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PMI VECTORLINK RWANDA 2019 ENTOMOLOGICAL MONITORING REPORT JULY 2018 – JUNE 2019

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PMI VECTORLINK RWANDA 2019 ENTOMOLOGICAL MONITORING REPORT

JULY 2018–JUNE 2019

CONTENTS

LIST OF TABLES

LIST 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
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

We acknowledge the invaluable contributions and support from the many people and institutions that contributed to the collection and analysis of the entomological data reported here. Particularly, we thank the Rwanda Ministry of Health through Rwanda Biomedical Center's Malaria and Other Parasitic Diseases Division for their fruitful collaboration in conducting the entomology monitoring activities including field data collection, supervision, and review of this report.

EXECUTIVE SUMMARY

During the year-long reporting period July 2018–June 2019, monthly entomological data collection took place in five districts: Nyagatare, Kirehe, Bugesera, Ngoma, and Kamonyi. Nyagatare, Kirehe, and Ngoma were surveyed throughout the year (12 months), Bugesera for nine months (July 2018-March 2019), and Kamonyi for three months (April-June 2019). Ngoma served as the control for nine months (July 2018-March 2019) but was replaced by Kamonyi when it (Ngoma) was sprayed in March 2019. Adult mosquitoes were sampled using pyrethrum spray catch (PSC) and human landing catch (HLC) methods, to assess vector species composition, seasonality, and behavior. World Health Organization (WHO) cone bioassays were conducted in two districts (Nyagatare and Kirehe) 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 *Anopheles gambiae* s.l. was identified using the polymerase chain reaction (PCR) method.

During the reporting period, a total of 7,133 adult female *Anopheles* mosquitoes were collected, 5,820 using HLC and 1,313 using PSC. Among the *Anopheles* mosquitoes collected, 90.2% were *An. gambiae* s.l., 5.44% *An. pharoensis*, 3.28% *An. ziemanni*, and the remaining percentage was shared by *An. maculipalpis*, *An.coustani*, *An. funestus* group, and *An. rufipes*. All *Anopheles* collected by PSC were *An. gambiae* s.l.

A subsample of *An. gambiae* s.l. were identified using the PCR method, and 66% were *An. arabiensis*. *An. arabiensis* was dominant in most of the sites where the spraying took place, except in Remera and Zaza sites in Ngoma district, which was sprayed in March 2019 for the first time and where *An. gambiae* s.s. was more prevalent. At the Musambira site (control), *An. gambiae* s.s. was more dominant, and the difference was statistically significant ($p<0.05$).

An. gambiae s.l. generally showed a slightly more exophagic than endophagic tendency in all sites surveyed. The difference between indoor and outdoor collections was statistically significant in all sites that were sprayed except the sites in Bugesera district. The difference between the indoor and outdoor landing collections in Kamonyi district (control) was not statistically significant ($p>0.05$).

The peak of *An. gambiae* s.l. bites was observed very early in the evening (18:00-20:00), both indoors and outdoors, in Nyagatare and Kirehe districts. In other districts, the bites started to peak around midnight, both indoor and outdoor.

Although vector density varied over the months of the reporting period, Ngoma district showed a higher average vector density among the IRS districts (0.47 *An. gambiae* s.l./house/collection) than did the other IRS districts of Bugesera (0.4 *An. gambiae* s.l./house/collection), Nyagatare (0.29 *An. gambiae* s.l./house/collection), and Kirehe (0.17 *An. gambiae* s.l./house/collection). Kamonyi (control) district showed the highest density of 4.6 *An. gambiae* s.l./house/collection.

Ovary dissection of the *An. gambiae* s.l. collected through HLC was performed to determine parity rates. There was a significant difference ($p<0.05$) between the average number of parous *An. gambiae* s.l. in the Kamonyi control site and both sites in Nyagatare, Kirehe, and Bugesera districts and one site of Ngoma district, but the difference was not statistically significant in one site (Remera) of Ngoma district. The difference observed in other intervention sites could be attributed to the IRS. The similar parity rates in Remera site (Ngoma district) and Musambira in Kamonyi district (control) could be attributed to the fact that in Remera site, nine months of collections were done without IRS.

The overall sporozoite positivity rate was 0.22% (n=3,182). Among the positive mosquitoes, *An. gambiae* s.l. represented 85.7% of the total *Anopheles* mosquitoes that tested positive and *An. pharoensis* represented 14.3%. According to the location of collection, there was no significant difference between infectivity rates of mosquitoes collected indoors and outdoors in all surveyed sites.

The entomological inoculation rate (EIR) was calculated on a quarterly basis. The highest EIR, 1.1 infective bites/person/month, was observed in Nyagatare in the second quarter (October-December 2018). The EIR of *An. gambiae* s.l. based on collection location showed that the outdoor EIR is higher than the indoor EIR in October-December 2018 in Nyagatare, and in Kirehe in the third quarter.

A total of 147 blood-fed *An. gambiae* s.l. samples from the PSC collections made over the year-long reporting period were tested for vertebrate host blood source (human, bovine, and goat). Human blood indices were as follows: Kamonyi 86.4%, Ngoma 82.4%, Bugesera 50%, Nyagatare 41.6%, and Kirehe 18.8%. The results showed that a relatively high proportion of the vectors also fed on non-human hosts.

Cone bioassays conducted within one week after spraying to assess the quality of spraying showed 100% mortality of susceptible *An. gambiae* s.s., which is a proxy measure indicating that the quality of the spray operation was good. Subsequently, bio-efficacy of the sprayed insecticide was monitored monthly. Through June 2019 (nine months post IRS), the mortality rate was over 80% on all surface types.

The results for fumigant effect of pirimiphos-methyl 300CS 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 six months, it was below 20%.

1. INTRODUCTION

The U.S. 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 places where mosquitoes rest. In September 2017, PMI awarded Abt Associates the five-year PMI VectorLink Project. Working in 23 countries in sub-Saharan Africa as well as Cambodia, PMI VectorLink 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.

In 2018, VectorLink Rwanda sprayed two districts, Kirehe (12 sectors) and Nyagatare (14 sectors) between September 10 and October 2, 2018. Actellic 300CS was used for a third consecutive year starting 2016.

This report covers entomological monitoring activities conducted from July 1, 2018, to June 30, 2019. The entomological monitoring activities were aimed at:

- Assessing malaria vector density and species composition in intervention and selected control areas
- Understanding vector preference for feeding times and locations and estimating human biting rates (HBRs)
- 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 three IRS districts at a time, Bugesera (September 18-March 19) or Ngoma district (April-June 2019), Kirehe, and Nyagatare, and one non-IRS (control) district, Kamonyi (which replaced Ngoma district starting in April 2019, when Ngoma was sprayed in March 2019 (Figure 1). The MOPDD/NMCP decided to spray Ngoma in March 2019 after it served as a control district for 9 months. In each IRS district, two sites were selected as data collection sites. In the control district, one site was selected. Table 1(i) lists the data collection sites and their spray status, and (ii) shows the data collection schedule.

Figure 1: Data Collection Districts

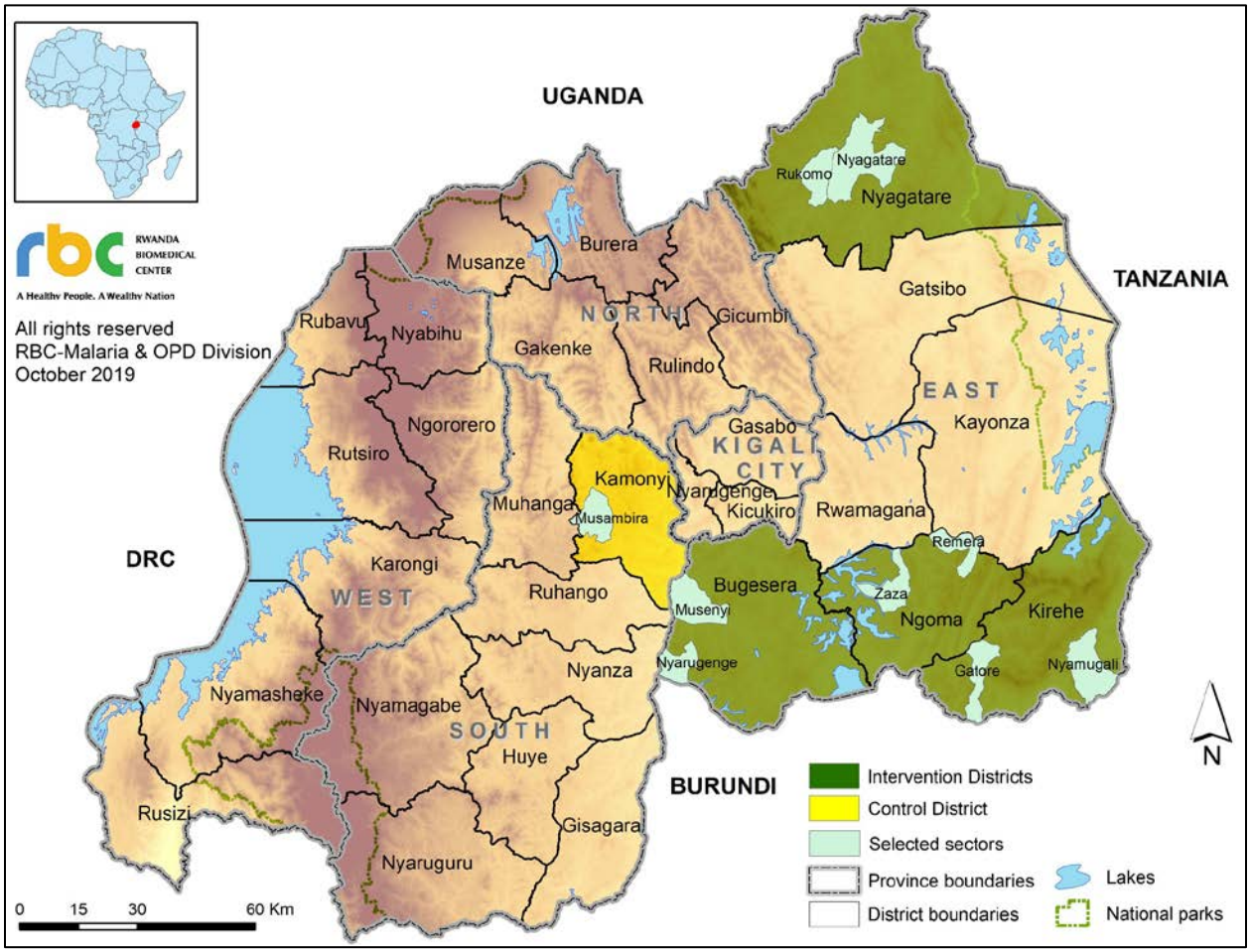


Table 1: Data Collection (Sentinel) Sites

(i) Spray Status of Sites

District	Data Collection Sites	Spray Status
Bugesera	Nyarugenge, Musenyi	Sprayed March 2019 using Actellic 300CS by Government of Rwanda
Ngoma	Remera, Zaza	Sprayed March-April 2019 using Actellic 300CS by Government of Rwanda
Nyagatare	Nyagatare, Rukomo	Sprayed Sep-Oct 2018 using Actellic 300CS with PMI support
Kirehe	Gatore, Nyamugali	Sprayed Sep-Oct 2018 using Actellic 300CS with PMI support
Kamonyi (control)	Musambira	Not sprayed

(ii) Data Collection Schedule

District	Site	Ento Monitoring Data												
		J	A	S	O	N	D	J	F	M	A	M	J	Comment
Nyagatare	Nyagatare	x	x	x	x	x	x	x	x	x	x	x	x	12 months of data
	Rukomo	x	x	x	x	x	x	x	x	x	x	x	x	
Kirehe	Gatore	x	x	x	x	x	x	x	x	x	x	x	x	
	Nyamugali	x	x	x	x	x	x	x	x	x	x	x	x	
Bugesera	Nyarugenge	x	x	x	x	x	x	x	x	x	NA	NA	NA	Dropped as IRS/data collection district effective April 2019
	Musenyi	x	x	x	x	x	x	x	x	x	NA	NA	NA	
Ngoma	Remera	c	c	c	c	c	c	c	c	c	x	x	x	Gov't of Rwanda decided to spray district starting March (Remera spent 9 months as a control and 3 as IRS district; Zaza is a new site)
	Zaza	NA	NA	NA	NA	NA	NA	NA	NA	NA	x	x	x	
Kamonyi	Musambira	NA	NA	NA	NA	NA	NA	NA	NA	NA	c	c	c	New control district starting in April 2019

Note: x=IRS site, c=control site, NA=not applicable

2.2 Data Collection Methods

Blood-seeking and indoor-resting adult mosquito collections were conducted each month in every site using human landing catch (HLC) and pyrethrum spray catch (PSC) methods, respectively.

Spray quality was assessed in four sites (two in Kirehe and Nyagatare district) using World Health Organization (WHO) standard protocol (WHO 2006) cone/wall bioassays, which were conducted within one week after 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. The same houses were used for HLC each month. A team of collectors was composed of four people per house per night; two collectors per house, one indoors and another outdoors, collected mosquitoes from 18:00 to 24:00h and two

others collected from 24:00 to 6:00h. In each site, the collectors switched places (outdoors vs indoors) 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. The same houses were sampled each month. Collections were carried out in the morning between 06:00 and 09:00h. Before the performance of PSC, all occupants were politely asked for their consent to remove food stuff and other items 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 aerosol (BOP) 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, fully 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. was identified to species level by the polymerase chain reaction (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 50µl of grinding buffer; then another 200µl of grinding buffer was added, bringing the final volume to 250µl. Fifty-microliter aliquots were tested by Enzyme-linked Immunosorbent Assay (ELISA) using monoclonal antibodies to detect circumsporozoite proteins of *Plasmodium falciparum* received for free from BEI resources (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 fully 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 50µl phosphate buffered saline (PBS) was added; the mixture was ground with a pestle, and another 950µl 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 l99l).

2.6 Molecular Identification of *Anopheles Gambiae* s.l.

A subsample of *An. gambiae* s.l. collected by HLC and PSC was used in molecular tests (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 the CTAB (Cetyl Trimethyl Ammonium Bromide)

method and DNA was amplified using primers specific to *An. gambiae* s.s., *An. arabiensis*, *An. merus*, *An. quadriannulatus*, universal primer, and Taq polymerase. 1×TAE running buffer was 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 TAE at 100 volts for 1 hour. The bands were visualized under ultraviolet 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 the 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 *An. gambiae* s.s. were put in a small cage (15 cm x 10 cm) covered with an untreated polyester net. Cages were placed approximately 10 cm from a sprayed wall and about one meter above the floor. Mosquitoes were exposed for 30 minutes and then transferred to paper cups in which they were provided with 10% glucose soaked 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.

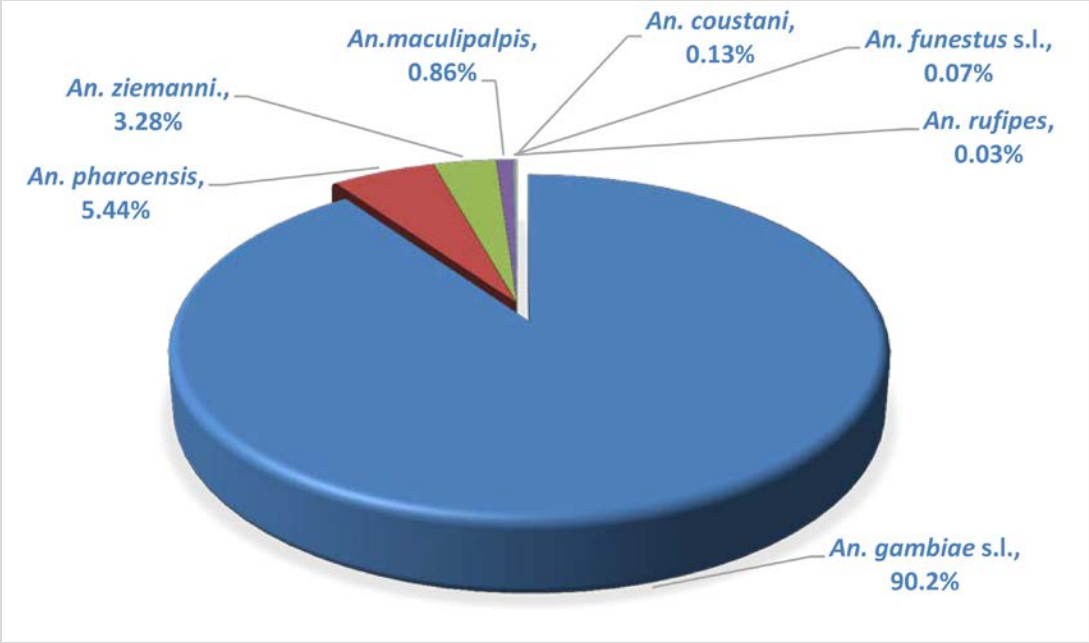
3. Results, Discussion, and Conclusions

3.1 Species Composition and Vector Seasonality

3.1.1 Species Composition

During the reporting period July 2018 to June 2019, a total of 7,133 adult female *Anopheles* mosquitoes were collected; 5,820 were collected using HLC, 1,313 using PSC. As shown in Figure 2, among the *Anopheles* mosquitoes collected, 90.2% were *An. gambiae* s.l., 5.44% *An. pharoensis*, 3.28% *An. ziemanni*, and the remaining percentage was shared by *An. maculipalpis*, *An.coustani*, *An. funestus* s.l., and *An. rufipes*. All *Anopheles* collected by PSC were *An. gambiae* s.l. In addition, 38,517 *Culex* mosquitoes were collected. Only *An. gambiae* s.l. and the *An. funestus* s.l. are known as primary vectors of malaria in Rwanda.

Figure 2: Anopheles Species Composition



A subsample of *An. gambiae* s.l. (n=597) was identified using molecular technique; 66% were *An. arabiensis*.

An. arabiensis was dominant in all of the sprayed sites except in Remera and Zaza sites, both in Ngoma district, which was sprayed for the first time in March 2019; in those two Ngoma sites, *An. gambiae* s.s. was dominant (Table 2). The difference in proportion between *An. arabiensis* and *An. gambiae* s.s. in all sites was statistically significant. In Musambira site (control site starting April 2019), *An. gambiae* s.s. was dominant.

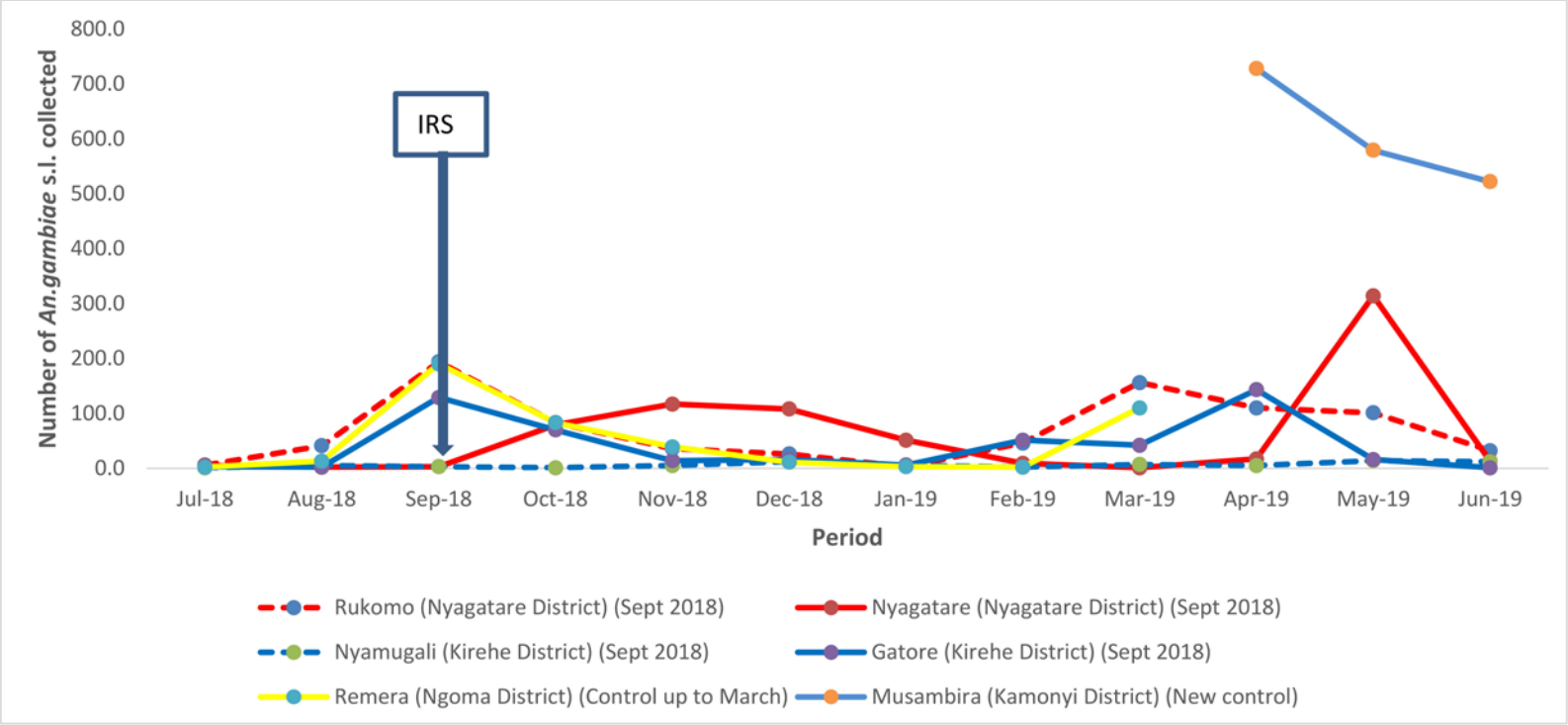
Table 2: *An. gambiae* s.l. Sibling Composition

District	Site	Spray Status/Time	*An. gambiae s.s.*	*An. arabiensis*	P-value	Significance Status
Nyagatare	Nyagatare	Sep 2018	0%(0)	100%(57)	P<.001	S
	Rukomo	Sep 2018	0%(0)	100%(118)	P<.001	S
Kirehe	Gatore	Sep 2018	5.6%(4)	94.4%(68)	P<.001	S
	Nyamugali	Sep 2018	0%(0)	100%(17)	P<.001	S
Bugesera	Nyarugenge	March 2019	24%(18)	76%(57)	P<.001	S
	Musenyi	March 2019	29.5%(18)	70.5%(43)	0.001	S
Ngoma	Remera	March 2019	68.8%(77)	31.2%(35)	P<.001	S
	Zaza	March 2019	90.5%(19)	9.5%(2)	0.0002	S
Kamonyi	Musambira	Control	100%(64)	0%(0)	P<.001	S

3.1.2 VECTOR SEASONALITY

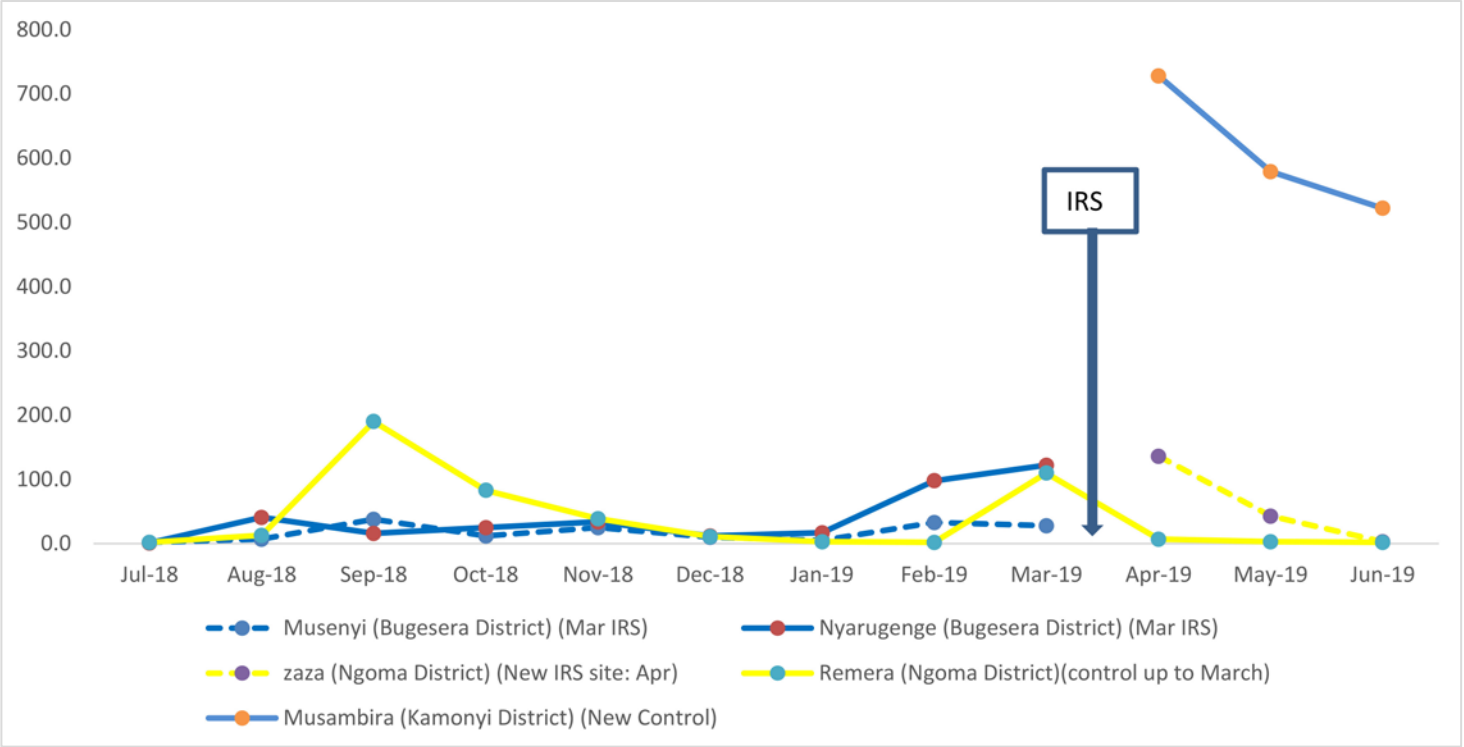
An. gambiae s.l. was the most prevalent malaria vector collected by HLC throughout the data collection period in both the intervention and control sites. As Figure 3A shows, the number of *An. gambiae* s.l. collected in Kirehe (Gatore) was high in the district's pre-IRS months, September 2018 in particular, but decreased after the September-October IRS. The numbers at Nyamugali site were low before and after spray. In Nyagatare district, the number of mosquitoes was high in Rukomo site pre-IRS and decreased after IRS, but the opposite happened in the Nyagatare site of Nyagatare district. In Bugesera district (Nyarugenge and Musenyi) (Figure 3B), the numbers were low until January 2019 and started to increase in February and March 2019; probably as a result of the waning of the residual efficacy of the insecticide that was sprayed in March 2018. No data was collected after March 2019 as the district was dropped as a sentinel site. There was also some decrease in the control site of Remera (Ngoma district) starting in September 2018 and lasting into February 2019 which could also be due to the seasonal fluctuation in mosquito populations. In March 2019, Ngoma became an intervention district, and Bugesera district was dropped to balance the budget. Ngoma district was sprayed in March, and number sharply declined after spray in both sentinel sites (Remera and Zaza). Musambira site (Kamonyi district) was chosen to replace Remera as the new control site. It was the first time mosquitoes were collected in Musambira and the numbers collected in the 3 months post spray (Apr-Jun) were much higher in this new control site than in the IRS sites.

Figure 3a: Number of *An. gambiae* s.l. Collected by Month for September 2018 IRS Campaign



Nyagatare and Kirehe were sprayed in Sept-Oct 2018, whereas Remera (Ngoma District) was used as control up to March 2019 (Figure 3a), and then sprayed in March 2019 where it replaced Bugesera district as intervention (figure 3b).

Figure 3b: Number of An. gambiae s.l. Collected by Month for March 2019 IRS Campaign



Nyagatare and Kirehe were sprayed in Sept-Oct 2018, whereas Remera (Ngoma District) was used as control up to March 2019 (Figure 3a), and then sprayed in March 2019 where it replaced Bugesera district as intervention (figure 3b).

3.2 VECTOR FEEDING TIME AND LOCATION

An. gambiae s.l. generally showed slightly more exophagic than endophagic tendency in all sites surveyed (Table 3). The average district percentage endophagy / exophagy was as follows: Nyagatare: 40.6%/59.4%, Kirehe: 29.2%/70.8%, Bugesera: 44.7%/55.3%, Ngoma: 38.8%/61.2%, and Kamonyi: 49%/51%. The difference between indoor and outdoor collections was statistically significant in all sites where the IRS was implemented except the sites in Bugesera district, which may be due to the fact that the collections were done before the February-March 2019 spray round (at which point, this district was replaced, as explained above). A chi-square test showed that the difference between the indoor and outdoor landing collections in Kamonyi district (control) was not statistically significant ($p>0.05$).

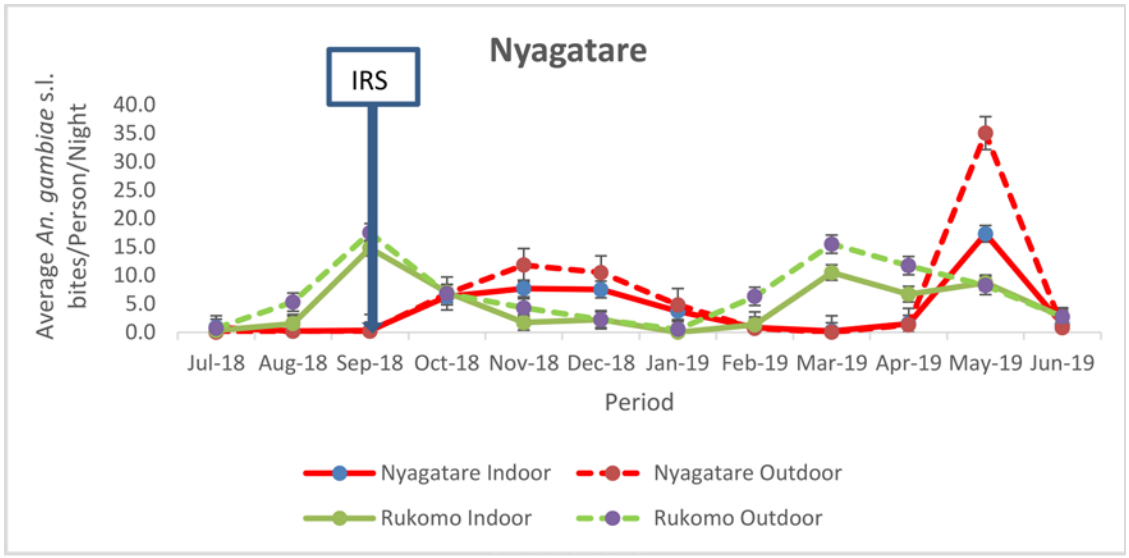
Table 3: Indoor and Outdoor Biting by *An. gambiae* s.l.

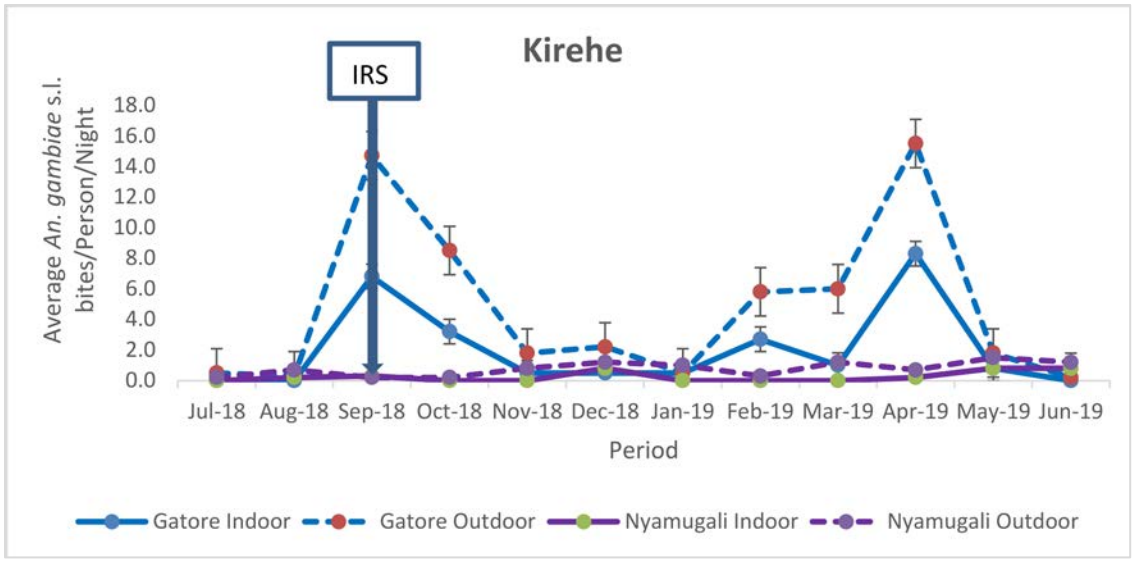
District	Site	In	Out	In: Out Ratio	P-value	Result
Nyagarare	Nyagatare	286	433	40:60	P<.001	S
	Rukomo	344	490	41:59	P<.001	S
Kirehe	Gatore	146	347	30:70	P<.001	S
	Nyamugali	19	54	26:64	P<.001	S
Bugesera	Nyarugenge	165	201	45:55	0.0599	NS
	Musenyi	70	90	44:56	0.1138	NS
Ngoma	Remera	197	268	42:58	P<.001	S
	Zaza	54	128	30:70	P<.001	S
Kamonyi	Musambira	897	932	49:51	0.413	NS

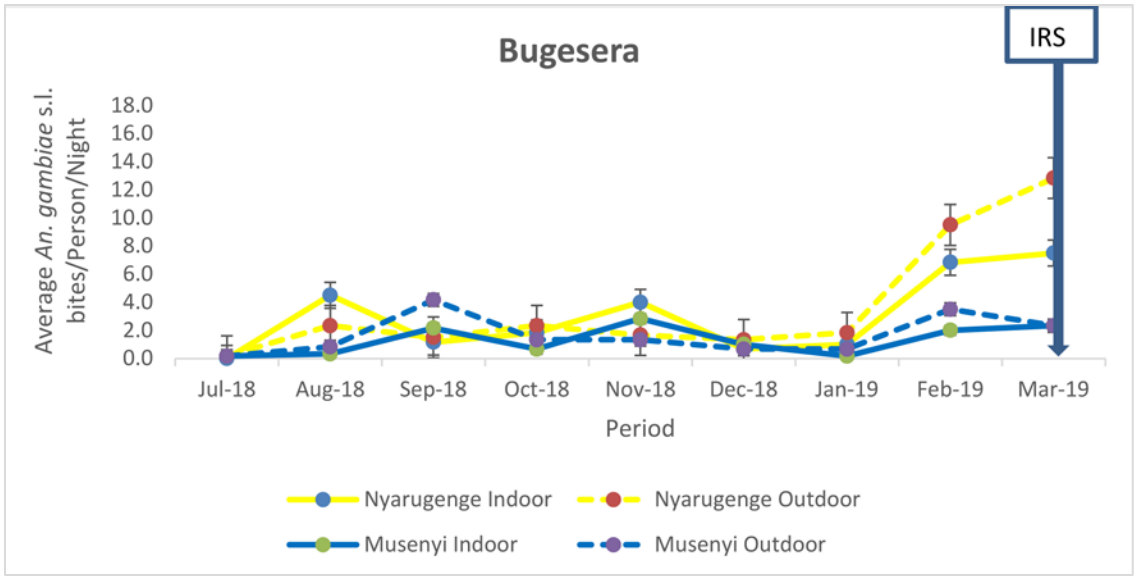
S: Statistically significant, **NS**: not statistically significant

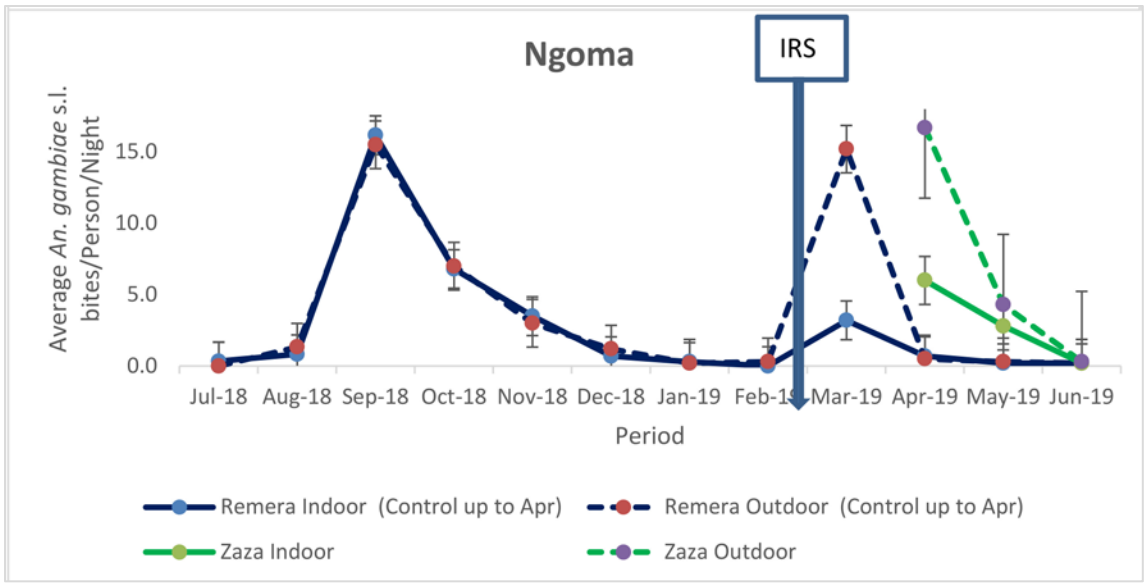
As Figure 4 shows, there are two peak biting seasons, September-October and March-April, but the HBR is higher in April-May in all intervention sites except Ngoma district , because it was sprayed later in the February-March spray round than were the other sites. In general the timing of the spray seems to be late as by the time the spray campaign starts, both in September/October and February/March cycle, the mosquito population has started or already peaked. The highest bites/person/night (b/p/n) in intervention districts was observed in Nyagatare site outdoors, but the highest overall b/p/n was observed in Musambira site (control). In general, there was more biting outdoors than indoors.

Figure 4: *An. gambiae* s.l. Average Monthly Biting Trends, by District






Bugesera
IRS
Average An. gambiae s.l. bites/Person/Night
18.0
16.0
14.0
12.0
10.0
8.0
6.0
4.0
2.0
0.0
Jul-18
Aug-18
Sep-18
Oct-18
Nov-18
Dec-18
Jan-19
Feb-19
Mar-19
Period
Nyarugenge Indoor
Nyarugenge Outdoor
Musenyi Indoor
Musenyi Outdoor


Ngoma
IRS
Average An. gambiae s.l. bites/Person/Night
15.0
10.0
5.0
0.0
Jul-18
Aug-18
Sep-18
Oct-18
Nov-18
Dec-18
Jan-19
Feb-19
Mar-19
Apr-19
May-19
Jun-19
Period
Remera Indoor (Control up to Apr)
Remera Outdoor (Control up to Apr)
Zaza Indoor
Zaza Outdoor

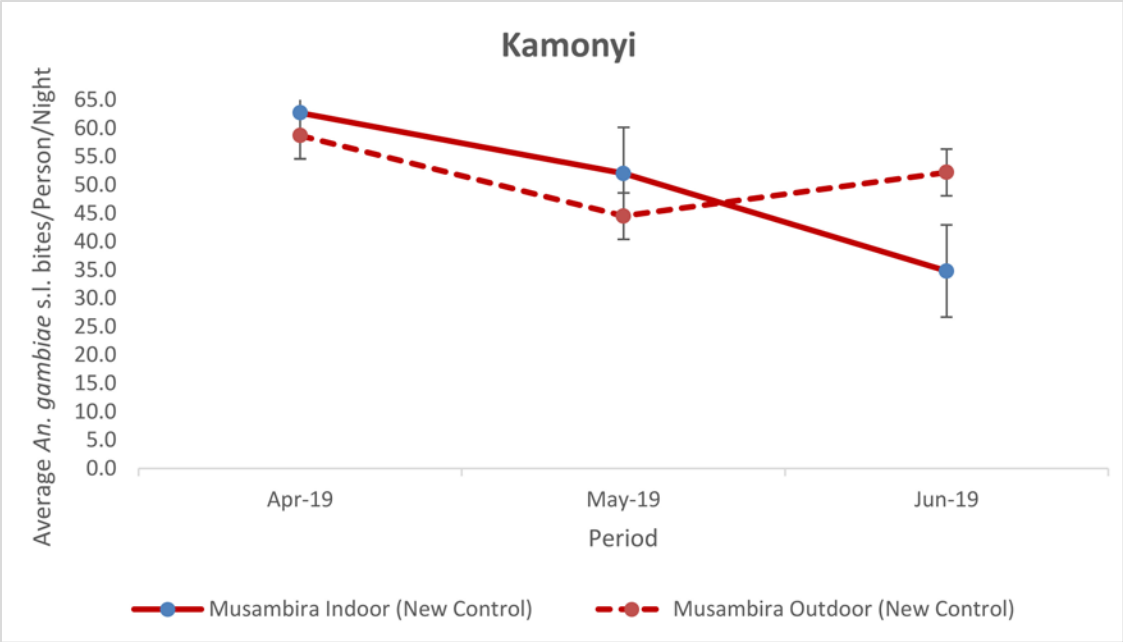

Kamonyi
Average An. gambiae s.l. bites/Person/Night
65.0
60.0
55.0
50.0
45.0
40.0
35.0
30.0
25.0
20.0
15.0
10.0
5.0
0.0
Apr-19
May-19
Jun-19
Period
Musambira Indoor (New Control)
Musambira Outdoor (New Control)

Figure 5: Hourly Biting of *An,gambiae* s.l., by District

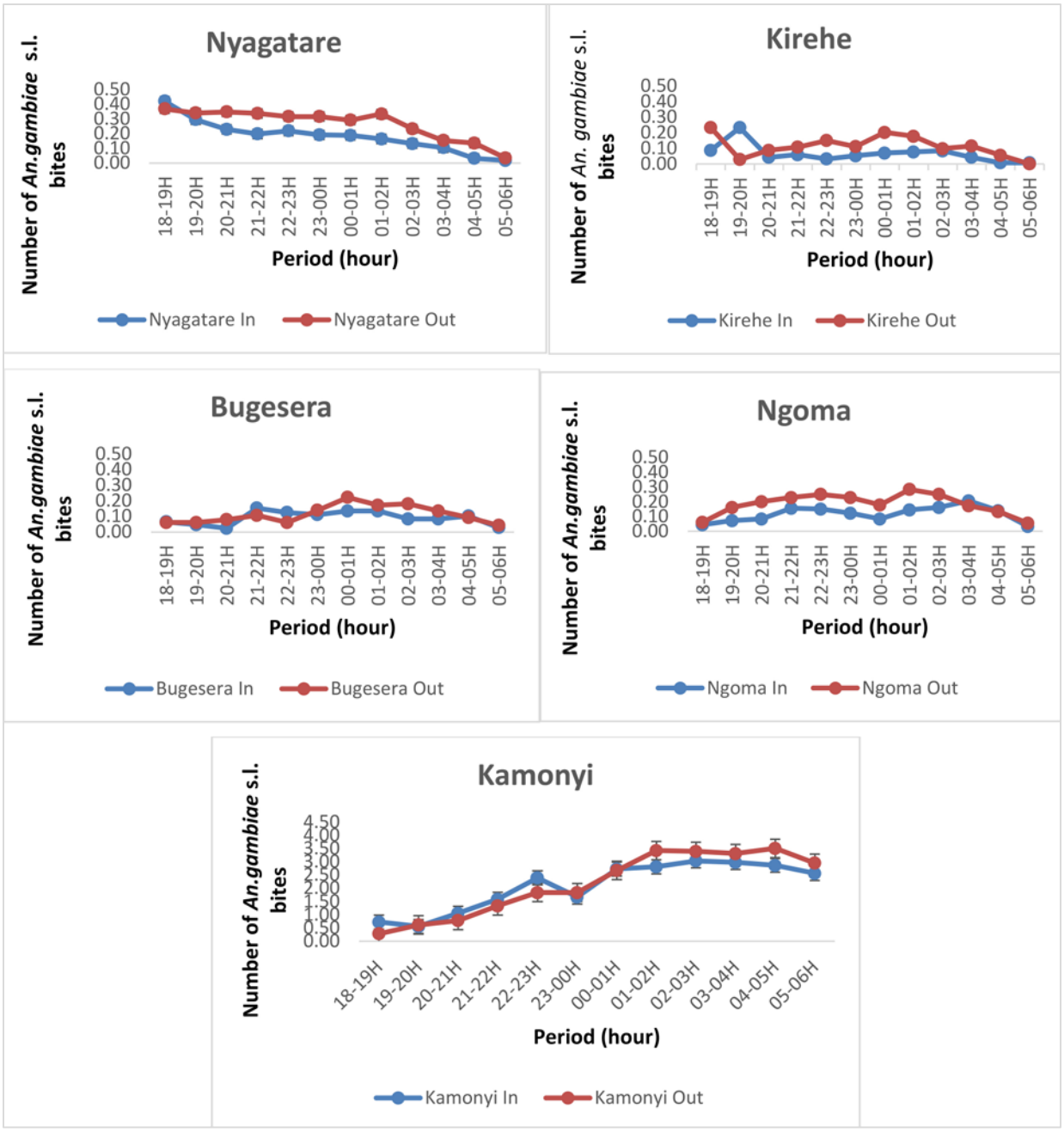


Figure 5 shows average bites per person per hour through the night across the five districts. As the figure shows, more biting took place outdoors. Within an average day, *An. gambiae* s.l. biting peaked very early in evening (18:00-20:00) both indoors and outdoors in Nyagatare and Kirehe districts. In other districts, biting started to peak around midnight both indoors and outdoors. If people do not go to bed and instead stay outdoors after nightfall, this pattern could have implications for the effectiveness of indoor-based vector control interventions.

3.3 INDOOR RESTING DENSITY

As noted above, a total of 1,313 female indoor-resting *An. gambiae* s.l. were collected using PSC in the four IRS districts and the control district over the July 2018 to June 2019 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

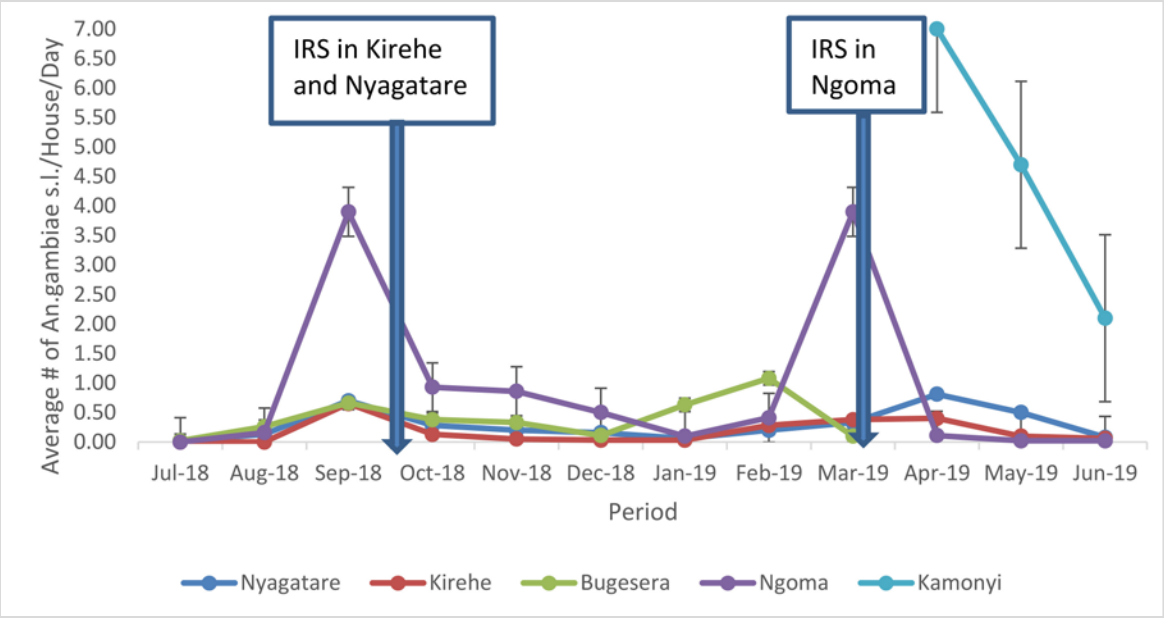
District	Nyagatare		Kirehe		Bugesera		Ngoma		Kamonyi (Control)	
	Total Collected	d/h/d	Total Collected	d/h/d	Total Collected	d/h/d	Total Collected	d/h/d	Total Collected	d/h/d
Jul-18	1	0.02	0	0	1	0.02	0	0	NA	NA
Aug-18	8	0.13	0	0	16	0.26	5	0.16	NA	NA
Sep-18	42	0.7	39	0.65	40	0.66	117	3.9	NA	NA
Oct-18	17	0.28	8	0.13	23	0.38	28	0.93	NA	NA
Nov-18	12	0.2	3	0.05	20	0.33	26	0.86	NA	NA
Dec-18	10	0.16	2	0.03	7	0.11	15	0.5	NA	NA
Jan-19	4	0.06	2	0.03	38	0.63	3	0.1	NA	NA
Feb-19	12	0.2	17	0.28	65	1.08	25	0.41	NA	NA
Mar-19	20	0.33	23	0.38	6	0.1	117	3.9	NA	NA
Apr-19	49	0.81	24	0.4	NA	NA	7	0.11	210	7
May-19	30	0.5	6	0.1	NA	NA	1	0.02	141	4.7
Jun-19	5	0.08	4	0.06	NA	NA	1	0.02	63	2.1
Avg. monthly vector density	17.5	0.29	10.7	0.17	24	0.4	28.7	0.47	138	4.6
P-value	*P*<.001		*P*<.001		*P*<.001		*P*<.001		1	

*d/h/d: density/house/day

**Ngoma district was used as control up to March 2019, when it was replaced by Kamonyi, so the P-value was calculated between Kamonyi as control and the other, intervention districts.

In general there are two peaks of *An. gambiae* s.l. density, the first one in September, and the second one in March (Figure 6). The difference between average monthly *An. gambiae* s.l. density in all IRS districts and control district was statistically significant (<0.05). Although monthly vector density varied throughout the reporting period, Ngoma district showed the highest average vector density of the IRS districts (0.47 *An. gambiae* s.l./house/collection); this was slightly higher than Bugesera (0.4 *An. gambiae* s.l./house/collection) and appreciably higher than Nyagatare (0.29 *An. gambiae* s.l./house/collection) and Kirehe (0.17 *An. gambiae* s.l./house/collection). The high vector density observed in Ngoma and Bugesera districts may due to the fact that they were sprayed later in March 2019. Indoor resting density in Ngoma was very low, essentially zero, after the March IRS. The control (non-IRS) Kamonyi district showed the highest density, 4.6 *An. gambiae* s.l./house/collection.

Figure 6: *An. gambiae* s.l. Indoor Resting Density, by District



All 1,313 *An. gambiae* s.l. collected indoors in all sites using PSC were classified according to their blood digestion stages: 578 (44%) were unfed, 604 (46%) were fed, 105 (8%) were half-gravid, and 26 (2%) were gravid (Figure 7). The half-gravid and gravid were collected mostly in Kamonyi district (Figure 8), probably because this district was not sprayed. The high proportion of unfed mosquitoes collected indoors may indicate a high and sustained usage of long-lasting insecticide-treated nets in the communities. Further observation is required.

Figure 7: Blood Digestion Stages of All *An. gambiae* s.l. Collected Using PSC

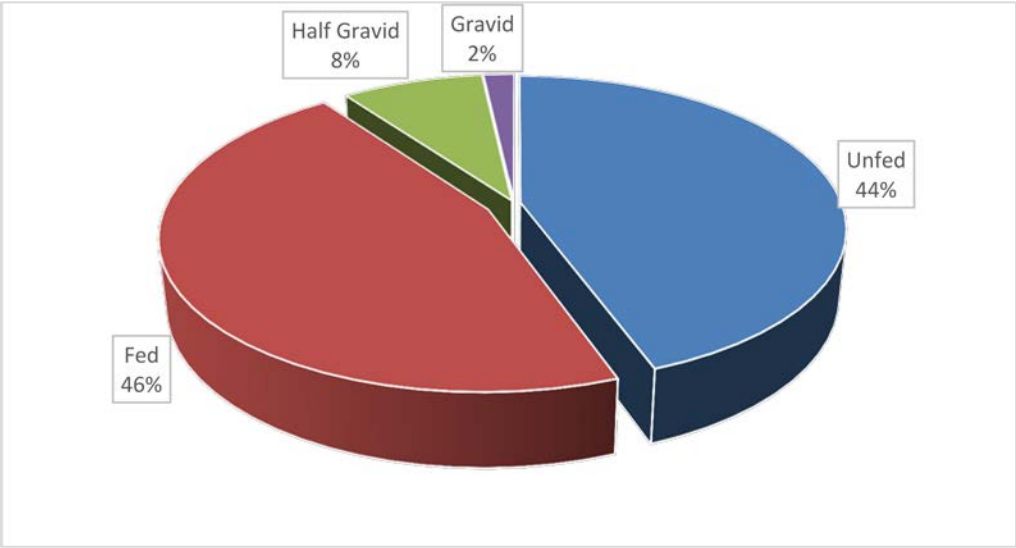
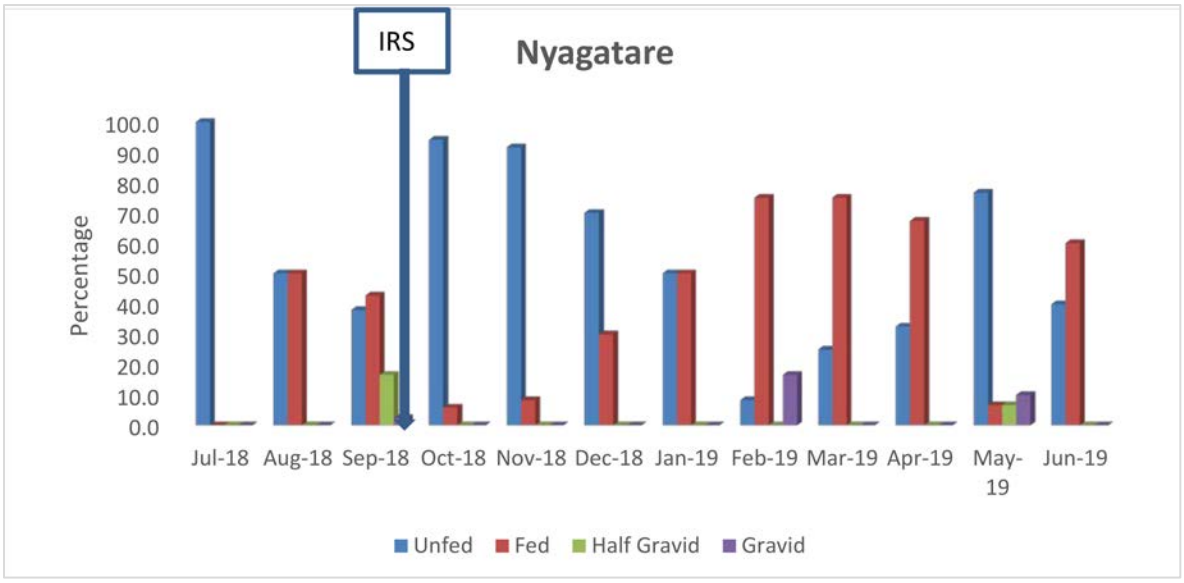
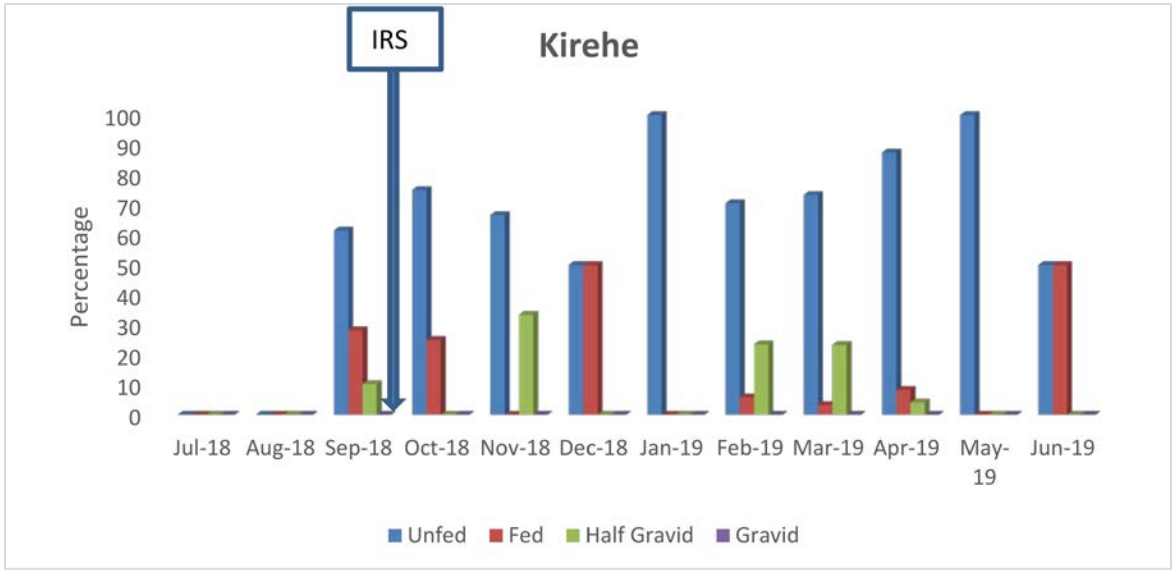
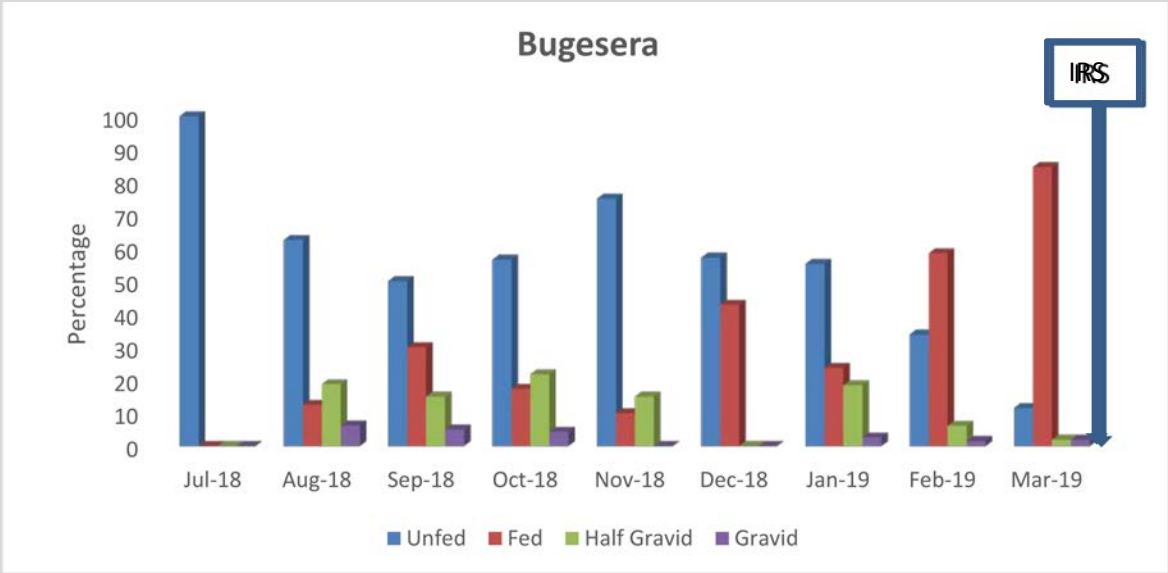
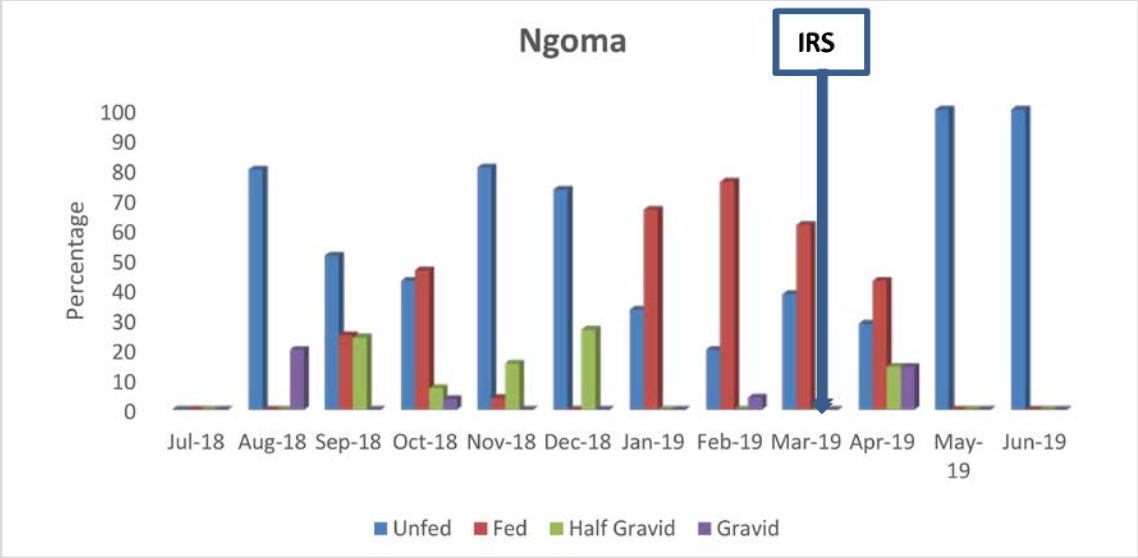


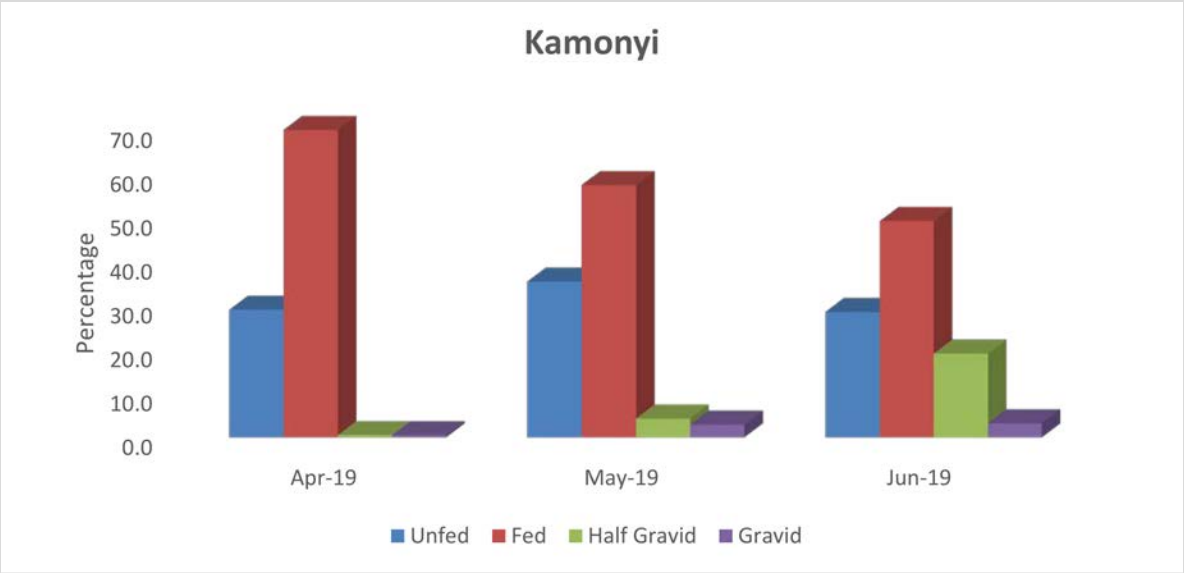
Figure 8: Blood Digestion Stages of An. gambiae s.l. Collected using PSC, by District and Month






Bugesera
IRS
Percentage
100
90
80
70
60
50
40
30
20
10
0
Jul-18
Aug-18
Sep-18
Oct-18
Nov-18
Dec-18
Jan-19
Feb-19
Mar-19
Unfed
Fed
Half Gravid
Gravid


Ngoma
IRS
Percentage
100
90
80
70
60
50
40
30
20
10
0
Jul-18
Aug-18
Sep-18
Oct-18
Nov-18
Dec-18
Jan-19
Feb-19
Mar-19
Apr-19
May-19
Jun-19
Unfed
Fed
Half Gravid
Gravid



3.4 DETERMINATION OF PARITY

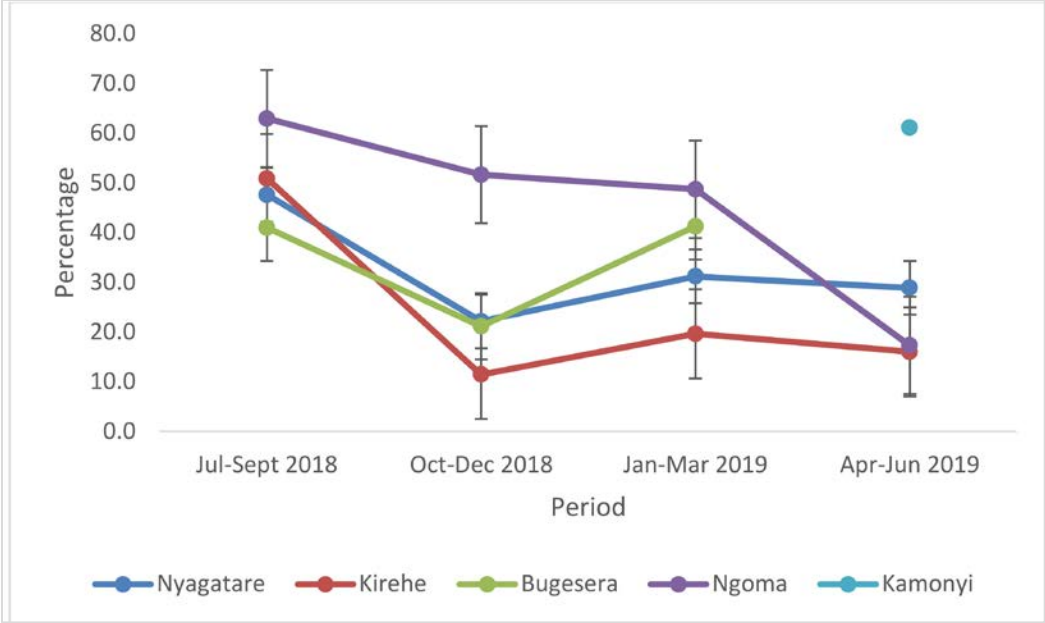
Ovary dissection of the *An. gambiae* s.l. collected through HLC was performed to determine parity rates. Table 5 shows average percentage parity from July 2018 to June 2019. A Z-test of proportions showed that there was a significant difference ($p<0.05$) between the proportion of parous *An. gambiae* s.l. in the Kamonyi control site and in both sites in Nyagatare, Kirehe, and Bugesera districts and in one site of Ngoma district; the difference was not statistically significant in one site (Remera) of Ngoma district. The difference observed in the other intervention sites could be attributable to the IRS. The similar parity rates in Remera site (Ngoma intervention district) and Musambira (Kamonyi control district) could be attributable to the fact that nine months of collection in Remera were done before IRS.

Table 5: Parity

District	Site	Total Collected	Total *An. gambiae* s.l. Dissected	# Parous	% Parity	Confidence Interval	P-value	Result
Nyagatare	Nyagatare	719	225	63	28.0	22.1-33.9	P<.001	S
	Rukomo	834	310	98	31.6	26.4-36.8	P<.001	S
Kirehe	Gatore	493	219	54	24.7	18.9-30.4	P<.001	S
	Nyamugali	73	39	6	15.4	4.1-26.7	P<.001	S
Bugesera	Nyarugenge	366	153	56	36.6	29.0-44.2	P<.001	S
	Musenyi	160	119	39	32.8	24.3-41.2	P<.001	S
Ngoma	Remera	465	159	85	53.5	45.7-61.2	0.1314	NS
	Zaza	182	69	12	17.4	8.4-26.3	P<.001	S
Kamonyi	Musambira	1829	234	143	61.1	54.9-67.4	1	

When the parity data are organized by quarter, it shows that the parity rate decreased significantly in the sprayed sites after IRS, compared with the control site (Figure 9). Ngoma district showed the highest reduction after the spray in March 2019. See Annex A for more detailed data on parity.

Figure 9: Parity Rate



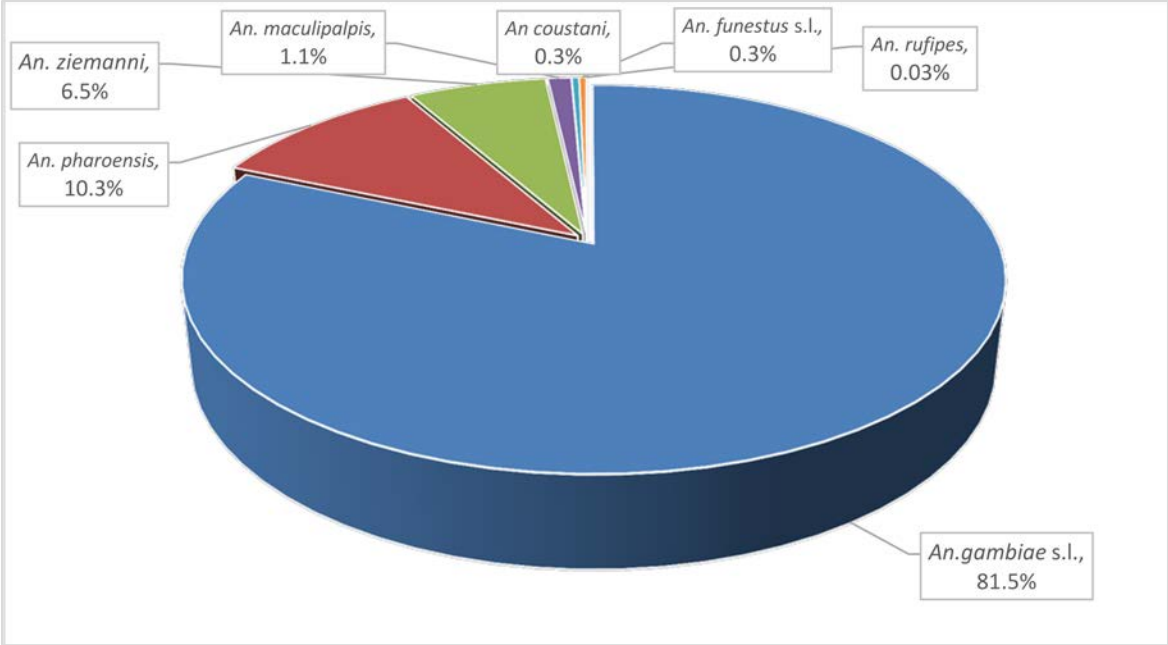
Note: Nyagatare and Kirehe were sprayed in Sept-Oct 2018, Ngoma was sprayed in March 2019, Kamonyi was used as control from April 2019.

3.5 Enzyme-Linked Immunosorbent Assay

3.5.1 Sporozoite ELISA

Mosquitoes collected through HLC and PSC were tested for infection using ELISA. A total of 3,182 mosquitoes collected from July 2018 to June 2019 from the five districts were tested for the *Plasmodium falciparum* circumsporozoite protein. Different *Anopheles* species were tested; *An. gambiae* s.l. were dominant (82%) (Figure 10).

Figure 10: *Anopheles* Mosquitoes Tested, by Species



The overall sporozoite positivity rate was 0.22% (n=3,182). Table 6 (i) and (ii) show the numbers of mosquitoes tested and found positive, by species and by monitoring site. Among the positive mosquitoes, 85.7% were *An. gambiae* s.l., and 14.3% were *An. pharoensis*. The testing was done on a quarterly basis (July–September 2018, October–December 2018, January–March 2019, and April–June 2019). In the first quarter, 670 mosquitoes were tested and only two tested positive, one from Rukomo site (Nyagatare district) and one from Nyarugenge site (Bugesera district) (Table 6(iii)). In the second quarter, 1,001 mosquitoes were tested and four were found to be positive, two from Nyagatare site (Nyagatare district), one from Gatore site (Kirehe district), and one from Remera (Ngoma district). In the third quarter, 751 mosquitoes were tested and one, from Gatore site, was positive. In the final quarter, 760 mosquitoes were tested and none was found to be positive.

According to the location of collection, there was no significant difference in infectivity rates of indoor- and outdoor-collected mosquitoes (Table 6 (iv)). The infection rate was too low to make any meaningful comparisons and analysis. The sporozoite rate per month per district is included in Annex B.

Table 6: Sporozoite Rates by Species

Species	Number Tested	Number Positive	% Positive
An. gambiae s.l.	2592	6	0.23
An. pharoensis	327	1	0.31
An. ziemanni	206	0	0.00
An. maculipalpis	35	0	0.00
An. coustani	11	0	0.00
An. funestus	10	0	0.00
An. rufipes	1	0	0.00
Total	3182	7	0.22

By Site

District	Site	# Tested	Number Positive	% Positive
Nyagatare	Nyagatare	361	2	0.55
	Rukomo	529	1	0.19
Kirehe	Gatore	526	2	0.38
	Nyamugali	77	0	0
Bugesera	Nyarugenge	365	1	0.27
	Musenyi	472	0	0
Ngoma	Remera	504	1	0.2
	Zaza	99	0	0
Kamonyi	Musambira	249	0	0

By Quarter

District	Site	Jul–Sep 2018			Oct–Dec 2018			Jan-Mar 2019			Apr-Jun 2019		
		# Tested	# Positive	% Positive	# Tested	# Positive	% Positive	# Tested	# Positive	% Positive	# Tested	# Positive	% Positive
Nyagatare	Nyagatare	18	0	0	170	2	1.17	66	0	0	107	0	0
	Rukomo	178	1	0.56	162	0	0	86	0	0	103	0	0
Kirehe	Gatore	133	0	0	143	1	0.7	124	1	0.8	126	0	0
	Nyamugali	9	0	0	15	0	0	18	0	0	35	0	0
Ngoma	Remera	132	0	0	201	1	0.49	130	0	0	41	0	0
	Zaza	NA	NA	NA	NA	NA	NA	NA	NA	NA	99	0	0
Kamonyi	Musambira	NA	NA	NA	NA	NA	NA	NA	NA	NA	249	0	0
Bugesera	Nyarugenge	103	1	0.97	118	0	0	144	0	0	NA	NA	NA
	Musenyi	97	0	0	192	0	0	183	0	0	NA	NA	NA

Significance

District	Sites	Indoor Collection			Outdoor Collection			P-value*	Results
		Total Tested	# Positive	% Positive	Total Tested	# Positive	% Positive		
Nyagatare	Nyagatare	182	1	0.55	179	1	0.55	0.9905	NS
	Rukomo	284	1	0.35	245	0	0	0.3525	NS
Kirehe	Gatore	189	0	0	337	2	0.59	0.2886	NS
	Nyamugali	17	0	0	60	0	0	NA	-
Bugesera	Nyarugenge	233	1	0.43	132	0	0	0.451	NS
	Musenyi	301	0	0	171	0	0	NA	-
Ngoma	Remera	301	1	0.33	203	0	0	0.411	NS
	Zaza	43	0	0	56	0	0	NA	-
Kamonyi	Musambira	156	0	0	93	0	0	NA	-

3.5.2 Blood Meal ELISA

Blood-fed samples from the collections made July 2018 to June 2019 were also assayed to determine the source of the blood meal. A total of 147 *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 (Table 7). A higher proportion of *An. gambiae* s.l. specimens that fed on human were observed in Ngoma, Bugesera, and Kamonyi. Ngoma and Kamonyi were control districts (Ngoma from July 2018 to March 2019 and Kamonyi from March 2019 onward), which explains the high anthropophagic behavior of *An. gambiae* s.l. in those districts. The results show that a relatively high proportion of the vectors also fed on non-human hosts especially in IRS districts.

Table 7: Blood Meal Source

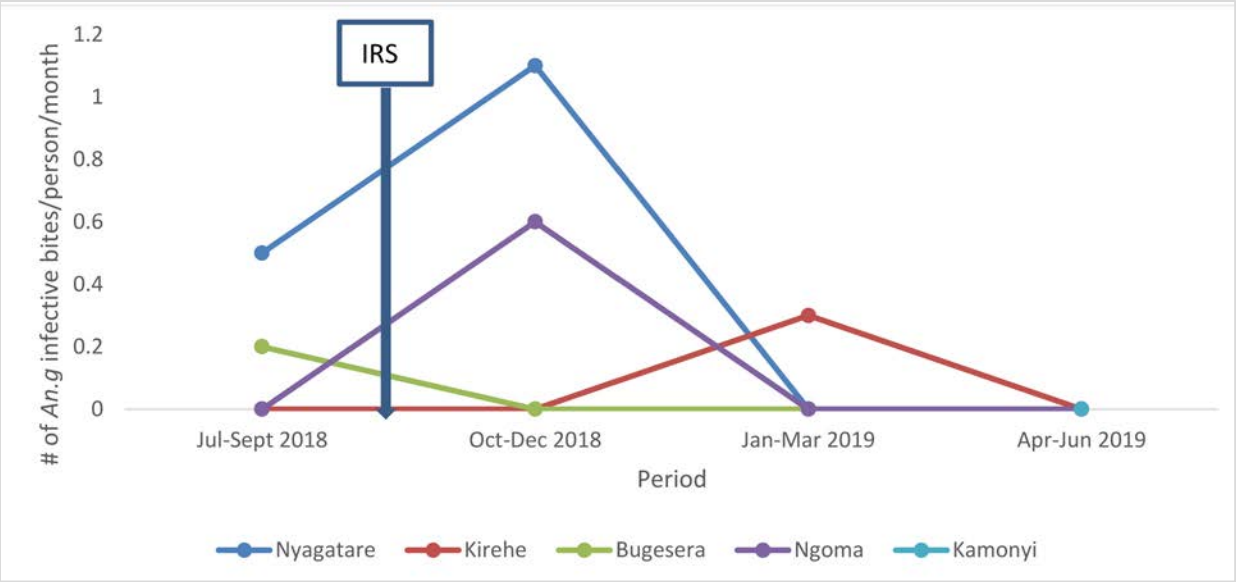
Site	Number Tested	Results					
		Human	Bovine	Goat	Human and Other	Goat and Bovine	No Specified Host
Nyagatare	36	15(41.6%)	16(44.4%)	1(2.8%)	0(0%)	1(2.8%)	3(8.4%)
Kirehe	16	3(18.8%)	9(56.2%)	1(6.3%)	1(6.3%)	1(6.3%)	1(6.3%)
Bugesera	32	16(50%)	14(43.7%)	1(3.1%)	0(0%)	0(0%)	1(3.1%)
Ngoma	41	34(82.9%)	1(2.4%)	0(0%)	0(0%)	0(0%)	6(14.7%)
Kamonyi	22	19(86.4%)	3 (13.6%)	0(0%)	0(0%)	0(0%)	0(0%)
Total	147	87(59.2%)	43(29.2%)	3(2%)	1(0.7%)	2(1.4%)	11(7.5%)

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 EIR was calculated on quarterly basis from July 2018 to June 2019. The highest EIR was observed in Nyagatare in the second quarter (October–December 2018) at 1.1 infective

bites/person/month. (See Figure 11 and Annex C). The EIR of *An. gambiae* s.l. based on collection location showed that the outdoor EIR was higher than the indoor EIR in the second quarter in Nyagatare, and in the third quarter in Kirehe (Figure 12). However, these results need to be cautiously interpreted as the EIR was generally low.

Figure 11: Entomological Inoculation Rates of *An. gambiae* s.l.



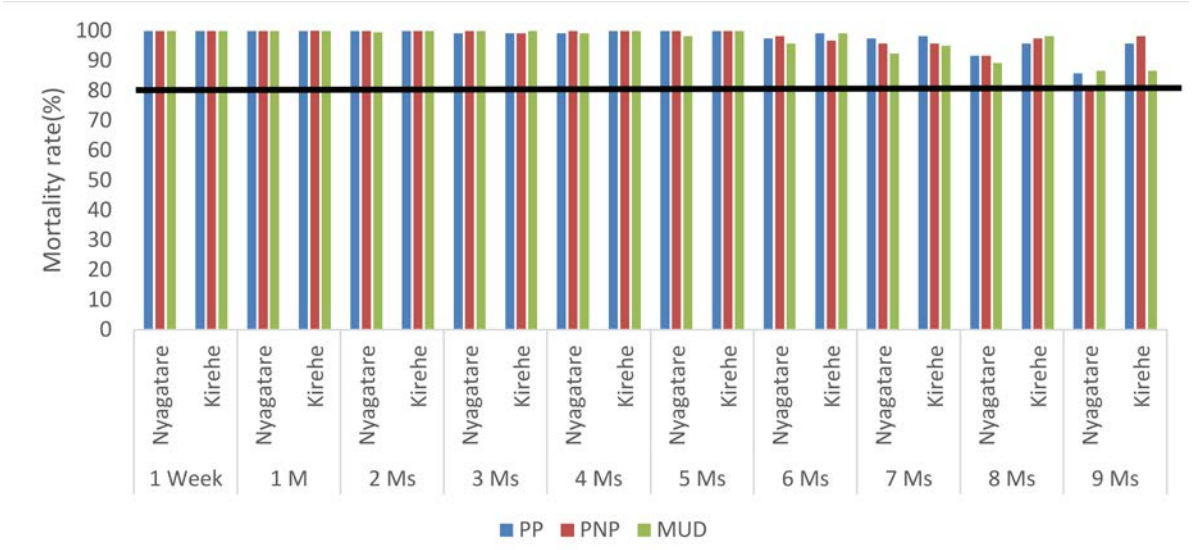
3.6 QUALITY OF SPRAY, INSECTICIDE DECAY RATE AND FUMIGANT EFFECT

3.6.1 QUALITY OF SPRAYING AND INSECTICIDE DECAY RATE

As noted above, VectorLink Rwanda sprayed Nyagatare and Kirehe districts in September 2018, using Actellic 300CS. It then carried out WHO cone wall bioassays to assess the quality of spraying. The first were carried out within one week after spraying, in 24 of the sprayed structures. Two sites were sampled in each district. In each site, six structures were sampled, two each of different wall surface types (mud, plastered not painted (PNP), and plastered and painted (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).

These first cone bioassays showed 100% mortality of susceptible *An. gambiae* s.s., a proxy measure indicating the spraying was of good quality. Subsequent bioassays were done each month to monitor the bioefficacy of the sprayed walls. Through June 2019, the mortality rate was over 80% on all wall surface types (Figure 13).

Figure 12: Wall Bioassay Test Results (September 2018–June 2019)

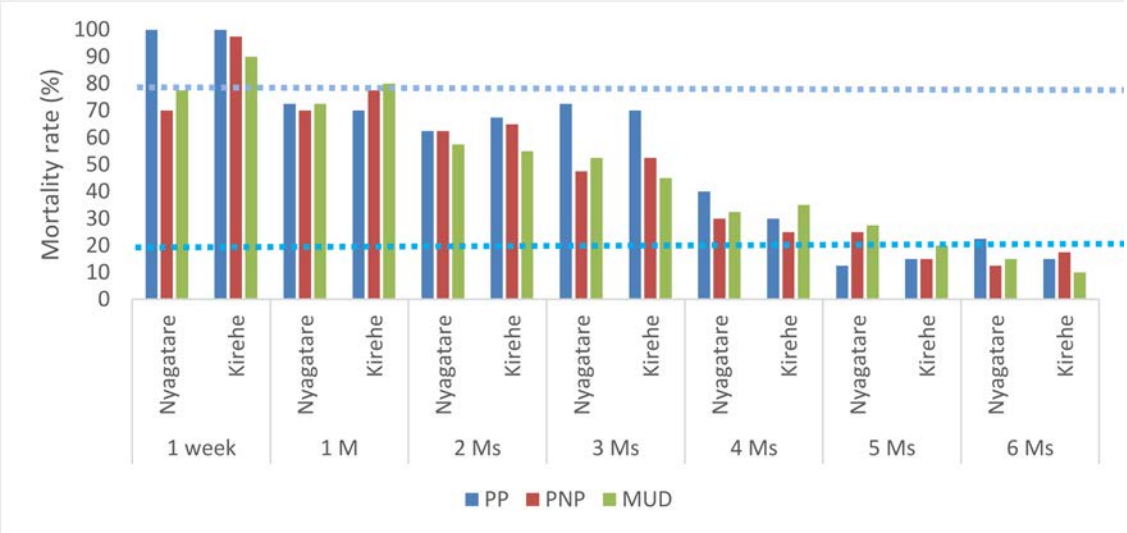


Ms: Months

3.6.2 FUMIGANT EFFECT OF PIRIMIPHOS-METHYL 300CS

The fumigant effect of pirimiphos-methyl 300CS was tested in the same sprayed houses where the cone wall bioassay was conducted. The results showed that the average mortality rate was high for the first week and the first month after spraying. It then started decreasing, and at six months, it was below 20% (Figure 14).

Figure 13: Fumigant Effect Results



3.7 CONCLUSIONS

- *An. gambiae* s.l. is the major malaria vector in all surveyed districts; based on molecular identification, *An. arabiensis* comprised the highest percentage (66%) of tested *An. gambiae* s.l. *Anopheles gambiae* s.l. was the most prevalent malaria vector throughout the data collection period in both the intervention and control sites. There was a reduction in mosquito numbers after the 2018 spray in most of the sites except in Nyagatare, where a slight increase was observed. Similarly, there was a slight increase and then dip in numbers after the March 2019 spray. The increase in malaria vectors in March to May may be attributed to the rains in that period, which created many breeding sites. It also seems that the September spray was done after the mosquito population had peaked; it would have been better to have completed spraying before August.
- The number of *An. arabiensis* collected from sprayed sites was significantly higher than *An. gambiae* s.s. It was the opposite for the control site and for Ngoma district that was sprayed in March 2019 for the first time. Therefore, IRS is probably having more impact on reducing the *An. gambiae* s.s. population than on reducing *An. arabiensis*.
- *An. gambiae* s.l. showed more exophagic than endophagic behavior in all surveyed districts. In all intervention districts except Bugesera, the difference between indoor and outdoor collection was statistically significant. The difference was not statistically significant in Kamonyi district (control). This implies that IRS might result in the mosquitoes biting more outdoors than indoors.
- The peak of *An. gambiae* s.l. bites was observed very early in the evening (18:00-20:00) both indoors and outdoors in Nyagatare and Kirehe districts. In other districts, biting started to increase around midnight both indoors and outdoors. Hourly biting was slightly higher outdoors than indoors in all sites. The early biting behavior of the mosquito in some sites may have an implication on the effectiveness of bednets as an intervention.
- In general, there are two peaks of *An. gambiae* s.l. density, the first one in September-October, and the second one in February-April. The difference between average monthly *An. gambiae* s.l. density in all IRS districts and the control district was statistically significant ($p<0.05$). Ngoma district showed the highest average vector density of the IRS districts (0.47 *An. gambiae* s.l./house/collection) until it was sprayed in March 2019, when indoor resting density there fell almost to zero. The control (non-IRS) Kamonyi district showed the highest density, 4.6 *An. gambiae* s.l./house/collection. These findings indicate the impact IRS has on lowering indoor resting density.

- Parity was lower in seven of the eight sites sprayed in 2018 and 2019 than in the control site. There was a significant difference ($p<0.05$) of parity in *An. gambiae* s.l. between Kamonyi (control site) and all sprayed sites except in one site of Ngoma district (Remera), where the difference was not statistically significant. This is also most likely due to the impact of IRS on longevity of the vector.
- The overall sporozoite positivity rate was low, 0.22% (n=3,182). The rate for *An. gambiae* s.l., was 0.23% (2,592) and that of *An. pharoensis* was 0.31% (1/327). There was no significant difference in infectivity rates of indoor- and outdoor-collected mosquitoes. However, the rate is too low to make any meaningful comparisons. The finding of *An. pharoensis* infected with *P. falciparum* sporozoite in Gotore site (Kirehe) indicates the need for further attention to the role this vector plays in malaria transmission.
- The EIR was calculated on a quarterly basis. A high EIR was observed in Nyagatare in the second quarter (October-December 2018) with infective bites per person per month (1.1 infective bites/person/month). The EIR of *An. gambiae* s.l. based on collection location showed that the outdoor EIR was higher than the indoor EIR in Nyagatare in the second quarter, and in Kirehe in the third quarter. These results should be cautiously interpreted because of the low numbers of infected mosquitoes.
- A total of 147 blood-fed *An. gambiae* s.l. samples from the collections made over the year-long reporting period (July 2018–June 2019) were tested for vertebrate host blood source (human, bovine, and goat). Human blood indices were as follows: Kamonyi 86.4%, Ngoma 82.4%, Bugesera 50%, Nyagatare 41.6%, and Kirehe 18.8%. 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 2019, the mortality rate was at least 80% on all surface types.
- The results for the fumigant effect of pirimiphos-methyl 300CS 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 six months the mortality was below 20%.

4. Support for Rwanda Biomedical Center

4.1 Insectary Maintenance and Associated Vector Control Laboratory Support

PMI VectorLink Rwanda supports the maintenance of the insectary and associated vector laboratory at the RBC Malaria and Other Parasitic Diseases Division. This support includes procuring supplies to sustain the established *An. gambiae* s.s. susceptible colony used for bioassays. These supplies include reagents, and materials for entomology monitoring and general laboratory activities. VectorLink Rwanda also provides technical support to the entomology laboratory. PMI VectorLink Rwanda also supported the operations of the MOPDD's 12 entomology sentinel sites for 3 months (July to September 2018), whose activities include malaria vector insecticide resistance testing, vector behavior assessments, and determination of vector density/distribution. The VectorLink entomology coordinator participated in the annual conferences of the Pan African Mosquito Control Association (PAMCA) and American Society of Tropical Medicine and Hygiene (ASTMH). VectorLink Rwanda supported the review and updating of the Rwanda Insecticide Resistance Monitoring and Management Plan (2019-2024) and the insecticide susceptibility study on new molecules (clothianidin and chlorfenapyr) that will inform decisions on insecticide rotation.

5. Challenges and Recommendations

- Detection of insecticide resistance mechanism is needed at Kicukiro entomology laboratory. This requires the purchase of the RealTimePCR machine.
- The government of Rwanda decided to spray the control district Ngoma in March, which prevented the project from collecting 12 months of data for comparison between sprayed and non-sprayed areas for some of the indicators.
- All insecticide resistance and related tests are performed by the Rwanda Biomedical Center's Malaria and Other Parasitic Diseases Division and are not part of this report.
- Review the timing of IRS for 2020, considering malaria case data and other data sources, as the entomological data suggests that it may be beneficial to spray Kirehe and Nyagatare in August, rather than in September.

ANNEX A. PARITY

	Nyagatare District				Kirehe District				Bugesera District				Ngoma District				Kamonyi 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	Total collected	Total *An. gambiae* s.l. Dissected	# parous	% parity
Jul-18	11	7	2	28.6	4	3	2	66.7	3	3	0	0	2	2	1	50				
Aug-18	43	26	10	38.5	7	4	1	25	48	25	12	48	13	8	2	25				
Sept-18	197	51	28	55	132	48	25	52	54	33	13	39.4	190	44	31	70.4				
Oct-18	162	62	16	25.8	71	33	5	15.1	37	18	1	5.5	83	37	18	48.6				
Nov-18	153	61	13	21.3	19	13	0	0	59	20	6	30	39	17	10	58.8				
Dec-18	134	53	10	18.9	28	15	2	13.3	22	47	11	23.4	11	6	3	50				
Jan-19	54	31	10	32.2	12	6	2	33.3	22	11	5	45.4	3	3	2	66.7				

	Nyagatare District				Kirehe District				Bugesera District				Ngoma District				Kamonyi 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	Total collected	Total *An. gambiae* s.l. Dissected	# parous	% parity
Feb-19	55	25	10	40	53	27	6	22.2	131	51	16	31.4	2	2	2	100				
Mar-19	157	53	14	26.4	49	28	4	14.3	150	64	31	48.4	110	34	15	44.1				
Apr-19	127	58	18	31	148	57	9	15.8					143	49	11	22.4	728	80	46	57.5
May-19	415	83	23	27.7	30	17	3	17.6					46	23	2	8.7	579	74	49	66.2
Jun-19	45	25	7	28	13	7	1	14.3					5	3	0	0	522	80	48	60
Total	1553	535	161	30.1	566	258	60	23.3	526	272	95	34.9	647	228	97	42.5	1829	234	143	61.1

ANNEX B: SPOROZOITE RATES

	Nyagatare			Kirehe			Bugesera			Ngoma			Kamonyi		
	Total tested	# positive	% positive	Total tested	# positive	% positive	Total tested	# positive	% positive	Total tested	# positive	% positive	Total tested	# positive	% positive
Jul-18	16	0	0	8	0	0	10	0	0	9	0	0			
Aug-18	49	0	0	8	0	0	89	1	1.12	17	0	0			
Sept-18	131	1	0.76	126	0	0	101	0	0	106	0	0			
Oct-18	152	0	0	59	0	0	95	0	0	70	0	0			
Nov-18	55	0	0	40	1	2.5	113	0	0	60	0	0			
Dec-18	125	2	1.6	59	0	0	102	0	0	71	1	1.4			
Jan-19	42	0	0	20	0	0	84	0	0	20	0	0			
Feb-19	56	0	0	66	0	0	134	0	0	25	0	0			
Mar-19	54	0	0	56	1	1.8	109	0	0	85	0	0			
Apr-19	67	0	0	82	0	0				73	0	0	92	0	0
May-19	88	0	0	46	0	0				43	0	0	68	0	0
Jun-19	55	0	0	33	0	0				24	0	0	89	0	0
Total	890	3	0.33	603	2	0.33	837	1	0.12	603	1	0.16	249	0	0

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

Month	*Nyagatare*					*Kirehe*				
	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-18	11	0.5	0	0	0	4	0.2	0	0	0
Aug-18	43	1.8	0	0	0	7	0.3	0	0	0
Sept-18	197	8.2	0.76	0.06	1.87	132	5.5	0	0	0
Oct-18	162	6.8	0	0	0	71	3.0	0	0	0
Nov-18	153	6.4	0	0	0	19	0.8	0	0	0
Dec-18	134	5.6	1.6	0.09	2.68	28	1.2	0	0	0
Jan-19	54	2.3	0	0	0	12	0.5	0	0	0
Feb-19	55	2.3	0	0	0	53	2.2	0	0	0
Mar-19	157	6.5	0	0	0	49	2.0	2.17	0.04	1.3
Apr-19	127	5.3	0	0	0	148	6.2	0	0	0
May-19	415	17.3	0	0	0	30	1.3	0	0	0
Jun-19	45	1.9	0	0	0	13	0.5	0	0	0

Month	*Bugesera*					*Ngoma*				
	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-18	3	0.1	0	0	0	2	0.2	0	0	0
Aug-18	48	2.0	1.23	0.024	0.74	13	1.1	0	0	0
Sept-18	54	2.3	0	0	0	190	15.8	0	0	0
Oct-18	37	1.5	0	0	0	83	6.9	0	0	0
Nov-18	59	2.5	0	0	0	39	3.3	0	0	0
Dec-18	22	0.9	0	0	0	11	0.9	2	0.018	0.54
Jan-19	22	0.9	0	0	0	3	0.3	0	0	0
Feb-19	131	5.5	0	0	0	2	0.2	0	0	0
Mar-19	150	6.3	0	0	0	110	9.2	0	0	0
Apr-19						143	6.0	0	0	0
May-19						46	1.9	0	0	0
June-19						5	0.2	0	0	0

Month	**Total *An. gambiae* s.l. collected**	**Biting rate**	**SPZ rate**	**Nightly EIR**	**Monthly EIR**
Apr-19	728	60.67	0	0	0
May-19	579	48.25	0	0	0
June-19	522	43.50	0	0	0

ANNEX D. REFERENCES

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