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RWANDA: 2018 ENTOMOLOGICAL MONITORING REPORT

JULY 2017-JUNE 2018

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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
HLC	Human Landing Catch
IRS	Indoor Residual Spray
ITN	Insecticide-treated Net
OP	Organophosphate
PBS	Phosphate Buffered Solution
PCR	Polymerase Chain Reaction
PMI	President's Malaria Initiative
PNP	Plastered Not Painted
PP	Plastered and Painted
PSC	Pyrethrum Spray Catch
USAID	United States Agency for International Development
WG	Wettable Granules
WHO	World Health Organization
WP	Wettable Powder

ACKNOWLEDGMENTS

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EXECUTIVE SUMMARY

During the year-long reporting period (July 2017–June 2018), monthly entomological data collection was conducted in three indoor residual spray (IRS) districts, Nyagatare, Kirehe, and Bugesera, and one non-IRS (control) district, Ngoma. Adult mosquitoes were sampled using pyrethrum spray collection (PSC) and human landing catch (HLC) to assess vector species composition, seasonality, and behavior. World Health Organization (WHO) cone bioassays were conducted in three districts (Nyagatare, Kirehe and Gisagara) to assess the quality of spraying as well as to 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 morphologically and a subsample of *An. gambiae* s.l. were identified using polymerase chain reaction (PCR).

During the reporting period, a total of 7,600 adult female *Anopheles* mosquitoes were collected, 6,245 using HLC and 1,355 using PSC. Among the *Anopheles* mosquitoes collected by HLC, 87.9% were *An. gambiae* s.l., 8% *An. pharoensis*, 3.4% *An. ziemanni*, and the remaining 0.7 percentage was shared by *An. coustani*, *An. maculipalpis* and *An. funestus* group. All *Anopheles* collected by PSC were *An. gambiae* s.l.

A subsample of *An. gambiae* s.l. were identified using molecular technique, and 65% were *An. arabiensis*. *An. arabiensis* was dominant in most of the sites where the spraying took place, except in Nyarugenge site where *An. gambiae* s.s. was collected in higher numbers, and the difference was statistically significant in all sites except in the Musenyi site. At the Remera site (control), *An. gambiae* s.s. was more dominant, and the difference was statistically significant ($p<0.05$).

An. gambiae s.l. generally showed slightly more exophagic than endophagic tendency in the intervention districts, but in control district the endophagic behavior was higher than the exophagic. The difference between indoor and outdoor collection was statistically significant in Nyagatare and Kirehe, but not significant in Bugesera and Ngoma district ($p>0.05$).

The average biting rates per person per hour (b/p/h) varied across the four districts. In Nyagatare district the biting was high at 18:00-19:00, which may have a negative impact on indoor based interventions that are used at night in the area. There are two distinct peak seasons in human biting, March and September.

Although vector density varied over the months of the reporting period, Bugesera district showed a higher average vector density among the IRS districts (0.86 *An. gambiae* s.l./house/collection) than did the other two IRS districts of Nyagatare (0.28 *An. gambiae* s.l./house/collection) and Kirehe (0.26 *An. gambiae* s.l./house/collection). Ngoma (control) district showed the highest density of 1.01 *An. gambiae* s.l./house/collection.

The parity rate was lower three months post IRS in intervention areas while no reduction was observed in the control site. There was a significant difference ($p<0.05$) between the proportion of parous *An. gambiae* s.l. in the Ngoma control site and both sites in Nyagatare and Kirehe districts and one site of Bugesera district.

The overall sporozoite positivity rate was 0.62% (n=3,837). Among the positive mosquitoes, *An. gambiae* s.l. represented 70.8% of the total *Anopheles* mosquitoes that tested positive. The general comparison of positivity between the intervention and control sites post IRS showed that the difference was not statistically significant ($p>0.05$). However, the increase in sporozoite positivity rate during post spray compared to pre spray, which is also the peak mosquito and malaria transmission season, was higher in the control than in the IRS intervention sites.

According to the location of collection, there was a significant difference between infectivity rates of indoor and outdoor collected mosquitoes in Gatore site (Kirehe district) and in Nyarugenge site (Bugesera district). In the remaining sites, there was no significant difference in infectivity rates of indoor- and outdoor-collected mosquitoes.

EIR was higher outdoors than indoors in Nyagatare and Ngoma districts. The pre-IRS period in Nyagatare showed higher infective bites (0.32 infective bites per person per month) relative to the other intervention districts and control district. Three months post IRS the infective bites per person per month was highest (1.5 infective bites/person/month) in the control district compared with the intervention districts.

A total of 215 blood-fed *An. gambiae* s.l. samples from the collections made over the year-long reporting period (July 2017–June 2018) were tested for vertebrate host blood source (human, bovine, and goat). Human blood indices were as follows: Ngoma 56%, Nyagatare 50%, Kirehe 50%, and Bugesera 39%. The results showed that a relatively high proportion of the vectors also fed on non-human hosts.

Cone bioassays conducted within one week of spraying to assess the quality of spraying showed 100% mortality of susceptible *An. gambiae* s.s., indicating that the quality of the spraying operation was good. Subsequently, bio-efficacy of the sprayed insecticide was monitored monthly. Through June 2018, the average mortality rate was over 80% on all surface types in all districts, except on mud in Gisagara district where the mortality rate after eight months was 78.3%.

The results for fumigant effect of pirimiphos-methyl 300 CS showed that the average mortality rate was high within one week and one month after spraying (mortality of 90%) in Gisagara and Nyagatare. At three months in the Gisagara district, the mortality rate was below 10%, but in Nyagatare district the average mortality remained high (43.3%) up to 5 months.

1. INTRODUCTION

The President's Malaria Initiative (PMI) has protected millions of people in Africa from malaria through indoor residual spraying (IRS), which kills the mosquitoes that transmit malaria, by spraying insecticide on the walls, ceilings, and other indoor resting places of those mosquitoes. In September 2014, Abt Associates was awarded the three-year Africa Indoor Residual Spraying (AIRS) Project, IRS2 Task Order 6, which is funded by the United States Agency for International Development (USAID) under the United States President's Malaria Initiative (PMI). In September 2017, PMI launched the five-year PMI VectorLink Project. Working across 23 countries in sub-Saharan Africa as well as Cambodia, the PMI VectorLink Project is equipping countries to plan and implement safe, cost-effective and sustainable IRS programs and other proven life-saving malaria vector control interventions with the overall goal of reducing the burden of malaria.

Since 2012, AIRS Rwanda has implemented two spray campaigns (February/March and September/October) annually. 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, and from two to one round of spray per year. AIRS Rwanda transitioned to PMI VectorLink Rwanda in March 2018.

This report covers entomological monitoring activities conducted from July 1, 2017 to June 30, 2018. 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
- Assessing the impact of IRS on lifespan of malaria vectors through ovary dissection for parity
- Monitoring the quality of insecticide application and insecticide decay rates
- Determining sporozoite rates, blood meal source and entomological inoculation rates (EIRs).

2. Data Collection Sites and Methods

2.1 Study Sites

Data collection was conducted on a monthly basis in four IRS districts, Bugesera, Gisagara, Kirehe, and Nyagatare, and one non-IRS (control) district, Ngoma (Figure 1). In each IRS district except Gisagara, two sectors (sites) were selected as data collection sites. In the control district and Gisagara district one sector was selected. Table 1 lists the data collection sites and their spray status.

FIGURE 1: MAP OF RWANDA SHOWING DATA COLLECTION DISTRICTS

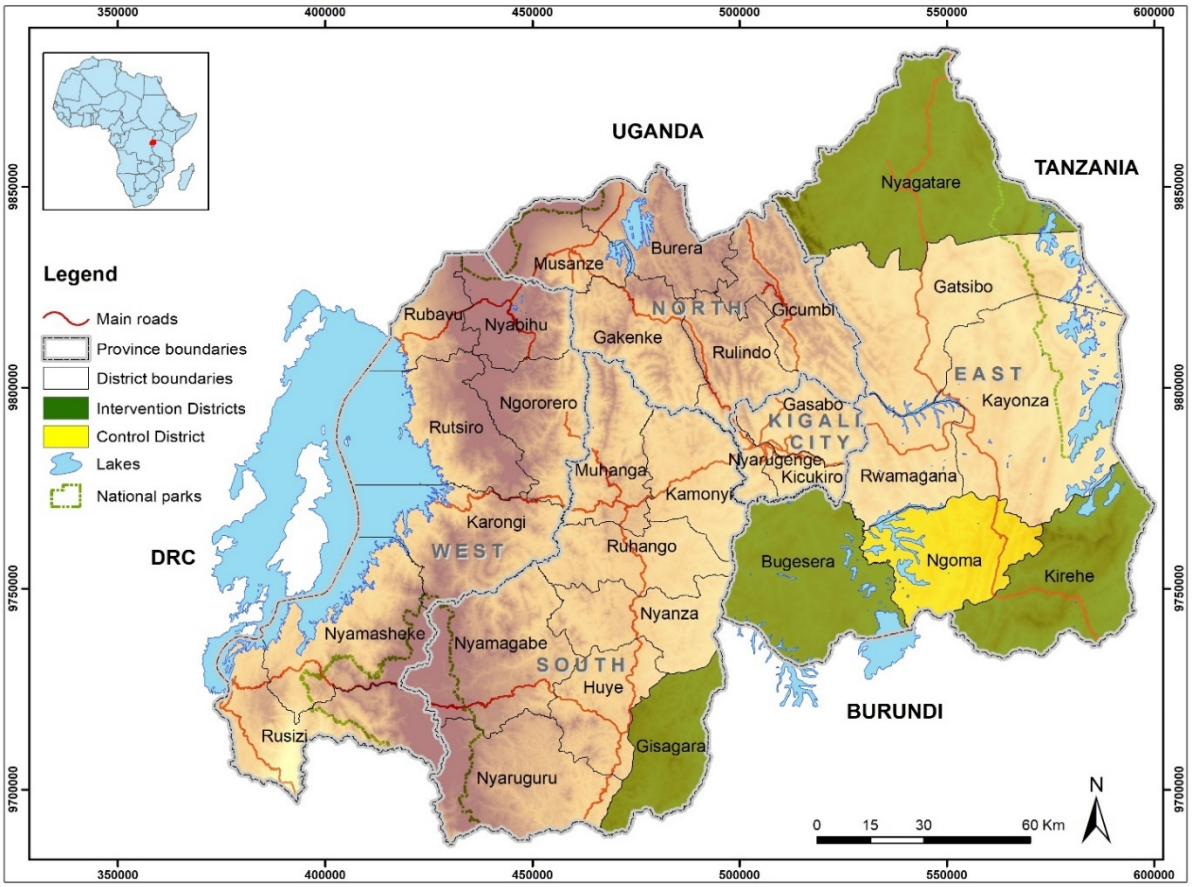


TABLE 1: DATA COLLECTION (SENTINEL) SITES

District	Data Collection Sites (sectors)	Spray Status
Bugesera	Nyarugenge, Musenyi	Sprayed in February/March 2018 using Actellic 300CS (OP) with Government of Rwanda support
Gisagara	Kansi	Sprayed in October/November 2017 using Actellic 300 CS with PMI and Government of Rwanda (GOR) support
Nyagatare	Nyagatare, Rukomo	Sprayed in Sep/Oct 2017 using Actellic 300 CS with PMI support
Kirehe	Gatore, Nyamugali	Sprayed in Sep/Oct 2017 using Actellic 300 CS with PMI support
Ngoma (control)	Remera	Not sprayed

2.2 DATA COLLECTION METHODS

Blood-seeking and indoor-resting adult mosquito collections were conducted each month in the seven sites (two sites in Bugesera, Kirehe and Nyagatare and one site in Ngoma district) using HLC and PSC methods, respectively.

Spray quality was assessed in five sites (two in Kirehe and Nyagatare and one in Gisagara district) using World Health Organization (WHO) standard protocol (WHO 2013) cone/wall bioassays, which were conducted within one week of the start of the spray campaign. Then, cone bioassays continued on a monthly basis after the spray round to assess the rate of insecticide decay.

2.2.1 HUMAN LANDING CATCH

HLC was done in three households in each site for two consecutive nights per month. A team of collectors was composed of four people per house per night; two collectors, one indoor and another outdoor, per house collected mosquitoes from 18:00 to 24:00 and two others collected from 24:00 to 6:00. In each site, 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 their 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 (Gillies and Coetzee 1987).

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 06:00 and 09:00 hours. Before the performance of PSC, all occupants were politely asked for their consent to move out of the house. The floor was covered with white sheets. Windows and other mosquito escape routes around the house were sealed 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, mosquitoes that had been knocked down were collected and sorted by species. The abdominal status of all female *Anopheles* 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 morphologically identified, and a sample of *An. gambiae* s.l. were identified to species level by PCR method.

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).

2.5 ELISA TEST

2.5.1 ELISA FOR SPOROZOITE INFECTION

The *Anopheles* mosquitoes were cut transversely between the thorax and the abdomen, and the head-thorax was placed in a vial labeled by mosquito number. The head-thorax of each individual mosquito was ground using 50 µl of grinding buffer; then another 200 µl of grinding buffer was added, bringing the final volume to 250 µl. Fifty-microliter aliquots were tested by Enzyme-linked Immunosorbent Assay (ELISA) using monoclonal antibodies to detect circumsporozoite proteins of *Plasmodium falciparum* (Wirtz et al. 1987). The results were read visually (Beier and Koros 1991).

2.5.2 ELISA FOR BLOOD MEAL SOURCE

Wild-caught half-gravid to freshly-fed mosquitoes were cut transversely at the thorax between the first and third pairs of legs. The abdomens were placed in a labeled tube, and 50 µl phosphate buffered saline (PBS) was added; the mixture was ground with a pestle, and another 950 µl of PBS was added after grinding. Samples diluted (1:50) with PBS were frozen at –20°C until testing. Blood meals were identified by direct ELISA using anti-host (IgG) conjugate against goat and human blood in a single-step assay (Beier et al. 1988). The non-reacting samples were then tested subsequently using bovine IgG. ELISA results were visually read (Beier and Koros 199l).

2.6 MOLECULAR IDENTIFICATION OF *ANOPHELES GAMBIAE* S.L.

A sub sample of *An. gambiae* s.l. collected by HLC and PSC was used in molecular test (PCR). *An. gambiae* s.l. mosquitoes were cut transversely at the thorax between the first and third pair of legs. The legs and wings were placed in a labeled vial. DNA was extracted by CTAB (Cetyl Trimethyl Ammonium Bromide) method and DNA was amplified using primers specific *for An. gambiae* s.s., *An. arabiensis*, *An. merus*, *An. quadriannulatus*, universal primer and Taq polymerase. 1×TBE running buffer were used to prepare 2% gel and the gel was stained with Syber safe. After amplification, seven microliters of amplified PCR product mixed with loading dye was loaded in gel and subjected to electrophoresis with 1x TBE at 100 volt for 1 hour. The bands were visualized under ultraviolet (UV) light and recorded according to ladder and positive control of *An. gambiae* s.s. and *An. arabiensis* (Scott, et Al, 1993).

2.7 QUALITY OF SPRAY AND INSECTICIDE DECAY RATE

Quality of spraying and insecticide decay rates were assessed using the WHO-approved protocol (WHO 1998). Test cones were placed at three different heights on sprayed wall surfaces, while the control cone 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) reared at Rwanda Biomedical Center (RBC) insectary, 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 of 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.

For bioassays to determine the fumigant effect of Actellic sprayed in houses, 10 female *Anopheles gambiae* s.s. were put in a small cage (15 cm x 10 cm) covered with an untreated polyester net. We placed the net approximately 10 cm from a sprayed wall and about one meter above the floor. We exposed the mosquitoes for 30 minutes and then transferred them to paper cups in which we fed them 10% glucose impregnated in cotton. We observed knockdown and recorded the data after the 30-minute exposure. We observed mortality after a 24-hour holding period. A control cage was set up outside under a tree in the shade.

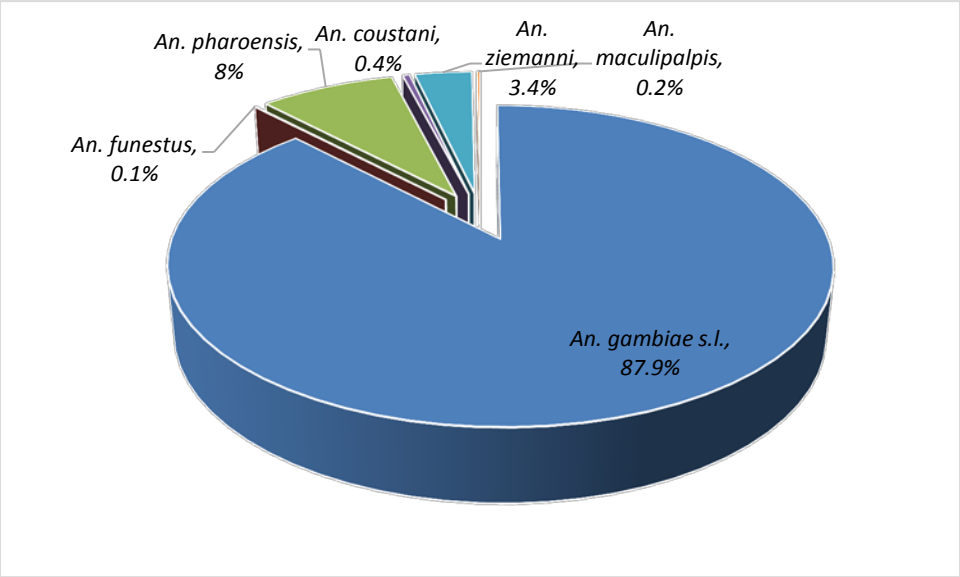
3. Results, Discussion, and Conclusions

3.1 Species Composition and Vector Seasonality

3.1.1 Species Composition

During the reporting period July 2017 to June 2018, a total of 7,600 adult female *Anopheles* mosquitoes were collected; 6,245 were collected using HLC, 1,355 using PSC. As shown in Figure 2, among the *Anopheles* mosquitoes collected by HLC; 87.9% were *An. gambiae* s.l., 8% *An. pharoensis*, 3.4% *An. ziemanni*, the remaining percentage was shared by *An. coustani*, *An. maculipalpis* and *An. funestus* group. All *Anopheles* collected by PSC were *An. gambiae* s.l. In addition, 39,116 Culcinae were collected. Only *An. gambiae* s.l. and the *An. funestus* group are known as primary vectors of malaria in Rwanda.

FIGURE 2: ANOPHELES SPECIES COMPOSITION



A subsample of *An. gambiae* s.l. (n=637) was identified using molecular technique and 65% were *Anopheles arabiensis*.

An. arabiensis was dominant in most of the sites where the spraying took place except in Nyarugenge site, and the difference was statistically significant in all sites except in the Musenyi site. At the Remera site (control), *An. gambiae* s.s. was more dominant, and the difference was statistically significant as shown in Table 2.

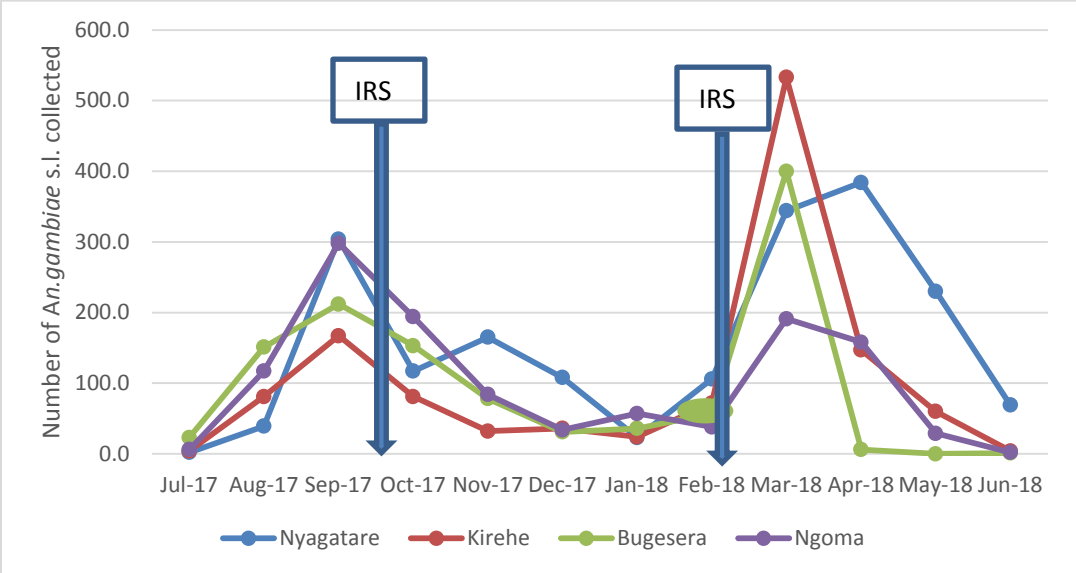
TABLE 2: *AN. GAMBIAE* S.L. SIBLING COMPOSITION

Sites	*An. gambiae* s.s	*An. arabiensis*	P-value	Significance status
Nyagatare	3%(2)	97%(62)	$6.38*10^{-14}$	S
Rukomo	1%(1)	99%(106)	$3.29*10^{-24}$	S
Gatore	8.4%(10)	91.6%(109)	$1.13*10^{-19}$	S
Nyamugari	6.5%(2)	93.5%(29)	$1.24*10^{-06}$	S
Nyarugenge	63%(58)	37%(34)	0.012343	S
Musenyi	46%(36)	54%(42)	0.4969	NS
Remera	77%(113)	23%(33)	$3.57*10^{-11}$	S

3.1.2 VECTOR SEASONALITY

Based on HLC collections, *An. gambiae* s.l. was the most prevalent malaria vector throughout the data collection period in both the intervention and control sites. As Figure 3 shows, the number of *An. gambiae* s.l. collected in Kirehe and in Nyagatare districts was high in pre-IRS August to mid-September 2017. This mosquito peak and drop in November is probably partly due to the seasonal fluctuation in mosquito populations. As it seems that the seasonal peak of the mosquito population is around September, it would be worth discussing whether it makes sense to shift the timing of IRS to come before September. The number remained low until February 2018, when it began to rise and peak in March 2018 and then gradually dropped until June 2018. The same trend was also observed in Bugesera district which was sprayed February-March 2018 and in the control district. The increase in malaria vectors in March may be attributed to the rains in that period, which creates many breeding sites.

FIGURE 3: NUMBER OF *AN. GAMBIAE* S.L. COLLECTED, BY SEASON



Nyagatare and Kirehe were sprayed in Sept-Oct 2017, whereas Bugesera was sprayed in Feb-March 2018.

3.2 VECTOR FEEDING TIME AND LOCATION

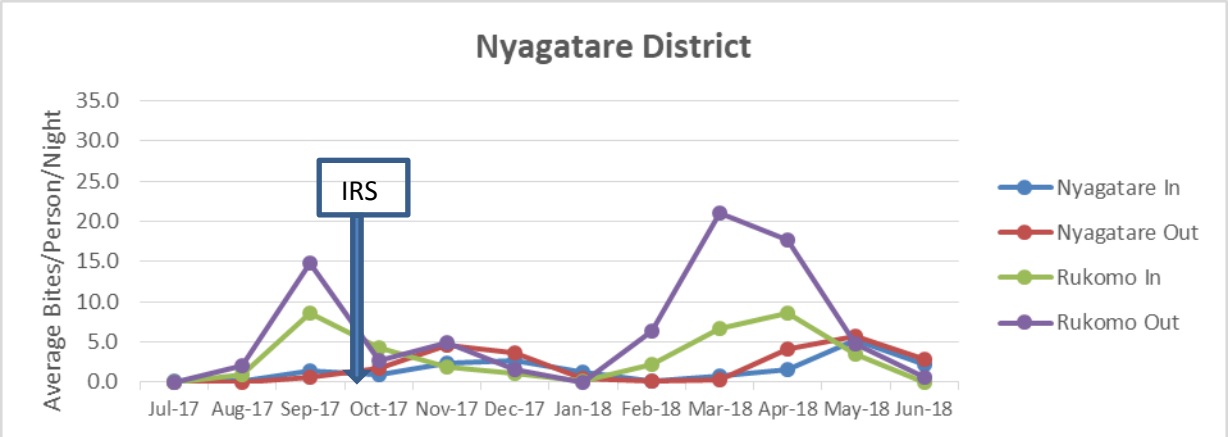
An. gambiae s.l. generally showed slightly more exophagic than endophagic tendency. In the intervention districts, there was no significant difference between the indoor and outdoor landing collections in the two sites in Bugesera district and one site in Ngoma (control) district ($p>0.05$) (Table 3). There was a significant difference ($p<0.05$) between the indoor and outdoor landing collections in the four sites in Kirehe and Nyagatare districts. The percentage endophagy/exophagy was recorded as follows: Bugesera: 46.7%/53.3%, Kirehe: 25.9%/74.1%, Nyagatare: 36.2%/63.8%, and Ngoma: 51.3%/48.7%.

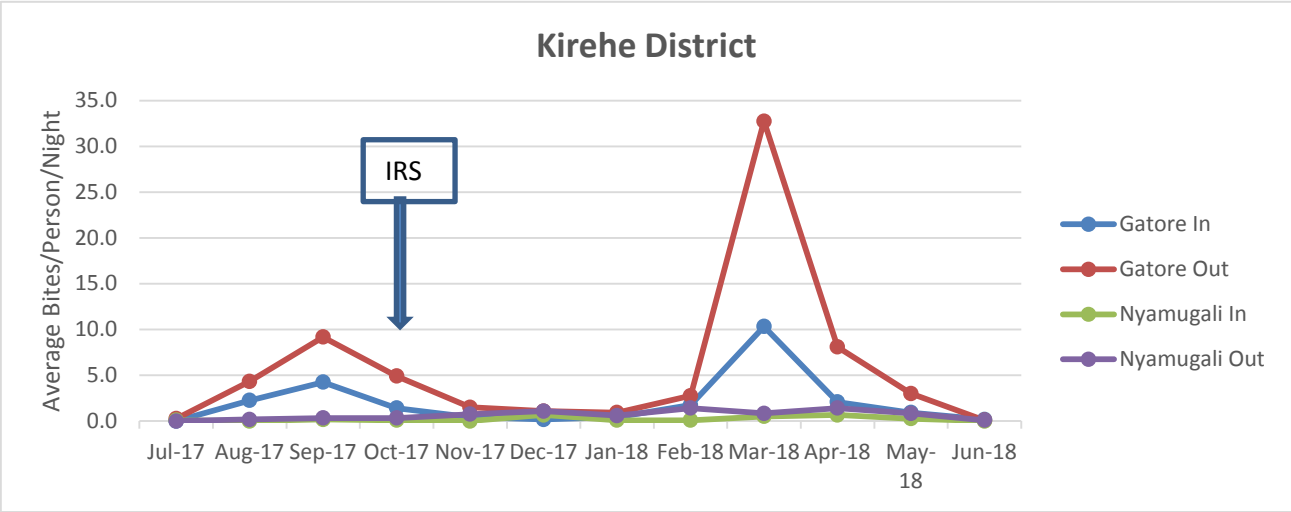
TABLE 3: INDOOR AND OUTDOOR BITING

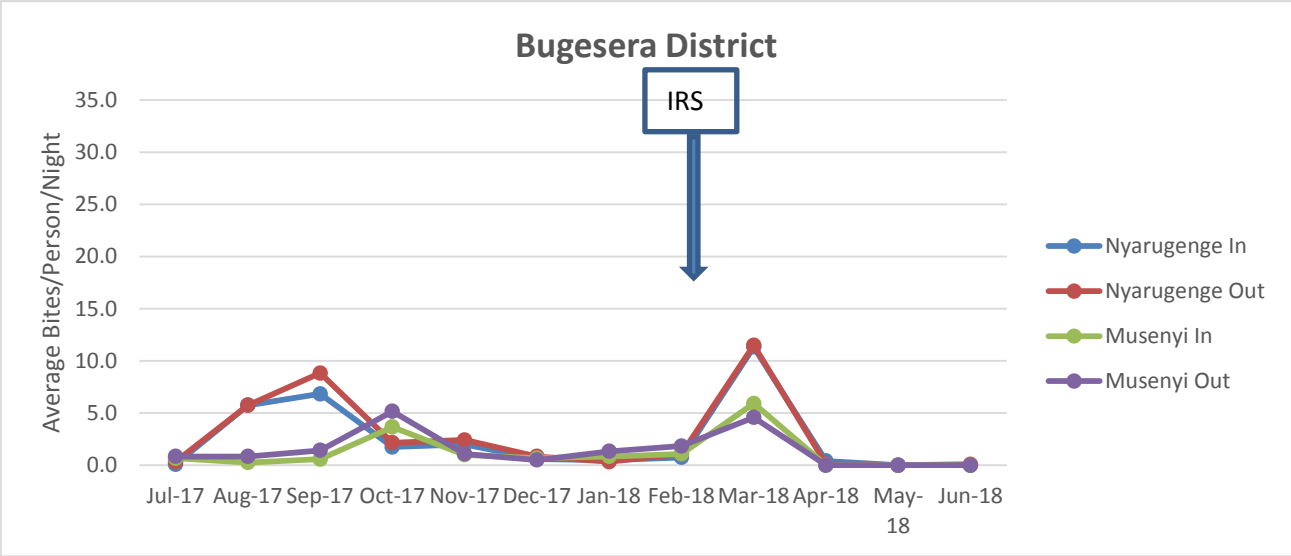
District	Site	In	Out	In: Out Ratio	P-value	Result
Nyagarare	Nyagatare	228	290	44:56	0.006	S
	Rukomo	456	917	33.2:66.8	0.001	S
Kirehe	Gatore	290	826	26:74	0.001	S
	Nyamugali	31	94	24.8:75.2	0.001	S
Bugesera	Nyarugenge	360	401	47.3:52.7	0.137	NS
	Musenyi	176	211	45.5:54.5	0.075	NS
Ngoma	Remera	620	588	51.3:48.7	0.357	NS

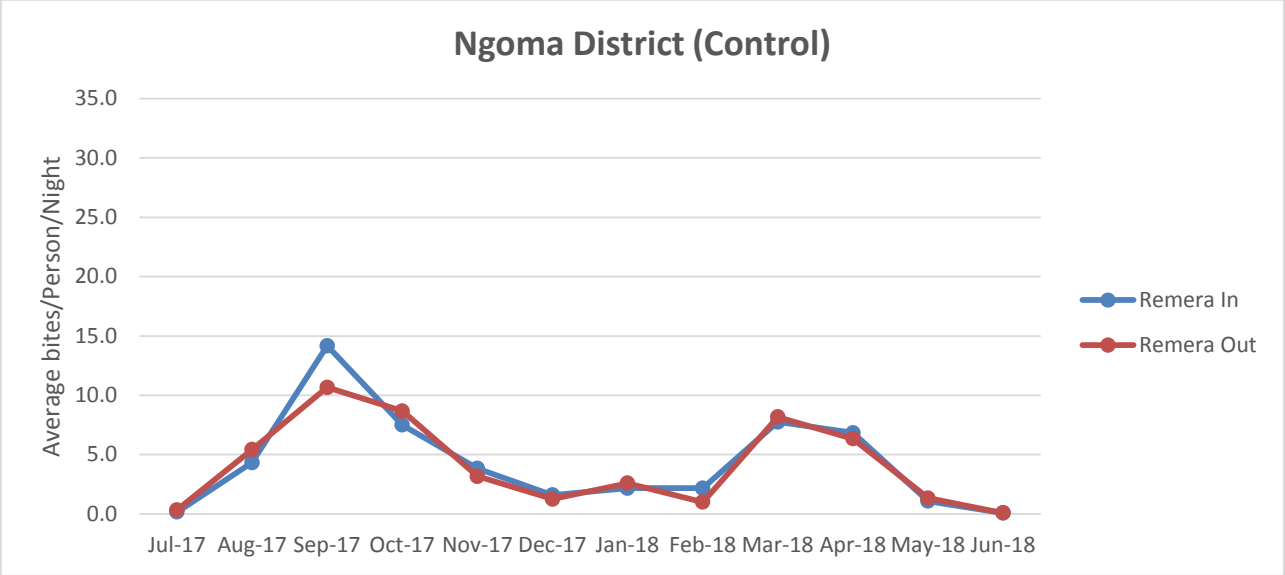
As Figure 4 shows, there are two peak biting seasons, namely, September–October and March–April. The highest bites/person/night (b/p/n) occurred in Gatore site of Kirehe district and more biting occurred outdoors than indoors. Even though there seem to be two distinct peak seasons for human biting rates, the b/p/n was higher around March than around September for Nyagatare and Kirehe. Human biting rates were 32.8 vs 10.3 b/p/n for outdoor vs indoor respectively in March and 9.2 vs 4.3 b/p/n outdoor vs indoor respectively in September.

FIGURE 4: *AN. GAMBIAE* S.L. AVERAGE MONTHLY BITING TRENDS



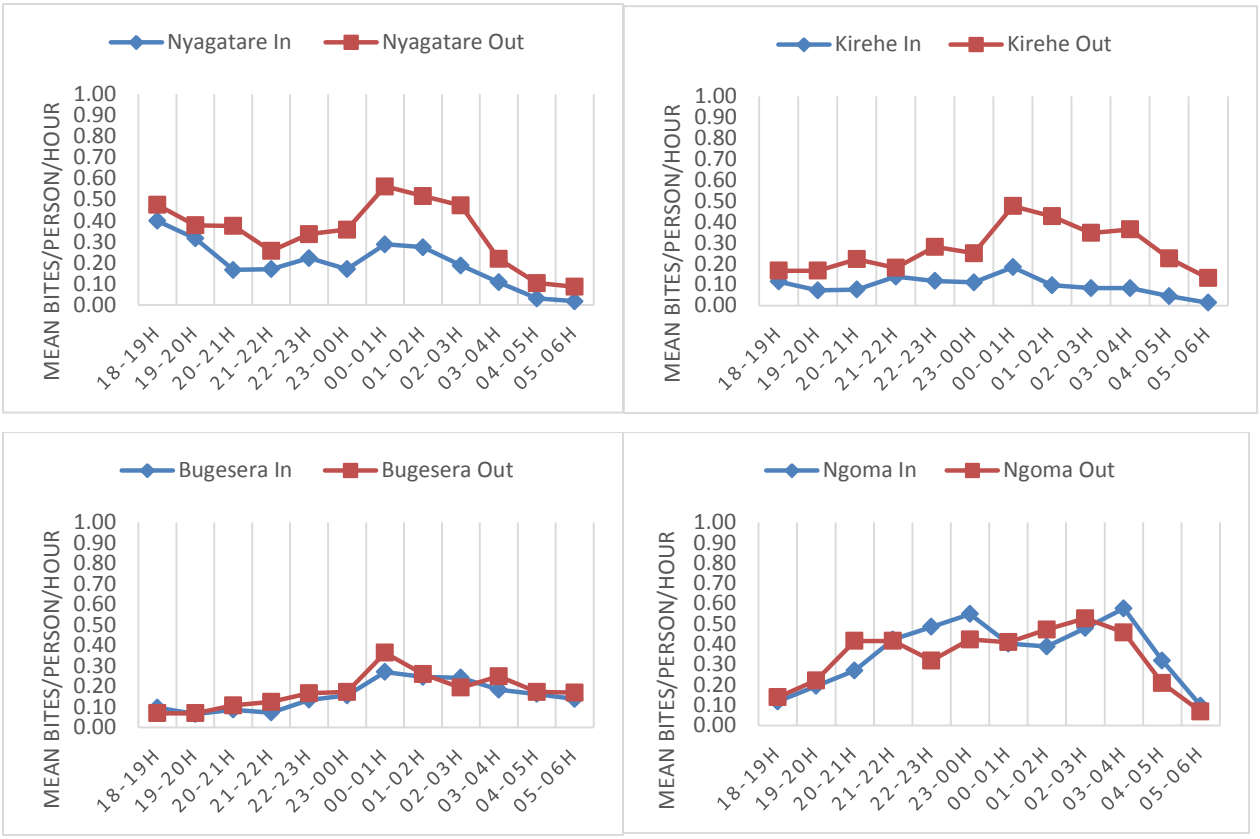

Kirehe District
Average Bites/Person/Night
35.0
30.0
25.0
20.0
15.0
10.0
5.0
0.0
IRS
Jul-17
Aug-17
Sep-17
Oct-17
Nov-17
Dec-17
Jan-18
Feb-18
Mar-18
Apr-18
May-18
Jun-18
Gatore In
Gatore Out
Nyamugali In
Nyamugali Out


Bugesera District
Average Bites/Person/Night
35.0
30.0
25.0
20.0
15.0
10.0
5.0
0.0
IRS
Jul-17
Aug-17
Sep-17
Oct-17
Nov-17
Dec-17
Jan-18
Feb-18
Mar-18
Apr-18
May-18
Jun-18
Nyarugenge In
Nyarugenge Out
Musenyi In
Musenyi Out


Ngoma District (Control)
Average bites/Person/Night
35.0
30.0
25.0
20.0
15.0
10.0
5.0
0.0
Jul-17
Aug-17
Sep-17
Oct-17
Nov-17
Dec-17
Jan-18
Feb-18
Mar-18
Apr-18
May-18
Jun-18
Remera In
Remera Out

The average *An. gambiae* s.l. bites per person per hour (b/p/h) varied across the four districts during the reporting time. Figure 5 shows average bites per person per hour through the night across the four districts. Biting in Nyagatare seems to start early (18H), though it gradually dipped towards mid night and then peaks up again after 00H. Biting is low in the morning hours between 04 and 06Hs. More biting seems to take place outdoors as long as people stay outside. This pattern could have implications to indoor-based interventions as people may not go to bed and may stay outdoors during the early hours of the night. There is no data on sleeping behavior of people, but observations suggest that most people often go to bed between 20 and 21Hs. Early biting seems to be less in the other three sentinel sites and slightly more biting occurs later than the first half of the night.

FIGURE 5: HOURLY BITING



3.3 INDOOR RESTING VECTOR DENSITY

As noted above, a total of 1,355 female indoor-resting *An. gambiae* s.l. were collected using PSC in the three IRS districts and the control district over the July 2017 to June 2018 reporting period. Table 4 shows the disaggregation of the collections and density in the districts.

TABLE 4: *AN. GAMBIAE* S.L. INDOOR RESTING DENSITY FROM PSC COLLECTIONS

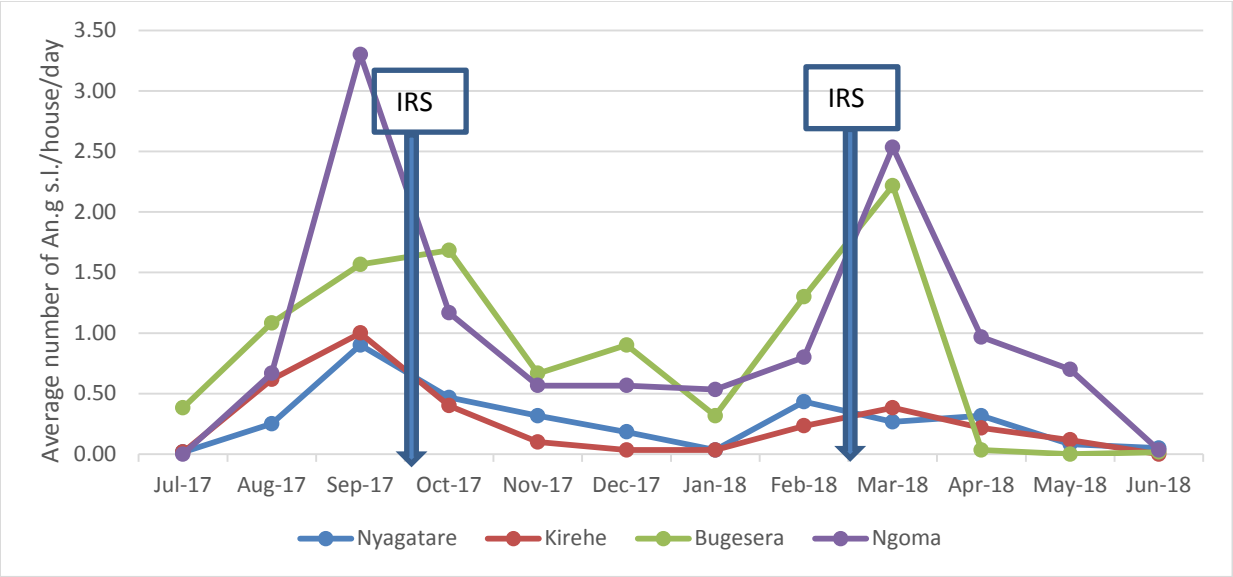
District	Nyagatare		Kirehe		Bugesera		Ngoma (control)	
	Total Collected	Vector Density /house/collection	Total Collected	Vector Density house/collection	Total Collected	Vector Density /house/collection	Total Collected	Vector Density /house/day
Jul-17	1	0.02	1	0.02	23	0.38	2	006
Aug-17	15	0.25	37	0.62	65	1.08	20	0.67
Sep-17	54	0.9	60	1	94	1.57	99	3.3
Oct-17	28	0.47	24	0.4	101	1.68	35	1.17
Nov-17	19	0.32	6	0.1	40	0.67	17	0.57
Dec-17	11	0.18	2	0.03	54	0.9	17	0.57
Jan-18	2	0.03	2	0.03	19	0.32	16	0.53
Feb-18	26	0.43	14	0.23	78	1.3	24	0.8
Mar-18	16	0.27	23	0.38	133	2.22	76	2.53
Apr-18	19	0.32	13	0.22	2	0.03	29	0.97
May-18	5	0.08	7	0.12	0	0	21	0.7
Jun-18	3	0.05	0	0	1	0.2	1	0.3
Avg. monthly vector density	16.58	0.28	15.75	0.26	50.83	0.86	29.75	1.01
p-value	0.053005 (NS)		0.03794 (S)		0.018858 (S)		1	

* Comparison of average collections in IRS districts and in the control district, July 2017–June 2018.

There was a significant difference in average monthly vector PSC collections between Kirehe and Bugesera districts and the control district ($p<0.05$). But, the difference between Nyagatare district and the control district was not significant. Although monthly vector density varied throughout the reporting period, Bugesera district showed the highest average vector density of the IRS districts (0.86 *An. gambiae* s.l./house/collection), higher than Nyagatare (0.28 *An. gambiae* s.l./house/collection) and Kirehe (0.26 *An. gambiae* s.l./house/collection). This is probably because Bugesera was sprayed about five months later than the other two. Indoor resting density in Bugesera dropped to almost zero after the spray in Feb-March. The control (non-IRS) Ngoma district showed the highest density, 1.01 *An. gambiae* s.l./house/collection.

The results also show that there are two vector density peaks during the year: February-April and September-October (Figure 6).

FIGURE 6: *AN. GAMBIAE* S.L. DENSITIES



Nyagatare and Kirehe were sprayed in Sept-Oct 2017, whereas Bugesera was sprayed in Feb-March 2018.

All 1,355 *An. gambiae* s.l. collected in four sites using PSC were classified according to their blood digestion stages: 583 (43%) were unfed, 612 (45%) were freshly fed, 101 (8%) were half-gravid, and 59 (4%) were gravid (see Figure 7). The half-gravid and gravid were mostly collected in Bugesera district compared to other district (see Figure 8) probably because this district was not sprayed until Feb-Mar. The high proportion of unfed mosquitoes collected indoors may indicate a high and sustained usage of long-lasting insecticide-treated nets (LLINs) in the communities. Further observation is required.

FIGURE 7: BLOOD DIGESTION STAGES OF ALL *AN. GAMBIAE* S.L. COLLECTED USING PSC

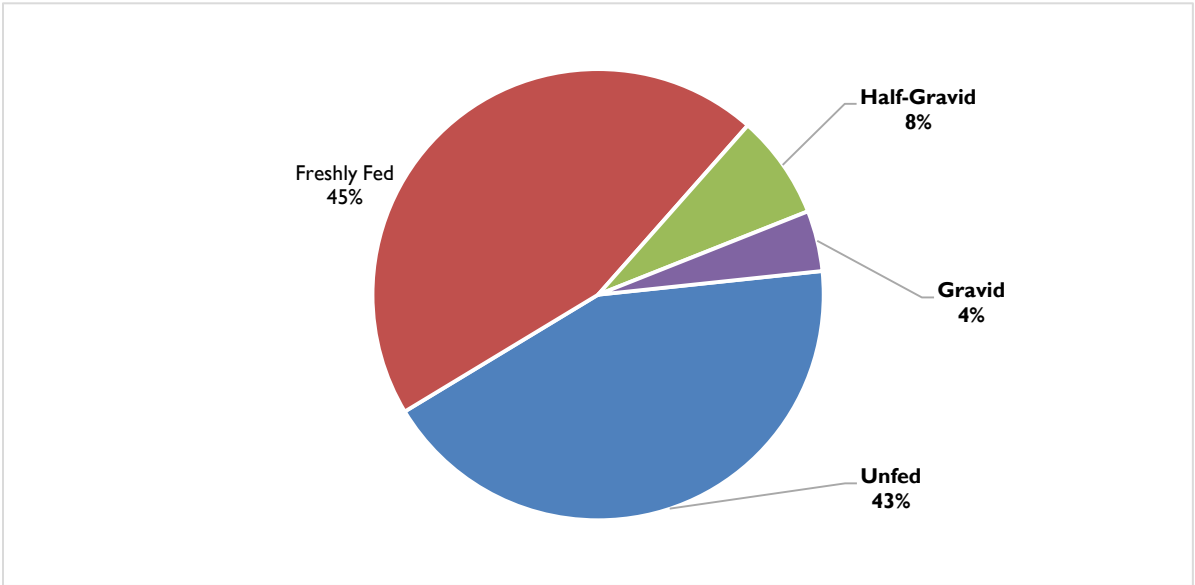
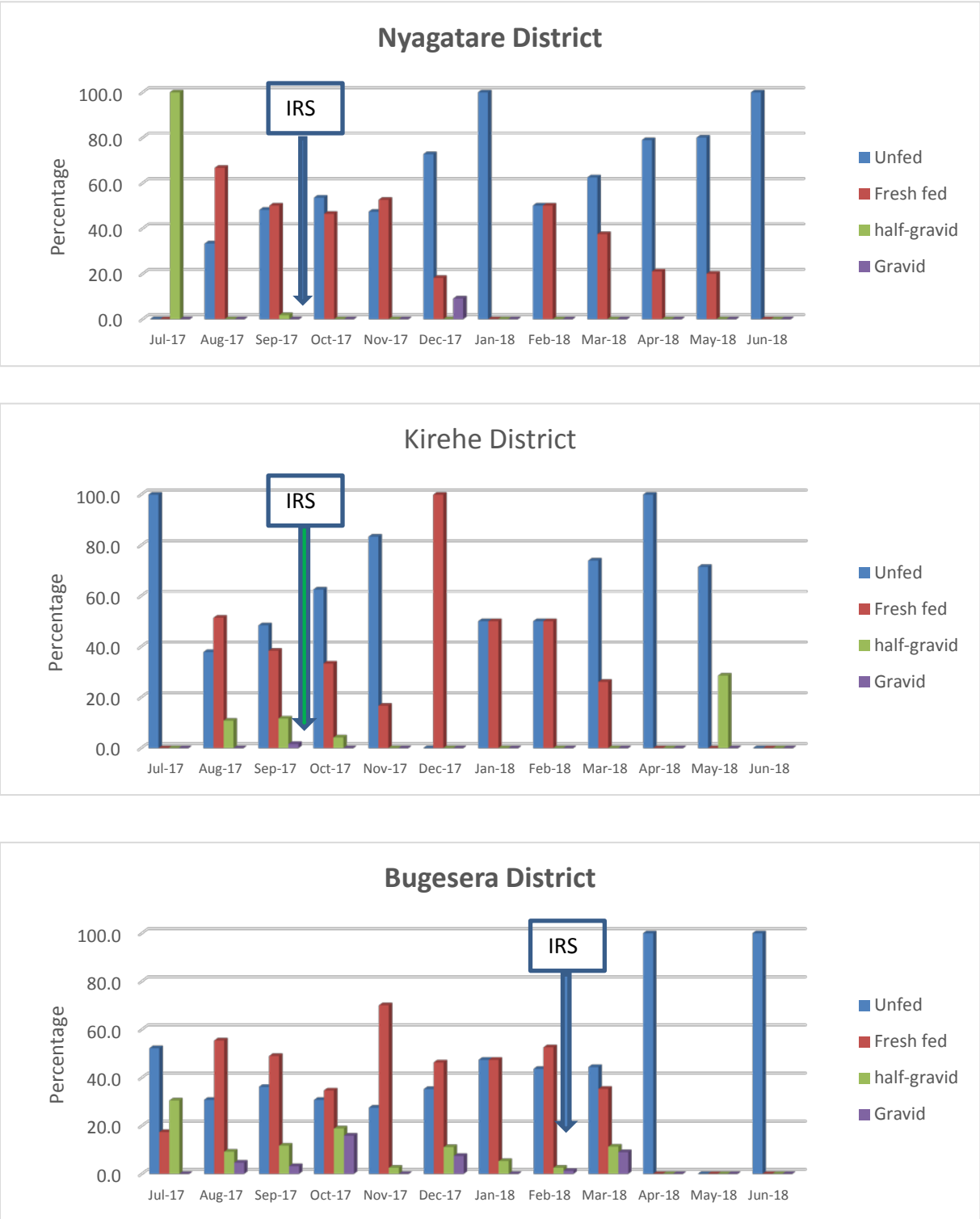
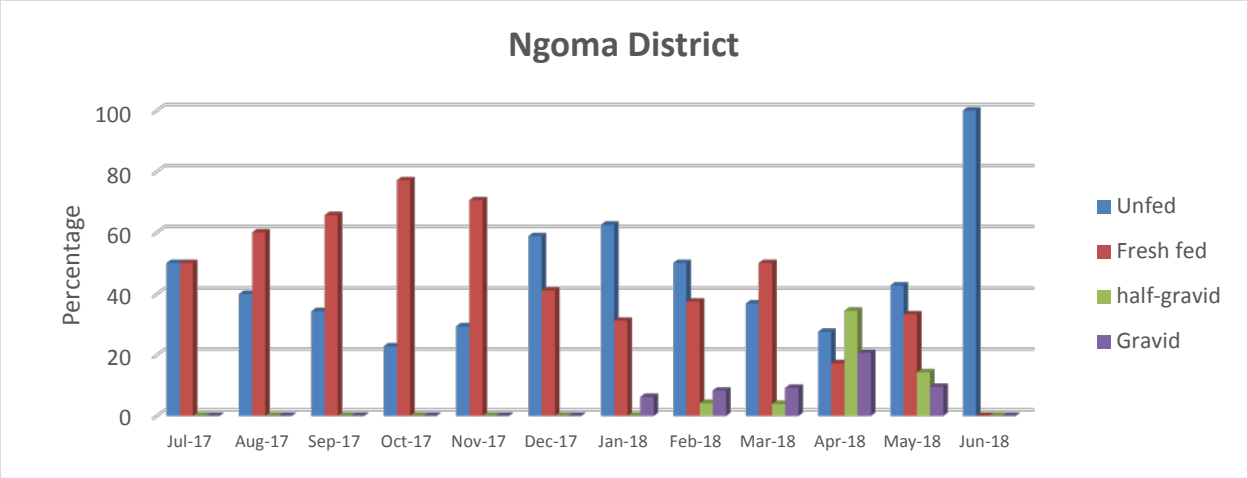


FIGURE 8: BLOOD DIGESTION STAGES OF *AN. GAMBIAE* S.L. COLLECTED USING PSC, BY DISTRICT AND MONTH




Ngoma District
Percentage
100
80
60
40
20
0
Jul-17
Aug-17
Sep-17
Oct-17
Nov-17
Dec-17
Jan-18
Feb-18
Mar-18
Apr-18
May-18
Jun-18
Unfed
Fresh fed
half-gravid
Gravid

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 from July 2017 – June 2018. There was a significant difference ($p<0.05$) between the average number of parous *An. gambiae* s.l. in the Ngoma control site and both sites in Nyagatare and Kirehe districts and one site of Bugesera district, but the difference was not statistically significant in one site (Musenyi) of Bugesera district (Table 5). The difference observed in Nyagatare and Kirehe could be attributed to spraying with Actellic 300CS, which lasts up to 10 months. The similar parity rates in Bugesera and Ngoma (control) could be attributed to later spraying, in February-March 2018.

TABLE 5: PARITY

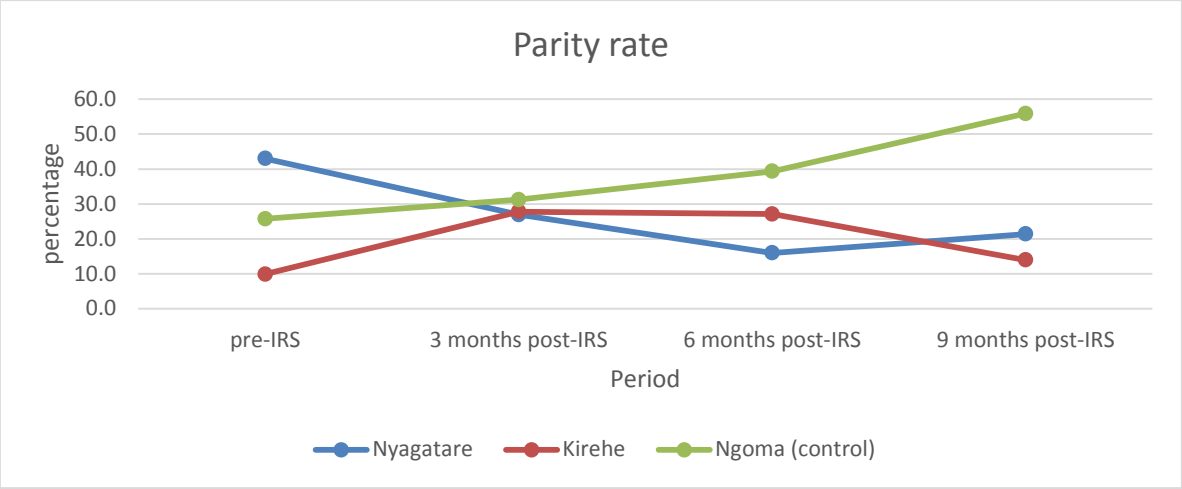
District	Sector	Total Collected	Total *An. gambiae* s.l. Dissected	# Parous	% Parity	Confidence interval	P-value	Result
Nyagatare	Nyagatare	518	232	59	25.4	19.8-31	0.00523	S
	Rukomo	1373	376	93	24.7	20.4-29.1	0.00059	S
Kirehe	Gatore	1116	332	63	19	14.8-23.2	$3.54*10^{-07}$	S
	Nyamugali	125	99	20	20.2	12.3-28.1	0.002365	S
Bugesera	Nyarugenge	761	204	48	23.5	17.7-29.4	0.001599	S
	Musenyi	387	152	49	32.2	24.8-39.7	0.363976	NS
Ngoma	Remera	1208	354	129	36.4	31.4-41.5	1	

Note: NS=not significant, S=significant

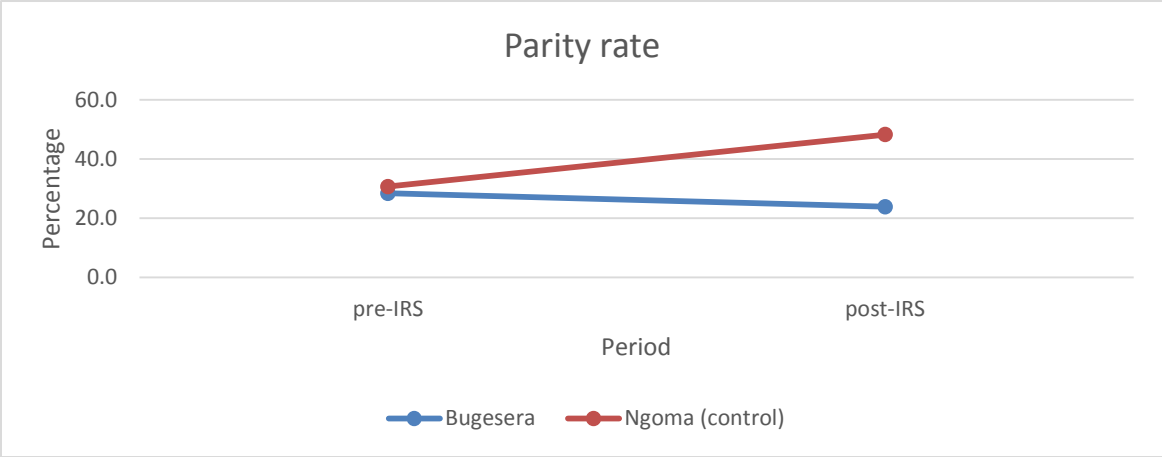
When the parity data of 3 months pre-spray and post-IRS was aggregated and compared between intervention and control sites, the parity rate showed a decreasing trend in Nyagatare and Bugesera (IRS district), little difference in Kirehe, and an increase in the control site after IRS. (See Figure 9 and Annex A).

FIGURE 9: PARITY

(i)



(ii)

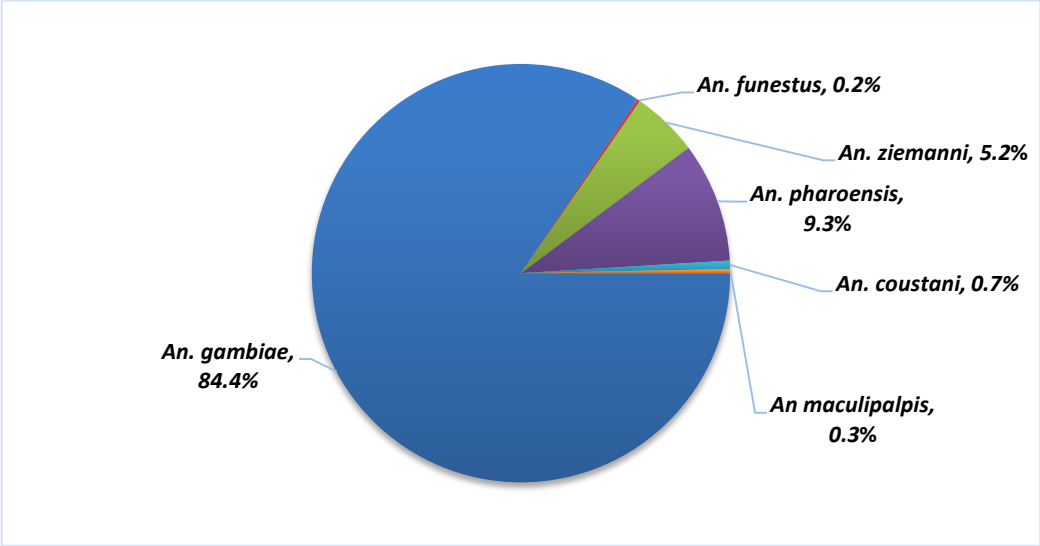

Parity rate
60.0
40.0
20.0
0.0
Percentage
pre-IRS
post-IRS
Period
Bugesera
Ngoma (control)

3.5 ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA)

3.5.1 SPOROZOITE ELISA

Mosquitoes collected through HLC and PSC were used for the ELISA. A total of 3,837 mosquitoes collected from July 2017 to June 2018 from the four districts were tested for the *Plasmodium* circumsporozoite protein. Different *Anopheles* species were tested; *An. gambiae* s.l. were dominant (84.4%) (Figure 10).

FIGURE 10: *ANOPHELES* MOSQUITOES TESTED, BY SPECIES



The overall sporozoite positivity rate was 0.62% (n=3,837). Among the positive mosquitoes, 70.8% were *An. gambiae* s.l., 12.5% were *An. ziemanni*, and 8.3% were *An. pharoensis* and *An. maculipal*pis (Table 6 (i)). Pre-IRS (July 2017–September 2017), 669 mosquitoes collected in Nyagatare, Kirehe, and Ngoma districts were tested for *Plasmodium* circumsporozoite proteins, and only one was found positive (positivity rate of 0.15%), from a sample collected in Rukomo site Nyagatare district. Bugesera district was sprayed in February/March 2018 so its pre-IRS is different from other intervention districts; a total of 1,063 samples collected in this district (July 2017–February 2018) were tested and five (0.47%) were positive for *Plasmodium* circumsporozoite proteins.

Post-IRS (Oct 2017–June 2018), 1,853 mosquitoes collected from Nyagatare, Kirehe, and Ngoma districts were tested for *Plasmodium* circumsporozoite proteins, and 14 samples were found positive (positivity rate of 0.75%): five out of 770 (0.65%) samples tested from Nyagatare district, two out of 513 (0.4%) tested from Kirehe district, and seven of 570 (1.22%) from Ngoma (control) district. The post-spray period collection and test for Bugesera district was conducted from March 2018 to June 2018; in this period, 199 samples collected in this district were tested and two (1%) were positive. There was an increase in percentage of *Plasmodium*-infected *Anopheles* mosquitoes when comparing pre-spraying and post-spraying in all intervention districts. However, this is expected given that the post spray period also coincides with the peak mosquito and malaria transmission season. Though the infection rates were generally too low to make any meaningful comparisons, the increase in the sporozoite rate from zero during the pre-spray period to 1.22% in the post spray period in the control district was much higher than the increase observed in the IRS districts.

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 all the intervention sites (Table 6 (ii)). Even though the difference in positivity between the samples tested from the control site and the intervention sites of Nyagatare and Kirehe districts in the post-spray period collections was not statistically significant, the increase in sporozoite positivity rate during post spray compared to pre-spray, which is also the peak mosquito and malaria transmission season, was higher in the control than in the IRS intervention sites. (Table 6 (iii, iv)). According to the location of collection, there was no significant difference in infectivity rates of indoor- and outdoor-collected mosquitoes (Table 6 (v)). The sporozoite rate per month per district is included in Annex C.

TABLE 6: SPOROZOITE RATES IN ALL SURVEYED SITES

(i)

Species	# tested	Positive
An. gambiae s.l.	3239	17
An. funestus	8	0
An. ziemanni	198	3
An. pharoensis	355	2
An. coustani	25	0
An. maculipalpis	12	2
Total	3837	24

(ii)

District	Sector	Total Tested	Number Positive	% positive	P-value	Result
Nyagatare	Nyagatare	356	3	0.84	0.938815	NS
	Rukomo	640	3	0.47	0.344374	NS
Kirehe	Gatore	579	2	0.34	0.220043	NS
	Nyamugali	159	0	0	0.232918	NS
Bugesera	Nyarugenge	383	4	1.04	0.795077	NS
	Musenyi	932	5	0.54	0.382404	NS
Ngoma	Remera	788	7	0.9	1	

(iii)

District	Sector	Pre-spray (Jul–Sep 2017)			Post-spray (Oct 2017–June 2018)				
		Total Tested	Number Positive	% Positive	Total # Tested	Total # Positive	% Positive	P-value*	Result
Nyagatare	Nyagatare	45	0	0	311	3	0.96	0.724269	NS
	Rukomo	181	1	0.55	459	2	0.43	0.174819	NS
Kirehe	Gatore	204	0	0	375	2	0.53	0.282018	NS
	Nyamugali	21	0	0	138	0	0	0.19077	NS
Ngoma	Remera	218	0	0	570	7	1.22	1	

*Calculated by using the monthly averages of the post-IRS collections. Comparison is between post- IRS infectivity and Ngoma control.

(iv)

District	Sector	Pre-spray (Jul 2017–Feb 2018)			Post-spray (March–June 2018)				
		Total Tested	Number Positive	% Positive	Tota. # Tested	Total # Positive	% Positive	P-value*	Result
Bugesera	Nyarugenge	291	3	1.03	92	1	1.08	0.844	NS
	Musenyi	825	4	0.48	107	1	0.93	0.738	NS
Ngoma (control)	Remera	492	3	0.61	296	4	1.35	1	

*Calculated by using the monthly averages of the post-IRS collections. Comparison is between post- IRS infectivity and Ngoma control.

(v)

Sites	Indoor Collection			Outdoor Collection			P-value*	Results
	Total Tested	# Positive	% Positive	Total Tested	# Positive	% Positive		
Nyagatare	178	1	0.56	178	2	1.12	0.56	NS
Rukomo	305	3	0.98	335	0	0	0.068	NS
Gatore	264	2	0.76	315	0	0	0.121	NS
Nyamugali	57	0	0	102	0	0	-	NA
Nyarugenge	261	2	0.76	122	2	1.64	0.433	NS
Musenyi	517	2	0.38	415	3	0.72	0.485	NS
Remera	494	3	0.6	294	4	1.36	0.275	NS

*Calculated by indoor and outdoor collections. Comparison is between indoor and outdoor infectivity.

3.5.2 Blood Meal ELISA

Blood-fed samples from the collections made July 2017 to June 2018 were also assayed to determine the source of the blood meal. A total of 215 *An. gambiae* s.l. specimens were tested for vertebrate host blood source (human, bovine, and goat). *An. gambiae* s.l. fed on all three blood sources. A higher proportion of *An. gambiae* s.l. specimens in all the sites, including the control site, were positive for a human blood meal, at 56%, 50%, 50% and 39%, for Ngoma, Nyagatare, Kirehe and Bugesera, respectively. 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 Other	Goat and bovine	No Specified Host
Nyagatare	26	13(50%)	6(23%)	0(0%)	2(8%)	0(0%)	5(19%)
Kirehe	18	7(39%)	7(39%)	0(0%)	0(0%)	1(5%)	3(17%)
Bugesera	121	61(50%)	14(12%)	2(1.5%)	2(1.5%)	4(3%)	38(32%)
Ngoma	50	28(56%)	5(10%)	0(0%)	2(4%)	0(0%)	15(30%)
Total	215	109(50.7%)	32(15%)	2(1%)	6(2.8%)	5(2%)	61(28.5%)

3.5.3 Entomological Inoculation Rates

The EIR for *An. gambiae* s.l. was calculated using human biting and sporozoite rate data acquired from HLCs and the ELISA tests, respectively. The data cover the period July 2017 to June 2018. During the pre-IRS period, Nyagatare showed the highest infective biting (0.32 infective bites per person per month) relative to the other intervention districts and control district. Three months post IRS the infective bites per person per month was highest (1.5 infective bites/person/month) in the control district compared to intervention districts. (See Figure 11 and Annex C). The EIR of *An. gambiae* s.l. based on collection location showed that, in Nyagatare and Ngoma districts, the outdoor EIR is higher than the indoor EIR (Figure 12).

FIGURE 11: HUMAN BITING AND ENTOMOLOGICAL INOCULATION RATES WITH *AN. GAMBIAE* S.L. MOSQUITOES

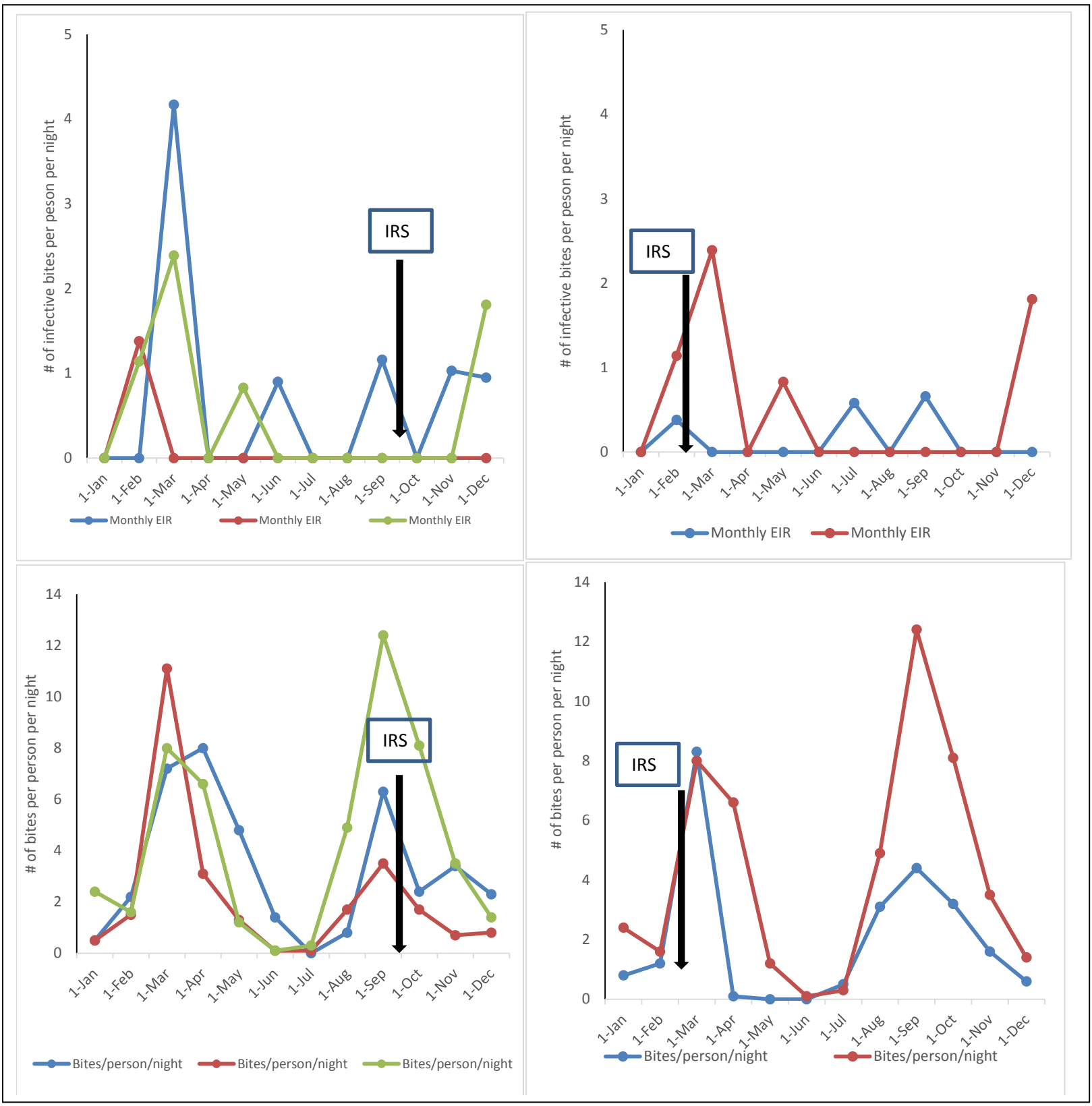
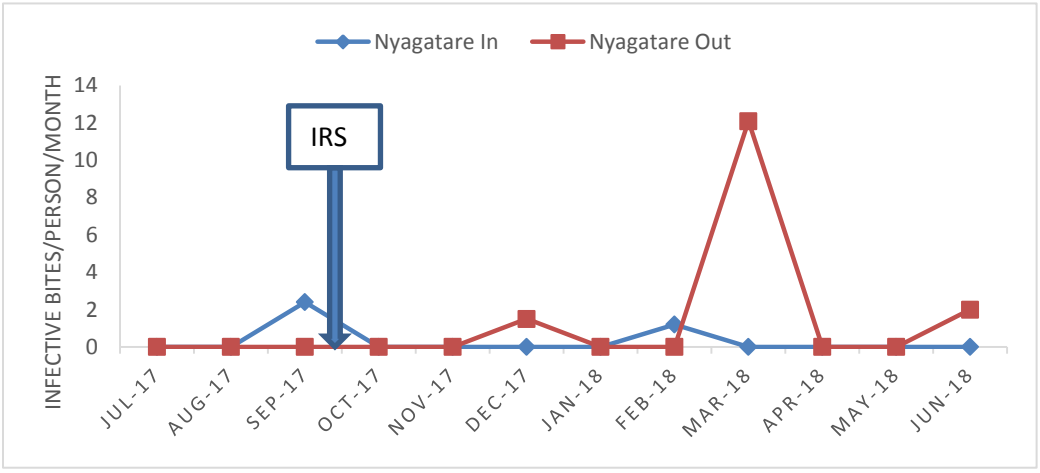
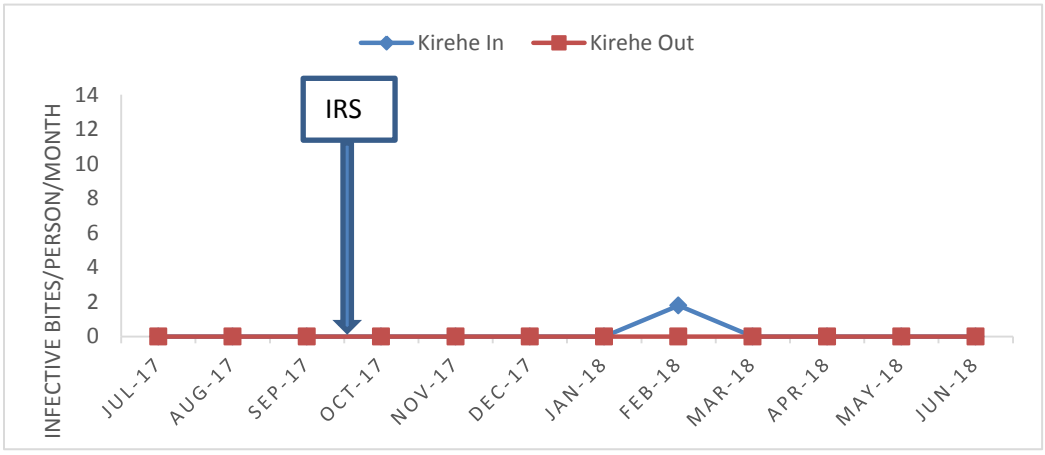
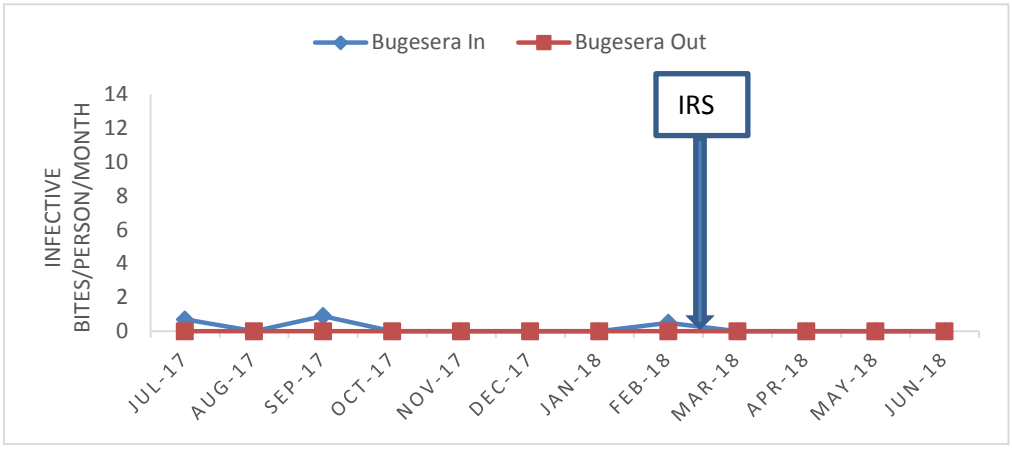
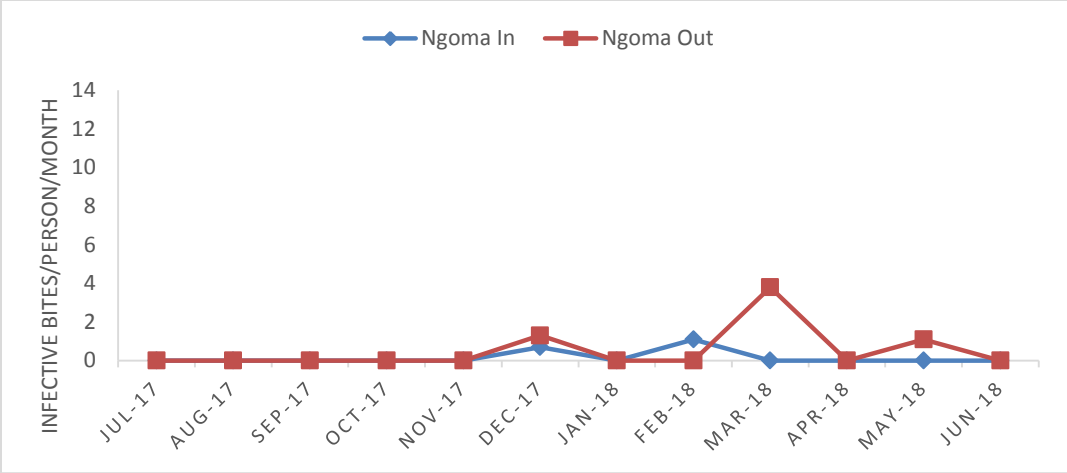


FIGURE 12: INDOOR VS OUTDOOR ENTOMOLOGICAL INOCULATION RATES OF *ANOPHELES GAMBIAE* S.L.









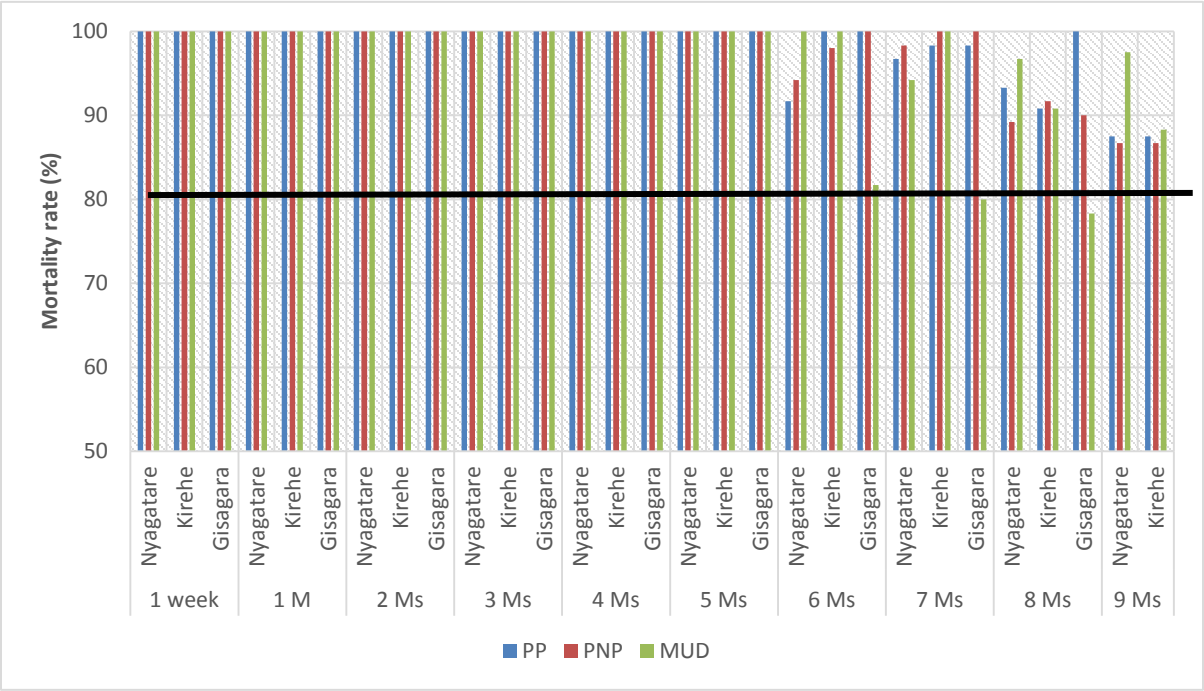
3.6 Quality of Spray, Insecticide Decay Rate and Fumigant Effect

3.6.1 Quality of Spraying and Insecticide Decay Rate

During the AIRS Rwanda spray in September (Nyagatare and Kirehe) and October (Gisagara) 2017 using pirimiphos-methyl (Actellic 300CS), WHO cone wall bioassays were carried out within one week in 30 of the sprayed structures. Three different wall surfaces (mud, plastered not painted (PNP), and plastered and painted (PP)) were tested in the districts. In each district (Nyagatare and Kirehe), two different sectors were sampled, but in Gisagara district only one sector was sampled. In each sector six structures were sampled, and out of the six structures in each sector, two each were of the different wall surface types (mud, PNP, and PP). Control tests were conducted on surfaces that were known to have no insecticide. The cone bioassays were conducted using susceptible *An. gambiae* s.s. (Kisumu colony).

Cone bioassays conducted within one week of spraying to assess the quality of spraying showed 100% mortality of susceptible *An. gambiae* s.s., indicating quality spraying took place. Subsequently, bio-efficacy of the sprayed insecticide was monitored monthly. Through June 2018, the mortality rate was over 80% on all surface types, except on mud in Gisagara district where the mortality rate at eight months was 78.3% (Figure 13).

FIGURE 13: WALL BIOASSAY TEST RESULTS (SEPTEMBER 2017–JUNE 2018)



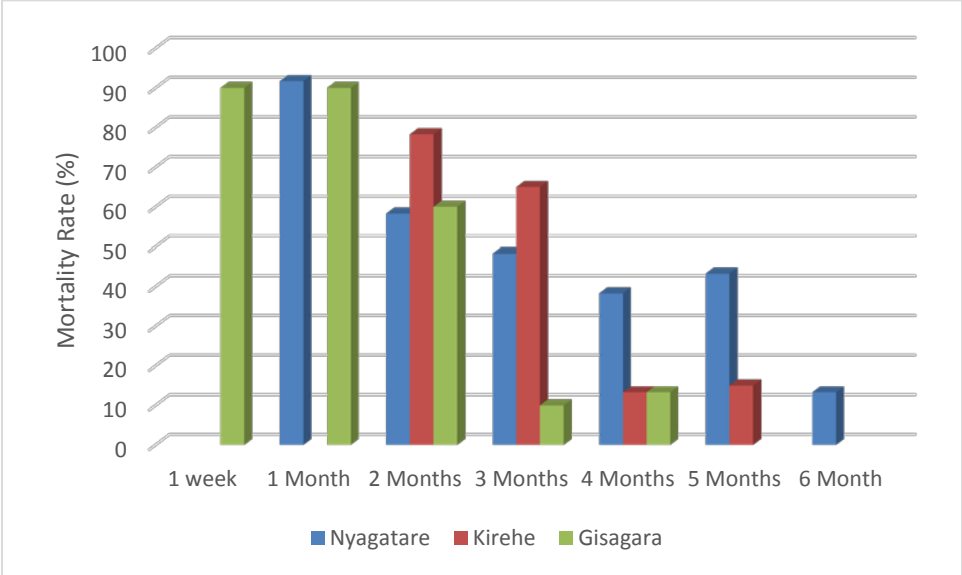
Ms: Months

3.6.2 FUMIGANT EFFECT OF PIRIMIPHOS-METHYL 300CS

We tested the fumigant effect of pirimiphos-methyl CS300 in sprayed houses. We performed the test in the same houses where we conducted the cone wall bioassay.

The results for fumigant effect of pirimiphos-methyl 300 CS showed that the average mortality rate was high within one week and one month after spraying. The mortality rate started decreasing in the following months. At three months in the Gisagara district, the mortality was below 10%, but in Nyagatare district the average mortality remained relatively higher (38-48%) up to 5 months (Figure 14).

FIGURE 14: FUMIGANT EFFECT RESULTS



3.7 CONCLUSIONS

An. gambiae s.l. is the major malaria vector in all surveyed districts; based on molecular identification *Anopheles arabiensis* comprised the highest percentage (65%) of tested *Anopheles gambiae* s.l. The number of collected *An. gambiae* s.l. was high in the pre-IRS period (July–mid-September 2017) in Nyagatare and Kirehe districts and dropped just after the September–October 2017 spray campaign, but the same trend was observed even in the control district. The peak was observed in March 2018 in all surveyed districts

An. gambiae s.l. showed more exophagic than endophagic behavior in the intervention districts (Nyagatare, Kirehe and Bugesera), and more endophagic behavior in the control district. The difference between indoor and outdoor collection was not statistically significant in Bugesera and Ngoma districts. The difference was statistically significant in Nyagatare and Kirehe districts.

Only *An. gambiae* were collected using the PSC method. None of the other *Anopheles* mosquitoes collected were resting indoors (PSC), indicating their exophilic behavior.

The average *An. gambiae* s.l. hourly biting rates per person (b/p/h) varied across the four districts; they were highest in Ngoma (control district) followed by Nyagatare. It was also higher in Ngoma than Bugesera; Kirehe showed the lowest b/p/h. The higher rate in Nyagatare could be attributed to the presence of rice paddies in those areas, which created more vector breeding sites, in addition to the rains.

Hourly biting was slightly higher outdoors than indoors in all sites except in Ngoma district.

Overall, the control (non-IRS) Ngoma district showed the highest density, 1.01 *An. gambiae* s.l./house/collection compared to IRS districts, Nyagatare,0.28 *An. gambiae* s.l./house/collection, and Kirehe, 0.26 *An. gambiae* s.l./house/collection. Mean average indoor resting density in Bugesera, was 0.86 *An. gambiae* s.l./house/collection and was low compared to the control district but higher than in the other two IRS districts. This is could be because it was sprayed late in Feb.

When the parity data of 3 months pre-spray and post-IRS was aggregated and compared between intervention and control sites, the parity rate showed a decreasing trend in Nyagatare and Bugesera (IRS district), little difference in Kirehe, and an increase in the control site after IRS.

The overall sporozoite positivity rate was 0.62%; among the positive *Anopheles*, 70.8% were *An. gambiae* s.l, 12.5% were *An.ziemanni*, 8.3% *An. pharoensis* and 8.3% *An. maculipalpis*.

Comparison of *Anopheles* spp. sporozoite positivity between the intervention and control sites during the post-IRS period showed that the difference was not statistically significant ($p>0.05$). However, the increase in sporozoite positivity rate during post spray compared to pre-spray was higher in the control than in the IRS intervention sites.

According to the location of collection, there was no significant difference in infectivity rates of indoor- and outdoor-collected mosquitoes.

EIR was higher outdoors than indoors in Nyagatare and Ngoma districts. During the pre-IRS period, Nyagatare showed the highest infective biting (0.32 infective bites per person per month) relative to the other intervention districts and control district. Three months post-IRS the infective bites per person per month was highest (1.5 infective bites/person/month) in the control district compared with intervention districts.

An. gambiae s.l. showed opportunistic feeding behavior, that is, they fed on all three blood sources tested (human, bovine and goat). A higher proportion of *An. gambiae* s.l. specimens in all the sites, including the control site, was positive to human blood. The results showed that a relatively high proportion of the vectors also fed on non-human hosts.

Cone bioassays conducted in Nyagatare, Kirehe and Gisagara districts within one week of spraying to assess the quality of spraying showed 100% mortality of susceptible *An. gambiae* s.s., indicating quality spraying took place. Subsequently, bio-efficacy of the sprayed insecticide was monitored monthly. Through June 2018, the average mortality rate was over 80% on all surface types in all districts, except on mud in Gisagara district where the mortality rate at eight months was 78.3%.

The results for fumigant effect of pirimiphos-methyl 300 CS showed that the average mortality rate was high within one week and one month after spraying. The mortality rate started decreasing in the following months. At three months in the Gisagara district, the mortality was below 10%, but in Nyagatare district the average mortality remained relatively higher (43.3%) up to 5 months.

4. Support to Rwanda Bio-Medical Center/Malaria and Other Parasitic Diseases Division

4.1 Insectary Maintenance and Associated Vector Control Laboratory Support

PMI VectorLink supports the maintenance of the insectary and associated vector laboratory. Its support includes procuring supplies 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. VectorLink Rwanda is also providing technical support to the entomology laboratory.

4.2 Entomology Sentinel Sites Support

VectorLink Rwanda also supports the operations of the MOPDD's 12 entomology sentinel sites, whose activities include malaria vector insecticide resistance testing, vector behavior assessments, and determination of vector density/distribution.

Two trainings of sentinel site entomology technicians were organized and held in Bugesera district in January 22–24, and in May 3-5, 2018. A total of 58 technicians were trained during these training sessions as per the table below:

Training of Sentinel Sites Entomology Technicians		
	January 22–24, 2018	May 3-5, 2018
Number Participants	29	29
Names of the Sites	12	12

NB: In each training session, 23 technicians were from sentinel sites, and six technicians were from the teams which conduct the insecticide resistance test.

The topic of the training was "evaluation of insecticide resistance in vectors using CDC Bottle Bioassay, determination of resistance intensity and use of synergist to detect metabolic resistance".

5. CHALLENGES AND RECOMMENDATIONS

Detection of insecticide resistance mechanism is needed at Kicukiro entomology laboratory. If funding is available, this capacity should be put in place.

ANNEX A. PARITY

	Nyagatare District				Kirehe District				Bugesera District				Ngoma District			
	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
Jul-17	2	0	0	0	4	0	0	0	23	7	1	14.3	6	0	0	0
Aug-17	39	28	19	67.9	81	40	3	7.5	151	44	26	59.1	117	40	15	37.5
Sept-17	304	65	21	32.3	167	61	7	11.5	212	66	7	10.6	298	61	11	18
Oct-17	117	46	16	34.8	81	31	6	19.4	153	57	12	21.1	194	49	15	30.6
Nov-17	165	66	18	27.3	32	20	6	30	78	33	4	12.1	84	35	9	25.7
Dec-17	108	44	8	18.2	36	21	8	38.1	31	14	3	21.4	34	12	6	50
Jan-18	23	12	3	25	24	13	8	61.5	36	11	6	54.5	57	20	6	30
Feb-18	106	37	8	21.6	72	35	12	34.3	57	32	16	50	38	21	11	52.4
Mar-18	344	76	9	11.8	533	81	15	18.5	400	88	21	23.9	191	48	18	37.5
Apr-18	384	110	19	17.3	147	58	8	13.8	6	4	1	25	158	47	25	53.2
May-18	230	88	25	28.4	60	39	4	10.3	0	0	0	0	29	19	12	63.2
Jun-18	69	36	6	16.7	4	32	6	18.8	1	0	0	0	2	2	1	50
Total	1891	608	152	25	1241	431	83	19.3	1148	356	97	27.2	1208	354	129	36.4

ANNEX B: SPOROZOITE RATES

	Nyagatare			Kirehe			Bugesera			Ngoma		
	Total tested	# positive	% positive	Total tested	# positive	% positive	Total tested	# positive	% positive	Total tested	# positive	% positive
Jul-17	6	0	0	9	0	0	53	2	3.77	8	0	0
Aug-17	54	0	0	85	0	0	123	0	0	88	0	0
Sept-17	166	1	0.6	131	0	0	203	1	0.5	122	0	0
Oct-17	73	0	0	38	0	0	121	0	0	75	0	0
Nov-17	100	1	1	37	0	0	83	0	0	49	0	0
Dec-17	71	1	1.4	38	0	0	192	2	1.04	53	2	3.77
Jan-18	25	0	0	23	0	0	107	1	0.93	52	0	0
Feb-18	94	0	0	80	2	2.5	234	1	0.42	45	1	2.22
Mar-18	103	2	2	112	0	0	152	0	0	100	1	1
Apr-18	124	0	0	90	0	0	20	1	5	88	1	1.13
May-18	114	0	0	77	0	0	22	0	0	94	2	2.12
Jun-18	66	1	1.5	18	0	0	5	1	20	14	0	0
Total	996	6	0.6	738	2	0.27	1315	9	0.68	788	7	0.88

ANNEX C: ENTOMOLOGICAL INOCULATION RATES OF *AN. GAMBIAE* S.L.

	Nyagatare					Kirehe				
Month	Total *An. gambiae* s.l. collected	Bites/person/night	SPZ rate	Nightly EIR	Monthly EIR	Total *An. gambiae* s.l. collected	Bites/person/night	SPZ rate	Nightly EIR	Monthly EIR
Jul-17	2	0.0	0.00	0.00	0.00	4	0.1	0	0	0
Aug-17	39	0.8	0.00	0.00	0.00	81	1.7	0	0	0
Sept-17	304	6.3	0.61	0.04	1.16	167	3.5	0	0.00	0.00
Oct-17	117	2.4	0.00	0.00	0.00	81	1.7	0	0.00	0
Nov-17	165	3.4	1.00	0.03	1.03	32	0.7	0	0	0
Dec-17	108	2.3	1.41	0.03	0.95	36	0.8	0	0	0
Jan-18	23	0.5	0.00	0.00	0.00	24	0.5	0	0.00	0.00
Feb-18	106	2.2	0.00	0.00	0.00	72	1.5	3.07	0.05	1.38
Mar-18	344	7.2	1.94	0.14	4.17	533	11.1	0	0	0
Apr-18	384	8.0	0.00	0.00	0.00	147	3.1	0	0	0
May-18	230	4.8	0.00	0.00	0.00	60	1.3	0	0	0
Jun-18	69	1.4	2.08	0.03	0.90	4	0.1	0	0	0

	Bugesera					Ngoma (Control)				
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
Jul-17	23	0.5	4	0.02	0.58	6	0.3	0	0	0
Aug-17	151	3.1	0	0.00	0	117	4.9	0	0	0
Sept-17	212	4.4	0.5	0.02	0.66	298	12.4	0	0	0
Oct-17	153	3.2	0	0.00	0	194	8.1	0	0	0
Nov-17	78	1.6	0	0.00	0	84	3.5	0	0	0
Dec-17	31	0.6	0	0.00	0	34	1.4	4.25	0.06	1.81
Jan-18	36	0.8	0	0.00	0	57	2.4	0	0	0
Feb-18	57	1.2	1.07	0.01	0.38	38	1.6	2.4	0.04	1.14
Mar-18	400	8.3	0	0.00	0	191	8.0	1	0.08	2.39
Apr-18	6	0.1	0	0.00	0	158	6.6	0	0	0
May-18	0	0.0	0	0.00	0	29	1.2	2.3	0.03	0.83
June-18	1	0.0	0	0.00	0	2	0.1	0	0	0

ANNEX D. REFERENCES

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