3% (n=3), 86.4% (n=22), and 62.55% (n=8) in Lopary, Vohitrambato and Vavatenina, respectively. Post IRS this species was collected from five sites and parity rate ranged from 0% in Lopary to 91% in Vavatenina (Table 14 in annex).

Table 8. Parity Rates Comparison

Sentinel sites	*An. gambiae* s.l.						P-value(pre and post IRS parity comparison)
	Pre-IRS			Post IRS			
	# dissected	# parous	% parous	# dissected	# parous	% parous	
Manambbotra Sud (Farafangana) (Intervention South East)	9	8	89.9%	79	25	31.6%	<0.0001
Lanivo (Intervention South East)	19	16	84,2%	100	40	40%	<0.0001
Lopary (Vangaindrano): Control SE	9	8	89.9%	71	51	69%	0.04
Comparison between intervention and control (East Coast)	p=0.99			P<0.001			
Ambodifaho (Brickaville)	25	25	100.0%	208	120	57.7%	P<0.0001
Vohitram bato (Toamasina II)	8	8	100.0%	475	228	48%	P<0.0001
Mahambo (Fenerive Est)	6	6	100.0%	282	147	52.1%	P<0.0001
Total: East Coast (Intervention)	39	39	100%	965	495	51.3%	P<0.0001
Vavatenina: Control (East Coast)	32	28	87.5%	352	197	56.9%	P<0.0001

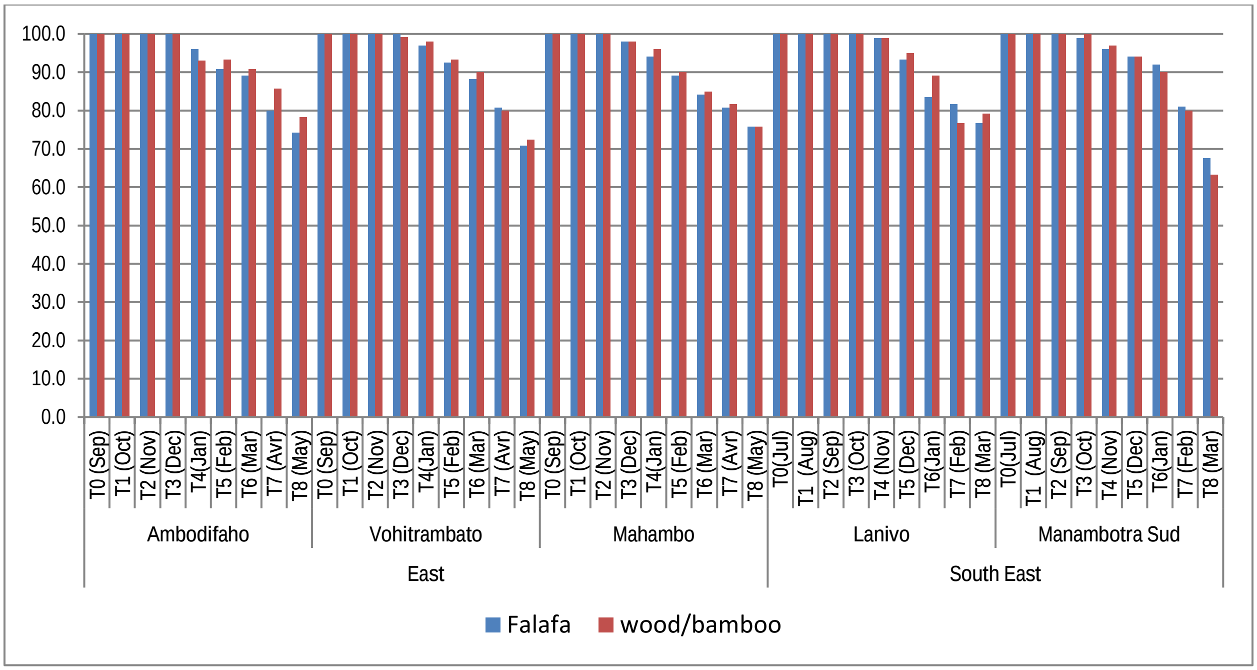
Comparison between intervention and control (East Coast)	P=0.037	P=0.429	

4.7. CONE BIOASSAY TEST RESULTS

During the first week of the IRS campaigns in the East Coast and in the South East, AIRS Madagascar conducted cone bioassay tests to assess whether the quality of the spraying was satisfactory. The results indicated that the spray quality, both in the East Coast and in the South East, was good, mortality being 100% for all the structures sampled at T0 and T1. AIRS Madagascar subsequently collected monthly cone bioassay data using the World Health Organization (WHO) procedure to assess the residual bio-efficacy of insecticides sprayed after the 2016 IRS campaign. The tests were conducted in the following sentinel sites: Ambodifaho (district of Brickaville), Vohitrambato (district of Toamasina II), and Mahambo (district of Fenerive Est) in the East Coast; Manambotra Sud (district of Farafangana) and Lanivo (district of Vohipeno) in the South East.

The monthly monitoring of the insecticide decay rate, for the insecticide used (Actellic 300 CS) showed pirimiphos-methyl lasted seven months on all sprayed surface types in the South East and in the East (Figure 6).

Figure 6: Residual Effectiveness Observed for Pirimiphos-Methyl CS 300 (Organophosphates) in the East Coast



4.8. INSECTICIDE SUSCEPTIBILITY TEST RESULTS

4.8.1 Results from WHO Tube Tests

Anopheles gambiae s.l. (Figure 7 below and Table 9 in the annex) has developed resistance to:

- Deltamethrin in three sites out of eleven: Ambodifaho, Vohitrambato, and Vavatenina.
- Permethrin in Ambodifaho, Vavatenina and Lanivo
- DDT in Imerina Imady, Vohimarina and Ankafina Tsarafidy

Possible resistance was observed for:

- DDT in Ambodifaho, Vohitrambato and Vavatenina
- Deltamethrin in Vohitrambato
- Permethrin in Ankafina Tsarafidy and Vohitrambato
- Lambda-cyhalothrin in Vohitrambato

An. funestus (Table 10) is susceptible to:

Pirimiphos-methyl and deltamethrin in Manambotra Sud (Farafangana District), Ambodifaho (Brickaville District), Vavatenina (Vavatenina District) and Vohitrambato (Toamasina II)

An. mascarensis (Table 11) is susceptible to:

Pirimiphos-methyl and deltamethrin in Manambotra Sud (Farafangana), Sahamatevina (Brickaville), Vavatenina (Vavatenina) and Mahambo (Fenerive Est)

Assessment of the resistance intensity (WHO tube test) (Table 12 in annex)

- The susceptibility test performed with permethrin at 5x diagnostic dose killed 100% of the mosquitoes tested in Ambodifaho, Vavatenina, and Lanivo.
- Deltamethrin 5x killed 100% of the mosquitoes tested in Ambodifaho, Vohitrambato and Vavatenina.
- Lambdacyhalothrin 5x killed 100% of the mosquitoes tested in Vohitrambato.

Synergists (WHO tube test) (Table 13 in annex)

Permethrin was tested with a synergist in four sites, and PBO restored susceptibility:

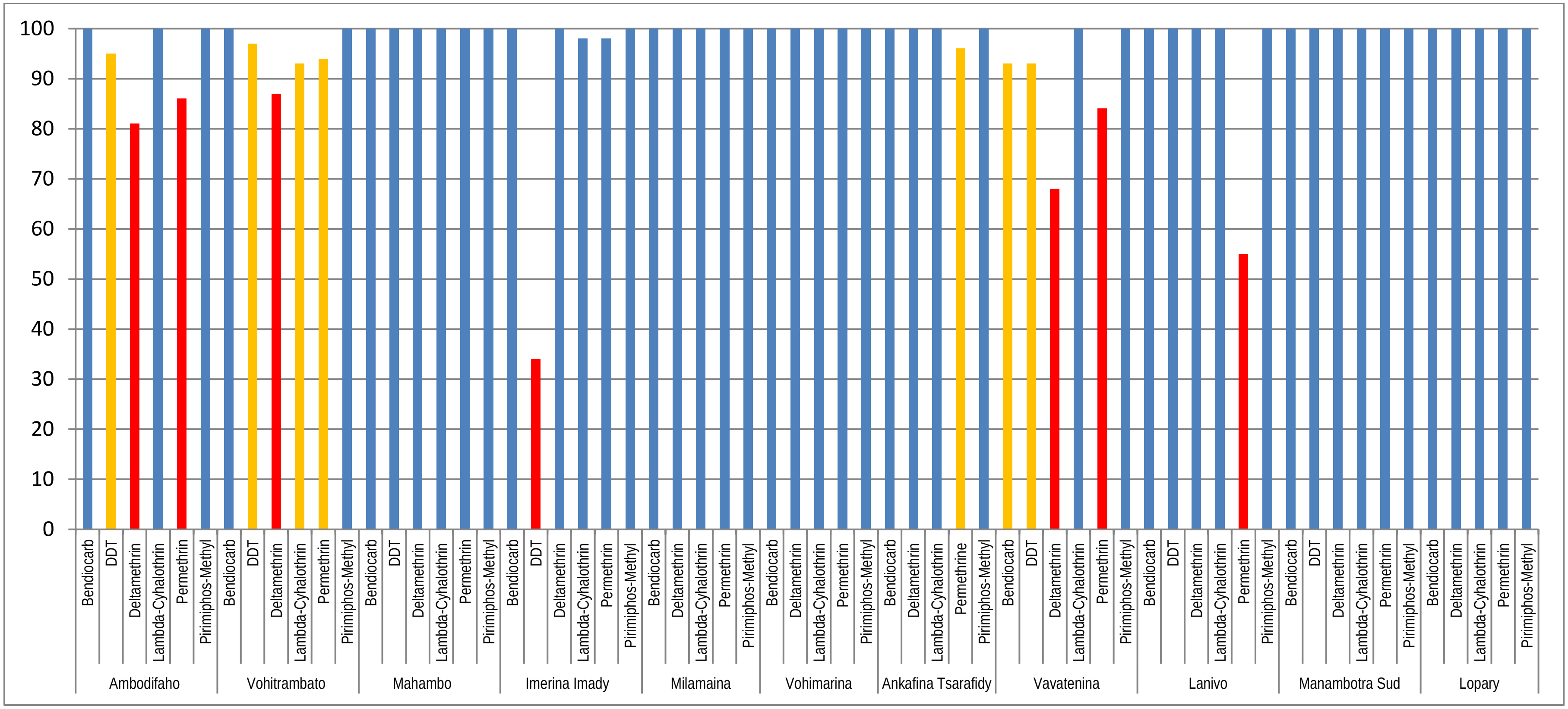
- Deltamethrin, tested in 3 sites became 100% susceptible after pre-exposure to PBO.
- Lambdacyhalothrin tested in one site became 100% susceptible after pre-exposure to PBO.

The emergence of insecticide resistance to pyrethroids is probably due to the wider use of long-lasting insecticide-treated nets (LLINs) for several years in the East Coast and the South East, IRS with alpha-cypermethrin/deltamethrin for several years in the Central High Lands (Imerina Imady, Ankafina

Tsarafidy, Milamaina and Vohimarina) in the past, and use of similar insecticides in agriculture, or a combination of those factors.

The results of the susceptibility tests using the WHO tubes tests are shown in Figures 8 below and Table 8 in the annex.

FIGURE 7. RESULTS OF INSECTICIDE SUSCEPTIBILITY TESTS USING THE WHO TUBE TEST FOR *An. gambiae* s.l.



4.8.2. Vector Susceptibility to a New Insecticide: Clothianidin (Sumishield®)

These insecticide susceptibility tests were carried out at ten sentinel sites selected from IRS targeted areas, in addition to the test with *An. gambiae*, Kisumu strain. The sites were: Ambodifaho (Brickaville district), Vohitrambato (Toamasina II), Mahambo (Fenerive Est), Vavatenina, Lanivo (Vohipeno), Manambotra Sud (Farafangana), Lopary (Vangaindrano), Mananjary, Manakara and Vondrozo

Results

- The results indicated full susceptibility of *An. gambiae* s.l. to clothianidin in the ten study sites. While poor knock down effect was observed everywhere, the 24h mortality ranges were from 46% in Ambodifaho (Brickaville) to 90% in Vondrozo, 100% mortality was observed starting at Day 4.
- In Vohitrambato (Toamasina II) and Vavatenina, 100% mortality was reached at Day 4. In six sites, this rate was observed at Day 5 (Ambodifaho, Lanivo, Manambotra Sud, Lopary, Manakaraand Vondrozo), in one site at Day 6 (Mananjary) and in one site at Day 7 (Mahambo)
- The test with *Anopheles gambiae*, Kisumu, showed 83% mortality at 24h and 100% mortality was reached at Day 5.
- In five sites and in the test using Kisumu strain, the control test mortality rate was less than five percent, so Abbot's correction was not used there. It was more than 5% but under 20% in five sites and Abbot's correction was calculated (Table 15 in annex).

5. CONCLUSIONS

The data collected indicates that *Anopheles gambiae* s.l., *Anopheles funestus* group, and *Anopheles mascarensis* vector species are present at different prevalence in various sentinel sites. *Anopheles gambiae* s.l. is the most common mosquito caught seeking human blood in the East Coast and the South East. High coverage with LLINs for more than three years might have contributed to the outdoor biting behavior. Mosquito samples are being sent to Institut Pasteur of Madagascar for molecular analysis (PCR identification and determination of the resistance mechanism) and ELISA test for sporozoite detection.

The results of the vector susceptibility tests indicate susceptibility of *Anopheles gambiae* s.l. to bendiocarb and pirimiphos-methyl in all spray areas. Based on the insecticide susceptibility data collected following the 2016-2017 IRS campaign, technically three of the four insecticide classes (except for organochlorines) approved by the WHO for IRS are potentially eligible for selection and use in Madagascar. In areas where LLIN coverage is still low and pyrethroid insecticide is still efficacious, there is a possibility that this class of insecticide can be considered for use.

Cone bioassay tests conducted during the first week of the IRS campaign indicated that the quality of spraying in the South East and East Coast was good with test mortality rates of 100 percent for all structures sampled and used for the testing within 24 hours and one month after structures were sprayed. The monthly monitoring of the insecticide decay rate for the insecticide used (Actellic 300 CS) showed pirimiphos-methyl lasted seven months on all sprayed surface types in the South East and in the East.

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ANNEX

Table 9. Results of *An. gambiae* s.l. Susceptibility Tests

Sentinel site	Insecticide tested	WHO tube tests			Test completion date
		N# mosquitos tested	24h % Observed mortality	Resistance status	
Ambodifaho (Brickaville	Bendiocarb	100	100	S	10/11/2016
	DDT	100	95	P	11/13/2016
	Deltamethrin	100	81	R	11/13/2016
	Lambda-Cyhalothrin	100	100	S	10/11/2016
	Permethrin	100	86	R	10/12/2016
	Pirimiphos-Methyl	100	100	S	09/11/2016
Vohitrambato (Toamasina II)	Bendiocarb	100	100	S	10/13/2016
	DDT	100	97	P	10/10/2016
	Deltamethrin	100	87	R	11/10/2016
	Lambda-Cyhalothrin	100	93	P	11/09/2016
	Permethrin	100	94	P	11/09/2016
	Pirimiphos-Methyl	100	100	S	01/24/2017
Mahambo (Fenerive Est)	Bendiocarb	100	100	S	10/10/2016
	DDT	100	100	S	10/10/2016
	Deltamethrin	100	100	S	11/08/2016
	Lambda-Cyhalothrin	100	100	S	11/05/2016
	Permethrin	100	100	S	11/11/2016
	Pirimiphos-Methyl	100	100	S	10/10/2016
Imerina Imady	Bendiocarb	100	100	S	01/18/2017
	DDT	100	34	R	01/16/2017
	Deltamethrin	100	100	S	01/20/2017
	Lambda-Cyhalothrin	100	98	S	01/20/2017
	Permethrin	100	98	S	01/16/2017
	Pirimiphos-Methyl	100	100	S	11/13/2016
Milamaina (Fandriana)	Bendiocarb	100	100	S	01/21/2017

Sentinel site	Insecticide tested	WHO tube tests			Test completion date
		N# mosquitos tested	24h % Observed mortality	Resistance status	
	Deltamethrin	100	100	S	01/20/2017
	Lambda-Cyhalothrin	100	100	S	01/21/2017
	Permethrin	100	100	S	01/20/2017
	Pirimiphos-Methyl	100	100	S	10/08/2016
Vohimarina (Fianarantsoa II)	Bendiocarb	100	100	S	02/11/2017
				S	
	Deltamethrin	100	100	S	02/16/2017
	Lambda-Cyhalothrin	100	100	S	02/16/2017
	Permethrin	100	100	S	02/16/2017
	Pirimiphos-Methyl	100	100	S	02/11/2017
Ankafina Tsarafidy	Bendiocarb	100	100	S	11/07/2016
	Deltamethrin	100	100	S	11/08/2016
	Lambda-Cyhalothrin	100	100	S	11/02/2016
	Permethrine	100	96	P	11/02/2016
	Pirimiphos-Methyl	100	100	S	11/07/2016
Vavatenina	Bendiocarb	100	93	P	09/03/2016
	DDT	100	93	P	09/03/2016
	Deltamethrin	100	68	R	12/06/2016
	Lambda-Cyhalothrin	100	100	S	02/14/2017
	Permethrin	100	84	R	12/06/2016
	Pirimiphos-Methyl	100	100	S	02/14/2017
Lanivo	Bendiocarb	100	100	S	10/24/2016
	DDT	100	100	S	11/23/2016
	Deltamethrin	100	100	S	11/22/2016
	Lambda-Cyhalothrin	100	100	S	10/25/2016
	Permethrin	100	55	R	10/23/2016
	Pirimiphos-Methyl	100	100	S	10/23/2016
Manambotra Sud,	Bendiocarb	100	100	S	01/23/2017
	DDT	100	100	S	01/22/2017

Sentinel site	Insecticide tested	WHO tube tests			Test completion date
		N# mosquitos tested	24h % Observed mortality	Resistance status	
Farafangana)	Deltamethrin	100	100	S	01/26/2017
	Lambda-Cyhalothrin	100	100	S	01/26/2017
	Permethrin	100	100	S	01/23/2017
	Pirimiphos-Methyl	100	100	S	01/22/2017
Lopary (Vangaindrano)	Bendiocarb	100	100	S	01/18/2017
	DDT				01/18/2017
	Deltamethrin	100	100	S	01/19/2017
	Lambda-Cyhalothrin	100	100	S	01/19/2017
	Permethrin	100	100	S	01/19/2017
	Pirimiphos-Methyl	100	100	S	01/19/2017

Table 10: Results Of Susceptibility Tests for *Anopheles funestus*

Sentinel site	Insecticide	N# mosquitos tested	24h % Observed mortality	Resistance status	Test completion dates
Manambotra Sud (Farafangana)	Deltamethrin	50	100	S	01/25/2017
	Pirimiphos methyl	50	100	S	01/25/2017
Vohitrambato	Deltamethrin	50	100	S	10/14/2016
	Pirimiphos methyl	50	100	S	11/11/2016
Ambodifaho Brickaville	Deltamethrin	50	100	S	11/12/2016
	Pirimiphos methyl	50	100	S	12/10/2016
Vavatenina	Deltamethrin	50	100	S	11/06/2016
	Pirimiphos methyl	50	100	S	11/08/2016

Table 11: Results Of Susceptibility Tests for *Anopheles mascarensis*

Sentinel sites		N# mosquitos tested	24h % Observed mortality	Resistance status	Test completion date
Manambotra Sud, Farafangana	Deltamethrin	50	100	S	02/20/2017
	Pirimiphos	50	100	S	02/20/2017

Sentinel sites		N# mosquitos tested	24h % Observed mortality	Resistance status	Test completion date
	methyl				
Sahamatevina, Brickaville	Permethrin	50	100	S	02/15/2017
	Pirimiphos methyl	50	100	S	02/15/2017
Vavatenina	Deltamethrin	50	100	S	03/20/2017
	Pirimiphos methyl	50	100	S	03/20/2017
Mahambo, Fenerive Est	Pirimiphos methyl	50	100	S	10/12/2016
	Deltamethrin	50	100	S	10/14/2016

Table 12: Resistance Intensity Observed for *An. gambiae* s.l.

Site	species tested	INSECTICIDES	# mosquitoes tested (T)	# mosquitos control (C)	% Kd 30mn		Test completion date
					T	C	
Vohitrambato, Toamasina II	*Anopheles gambiae* s.l.	Detamethrin 5x	100	50	100%	0%	05/25/2017
Vohitrambato, Toamasina II	*Anopheles gambiae* s.l.	Permethrin 5x	100	50	100%	0%	05/27/2017
Vohitrambato, Toamasina II	*Anopheles gambiae* s.l.	L-cyhalothrin 5x	100	50	100%	0%	05/27/2017
Vavatenina	*Anopheles gambiae* s.l.	Permethrin 5x	100	50	100%	0%	05/25/2017
Vavatenina	*Anopheles gambiae* s.l.	Deltamethine 5x	100	50	100%	0%	05/25/2017
Lanivo	*Anopheles gambiae* s.l.	Permethrin 5x	100	50	100%	0%	05/15/2017
Ambodifaho, Brickaville	*Anopheles gambiae* s.l.	Detamethrin 5x	100	50	100%	0%	05/29/2017
Ambodifaho, Brickaville	*Anopheles gambiae* s.l.	Permethrin 5x	100	50	100%	0%	05/29/2017

Table 13: Results Of Synergists Tests for Resistant or Possibly Resistant *Anopheles gambiae* s.l. (WHO tube test)

site	insecticide	#mosquitos PBO only		#mosquitos PBO + insecticide		#mosquitos insecticide only		Control		Test completion date
		T	24h % mortality	T	24h % mortality	T	24h % mortality	T	24h % mortality	
Ambodifaho	Deltamethrin	50	0	50	100	50	80	25	0	05/23/2017

Ambodifaho	Permethrin	50	0	50	100	50	84	25	0	05/23/2017
Vohitrambato	Deltamethrin	50	0	50	100	50	72	25	0	05/25/2017
Vohitrambato	Permethrin	50	0	50	100	50	76	25	0	05/27/2017
Vohitrambato	L-cyhalothrin	50	0	50	100	50	93	25	0	05/27/2017
Vavatenina	Deltamethrin	50	0	50	100	50	58	25	0	05/25/2017
Vavatenina	Permethrin	50	0	50	100	50	88	25	0	05/21/2017
Lanivo	Permethrin	50	0	50	100	50	55	25	0	05/16/2017

Table 14: Parity Rates

Month	Species	Manambonitra Sud Farafangana			Lopary Vangaindrano			Lanivo			Ambodifaho Brickaville			Vohitrambato Toamasina II			Mahambo Fenerive Est			Vavatenina		
		# dissected	# parous	Parity rate	# dissected	# parous	Parity rate	# dissected	# parous	Parity rate	# dissected	# parous	Parity rate	# dissected	# parous	Parity rate	# dissected	# parous	Parity rate	# dissected	# parous	Parity rate
June	*An.gambiae* sl	9.0	8.0	88.0	9.0	2.0	22.2	45.0	45.0	100.0												
	An.. funestus	0.0	0.0		5.0	0.0	0.0	21.0	21.0	100.0												
	An. mascariensis	10.0	8.0	80.0	0.0	0.0		0.0	0.0	0.0												
July*	*An.gambiae* sl	0.0	0.0		14.0	3.0	21.4	30.0	13.0	43.3												
	An.. funestus	2.0	1.0	50.0	16.0	7.0	43.8	15.0	6.0	40.0												
	An. mascariensis	2.0	1.0	50.0	1.0	0.0	0.0	0.0	0.0	0.0												
August*	*An.gambiae* sl	0.0	0.0		1.0	0.0	0.0	3.0	0.0	0.0	25.0	25.0	100.0	8.0	8.0	100.0	6.0	6.0	100.0	32.0	28.0	87.5
	An.. funestus	2.0	2.0	100.0	23.0	3.0	13.0	4.0	0.0	0.0	0.0	0.0	0.0	38.0	31.0	81.6	0.0	0.0		20.0	19.0	95.0
	An. mascariensis	0.0	0.0		0.0	0.0		0.0	0.0	0.0	0.0	0.0	0.0	63.0	50.0	79.4	12.0	9.0	75.0	12.0	12.0	100.0
September	*An.gambiae* sl	12.0	0.0	0.0	1.0	0.0	0.0	3.0	2.0	66.7	11.0	8.0	72.7	47.0	31.0	66.0				9.0	9.0	100.0
	An.. funestus	0.0	0.0		29.0	10.0	34.5	3.0	3.0	100.0	0.0	0.0	0.0	4.0	3.0	75.0				9.0	8.0	88.9
	An. mascariensis	0.0	0.0		0.0	0.0		2.0	0.0	0.0	0.0	0.0	0.0	14.0	11.0	78.6	5.0	5.0	100.0	8.0	8.0	100.0
October	*An.gambiae* sl	19.0	4.0	21.1	11.0	3.0	27.3	1.0	0.0	0.0	15.0	4.0	26.7	54.0	26.0	48.1				105.0	67.0	63.8
	An.. funestus	4.0	1.0	25.0	8.0	7.0	87.5	1.0	1.0	100.0	0.0	0.0	0.0	13.0	7.0	53.8	7.0	7.0	100.0	20.0	18.0	90.0
	An. mascariensis	0.0	0.0					0.0	0.0	0.0	0.0	0.0	0.0	1.0	0.0	0.0				22.0	19.0	86.4
November	*An.gambiae* sl	7.0	0.0	0.0	15.0	9.0	60.0	2.0	2.0	100.0	20.0	3.0	15.0	120.0	50.0	41.7				37.0	28.0	75.7
	An.. funestus	0.0	0.0					0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		9.0	5.0	55.6	1.0	1.0	100.0

	An. mascarensis	5.0	0.0	0.0				0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0							
December	*An.gambiae* sl	5.0	0.0	0.0	6.0	3.0	50.0	5.0	1.0	20.0	12.0	5.0	41.7	78.0	35.0	44.9	3.0	0.0	0.0	66.0	45.0	68.2
	An.. funestus	0.0	0.0		0.0	0.0		7.0	7.0	100.0	0.0	0.0	0.0	12.0	6.0	50.0				3.0	3.0	100.0
	An. mascarensis	0.0	0.0		0.0	0.0		0.0	0.0	0.0	0.0	0.0	0.0	2.0	1.0	50.0				2.0	1.0	50.0
January	*An.gambiae* sl	7.0	7.0	100.0	7.0	3.0	42.9	3.0	3.0	100.0	32.0	20.0	62.5	72.0	29.0	40.3	22.0	12.0	54.5	40.0	26.0	65.0
	An.. funestus	0.0	0.0		0.0	0.0		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		15.0	8.0	53.3
	An. mascarensis	0.0	0.0		0.0	0.0		1.0	1.0	100.0	0.0	0.0	0.0	0.0	0.0	0.0	3.0	0.0	0.0	2.0	1.0	50.0
February	*An.gambiae* sl	7.0	0.0	0.0	8.0	5.0	62.5	8.0	4.0	50.0	11.0	5.0	45.5	40.0	24.0	60.0				19.0	11.0	57.9
	An.. funestus	0.0	0.0		0.0	0.0		0.0	0.0	0.0	0.0	0.0	0.0	5.0	4.0	80.0				3.0	3.0	100.0
	An. mascarensis	0.0	0.0		0.0	0.0		1.0	1.0	100.0	0.0	0.0	0.0	2.0	2.0	100.0	23.0	14.0	60.9	10.0	5.0	50.0
March	*An.gambiae* sl	22.0	14.0	63.6	8.0	3.0	37.5	19.0	11.0	57.9	15.0	14.0	93.3	19.0	7.0	36.8				19.0	11.0	57.9
	An.. funestus	0.0	0.0		0.0	0.0		0.0	0.0	0.0	0.0	0.0	0.0	5.0	1.0	20.0				0.0	0.0	#DIV/0!
	An. mascarensis	0.0	0.0		0.0	0.0		0.0	0.0	0.0	0.0	0.0	0.0	1.0	1.0	100.0				7.0	3.0	42.9
April	*An.gambiae* sl										49.0	39.0	79.6	22.0	14.0	63.6				30.0	14.0	46.7
	An.. funestus										0.0	0.0	0.0	1.0	0.0	0.0	30.0	20.0	66.7	5.0	3.0	60.0
	An. mascarensis										0.0	0.0	0.0	3.0	3.0	100.0				5.0	2.0	40.0
May	*An.gambiae* sl										43.0	22.0	51.2	23.0	12.0	52.2				27.0	3.0	11.1
	An.. funestus										0.0	0.0	0.0	0.0	0.0					0.0	0.0	#DIV/0!
	An. mascarensis										0.0	0.0	0.0	0.0	0.0					0.0	0.0	#DIV/0!

*July baseline for South Eastern sites

August baseline for East coast sites

Table 15: Susceptibility of *Anopheles Gambiae* S.L. To Clothianidin

Sites	Test	# tested	KD after 10 min	KD after 15 min	KD after 20 min	KD after 30 min	KD after 40 min	KD after 50 min	KD after 60 min	dead after 24h	Dead day2	Dead day3	Dead day4	Dead day5	Dead day6	Dead day7	Corrected mortality (%)
Test with Kisumu strain	Total Test	100	0	0	0	13	24	31	41	83	83	90	92	100	100	100	
	Total Control	50	0	0	0	0	0	0	0	0	2	2	2	2	2	2	
Ambodifaho	Total Test	100	0	0	0	2	3	6	9	46	68	83	88	100	100	100	
	Total Control	50	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Vohitrambato	Total Test	100	0	3	3	8	9	11	14	66	86	97	100	100	100	100	100
	Total Control	50	0	0	0	0	0	0	0	0	0	0	2	2	2	3	
Mahambo	Total Test	100	0	0	0	2	0	0	7	56	82	88	91	93	97	100	100
	Total Control	50	0	0	0	0	0	0	0	2	2	2	3	3	4	4	
Vavatenina	Total Test	100	0	0	2	6	7	16	22	89	95	98	99	100	100	100	
	Total Control	50	0	0	0	0	0	0	0	0	0	0	0	1	2	2	
Vohipeno	Total Test	100	0	0	0	18	26	30	46	82	82	88	95	100	100	100	
	Total Control	50	0	0	0	0	0	0	0	0	2	2	2	2	2	2	
Manambotra Sud	Total Test	100	0	0	0	13	31	41	48	76	86	93	95	100	100	100	100
	Total Control	50	0	0	0	0	0	0	0	0	4	4	4	4	4	4	
Lopary	Total Test	100	0	0	0	24	28	35	44	83	91	92	95	100	100	100	100
	Total Control	50	0	0	0	0	0	0	0	0	3	3	3	3	3	3	
Mananjary	Total Test	100	0	1	8	12	19	22	26	57	82	88	93	98	100	100	100
	Total Control	50	0	0	0	0	0	0	0	2	2	2	2	2	3	3	
Manakara	Total Test	100	0	0	0	11	23	29	47	81	85	90	95	100	100	100	
	Total Control	50	0	0	0	0	0	0	0	0	0	0	2	2	2	2	
Vondrozo	Total Test	100	0	0	0	31	31	42	47	90	91	91	94	100	100	100	
	Total Control	50	0	0	0	0	0	0	0	0	0	0	1	2	2	2	

Table 16: Breakdown of Number of Mosquitoes Collected in Various Outdoor Resting Place.

Sites	Species	Pit shelters	Wall	Natural hole	Under floor	Tree hole	Cowshed	Garbage dump	Bush	Eave
Ambodifaho	*An. gambiae s.l.*	16	22	5						
Lanivo	*An. gambiae s.l.*	7	3	1						
	An. funestus	3			2					
	An. mascarensis			4						
Vohitrambato	*An. gambiae s.l.*	5	3		6	2	3	5	8	
	An. funestus	1				1	1			
	An. mascarensis		1		2	2				
	Other *Anopheles*	5		4	3	3	8	1	1	
	Other *Genus*	7	1		5	2	8	6		
Vavatenina	*An. gambiae s.l.*	4		2	7	3	4	10	1	
	An. funestus					1	1	1	2	1
	An. mascarensis	1				11		2	1	2
	Other *Anopheles*			1	1	4				2
	Other *Genus*	11	1		2	3	10	6		
Manambotra sud	*An. gambiae s.l.*	5								
	An. funestus									
	An. mascarensis	2		1						
	Other *Genus*	33			20					
Lopary	*An. gambiae s.l.*	5		1	2					
	An. funestus	4		1						
	Other *Anopheles*	3		4	10					
	Other *Genus*	1		8	31					
Mahambo	*An. gambiae s.l.*	7		40	47					
	An. mascarensis			2	3					
	Other *Anopheles*	13		19	21					
	Other *Genus*	19		75	63					