PMI | Africa IRS (AIRS) Project
Indoor Residual Spraying (IRS 2) Task Order Six

AIRS RWANDA 2016 ENTOMOLOGY ANNUAL REPORT

RWANDA:
2016 ENTOMOLOGICAL MONITORING

TABLE OF CONTENTS

LIST OF TABLES

TABLE OF FIGURES

ACRONYMS

AIRS	Africa Indoor Residual Spraying
An.	*Anopheles*
b/p/h	Bites/person/hour
CS	Capsule Suspension
EIR	Entomological Inoculation Rate
ELISA	Enzyme-linked Immunosorbent Assay
HBR	Human Biting Rate
HLC	Human Landing Catch
IRS	Indoor Residual Spray
LLIN	Long-lasting Insecticide-treated Nets
MOH	Ministry of Health
OP	Organophosphate
PCR	Polymerase Chain Reaction
PMI	President's Malaria Initiative
PNP	Plastered Not Painted
PP	Plastered and Painted
PSC	Pyrethrum Spray Catch
s.l.	sensu lato
s.s.	sensu stricto
WG	Wettable granules
WHO	World Health Organization
WP	Wettable Powder

EXECUTIVE SUMMARY

During the reporting period (October 2015-October 31 2016), entomological monthly data collection was conducted in three indoor residual spray (IRS) districts, Bugesera, Nyagatare, and Kirehe, and one non-IRS (control) district, Ngoma. Adult mosquitoes were sampled using pyrethrum spray catches (PSC) and human landing catches (HLC) to assess vector species composition, seasonality, and behavior. WHO cone bioassays were conducted to assess the quality of spraying as well as determine insecticide decay rates on sprayed surfaces. Tests were conducted on three wall surface types: mud, plastered not painted, and plastered and painted. Identification of malaria vectors was done both morphologically and using polymerase chain reaction (PCR).

A total of 12,347 *Anopheles* species were collected using HLC; 12,052 (97.7%) were identified morphologically as *An. gambiae* s.l. Molecular identification was conducted on 188 *An. gambiae* s.l. collected from January through March 2016. Collections showed 140 (74.5%) were *An. arabiensis* and the rest 48 (25.5%) were *An. gambiae* s.s. In all the IRS districts *An. arabiensis* constituted over 70% of the mosquitoes collected, while in the control district *An. gambiae* s.s constituted 55%.

In 2013, mosquito samples from two of the IRS districts (Nyagatare-Mimuli site and Bugesera-Mareba site) were sent to ICIPE, Nairobi for PCR identification. *Anopheles arabiensis* was the dominant species (76%) identified while the rest were identified as *An. gambiae* s.s. A further 1355 mosquito samples collected from 15 sampling sites within a one year period were sent to KEMRI (Kilifi, Kenya) for PCR – ID analysis in 2014. A total of 515 samples were either identified as *An. gambiae* s.s. (65.5%) or *An. arabiensis* (34.5%) and the remaining samples did not amplify. The mosquito samples were preserved in silica gel and stored for about a year before sent to KEMRI for laboratory analysis. Though the laboratory did not indicate the most likely reason/s why high percentage of the mosquito samples failed to amplify, degradation of DNA cannot be ruled out.

An. gambiae s.l. generally showed slightly more exophagic than endophagic tendency in the four districts, including the control district: Bugesera 44.7% endophagy vs 55.3% exophagy, Kirehe 30% endophagy vs 70% exophagy, Nyagatare 47.7% endophagy vs 52.6% exophagy, and Ngoma 39.8 endophagy vs 60.2% exophagy. There was no significant difference between the indoor and outdoor landing collections in either site of Bugesera district and the Rukomo site of Nyagatare district (p > 0.05). There was a significant difference between the indoor and outdoor landing collections in both sites of Kirehe district, Nyagatare site of Nyagatare district, and Ngoma (control district).

High biting tendency was observed as early as 1900-2000h in Kirehe and Nyagatare. In Nyagatare, approximately 4.5 bites per person per hour was recorded in the 1800 to 19:00 hour. An increase in biting was observed at around 2200h (4.25 bites/person/hour), peaked to

5.5 bites/person/hour (outdoors) at 0001h, and remained high until 0003h. In Kirehe, biting was 1.3 bites/person/hour at 1800h; it rose to 2.42 bites/person/hour and peaked at 0001h (4.5 bites/person/hour). In Bugesera, hourly biting rose at around 2100h, dropped in the next hour, and remained relatively constant through the rest of the night.

There was a significant difference in average monthly vector PSC collections in all the intervention districts and the control district ($p < 0.05$). Nyagatare district showed the highest average vector density (0.5 *An. gambiae* s.l./house/day) among the IRS districts (Bugesera was 0.4 *An. gambiae* s.l./house/day and Kirehe 0.3 *An. gambiae* s.l./house/day). The control (non-IRS) district showed the highest density, 1.0 *An. gambiae* s.l./house/day. Two vector density peak seasons were observed through the reporting period; one in February/March and the other in September/October.

No clear trend was observed in any district for parity at any time during the reporting period. Out of the 128 *An. gambiae* s.l. in the four districts which were tested in January (pre-spray) for *Plasmodium* circumsporozoite protein, none tested positive. During the post-spray period (April-August), 47 *An. gambiae* s.l. tested positive out of 1,400 *An. gambiae* s.l. from the four districts. In September (spray period), 464 *An. gambiae* s.l. were tested out of which 9 tested positive.

A total of 36 blood fed *An. gambiae* s.l. samples from the collections made in January-March were tested for vertebrate host blood source (human, bovine, and ovine). Human blood indices were as follows: Nyagatare 50%; Bugesera 57.1%, Ngoma 27.3%, and Kirehe 0%.

Cone bioassays conducted within one week of spraying with carbamate (bendiocarb 80WP) to assess the quality of spraying during the February spray campaign recorded 100% mortality of susceptible *An. gambiae* s.l., indicating quality spraying took place. Monthly cone bioassay tests showed that mortality of over 80% was observed on all the three wall surface types in the two districts (Kirehe and Nyagatare) up to four months after spraying.

Cone bioassays conducted within one week and one month (T_1) of OP insecticide (Actellic 300CS) spraying in September 2016 showed 100% mortality of susceptible *An. gambiae* s.s., demonstrating satisfactory application of the insecticide. Tests conducted two months post spray also showed 100% mortality of the test mosquitoes on all wall types. Monthly decay rate monitoring will continue until the efficacy of Actellic 300 CS drops below 80%.

1. INTRODUCTION

In September 2014, Abt Associates was awarded a three-year Africa-wide Indoor Residual Spraying (AIRS) project, IRS2 Task Order 6, which is funded by USAID under the United States President's Malaria Initiative (PMI). The IRS2 Task Order 6 has now been extended to September 2018. The President's Malaria Initiative Africa Indoor Residual Spraying (PMI AIRS) Project provides support in Indoor Residual Spraying (IRS) implementation, including technical support for entomological monitoring in 17 African countries.

Since 2012, AIRS Rwanda has implemented two spray campaigns (February/March and September/October) annually in Rwanda. In 2012 and part of 2013, IRS was conducted using a pyrethroid (deltamethrin 250WG). There was a shift to a carbamate (bendiocarb 80WP) from September 2013 to February/March 2016. In September/October 2016, there was another shift to an organophosphate (OP), Actellic 300CS.

This report covers entomological monitoring activities which were conducted during the period October 1, 2015 to October 31 2016. The entomological monitoring activities were aimed at:

- Assessing malaria vector density and species composition in intervention and selected control areas
- Understanding vector feeding times and locations
- Determination of parity of malaria vectors through ovary dissection
- Monitoring the quality of insecticide application and insecticide decay rates
- Determination of sporozoite rates and EIRs

2. DATA COLLECTION SITES AND METHODS

2.1 STUDY SITES

Data collection was conducted in three IRS districts, Bugesera, Nyagatare, and Kirehe, and one non-IRS (control) district, Ngoma (Figure 1). In each IRS district, two sectors were selected as data collection sites. Table 1 summarizes the data collection sites in the districts.

FIGURE 1: MAP OF RWANDA SHOWING DATA COLLECTION DISTRICTS
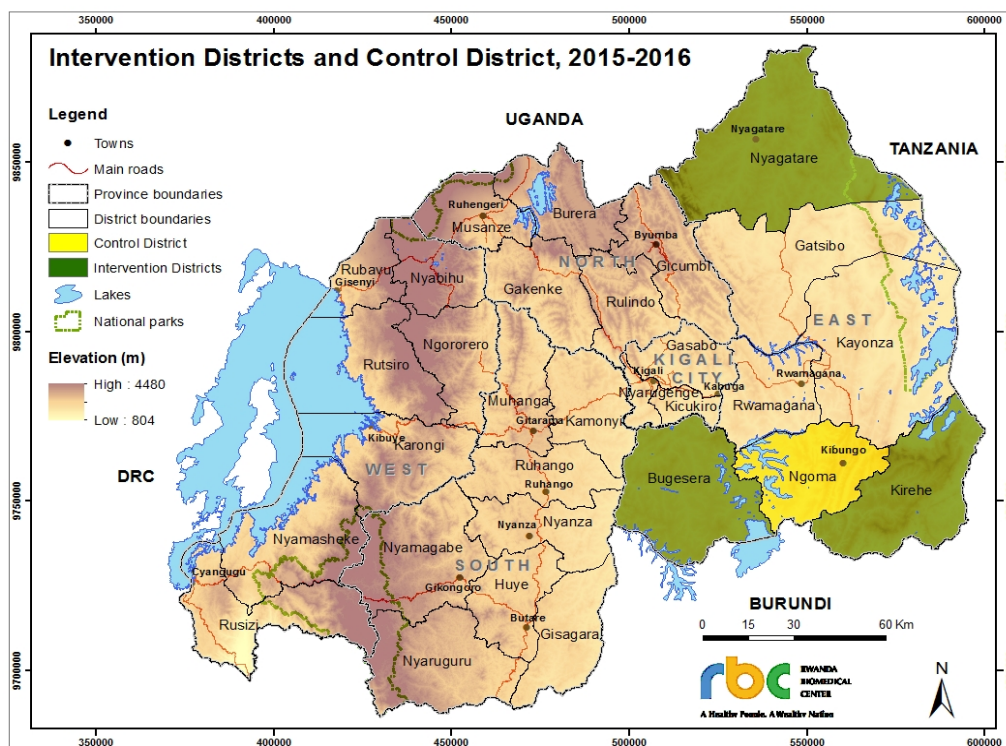
TABLE 1: DATA COLLECTION (SENTINEL) SITES

District	Data Collection Sites (sectors)	Spray Status
Bugesera	Nyarugenge, Musenyi	Sprayed
Nyagatare	Nyagatare, Rukomo	Sprayed
Kirehe	Gatore, Nyamugali	Sprayed
Ngoma (control)	Remera	Not sprayed

All intervention sites were sprayed with a carbamate (bendiocarb 80WP) in September/October 2015 and February/March 2016. In September/October 2016, two of the districts (Kirehe and Nyagatare)

were sprayed using an OP (Actellic 300CS), while in November 2016 Bugesera was sprayed with a carbamate (bendiocarb 80WP). Kirehe and Nyagatare are IRS PMI-supported districts while the Government of Rwanda supports Bugesera through the Ministry of Health (MOH).The control district was not sprayed during any of the rounds. Entomological data collection was done monthly in the three intervention districts and the control district.

2.2 DATA COLLECTION METHODS

Blood seeking and resting adult mosquito collections were conducted in two sites in each IRS district and one site in the control district using human landing catch (HLC) and pyrethrum spray catch (PSC) collection methods, respectively, on a monthly basis.

Spray quality was assessed using World Health Organization (WHO) cone/wall bioassays, which were conducted within one week of the start of the spray campaign. Cone/wall bioassays then were conducted on a monthly basis after the spray round to assess insecticide decay rate.

2.2.1 HUMAN LANDING CATCH

HLC was done in three households in each site for two consecutive nights per month; therefore, data were collected for four nights per district per month. A team of collectors was composed of four people per house per night; two collectors per house collected from 1800h to 2400h and two others collected from 2400h to 600h. In each of the sites, the collectors switched places (outdoor vs indoor) every hour. Outdoor mosquito collection was carried out about six meters from the door of each of the three sampled houses. Collectors adjusted their clothing so that the legs were exposed up to the knees. At the end of the collection, mosquitoes were transported to the field lab and were identified using taxonomic keys.[1]

2.2.2 PYRETHRUM SPRAY CATCH

PSC was used to sample indoor resting mosquitoes in 15 houses per day in each of the sites for two consecutive days every month. Collections were carried out in the morning between 0600h and 0900h. Before the PSC was performed, all occupants were politely asked to move out of the house. The floor was covered with white sheets. Windows and other mosquito escape routes around the house were closed and the house was sprayed with BOP insecticide that contains tetramethrin 0.30% w/w, cypermethrin 0.07% w/w and D-Allethrin 0.12%w/w. Ten minutes after spraying, collectors collected all the mosquitoes that were knocked downed from the sheets and sorted them by species. The abdominal status of all female anophelines was determined, and individuals were categorized according to their blood digestion stage (unfed, freshly fed, half-gravid, and gravid females).

2.3 IDENTIFICATION OF MALARIA VECTORS

Anopheles mosquitoes collected through HLC and PSC were identified to the species level morphologically. Polymerase chain reaction (PCR) was conducted on a sample of the *An. gambiae* s.l. collected during the period January-March 2016 for molecular identification.

---

[1] Gillies MT and Coetzee C. 1987. *A Supplement to the Anopheline of Africa South of the Sahara*. Johannesburg, SA: South African Institute for Medical Research.

2.4   DETERMINATION OF PARITY

Ovary dissections were conducted on a sample of females belonging to *An. gambiae* s.l., from HLC collections. The dissections were conducted under a dissecting microscope to determine the parity rate based on coiling of ovarian tracheoles (Detinova 1962).[2]

2.5   QUALITY OF SPRAY AND INSECTICIDE DECAY RATE

Quality of spraying and insecticide decay rates were assessed using the WHO-approved protocol.[3] Test cones were placed at three different heights on sprayed wall surfaces while the control tests were fixed on surfaces known to be free of insecticide. Batches of 10 mosquitoes, two to five day-old non-blood-fed female *An. gambiae* s.s. (Kisumu strain), were introduced into each of the cones. The mosquitoes were left in the cones exposed to the insecticide for 30 minutes, after which they were transferred to paper cups.

Knockdown was observed and recorded after 30 minutes exposure and mortality was recorded after a 24-hour holding period. When mortality in the control cones was between 5% and 20%, the results of the treated samples were corrected using Abbot's formula.

---

[2] Age-grouping methods in diptera of medical importance, with special reference to some vectors of malaria / T. S. Detinova ; [with] an Annex on the ovary and ovarioles of mosquitos (with glossary) by D. S. Bertram - See more at: http://apps.who.int/iris/handle/10665/41724#sthash.YVKq2sql.dpuf

[3] WHO. 1998. *Test procedures for insecticide resistance monitoring in malaria vectors, bio-efficacy and persistence of insecticides on treated surfaces*. Document WHO/CDS/MAL/98.12. Geneva.

3. RESULTS, DISCUSSION, AND CONCLUSIONS

3.1   SPECIES COMPOSITION AND VECTOR SEASONALITY

During the reporting period October 2015 to October 2016, a total of 12,347 adult female *An. mosquitoes* were collected using HLC. The species composition was 12,052 (97.7%) *An. gambiae* s.l., 19 *An. funestus group*, 112 *An. pharoensis*, 148 *An. ziemmani*, 13 *An. coustani,* and 3 *An. maculpalpis*. Figure 2 shows disaggregation of the anopheline species. Only *An. gambiae* s.l. and *The funestus* group are known to transmit malaria (vectors of malaria) in Rwanda. Other mosquito species collected are non-vectors of malaria.

FIGURE 2: *ANOPHELES* SPECIES COMPOSITION
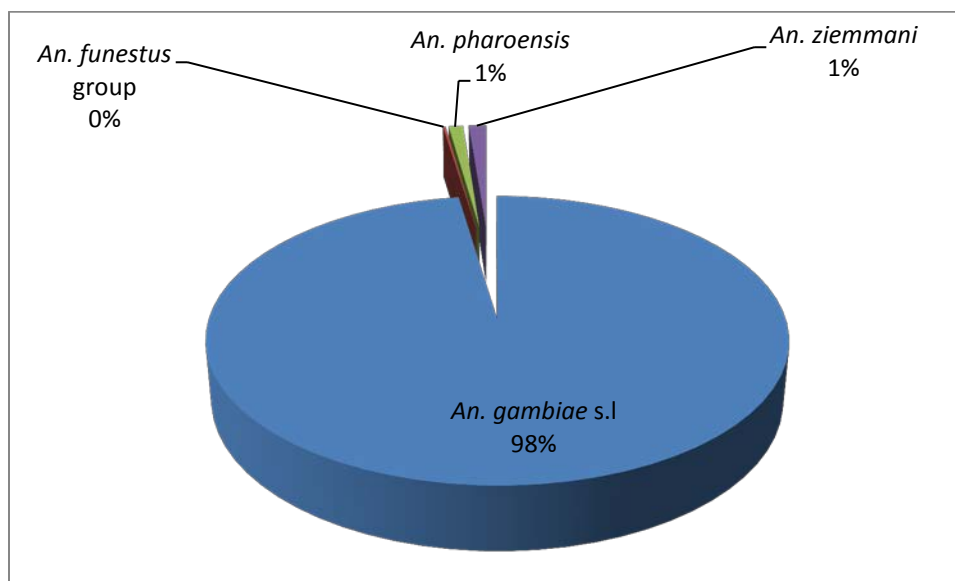
An. gambiae s.l. was the most prevalent malaria vector throughout the data collection period in both the intervention and control sites.

Molecular identification conducted on 188 *An. gambiae* s.l. that were sampled from January-March 2016 collections showed 140 (74.5%) were *An. arabiensis* and the remaining 48 (25.5%) were *An. gambiae* s.s. (Table 2).

13

TABLE 2: PCR IDENTIFICATION OF *AN. GAMBIAE* S.L.

District	Tested	*An. arabiensis*		*An. gambiae* s.s	
		#	%	#	%
Bugesera	24	19	79.2	5	20
Gisagara*	27	25	92.6	2	7.4
Kirehe	44	35	79.5	9	20.5
Ngoma	29	13	44.8	16	55.2
Nyagatare	65	48	75.0	16	25.0

*Gisagara District: IRS data collection district supported by PMI in 2015 but currently is supported by the MOH.

3.2 VECTOR FEEDING TIME AND LOCATION

Although *An. gambiae* s.l. generally showed slightly more exophagic than endophagic tendency in the four districts, including the control district, there was no significant difference between the indoor and outdoor landing collections in both sites of Bugesera district and the Rukomo site of Nyagatare district (p > 0.05). There was a significant difference between the indoor and outdoor landing collections in both sites of Kirehe district, Nyagatare site of Nyagatare district, and Ngoma (control district) (Table 3). The percentage endophagy/exophagy was recorded as follows: Bugesera 44.7% endophagy vs 55.3% exophagy, Kirehe 30% endophagy vs 70% exophagy, Nyagatare 47.7% endophagy vs 52.6% exophagy, and Ngoma 39.8 endophagy vs 60.2% exophagy (Figure 3). This could be attributed to the fact that a higher proportion of the *An. gambiae* s.l. were identified as *An. arabiensis,* whose tendency is to bite outdoors rather than indoors[4] (see Table 2 above).

TABLE 3: INDOOR AND OUTDOOR BITING

District	Site	In	Out	In: Out Ratio	P-value	
Bugesera	Nyarugenge	159	192	33:54.7	0.078	NS
	Musenyi	50	63	44.3:55.8	0.2213	NS
Kirehe	Gatore	1066	1218	46.7:53.3	0.0015	S
	Nyamugali	73	254	22.3:77.7	1.39×10^{-23}	S
Nyagatare	Rukomo	1960	1873	51.1:48.9	0.1599	NS
	Nyagatare	1535	2438	38.6:61.4	1.5×10^{-46}	S
Ngoma	Remera	465	579	44.5:55.5	0.000418	S

A general observation through the months shows that there are two peak biting seasons, namely; March to May and September to December (Figure 3). This is also reflected in the past years as seen in Annex A.

[4] http://www.publichealthjrnl.com/article/S0033-3506(16)30243-8/fulltext?rss=yes

Figure 3: *An. gambiae* s.l. Average Monthly Biting Trends
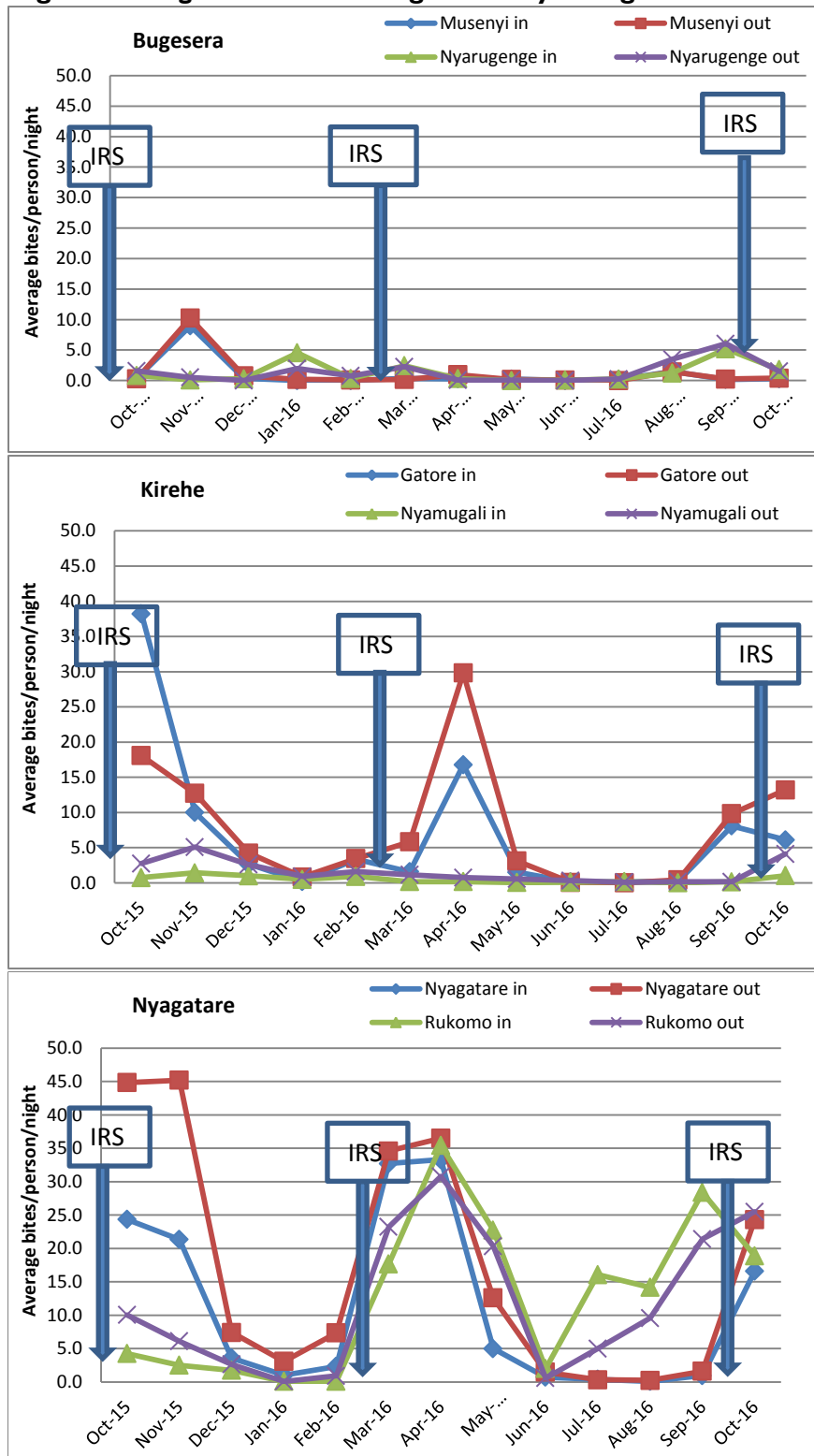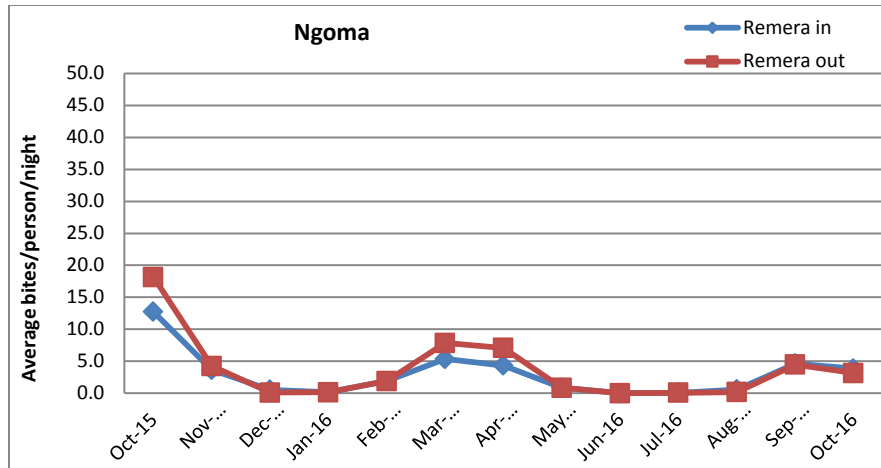
Ngoma

Hourly biting rates per person varied across the four districts; they were highest in Nyagatare (average 10.5 b/p/h indoors and 12.5 b/p/h outdoors), followed by Kirehe (average 3.7 b/p/h indoors and 4.4 b/p/h outdoors) and Ngoma (control district) (average 2.9 b/p/h indoors and 3.7 b/p/h outdoors). Bugesera showed the least mean bites/person/hour (average 0.7 b/p/h indoors and 0.9 b/p/h outdoors). The higher biting in Nyagatare and Kirehe could be due to the fact that, apart from the rains, there is considerably more rice farming in those two districts than in the other districts. This creates more breeding sites for the vector and consequently higher vector density, which increases the chances of biting. Despite the factors that favor increases in vectors, it would be expected that there would be a trend across the months corresponding to application of insecticide. This does not however seem to be the case.

Hourly biting was slightly higher outdoors than indoors in all four districts. In Nyagatare and Kirehe districts, biting was generally higher at 1900h and dropped at 2000h. In Nyagatare, approximately 4.5 bites per person per hour was recorded in the 1800 to 1900 hour. An increase in biting density was observed at around 2200h (4.25 bites/person/hour), peaked to 5.5 bites/person/hour (outdoors) at 0001h, and remained high until 0003h. In Kirehe, biting was 1.3 bites/person/hour at 1800h; it rose to 2.42 bites/person/hour and peaked at 0001h (4.5 bites/person/hour). In Bugesera, hourly biting rose at around 2100h, dropped in the next hour, and remained relatively constant through the rest of the night. High biting tendency observed as early as 1900-2000h in Kirehe and Nyagatare is worrying since most people go to bed between 2100h-2200h and thus would not be protected by long-lasting insecticide-treated nets (LLIN) use. It is, however, important to note that more biting was observed outdoors than indoors. It is, therefore, very important for community members to remain indoors during the night, and to sleep under a bed net. Figure 4 shows average *An. gambiae* s.l. bites per person per hour through the night across the four districts.

FIGURE 4: HOURLY BITING
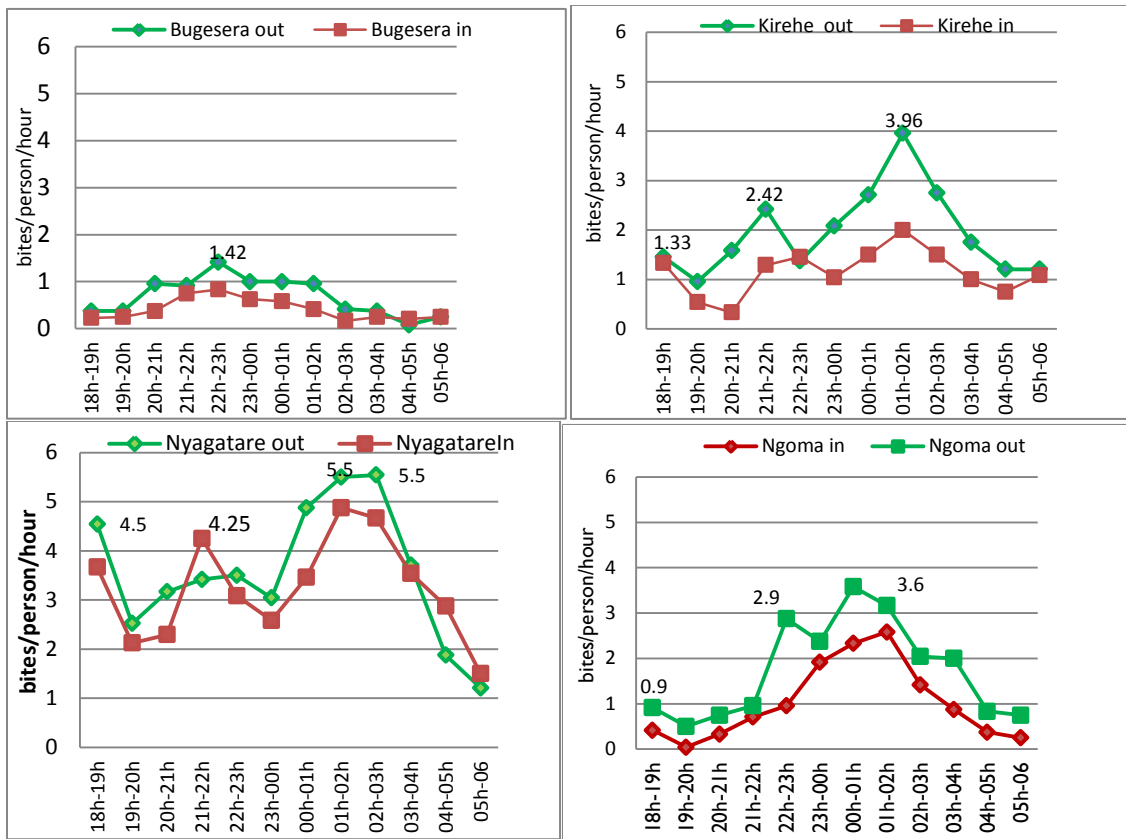
3.3   VECTOR DENSITY

A total of 1,382 female indoor-resting *An. gambiae* s.l. were collected using PSC in the IRS districts and the control districts during the period October 2015-October 2016. Table 4 shows the disaggregation of the collections and density in the districts.

TABLE 4: PSC COLLECTIONS AND VECTOR DENSITY

District	Nyagatare		Bugesera		Kirehe		Ngoma(control)	
	Total Collected	Vector Density	Total Collected	Vector Density	Total Collected	Vector Density	Total Collected	Vector Density
Oct-15	22	0.4	10	0.2	42	0.7	107	3.6
Nov-15	18	0.3	6	0.1	28	0.5	30	1.0
Dec-15	5	0.1	11	0.2	22	0.4	7	0.2
Jan-16	2	0.0	4	0.1	2	0.0	1	0.0
Feb-16	18	0.3	16	0.3	14	0.2	17	0.6
Mar-16	52	0.9	22	0.4	16	0.3	45	1.5
Apr-16	66	1.1	8	0.1	2	0.0	27	0.9
May-16	106	1.8	2	0.0	22	0.4	14	0.5
Jun-16	6	0.1	14	0.2	3	0.1	1	0.0
Jul-16	27	0.5	13	0.2	2	0.0	0	0.0
Aug-16	21	0.4	32	0.5	1	0.0	19	0.6
Sep-16	21	0.4	46	0.8	30	0.5	50	1.7
Oct-16	30	0.5	126	2.1	77	1.3	77	2.6
Av. monthly vector density	15	0.50	12	0.40	10	0.30	30	1.00
p –value*	0.025 (S)		0.005 (S)		0.0015 (S)		1	

*comparison between average collections (October 2015-October 2016) in IRS districts with the control district

There was a significant difference in average monthly vector PSC collections between all the intervention districts and the control district (p < 0.05). Although vector density varied through the months during the reporting season, Nyagatare district showed the highest average vector density among IRS districts (0.5 *An. gambiae* s.l./house/day), compared to Bugesera (0.4 *An. gambiae* s.l./house/day) and Kirehe (0.3 *An. gambiae* s.l./house/day). The control (non-IRS) district showed the highest density of one *An. gambiae* s.l./house/day relative to the intervention districts.

The results also show that there are two vector density peaks in the year: February-March and October November. Following the September 2015 spray, there was a gradual decrease in vector density over the months to average zero *An. gambiae* s. l. /per house/day in January 2016 in both intervention and control districts. Because the trend is seen in all four districts, it could be partly attributed to vector seasonality. Notwithstanding vector seasonality, the application of insecticide probably had an effect on the density, in that density in the control district was higher relative to the intervention districts through the reporting period except in Nyagatare in April-July 2016 and Bugesera in June-July 2016. In Nyagatare, the rise in vector population observed after the February spray could be a result of a combination of factors such as rice farming, which is practiced extensively in the district. It is also not clear if the insecticide resistance status of the malaria vectors to bendiocarb in Nyagatare could be a contributing factor. Insecticide resistance tests that were conducted in 2015 showed an average 90% mortality rate in one site, though 100% susceptibility was reported in two other sites in the district (see Figure 5 and Annex B). During the two-month spray season (September and October 2016), vector

density continued to rise. A significant drop in vector density is expected given the insecticide change to OP.

FIGURE 5: *AN. GAMBIAE* S.L. DENSITIES

Sep 2013-Oct 2014
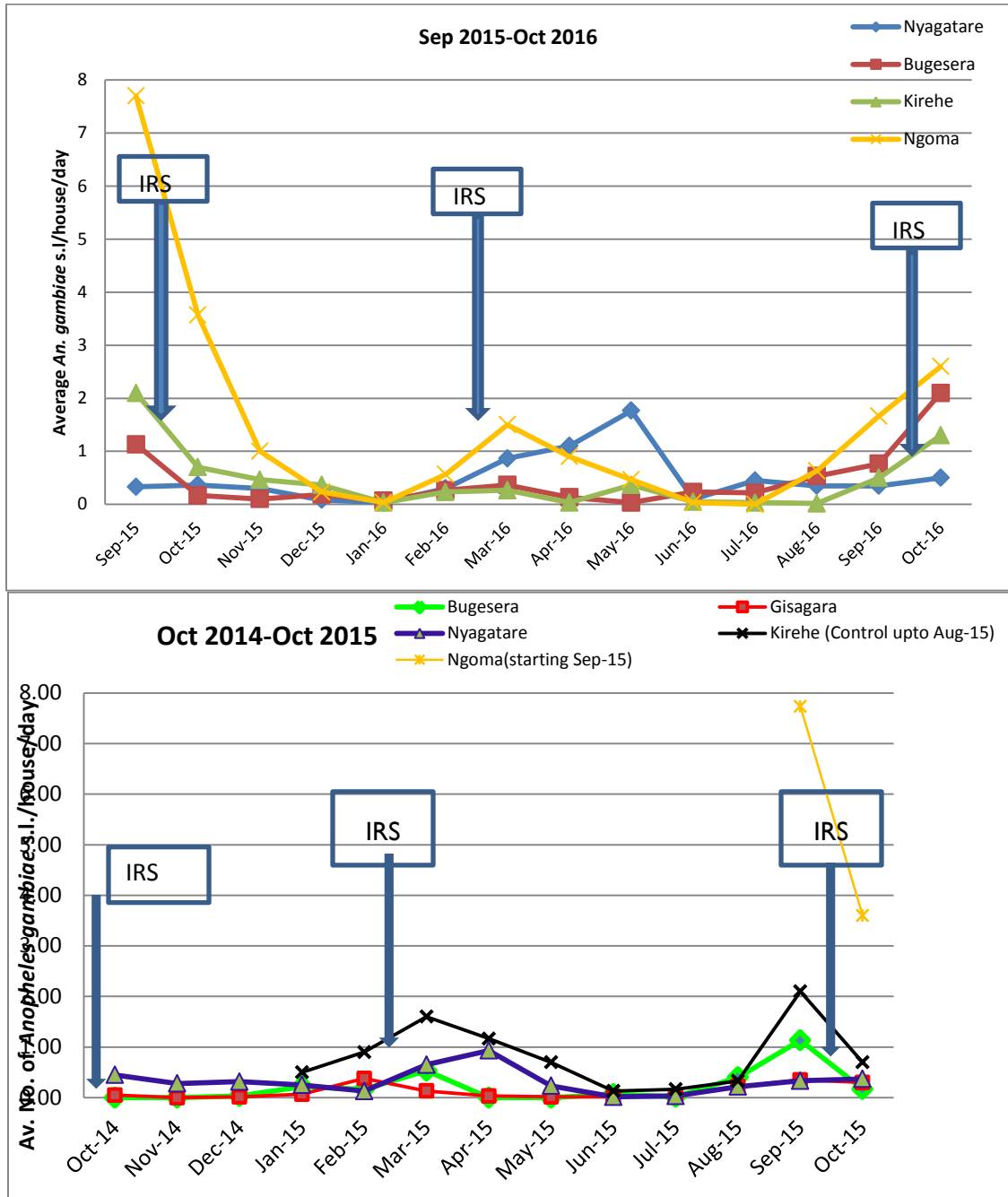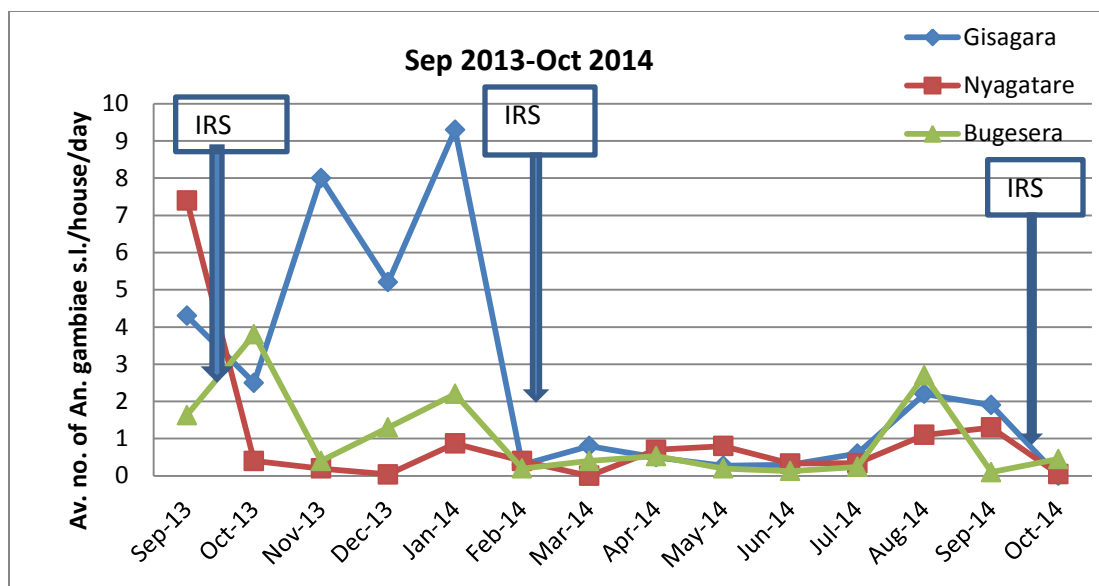
3.4 DETERMINATION OF PARITY

Ovary dissection of the *An. gambiae* s.l. collected through HLC was performed to determine parity rates. Table 5 shows average percentage parity during the period October 2015-October 2016. There was a significant difference ($p < 0.05$) between average number of parous *An. gambiae* s.l. in the control site and all intervention sites except Nyarugenge (Bugesera). While this difference could be attributed to spraying, it is not clear why there was no difference in parity status of mosquitoes collected in Nyarugenge and the control site.

TABLE 5: PARITY

District	Sector	Total Collected	Total *An. gambiae* s.l. Dissected	# Parous	% Parity	P-value	Result
Bugesera	Nyarugenge	351	133	43	32.33	0.294	NS
	Musenyi	110	50	9	18.00	0.0068	S
Kirehe	Gatore	2284	515	135	26.21	0.00027	S
	Nyamugali	325	162	19	11.73	1.6x10-9	S
Nyagatare	Nyagatare	3973	570	168	29.47	0.00933	S
	Rukomo	3833	609	163	26.77	0.00033	S
Ngoma	Remera	1237	415	155	37.35	1	

NS-Not Significant; S-Significant

Parity remained generally high with fluctuations through the months although there was no definitive trend observed in monthly percentage parity in the intervention sites or the control site. The effect of IRS on parity is not clear from these data and may be a result of the low numbers of mosquitoes analyzed during certain months (see Figure 6 and Annex C).

FIGURE 6: PARITY
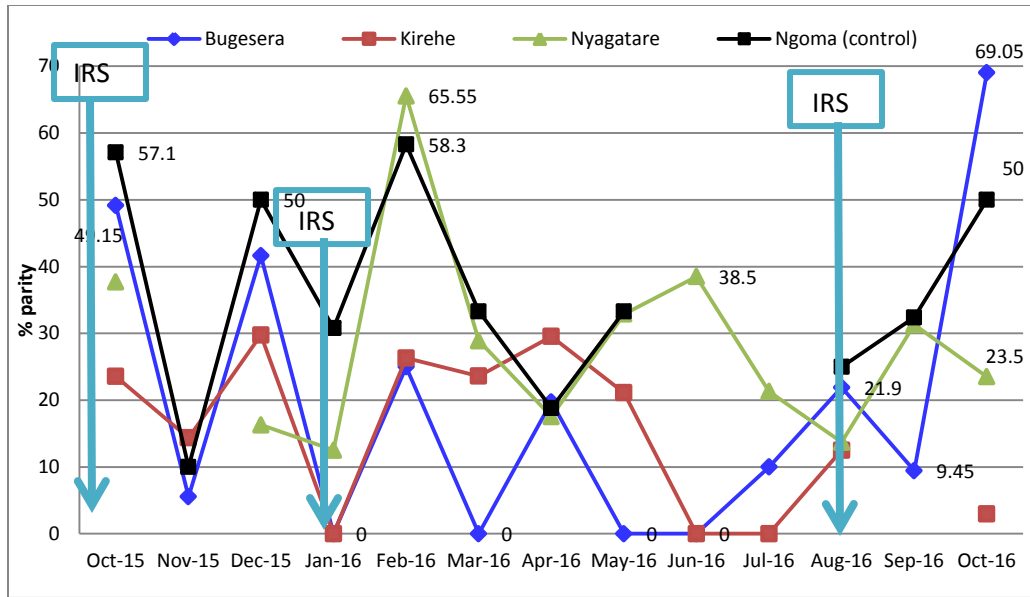
3.5   ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA)

3.5.1   SPOROZOITE ELISA

Mosquitoes collected through HLC and PSC were used for this test. A total of 128 *An. gambiae* s.l. were tested in the four districts in January (pre-spray) for *Plasmodium* circumsporozoite protein. None of the tested *An. gambiae* s.l. were positive (see Annex D).

A total of 586 *An. gambiae* s.l. specimens collected from the four districts during spray operations (February and March) were tested for *Plasmodium* circum-sporozoite proteins. In February, only two of the 22 samples from Bugesera were positive for *Plasmodium* circum-sporozoite proteins. In Nyagatare, four of the 50 tested were positive; in Kirehe, only one of the 78 tested was positive. In Ngoma (control) district, none of the 49 tested samples was positive. In March, nine samples from Nyagatare district and five from Kirehe tested positive while all samples tested in Bugesera and Ngoma tested negative (Table 6). The *Plasmodium*-infected anopheline mosquitoes were observed to increase with time in both Nyagatare and Kirehe districts.

During the post-spray period (April-August), 47 *An. gambiae* s.l. tested positive out of 1,400 *An. gambiae* s.l. from the four districts. In September (spray period), 464 *An. gambiae* s.l. were tested out of which 9 tested positive.

General comparison of positivity between the intervention and control sites showed that there was no significant difference (P>0.05) between positivity in the control site and four of the intervention sites (Table 6 (i)). There was also no significant difference in positivity between the samples tested from the control site and all the intervention sites on the post-spray period collections (Table 6 (ii)).

TABLE 6: SPOROZOITE RATES

21

(i)

District	Sector	Total Tested	Number Positive	% positive	P-value	Result
Bugesera	Nyarugenge	260	3	1.15	0.96538	NS
	Musenyi	92	3	3.26	0.1961	NS
Kirehe	Gatore	421	9	2.14	0.3721	NS
	Nyamugali	108	6	5.56	0.0154	S
Nyagatare	Nyagatare	549	29	5.28	0.0062	S
	Rukomo	849	24	2.83	0.1422	NS
Ngoma	Remera	251	3	1.20	1	

(ii)

District	Sector	Pre-Spray (January)			Post-Spray (April-August)				Result
		Total Tested	Number positive	% positive	Total. # Tested	Total # positive	% positive	P*-value	
Bugesera	Nyarugenge	16	0	0	77	2	2.60	1.00	NS
	Musenyi	8	0	0	60	1	1.39	0.81	NS
Kirehe	Gatore	29	0	0	198	8	4.04	0.80	NS
	Nyamugali	14	0	0	36	0	0.00	0.63	NS
Nyagatare	Nyagatare	51	0	0	350	13	4.29	0.76	NS
	Rukomo	5	0	0	602	19	3.16	0.91	NS
Ngoma	Remera	5	0	0	77	2	2.60	1.00	

Comparison is between pre- and post- IRS infectivity. *P-value was calculated by using the monthly averages of the post-IRS collections

3.5.2 BLOOD MEAL ELISA

Blood fed samples from the collections made in January-March were also assayed to estimate the source of blood meal. A total of 36 *An. gambiae* s.l. specimens were tested for vertebrate host blood source (human, bovine, ovine). *An. gambiae* s.l. showed opportunistic feeding behavior – they fed on all three sources. A higher proportion of *An. gambiae* s.l. specimens in three of the sites (Nyagatare, Bugesera, and Ngoma) was positive for a human blood meal; in Kirehe, the human blood index was 0%. The results show that a relatively high proportion of the vectors also fed on non-human hosts (Table 7).

TABLE 7: BLOOD MEAL SOURCE

Site	Number Tested	Results				
		Human	Bovine	Goat	Human and Bovine	No Specified Host
Nyagatare	14	50% (7)	28.6%(2)	14.3%(2)	0%(0)	3
Ngoma	11	27.3% (3)	27.3%(3)	9.1%(1)	0%(0)	4
Kirehe	4	0%(0)	75%(3)	0%(0)	25%(1)	0
Bugesera	7	57.1% (4)	42.9%(3)	0%(0)	0%(0)	0
Total	36	14	11	3	1	8

3.5.3 ENTOMOLOGICAL INOCULATION RATES

Entomological inoculation rate (EIR) was calculated using human biting and sporozoite rate data acquired from HLCs and the ELISA tests, respectively. The data cover only nine months (Jan-September 2016). Nyagatare showed more months (February, April, May, August, and September) of higher infective biting relative to the other control districts. Although the period of highest infectivity is not the same in the four districts, there seems to be some general trend during the months March-May in Nyagatare, Kirehe, and Ngoma districts (see Figure 7 and Annex E).

FIGURE 7: ENTOMOLOGICAL INOCULATION RATES
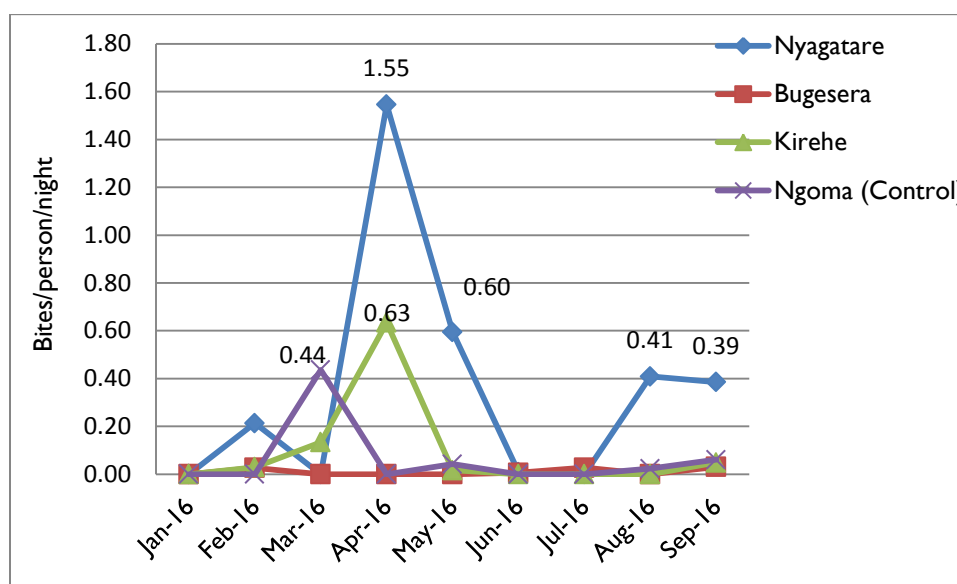
3.6 QUALITY OF SPRAY AND INSECTICIDE DECAY RATE

Following each of the spray campaigns (February and September 2016), AIRS Rwanda conducted quality control and monthly WHO cone bioassays in 24 sprayed structures in two IRS districts (Nyagatare and Kirehe). The tests were conducted on three different wall surfaces (mud, plastered not painted (PNP), and plastered and painted (PP)) in both IRS districts. In each district, two different sectors were sampled, and in each sector six structures were sampled. Out of the six structures in each sector, two were of each wall surface type (mud, PNP, PP). Control tests were conducted alongside on surfaces that were known to have no insecticide. The cone bioassays were conducted using susceptible *An. gambiae* s.l. (Kisumu colony).

The February cone bioassays conducted within one week of spraying to assess the quality of spraying showed 100% mortality of susceptible *An. gambiae* s.l., indicating quality spraying took place. Mortality of over 80% was observed on all the three surface types in the two districts up to four months after spraying. Plastered surfaces showed a longer retention of the insecticide with over 80% mortality at five months in Kirehe, while in Nyagatare mud surfaces showed over 80% mortality 5 months post IRS (Figure 8).

FIGURE 8: WALL BIOASSAY TEST RESULTS OF SURFACES SPRAYED WITH BENDIOCARB (FEBRUARY-JULY 2016)
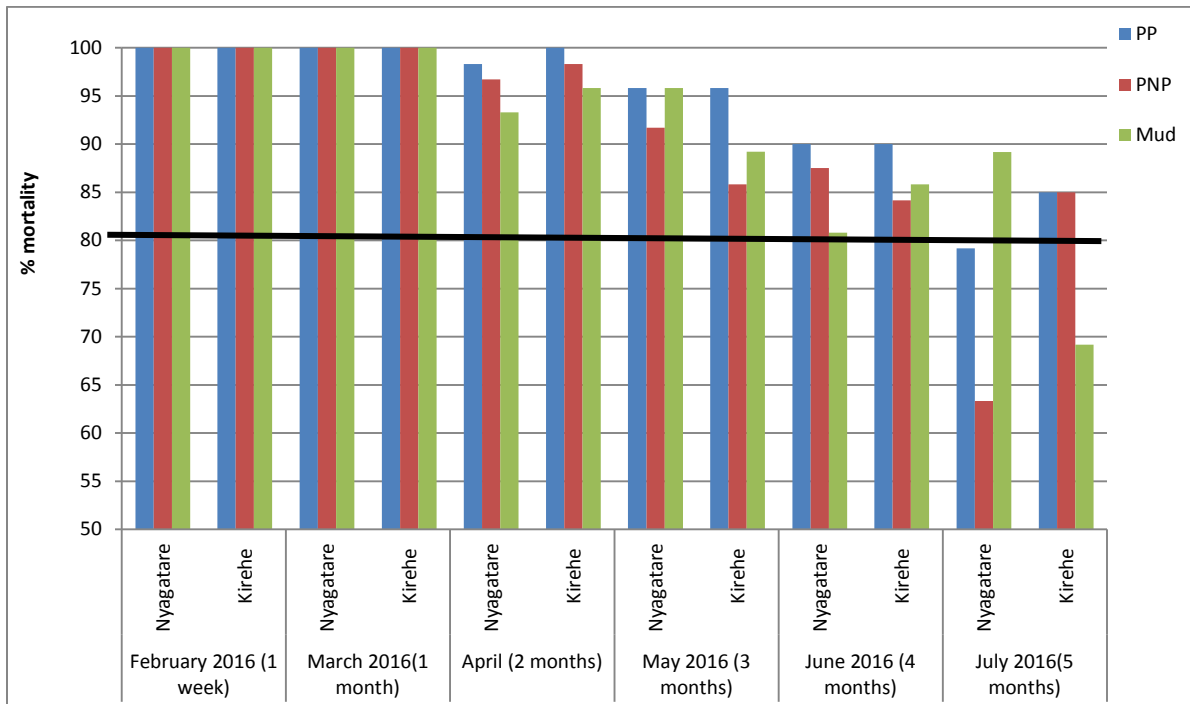
Cone bioassays conducted within one week and one month (T_1) of OP insecticide (Actellic 300CS) spraying in September 2016 showed 100% mortality of susceptible *An. gambiae* s.s., demonstrating satisfactory application of the insecticide. Tests conducted two months post-spray also showed 100% mortality of the test mosquitoes on all wall types, (Figure 9).

FIGURE 9: WALL BIOASSAY TEST RESULTS OF SURFACES SPRAYED WITH ACTELLIC 300 CS (SEPTEMBER-NOVEMBER, 2016)
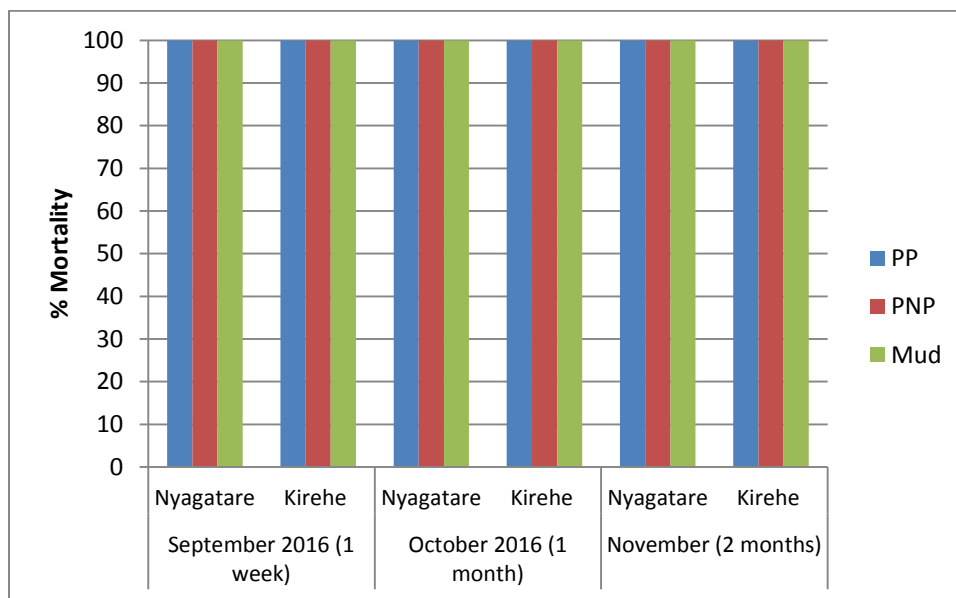
3.7   CONCLUSIONS

- A*n. gambiae* s.l. is the major malaria vector in Nyagatare, Bugesera, Kirehe, and Ngoma districts with *An. arabiensis* being proportionately higher (74.5%) than *An. gambiae* s.s. (25.5%).

- *An. gambiae* s.l. showed more exophagic than endophagic behavior in the four data collection districts. This could be attributed to the fact that a higher proportion (74.5%) of the *An. gambiae* s.l. were identified as *An. arabiensis,* whose tendency is to bite outdoors rather than indoors.

- Hourly biting rates per person varied across the four districts; they were highest in Nyagatare followed by Kirehe and Ngoma (control district); Bugesera showed the least mean bites/person/hour. The relatively higher biting in Nyagatare and Kirehe could be attributed to the presence of more vector breeding sites created by the rice paddies in addition to the rains.

- In Nyagatare and Kirehe districts, biting was higher at 1900h, and then dropped at 2000h. An increase in biting density was observed at around 2200h, peaked at 0100h and remained high until 0300h. In Bugesera, hourly biting rose at around 2100h and remained relatively constant through the rest of the night.

- Community members should be given information on and encouraged to stay indoors in the early hours of the night and to use LLINs while in bed.

- Vector density varied through the months across the districts during the reporting season; Nyagatare district showed the highest average vector density (0.5 *An. gambiae* s.l./house/day) of the three IRS districts (Bugesera 0.4 *An. gambiae* s.l./house/day and Kirehe-0.3 *An. gambiae* s.l./house/day). The control (non-IRS) district showed the highest density, of 1.0 *An. gambiae* s.l./house/day.

- There are two vector density peaks in the year: February-March and October November. Use of an insecticide with a residual period of less than six months such as carbamate requires that spraying is done twice, with each spray campaign starting prior to the peak seasons. With the change to an insecticide with longer residual efficacy, data from the insecticide decay assays will help to inform if the insecticide remains effective long enough to cover both vector density peaks.

- Biting rates were higher in Nyagatare than in the other districts (including the control district). Infectivity was also observed to be higher in Nyagatare, with 1.84 infective bites per night in March. General comparison of positivity between the intervention and control sites showed that there was no significant difference ($P>0.05$) between positivity in the control site and four of the intervention sites.

- There was also no significant difference in the infection rates between the samples tested from the control site and all the intervention sites on the post-spray period collections.

- There was no clear trend observed for parity even in the IRS districts relative to the control district.
- Bendiocarb 80WP showed a four-month efficacy period (≥80% mortality) on the three wall surface types tested; it however lasted up to five months with over 80% mortality on plastered wall surfaces in Kirehe and mud surfaces in Nyagatare.
- Wall assays conducted so far on wall surfaces sprayed with OP (Actellic 300CS) show 100% mortalities on all three wall types two months post spray.

4. SUPPORT TO RWANDA BIO-MEDICAL CENTER/MOPDD

4.1 INSECTARY MAINTENANCE AND ASSOCIATED VECTOR CONTROL LABORATORY SUPPORT

Through the support of USAID/ PMI, AIRS Rwanda provides support for the maintenance of the insectary and associated vector laboratory. The support includes procuring supplies needed for the sustenance of the established *An. gambiae* s.s. susceptible colony used for bioassays and reagents for molecular assays conducted in the vector control laboratory.

4.2 ENTOMOLOGY SENTINEL SITES SUPPORT

AIRS Rwanda also provided support for running the 12 entomology sentinel sites whose activities include malaria vector insecticide resistance testing, vector behavior assessments and determination of vector density/ distribution.

During the period May 23-28, 2016 two entomology sentinel site technicians attended the PMI/AIRS supported entomology regional training which was held in Dakar, Senegal. The training illustratively covered the following topics; mosquito sampling methods; species identification; insecticide resistance testing (including insecticide resistance intensity; assessment of the contribution of metabolic detoxification enzymes to phenotypic resistance using CDC bottle bioassays with synergists); ovary dissection; and preserving, storage, and shipment of mosquito samples for advanced laboratory works.

A three-day refresher training session which was scheduled for December 2016 was postponed to January 2017. The aim of the refresher training is to strengthen the skills of the sentinel site technicians on mosquito identification using taxonomic keys, mosquito ovary dissection and entomological data reporting. The trainees are drawn from the twelve entomology sentinel sites and the facilitators are drawn from MOPDD and AIRS Rwanda.

5. CHALLENGES AND RECOMMENDATIONS

- Sporozoite rate data for September and October 2015 and 2016 are not included in this report. In 2015, AIRS Rwanda had not yet begun conducting these tests; in 2016, the tests will be conducted in December, and data will be available in January.

- Insecticide resistance data for 2016 are not included in this report because the data have not been shared by The Malaria and Other Parasitic Diseases Division (MOPD). This will be done once the malaria technical working group has discussed the data. Wall bioassays will be conducted until the mortalities fall below the 80% threshold.

ANNEXES

ANNEX A: *AN. GAMBIAE* S.L. AVERAGE MONTHLY BITING TRENDS (SEP 2013-OCT 2015)
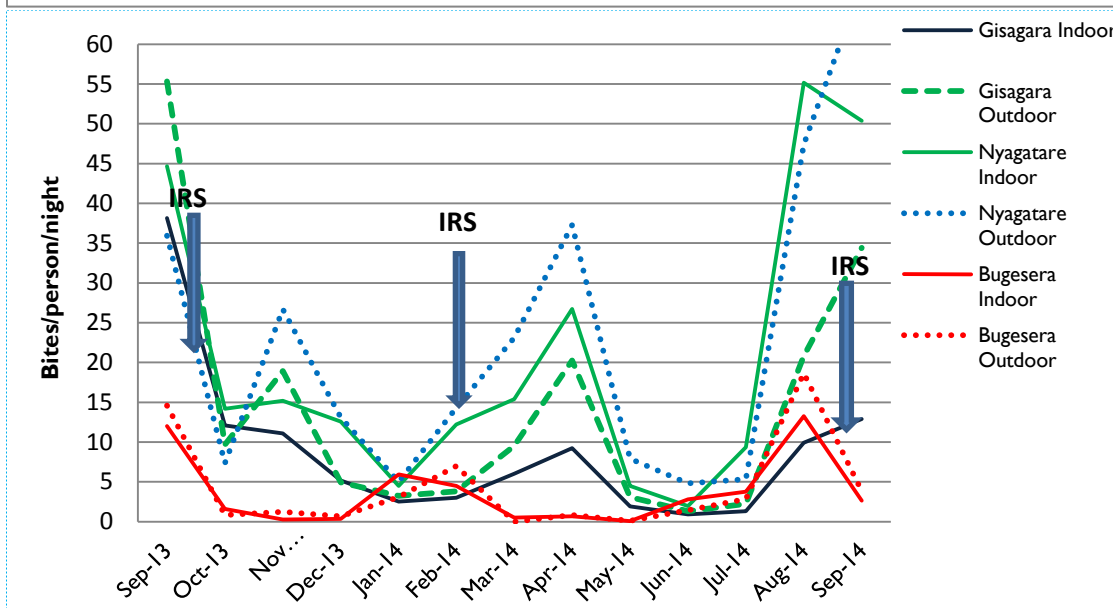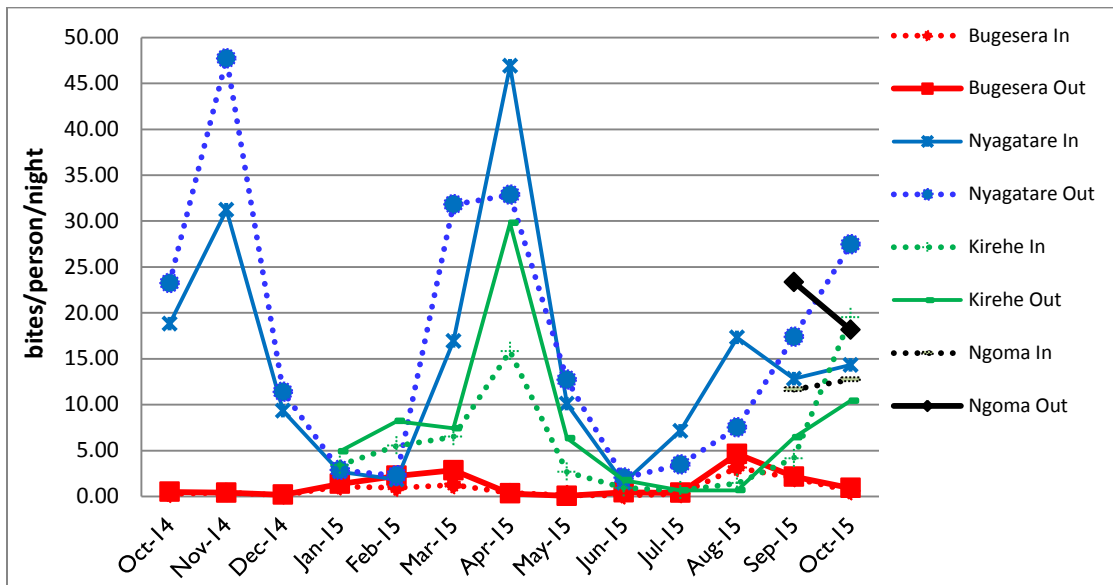
ANNEX B. 2015 INSECTICIDE RESISTANCE DATA

No	District	Sites	Period	Deltamethrin 0.05%	Permethrin 0.75%	Lambdacyh alothrin 0.05%	Pyrimiphos-methyl 0.25%	Bendiocarb 0.1%	Fenitrothion 1%	DDT 4%
1	Kirehe	Bukora	Jan-15	67	54	36	100	94	100	62
2	Bugesera	Mareba	Jan-15	67	63	43	100	100	100	80
3	Bugesera	Gashora	Jan-15	58	41	46	100	100	100	70
4	Nyanza	Busoro	May-15	63	47	62	100	100	100	
5	Nyagatare	Cyondo	Aug-15	93	91	96	100	100	100	99
6	Gatsibo	Ngarama	Sep-15	65	53	68	100	98	100	90
7	Nyagatare	Mimuli	Sep-15	58	43	44	100	90	100	71
8	Nyagatare	Nyagatare	Sep-15	100	87	91	100	100	100	99
9	Gisagara	Gakoma	Sep-15	94	93	89	100	100	100	97
10	Bugesera	Mwogo	Oct-15	73	46	78	100	98	100	88
11	Gisagara	Kirarambogo	Nov-15	78	83	85	100	96	100	81
12	Rwamagana	Nyagasambu	Nov-15	78	74	78	100	75	100	85
13	Kirehe	Bukora	Dec-15	99	81	89	100	100	100	100
14	Bugesera	Mareba	Dec-15	100	97	95	100	100	100	100

ANNEX C. PARITY

	Bugesera District								Nyagatare District								Kirehe District							
	Nyarugenge Site				Musenyi Site				Nyagatare Site				Rukomo Site				Gatore site				Nyamugali site			
	Total collected	Total *An. gambiae* s.l. Dissected	# parous	% parity	Total collected	Total *An. gambiae* s.l. Dissected	# parous	% parity	Total collected	Total *An. gambiae* s.l. Dissected	# parous	% parity	Total collected	Total *An. gambiae* s.l. Dissected	# parous	% parity	Total collected	Total *An. gambiae* s.l. Dissected	# parous	% parity	Total collected	Total *An. gambiae* s.l. Dissected	# parous	% parity
Oct-15	29	20	13	65	7	6	2	33.3	830	96	30	31.3	171	59	26	44.1	675	100	31	31	42	31	5	16.1
Nov-15	7	3	0	0	15	9	1	11.1	434	74	9	12.2	103	40	8	20.0	272	70	18	26	78	33	1	3.0
Dec-15	4	4	2	50	13	9	3	33.3	132	46	15	32.6	53	33	0	0.0	83	40	20	50	43	21	2	9.5
Jan-16	10	5	0	0		0	0		49	24	6	25.0	2	0	0	0.0	12	8	0	0	17	12	0	0.0
Feb-16	9	4	2	50		0	0		116	41	23	56.1	12	4	3	75.0	81	35	9	26	30	26	7	26.9
Mar-16	56	21	0	0	6	2	0	0.0	808	68	22	32.4	491	63	16	25.4	89	36	17	47	16	7	0	0.0
Apr-16	5	4	1	25	14	7	1	14.3	837	68	14	20.6	794	69	10	14.5	559	94	18	19	11	5	2	40.0
May-16	1	0	0	0	5	0	0	0	204	52	20	38.5	518	66	18	27.3	55	26	6	23	14	11	2	18.2
Jun-16	1	0	0	0	0	0	0	0	27	13	5	38.5	31	13	5	38.5	3	0	0	0	3	0	0	
Jul-16	6	5	1	20	2	0	0	0	9	6	1	16.7	244	54	14	25.9	0	0	0		3	0	0	
Aug-16	50	16	7	43.75	35	14	0	0	4	0	0	0.0	285	62	17	27.4	8	4	1	25	2	1	0	0.0
Sep-16	134	37	7	18.92	5	0	0	0	32	16	3	18.8	597	80	35	43.8	215	51	12	24	4	0	0	
Oct-16	39	14	10	71.43	8	3	2	66.667	491	66	20	30.3	532	66	11	16.7	232	51	3	6	62	16	0	0.0
	351	133	43	26.5	110	50	9	14.4	3973	570	168	27.1	3833	609	163	27.6	2284	515	135	23	325	163	19	11.4

ANNEX D. SPOROZOITE RATES

	Bugesera			Kirehe			Nyagatare			Ngoma		
	Total tested	number positive	% positive	Total tested	number positive	% positive	Total tested	number positive	% positive	Total tested	number positive	% positive
Jan-16	24	0	0.0	43	0	0.0	56	0	0.0	5	0	0.0
Feb 16	22	2	9.1	78	1	1.3	50	4	8.0	49	0	0.0
Mar 16	76	0	0.0	81	5	6.2	46	0	0.0	136	9	6.6
Apr-16	24	0	6.0	131	7	5.3	308	14	4.5	39	0	0.0
May-16	7	0	0.0	77	1	1.3	306	12	3.9	20	1	5.0
Jun-16	15	2	13.3	11	0	0.0	137	1	0.7	0	0	0.0
Jul-16	6	1	16.7	5	0	0.0	98	0	0.0	2	0	0.0
Aug-16	85	0	0.0	10	0	0.0	103	7	6.8	16	1	6.3
Sep-16	93	1	1.1	93	1	1.1	204	6	2.9	74	1	1.4

Annex E. Entomological Inoculation Rates

Month	Total *An. gambiae* s.l. collected	Biting Rate	SPZ Rate	Nightly EIR	Monthly EIR	Total *An. gambiae* s.l. collected	Biting Rate	SPZ Rate	Nightly EIR	Monthly EIR
	Nyagatare					Bugesera				
Jan-16	51	1.075	0.00	0.00	0.00	12	1.675	0.00	0.00	0.00
Feb-16	128	2.675	8.00	0.21	6.42	10	0.3	9.09	0.03	0.82
Mar-16	816	27.05	0.00	0.00	0.00	62	1.3	0.00	0.00	0.00
Apr-16	1631	34	4.55	1.55	46.36	19	0.4	0.00	0.00	0.00
May-16	722	15.175	3.92	0.60	17.85	6	0.12	0.00	0.00	0.00
Jun-16	58	1.2	0.73	0.01	0.26	1	0.045	13.33	0.01	0.18
Jul-16	253	5.4575	0.00	0.00	0.00	8	0.165	16.67	0.03	0.83
Aug-16	289	6.02	6.80	0.41	12.27	85	1.9	0.00	0.00	0.00
Sep-16	629	13.1	2.94	0.4	11.6	139	2.9	1.08	0.03	0.94
	Kirehe					Ngoma (Control)				
Jan-16	29	0.6	0.00	0.00	0.00	5	0.15	0.00	0.00	0.00
Feb-16	11	2.325	1.28	0.03	0.89	49	1.9	0.00	0.00	0.00
Mar-16	105	2.175	6.17	0.13	4.03	158	6.6	6.62	0.44	13.10
Apr-16	570	11.875	5.34	0.63	19.04	137	5.7	0.00	0.00	0.00
May-16	69	1.3	1.30	0.02	0.51	20	0.85	5.00	0.04	1.28
Jun-16	8	0.15	0.00	0.00	0.00	0	0	0.00	0.00	0.00
Jul-16	3	0.0875	0.00	0.00	0.00	2	0.08	0.00	0.00	0.00
Aug-16	10	0.205	0.00	0.00	0.00	9	0.375	6.25	0.02	0.70
Sep-16	219	4.55	1.08	0.05	1.47	110	4.55	1.35	0.06	1.84