PMI | Africa IRS (AIRS) Project

Indoor Residual Spraying (IRS 2) Task Order Four

RWANDA: 2017 ENTOMOLOGICAL MONITORING

JULY 2016–JUNE 2017

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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
PCR	Polymerase Chain Reaction
PMI	President's Malaria Initiative
PNP	Plastered Not Painted
PP	Plastered and Painted
PSC	Pyrethrum Spray Catch
s.l.	sensu lato
s.s.	sensu stricto
USAID	United States Agency for International Development
WG	Wettable granules
WHO	World Health Organization
WP	Wettable Powder

EXECUTIVE SUMMARY

During the year-long reporting period (July 2016–June 2017), entomological monthly data collection was conducted in three indoor residual spray (IRS) districts, Bugesera, Kirehe, and Nyagatare, and one non-IRS (control) district, Ngoma. Adult mosquitoes were sampled using pyrethrum spray catches (PSC) and human landing catches (HLC) to assess vector species composition, seasonality, and behavior. World Health Organization cone bioassays were conducted to assess the quality of spraying as well as determine insecticide decay rates on sprayed surfaces. Tests were conducted on three wall surface types: mud, plastered not painted, and plastered and painted. Identification of malaria vectors was done morphologically.

During the reporting period, a total of 9,426 adult female Anopheles mosquitoes were collected, 7,626 using HLC and 1,800 using PSC. The species composition was 9,174 (97.4%) *An. gambiae* s.l., 146 (1.51%) *An. pharoensis*, 93 (0.96%) *An. ziemanni*, 10 (0.1%) *An. coustani*, and 3 (0.03%) *An. funestus* group.

An. gambiae s.l. generally showed slightly more exophagic than endophagic tendency in the four districts, including the control district: Bugesera 47.6% endophagy vs 52.4% exophagy, Kirehe 32.8% endophagy vs 67.2% exophagy, Nyagatare 48.7% endophagy vs 51.3% exophagy, and Ngoma (control district) 47.4% endophagy vs 52.6% exophagy. Although the *An. gambiae* s.l. showed slightly more exophagic than endophagic tendency in the four districts overall, the difference between the indoor and outdoor landing collections in Bugesera and Nyagatare ($p>0.05$) was not significant. There was a significant difference ($p<0.05$) in Kirehe and Ngoma.

The average hourly biting rates per person (bites/person/hour, or b/p/h) varied across the four districts; they were highest in Nyagatare district (average 0.56 b/p/h indoors and 0.55 b/p/h outdoors), followed by Ngoma (control district) (average 0.41 b/p/h indoors and 0.45 b/p/h outdoors), and Kirehe (average 0.16 b/p/h indoors and 0.28 b/p/h outdoors). Bugesera showed the lowest rates (average 0.10 b/p/h indoors and 0.19 b/p/h outdoors). Hourly biting was slightly higher outdoors than indoors in all sites except the two in Nyagatare district. In Nyagatare at 00:00–01:00, indoor biting was higher (at 1.19 b/p/h) than outdoor biting (at 0.78 b/p/h); the biting rate was constant between 18:00 and 23:00, increased slightly to peak at 00:00–01:00, and then dropped to its lowest point at 5:00–6:00. In Kirehe district, the peak (0.26 b/p/h) for indoor biting was 18:00–19:00; for outdoor biting (0.46 b/p/h), it was 00:00–01:00. In Bugesera district, the peak for indoor biting (0.22 b/p/h) and outdoor biting (0.20 b/p/h) was 00:00–01:00. In Ngoma district, the peak for indoor biting (0.63 b/p/h) was 22:00–23:00 and for outdoor biting (0.74 b/p/h) 02:00–03:00.

There was a significant difference in average monthly vector PSC collections in all the intervention districts and the control district ($p<0.05$). Although vector density varied over the months of the reporting season, Bugesera district showed a higher average vector density (0.55 *An. gambiae* s.l./house/day) than did the other two IRS districts of Kirehe (0.43 *An. gambiae* s.l./house/day) and Nyagatare (0.33 *An. gambiae* s.l./house/day). Ngoma, the control (non-IRS) district, showed the highest density of 1.0 *An. gambiae* s.l./house/day. Two vector density peak seasons were observed in reporting period, one in October and the other in March/April.

No clear trend was observed in any district for parity rates at any time during the reporting period. There was a significant difference ($p<0.05$) between average number of parous *An. gambiae* s.l. in the control site and Nyagatare and Kirehe intervention sites but the different between the control site and Bugesera intervention site was not statistically significant.

Out of the 617 mosquitoes in the three districts (Nyagatare, Kirehe, and Ngoma) that were tested in July–September 2016 (pre-spray) for the Plasmodium circumsporozoite protein, 17 tested positive (2.8%). During the post-spray period (October 2016–June 2017), 15 (0.51%) mosquitoes tested positive out of 2,151 mosquitoes collected from the three districts. In Bugesera district, the 305 samples collected in the pre-spray period (July 2016–Nov 2016) were tested and only three (0.98%) were positive for Plasmodium circumsporozoite proteins; in the post-spray period (December 2016–June 2017), the 419 samples collected were tested and eight (1.9%) tested positive.

A total of 251 blood-fed *An. gambiae* s.l. samples from the collections made over the year-long reporting period (July 2016–June 2017) were tested for vertebrate host blood source (human, bovine, and goat). Human blood indices were as follows: Nyagatare 65.1%, Ngoma 39.4%, Kirehe 25.6%, and Bugesera 21.8%. The results show that a relatively high proportion of the vectors also fed on non-human hosts.

Biting rates were higher in Nyagatare than in the other districts (including the control district). Infectivity was also observed to be higher in Nyagatare, with 24.6 infective bites per person per month in August 2016. The outdoor entomological inoculation rate (EIR) was higher than the indoor EIR in Nyagatare and Kirehe.

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 2017 (nine months post spraying), the mortality rate was over 80% on mud and plastered and painted surfaces. In houses with plastered not painted surfaces, test mortality dropped below 80% in Nyagatare and Kirehe.

1. INTRODUCTION

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

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

This report covers entomological monitoring activities conducted from July 1, 2016 to June 30, 2017. 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
- Determining parity of malaria vectors through ovary dissection
- Monitoring the quality of insecticide application and insecticide decay rates
- Determining sporozoite rates and entomological inoculation rates (EIRs)

2. Data Collection Sites and Methods

2.1 Study Sites

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

FIGURE 1. MAP OF RWANDA SHOWING DATA COLLECTION DISTRICTS

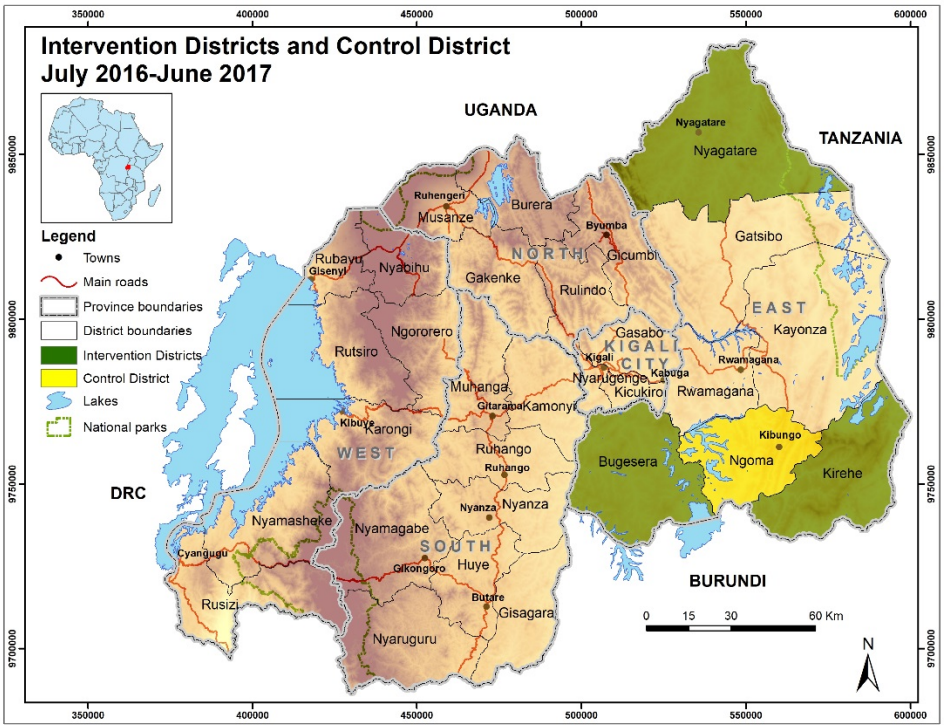


TABLE 1. DATA COLLECTION (SENTINEL) SITES

District	Data Collection Sites (sectors)	Spray Status
Bugesera	Nyarugenge, Musenyi	Sprayed in Nov 2016 using carbamate (with Government of Rwanda support)
Nyagatare	Nyagatare, Rukomo	Sprayed in Sep/Oct 2016 using OP (with PMI support)
Kirehe	Gatore, Nyamugali	Sprayed in Sep/Oct 2016 using OP (with PMI support)
Ngoma (control)	Remera	Not sprayed

All intervention sites were sprayed with a carbamate (bendiocarb 80WP) in September/October 2015 and February/March 2016. In September/October 2016, two of the districts (Kirehe and Nyagatare) were sprayed with an OP (Actellic 300CS), while in November 2016, Bugesera was sprayed with a carbamate (bendiocarb 80WP). Kirehe and Nyagatare are IRS PMI-supported districts; the Government of Rwanda supports Bugesera through the Ministry of Health. The control district was not sprayed during any of the rounds.

2.2 Data Collection Methods

Blood seeking and indoor resting adult mosquito collections were conducted each month in the seven sites using human landing catch (HLC) and pyrethrum spray catch (PSC) collection methods, respectively.

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

2.2.1 Human Landing Catch

HLC was done in three households in each site for two consecutive nights per month; therefore, data were collected for four nights per district per month. A team of collectors was composed of four people per house per night; two collectors per house collected from 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 the legs were exposed up to the knees. At the end of the collection, mosquitoes were transported to the field lab and were identified using taxonomic keys (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. Before the PSC was performed, all occupants were politely asked to move out of the house. The floor was covered with white sheets. Windows and other mosquito escape routes around the house were 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, collectors collected from the sheets all the mosquitoes that had been knocked down and sorted them by species. The abdominal status of all female anophelines was determined, and individuals were categorized according to their blood digestion stage (unfed, freshly fed, half-gravid, and gravid females).

2.3 Identification of Malaria Vectors

Anopheles mosquitoes collected through HLC and PSC were identified to the species level morphologically.

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 anopheline 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 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 antihost (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 read visually (Beier and Koros 1991).

2.6 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), 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.

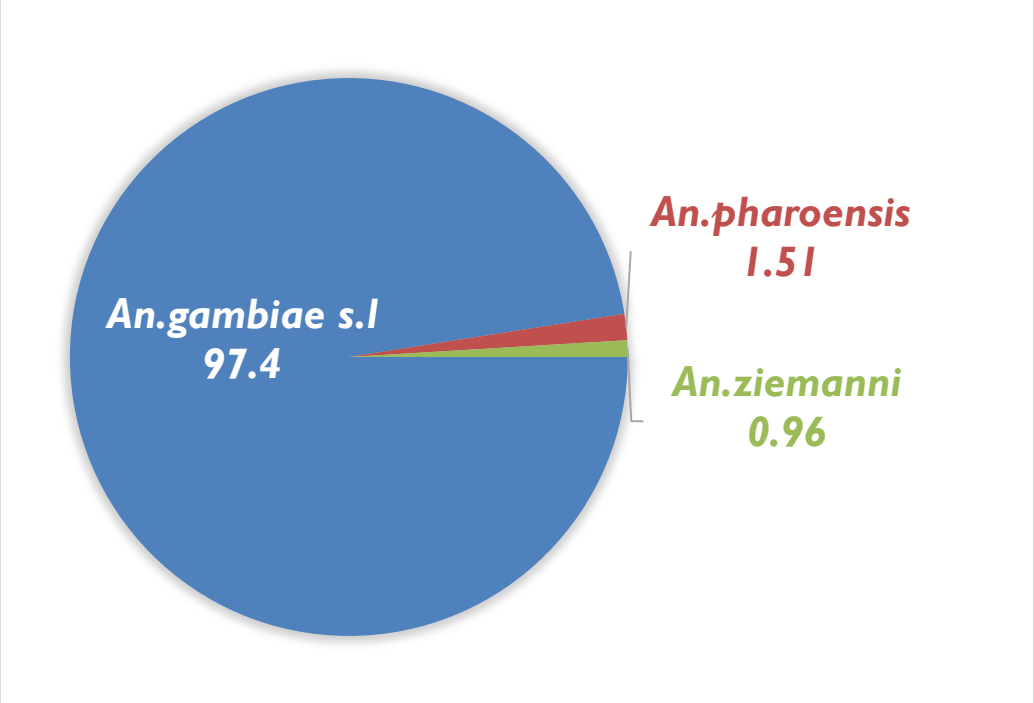
3. Results, Discussion, and Conclusions

3.1 Species Composition and Vector Seasonality

3.1.1 Species Composition

During the reporting period July 2016 to June 2017, a total of 9,426 adult female An. mosquitoes were collected; 7,626 were collecting using HLC, 1,800 using PSC. As shown in Figure 2, the anopheline species composition was 9,174 (97.4%) *An. gambiae* s.l., 146 (1.51%) *An. pharoensis*, 93 (0.96%) *An. ziemanni*, 10 (0.1%) *An. coustani*, and 3 (0.03%) *An. funestus* group. In addition, 39,587 Culcinae were collected. Only *An. gambiae* s.l. and the *An. funestus* group are known as vectors (transmitters) of malaria in Rwanda. Other mosquito species collected are non-vectors of malaria.

FIGURE 2. *ANOPHELES* SPECIES COMPOSITION*

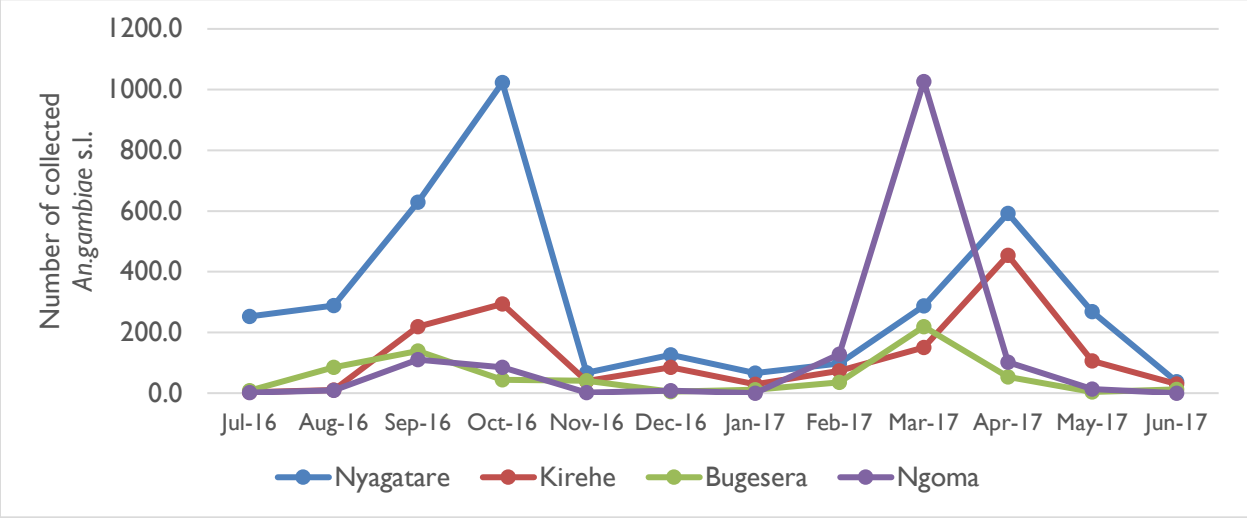


*An. funestus and An. coustani ≤ 0.1% each

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 had a peak in pre-IRS July to mid-September 2016, then dropped precipitously after the spray campaign of September–October 2016. The number remained low until February 2017, when it began to rise to another peak in March 2017 and then gradually dropped until June 2017, when collection ceased. The increase in the malaria vector in February–March may be attributed to the rains in that period, which create many breeding sites.

FIGURE 3. NUMBER OF *AN. GAMBIAE* s.l. COLLECTED, BY SEASON



3.2 Vector Feeding and Location

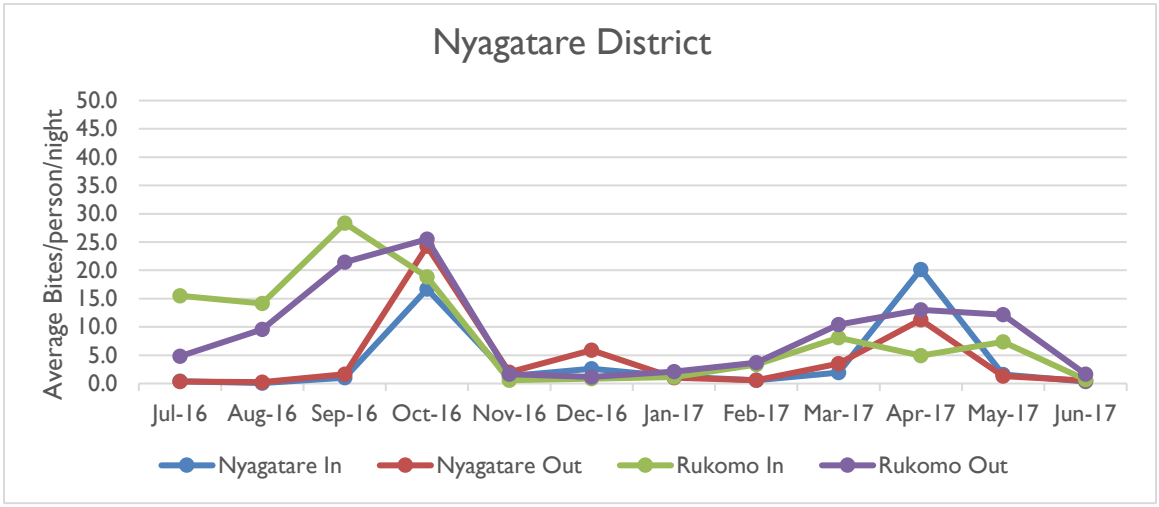
Although *An. gambiae* s.l. generally showed slightly more exophagic than endophagic tendency in the four districts, including the control district, there was no significant difference between the indoor and outdoor landing collections in the four sites in Bugesera district and Nyagatare district ($p>0.05$) (Table 2). There was a significant difference ($p<0.05$) between the indoor and outdoor landing collections in the two sites in Kirehe district, and in the one Ngoma district site (control). The percentage endophagy/ exophagy was recorded as follows: Bugesera 47.6% endophagy vs 52.4% exophagy, Kirehe 32.8% endophagy vs 67.2% exophagy, Nyagatare 48.7% endophagy vs 51.3% exophagy, and Ngoma 47.4% endophagy vs 52.6% exophagy.

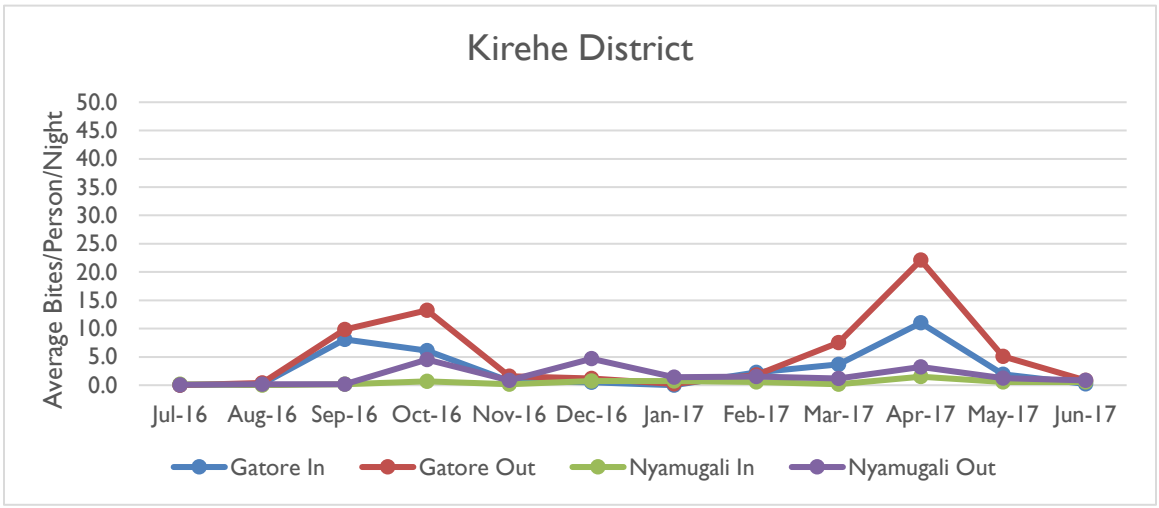
TABLE 2. INDOOR AND OUTDOOR BITING

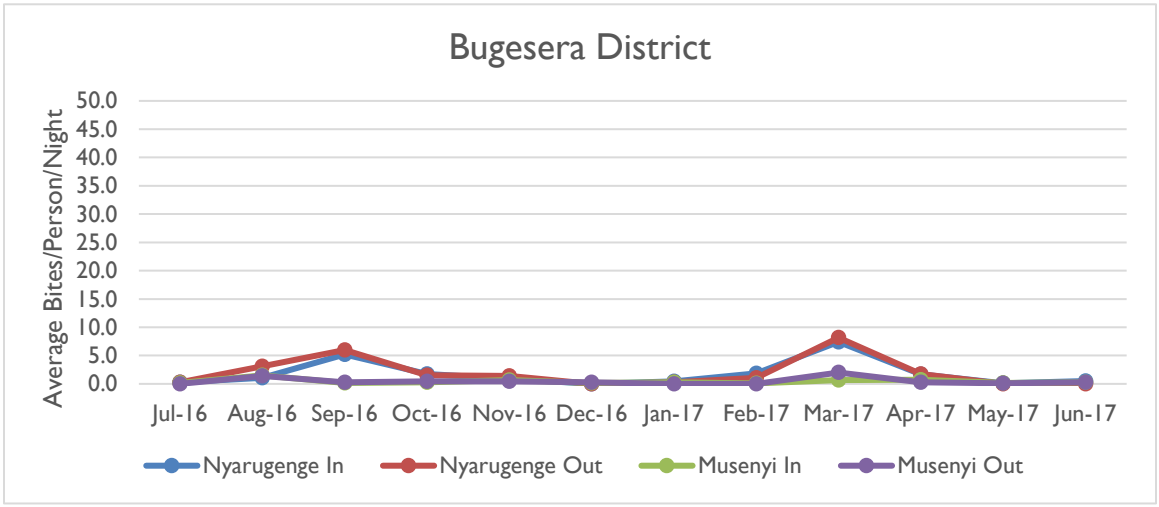
District	Site	In	Out	In: Out Ratio	P value	Result
Bugesera	Nyarugenge	253	282	47.3:52.7	0.209	NS
	Musenyi	61	64	48.8:51.2	0.788	NS
Kirehe	Gatore	417	766	35.2:64.8	$3.4*10^{-24}$	S
	Nyamugali	73	238	23.5:76.5	$8.2*10^{-21}$	S
Nyagatare	Rukomo	1245	1286	49.2:50.8	0.415	NS
	Nyagatare	575	631	47.7:52.3	0.106	NS
Ngoma	Remera	704	782	47.4:52.6	0.043	S

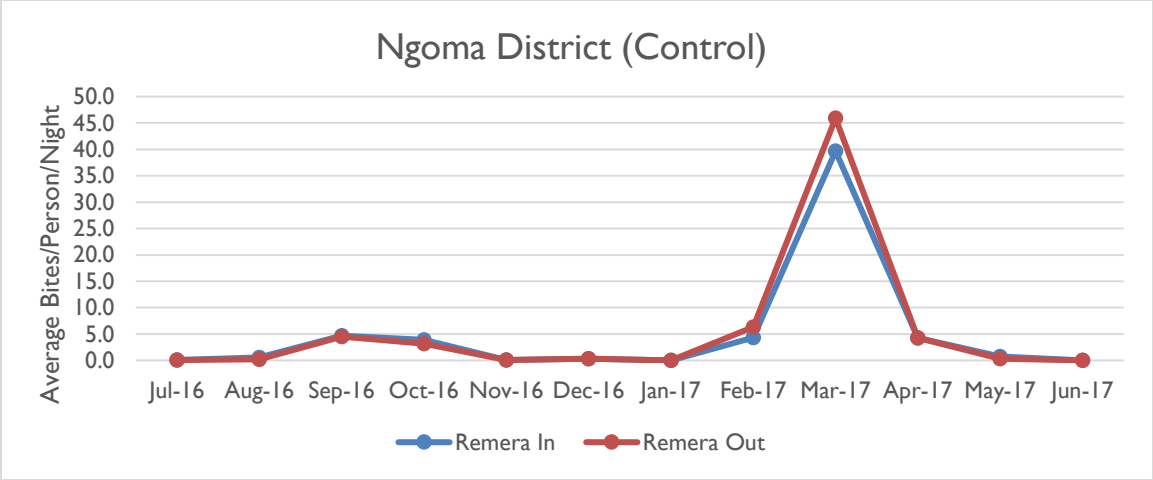
As Figure 4 shows, there are two peak biting seasons, namely, September–October and March–May.

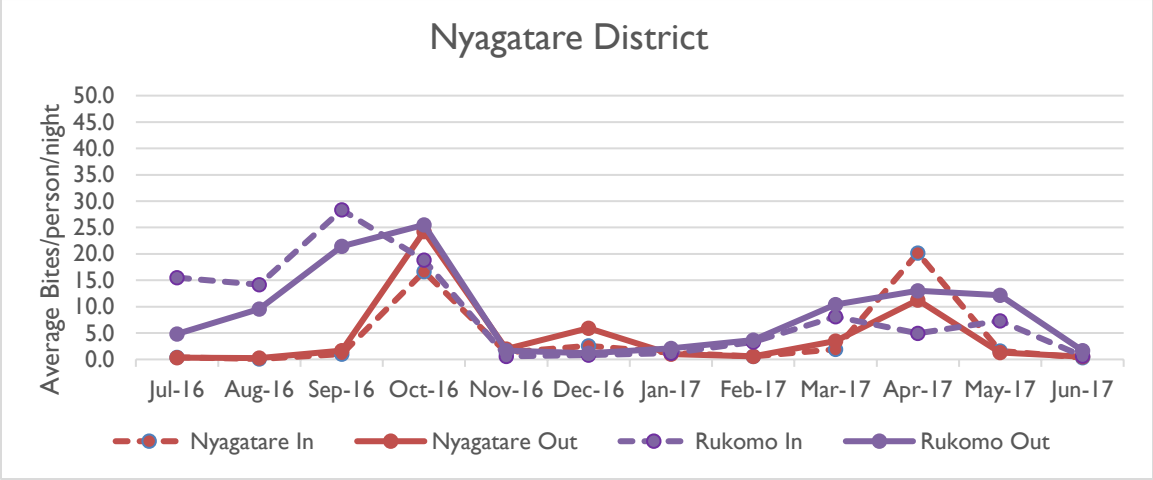
FIGURE 4. *AN. GAMBIAE* s.l. AVERAGE MONTHLY BITING TRENDS

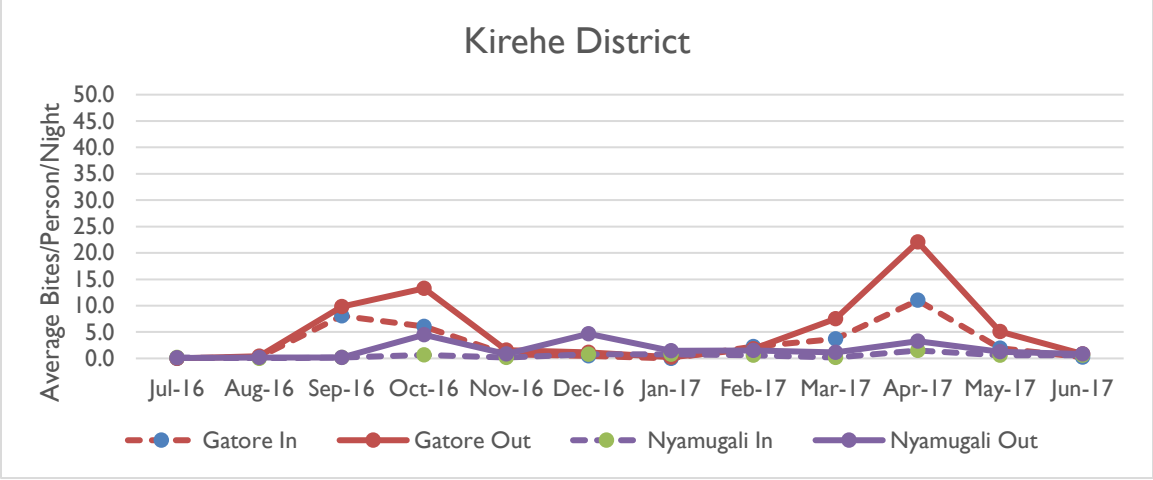


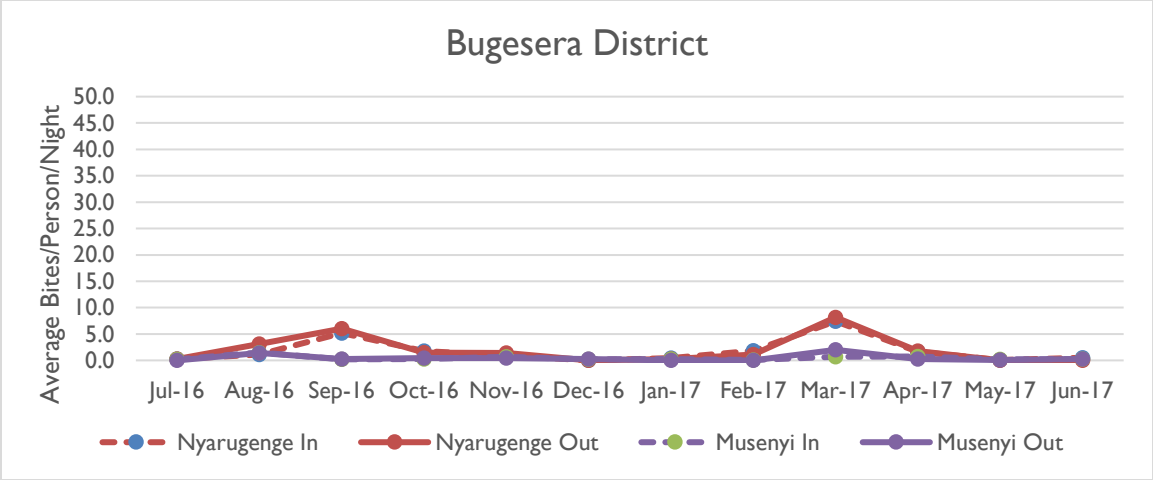


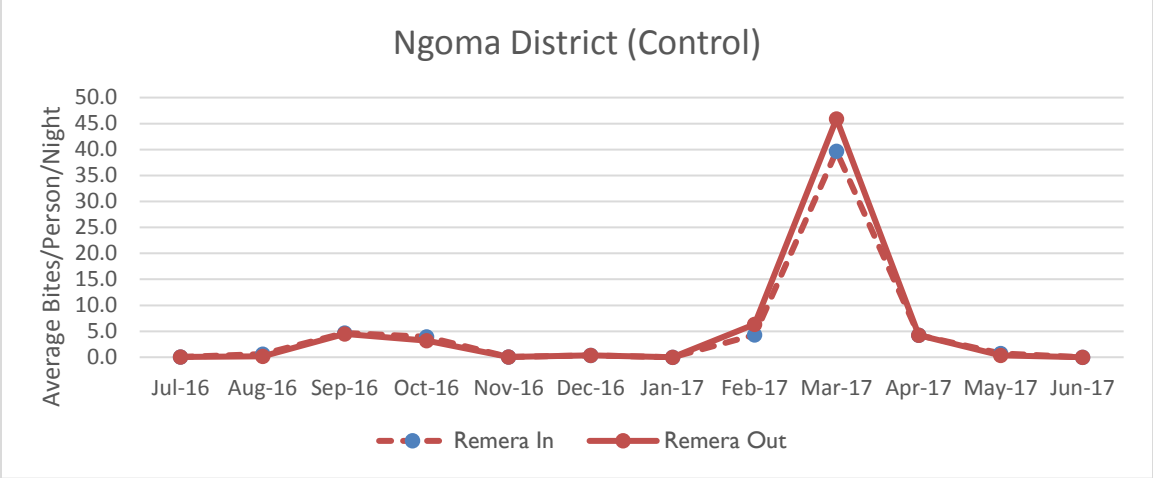



Ngoma District (Control)
Average Bites/Person/Night
50.0
45.0
40.0
35.0
30.0
25.0
20.0
15.0
10.0
5.0
0.0
Jul-16 Aug-16 Sep-16 Oct-16 Nov-16 Dec-16 Jan-17 Feb-17 Mar-17 Apr-17 May-17 Jun-17
Remera In
Remera Out


Nyagatare District
Average Bites/person/night
50.0
45.0
40.0
35.0
30.0
25.0
20.0
15.0
10.0
5.0
0.0
Jul-16 Aug-16 Sep-16 Oct-16 Nov-16 Dec-16 Jan-17 Feb-17 Mar-17 Apr-17 May-17 Jun-17
Nyagatare In
Nyagatare Out
Rukomo In
Rukomo Out


Kirehe District
Average Bites/Person/Night
50.0
45.0
40.0
35.0
30.0
25.0
20.0
15.0
10.0
5.0
0.0
Jul-16 Aug-16 Sep-16 Oct-16 Nov-16 Dec-16 Jan-17 Feb-17 Mar-17 Apr-17 May-17 Jun-17
Gatore In
Gatore Out
Nyamugali In
Nyamugali Out





The average *An. gambiae* s.l. hourly biting rates per person (bites/person/hour, or 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.

The bite rates were highest in Nyagatare (average 0.56 b/p/h indoors and 0.55 b/p/h outdoors), followed by Ngoma (control district) (average 0.41 b/p/h indoors and 0.45 b/p/h outdoors) and Kirehe (average 0.16 b/p/h indoors and 0.28 b/p/h outdoors). Bugesera showed the least mean b/p/h (average 0.10 b/p/h indoors and 0.19 b/p/h outdoors).

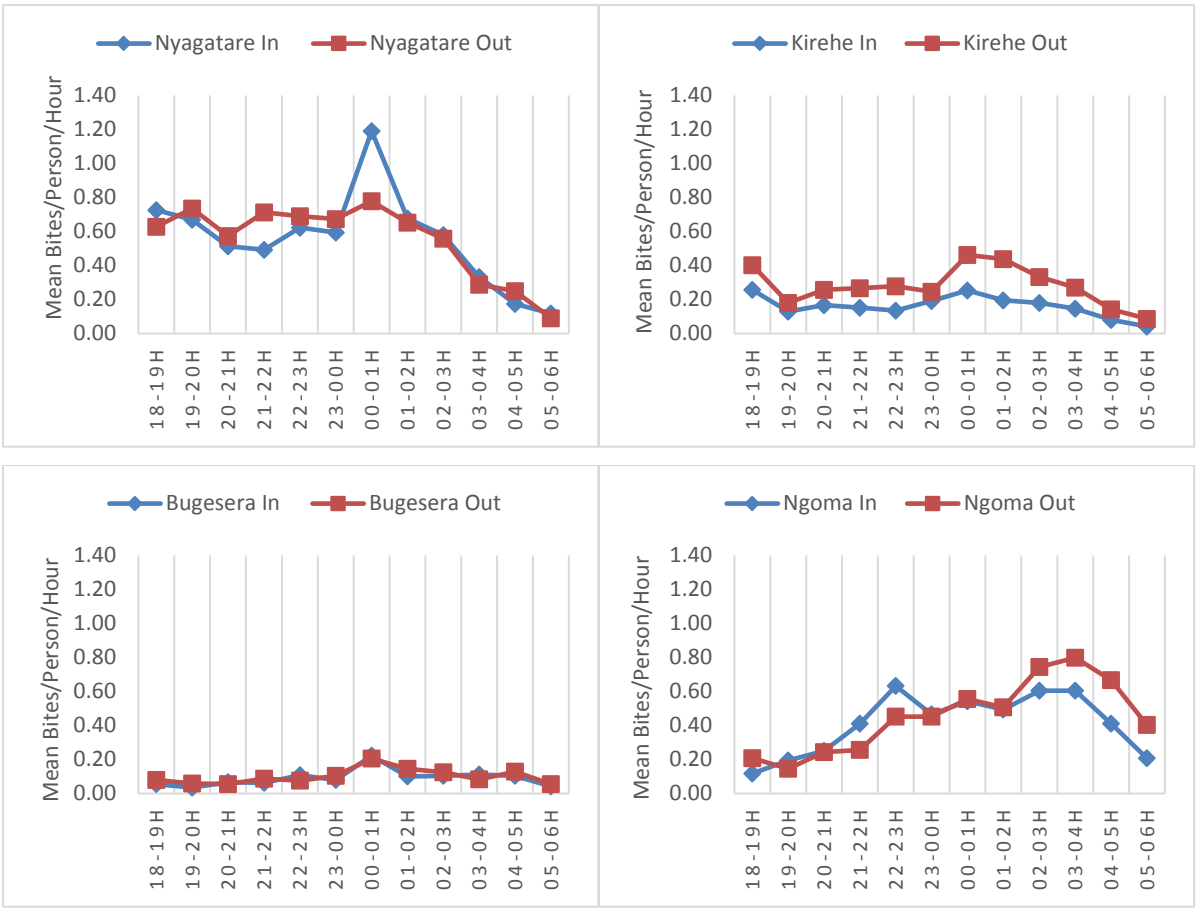
The higher biting in Nyagatare and Kirehe could be due to the fact that, apart from the rains, there is considerably more rice farming in those two districts than in the other two. This creates more breeding sites for the vector and consequently higher vector density, which increases the chances of biting.

As discussed above, hourly biting was slightly higher outdoors than indoors in all sites except in Nyagatare district. In Nyagatare site, indoor biting was higher (1.19 b/p/h) at 00:00–01:00 than outdoor biting (0.78 b/p/h) at the same hour; both indoor and outdoor biting rates were constant between 18:00 and 23:00, increased to peak at 00:00–00:01, and then dropped to their lowest at 5:00–6:00.

In Kirehe district, the peak (0.26 b/p/h) for indoor biting was at 18:00–19:00, and the peak for outdoor biting (0.46 b/p/h) was 00:00–01:00. In Bugesera district, the peak for indoor biting (0.22 b/p/h) and outdoor biting (0.20 b/p/h) was 00:00–01:00. In Ngoma district, the peak for indoor biting (0.63 b/p/h) was 22:00–23:00 and for outdoor biting (0.74 b/p/h) was 02:00–03:00.

Biting observed as early as 18:00–20:00 in Kirehe and Nyagatare is worrying since at this time the community members are still not in bed, which means they are not protected by insecticide-treated nets (ITNs). It is, however, important to note that more biting was observed outdoors than indoors. It is, therefore, very important for community members to remain indoors during the evening/night, and to sleep under an ITN.

FIGURE 5. HOURLY BITING



3.3 VECTOR DENSITY

As noted above, a total of 1,800 female indoor-resting *An. gambiae* s.l. were collected using PSC in the three IRS districts and the control district over the July 2016 to June 2017 reporting period. Table 3 shows the disaggregation of the collections and density in the districts.

TABLE 3. PSC COLLECTIONS AND VECTOR DENSITY

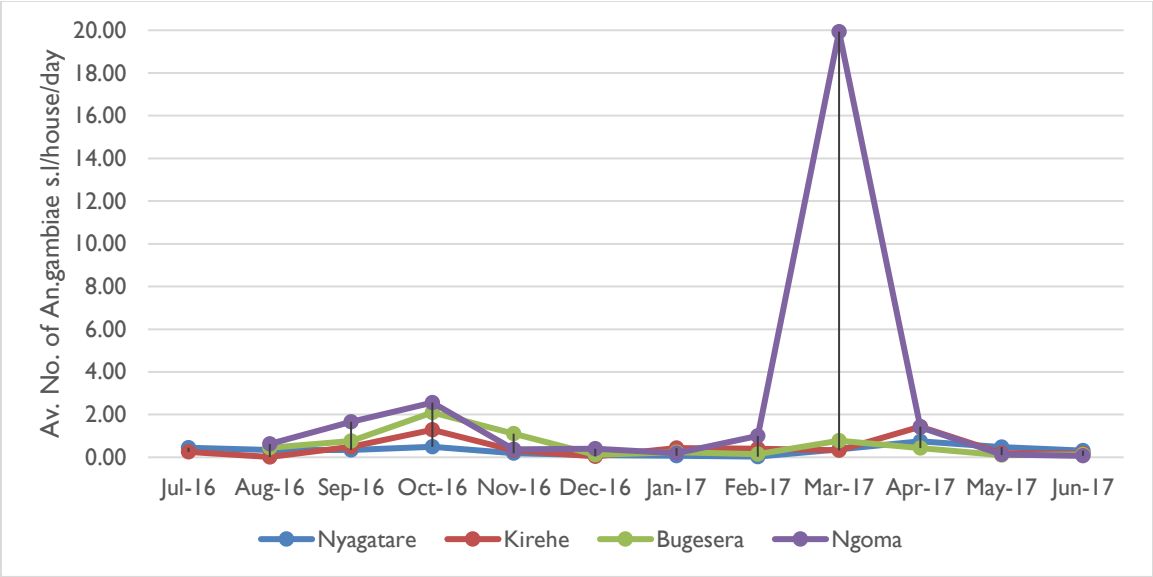
District	Nyagatare		Kirehe		Bugesera		Ngoma (control)	
	Total Collected	Vector Density	Total Collected	Vector Density	Total Collected	Vector Density	Total Collected	Vector Density
Jul-16	27	0.45	2	0.03	13	0.21	--	--
Aug-16	21	0.35	1	0.02	32	0.53	19	0.63
Sep-16	21	0.35	30	0.50	46	0.77	50	1.67
Oct-16	30	0.50	77	1.28	126	2.10	77	2.57
Nov-16	12	0.20	20	0.33	66	1.10	11	0.37
Dec-16	6	0.10	3	0.05	7	0.12	11	0.37
Jan-17	4	0.07	26	0.43	14	0.23	6	0.20
Feb-17	2	0.03	24	0.40	9	0.15	30	1
Mar-17	22	0.37	20	0.33	47	0.78	597	20
Apr-17	45	0.75	86	1.43	26	0.43	42	1.40
May-17	29	0.48	12	0.20	6	0.10	4	0.13
Jun-17	19	0.32	11	0.18	9	0.15	2	0.07
Avg. monthly vector density	20	0.33	26	0.43	33	0.55	77	2.56
p-value	$7.15*10^{-9}$ (S)		$9.44*10^{-7}$ (S)		$7.23*10^{-5}$ (S)		1	

* Comparison of average collections in IRS districts and in the control district, July 2016–June 2017.

There was a significant difference in average monthly vector PSC collections between all the intervention districts and the control district ($p<0.05$). Although monthly vector density varied throughout the reporting period, Bugesera district showed the highest average vector density of the IRS districts (0.55 *An. gambiae* s.l./house/day), higher than Kirehe (0.43 *An. gambiae* s.l./house/day) and Nyagatare (0.33 *An. gambiae* s.l./house/day). The control (non-IRS) Ngoma district showed the highest density, 2.56 *An. gambiae* s.l./house/day.

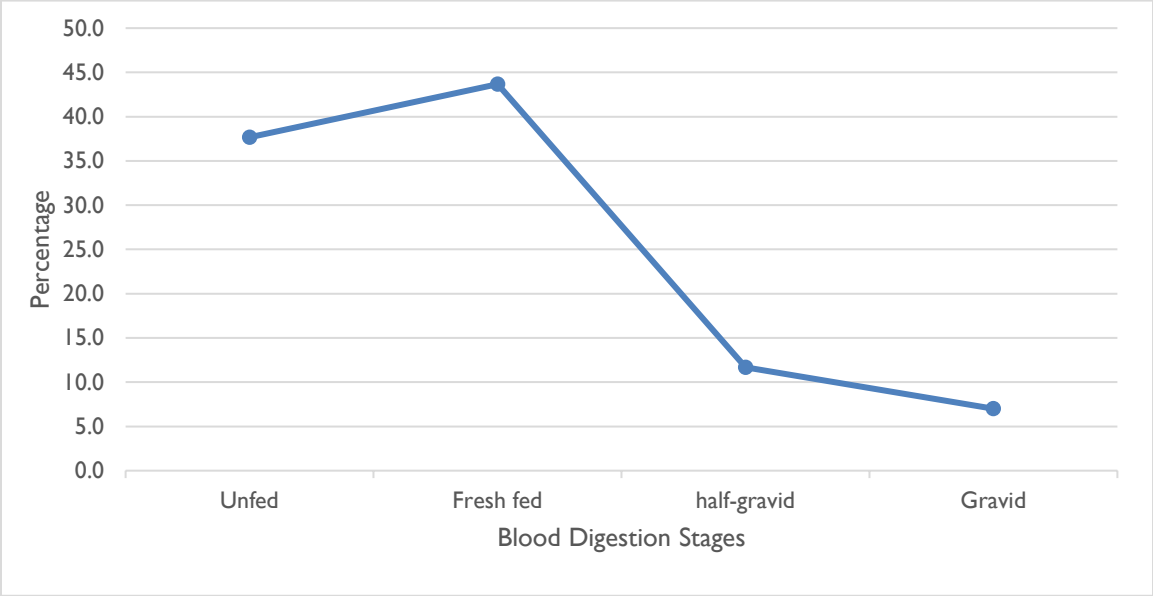
The results also show that there are two vector density peaks during the year: March–April and October. During September–October 2016, there was a gradual decrease in vector density in both intervention and control districts. A decrease observed in all four districts may be partly attributed to vector seasonality. Nevertheless, the density in the control district was higher than in the intervention districts throughout the reporting period except in Nyagatare in May–June 2017, in Kirehe in January and June 2017, and in Bugesera in November 2016 and June 2017. In Bugesera, the rise in vector density observed in November 2016 could be because the IRS was conducted late in this district.

FIGURE 6. *AN. GAMBIAE* s.l. DENSITIES



All 1,800 *An. gambiae* s.l. collected in four sites using PSC were classified according to their blood digestion stages: 678 (37.7%) were unfed, 786 (43.7%) were freshly fed, 210 (11.7%) were half gravid, and 126 (7%) were gravid (see Figure 7). The half gravid and gravid were mostly collected in the control district comparing to IRS district (see Figure 8).

FIGURE 7. BLOOD DIGESTION STAGES OF ALL *AN. GAMBIAE* s.l. COLLECTED USING PSC



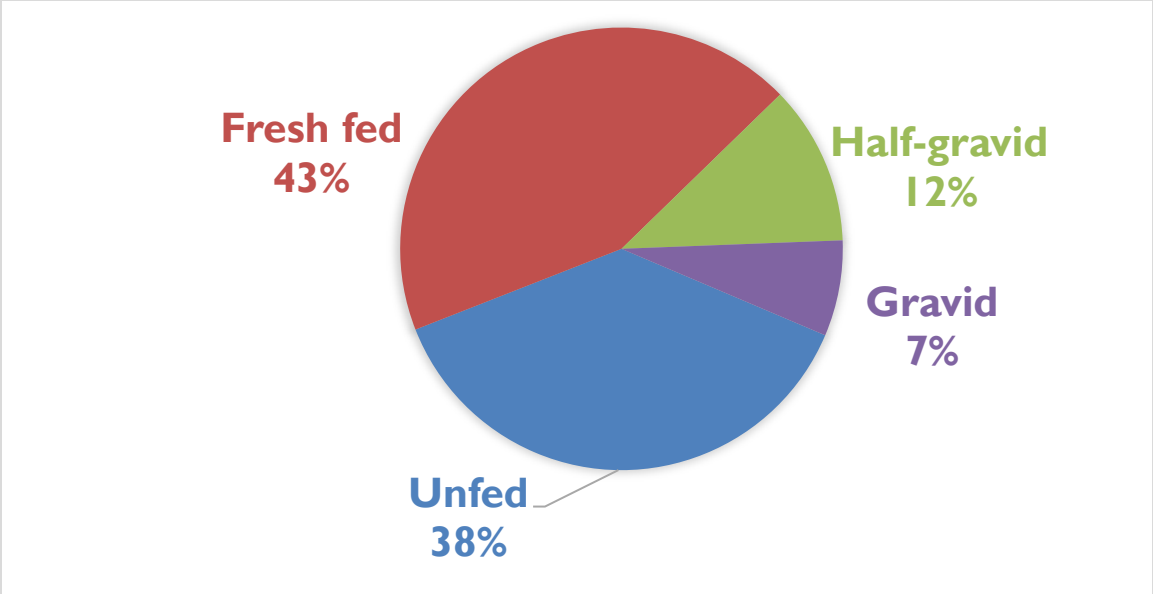
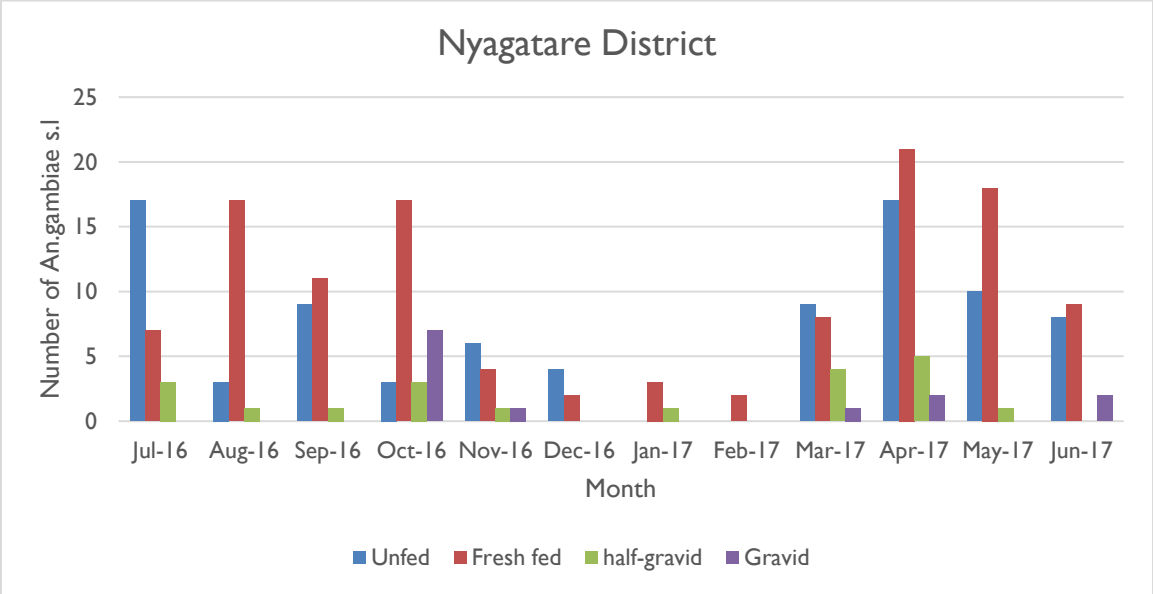
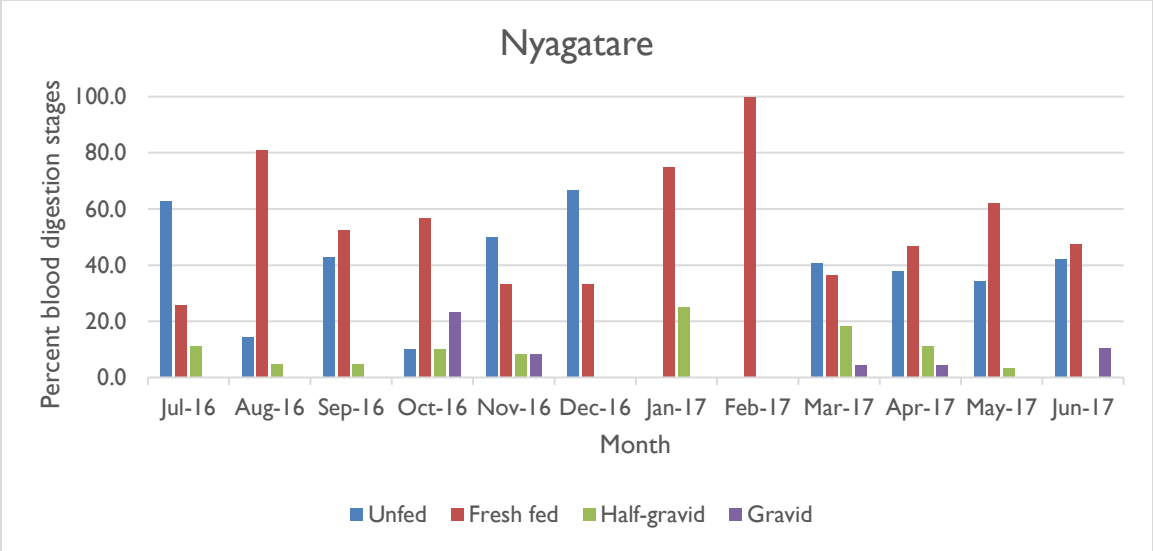
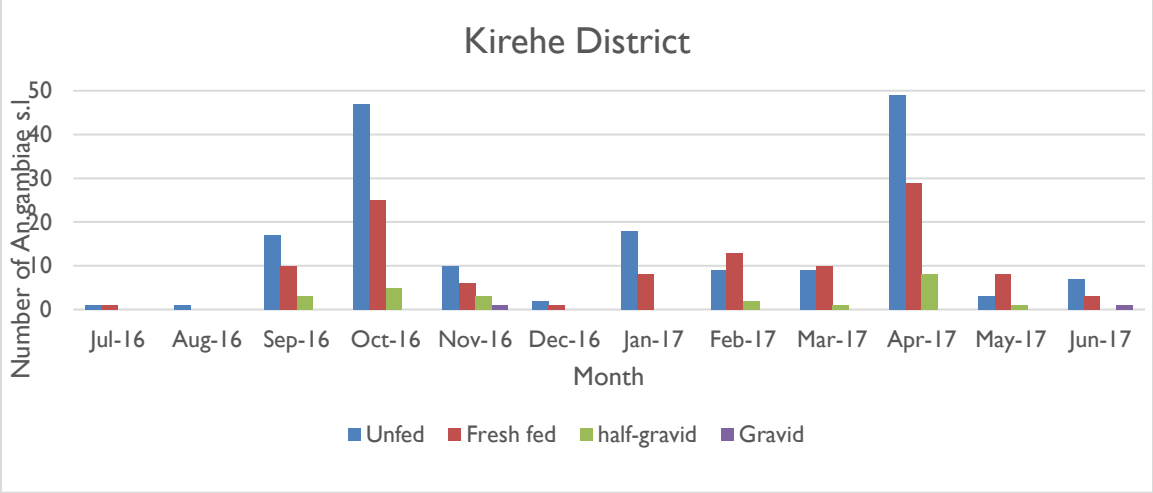
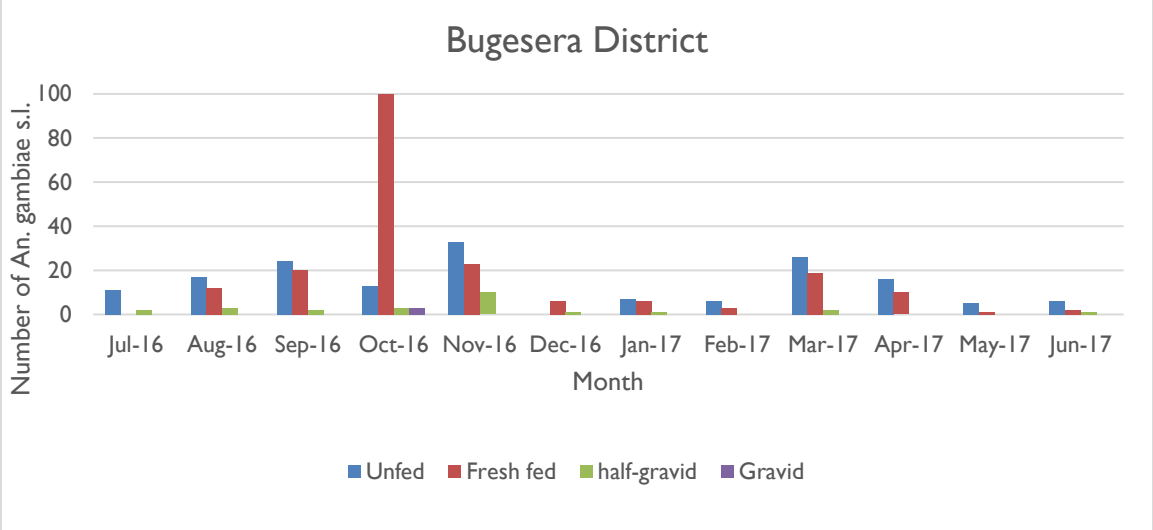


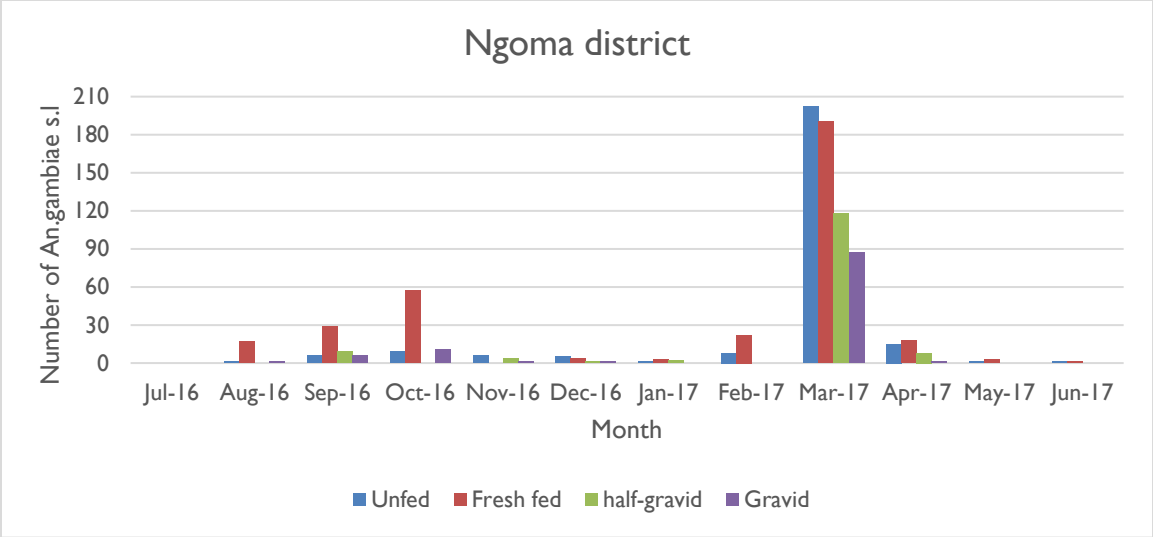
FIGURE 8. BLOOD DIGESTION STAGES OF *AN. GAMBIAE* s.l. COLLECTED USING PSC, BY DISTRICT AND MONTH

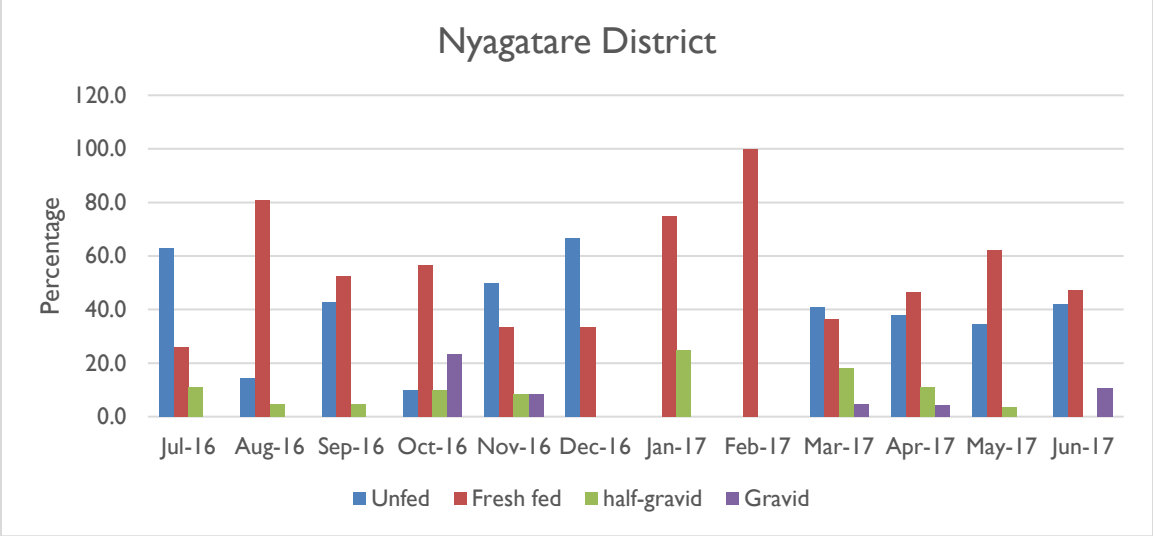


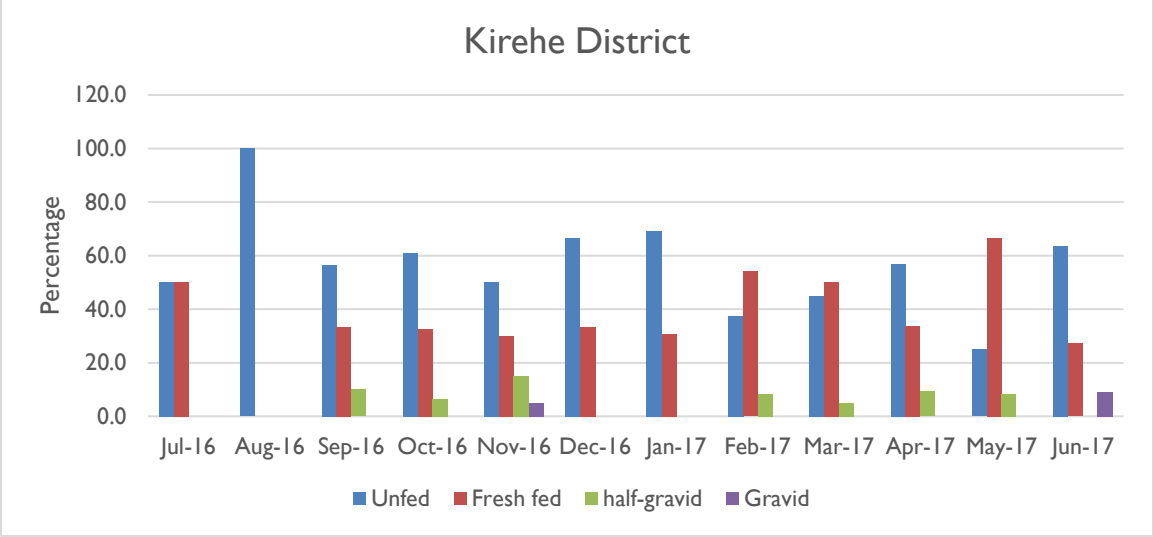

Nyagatare
Percent blood digestion stages
100.0
80.0
60.0
40.0
20.0
0.0
Jul-16
Aug-16
Sep-16
Oct-16
Nov-16
Dec-16
Jan-17
Feb-17
Mar-17
Apr-17
May-17
Jun-17
Month
Unfed
Fresh fed
Half-gravid
Gravid

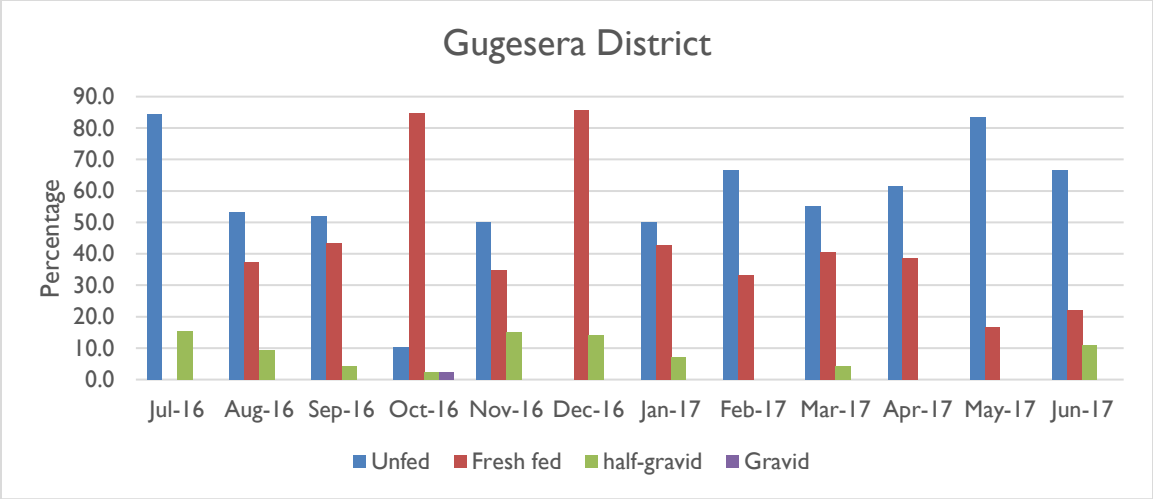

Kirehe District
Number of An.gambiae s.l.
50
40
30
20
10
0
Jul-16
Aug-16
Sep-16
Oct-16
Nov-16
Dec-16
Jan-17
Feb-17
Mar-17
Apr-17
May-17
Jun-17
Month
Unfed
Fresh fed
half-gravid
Gravid

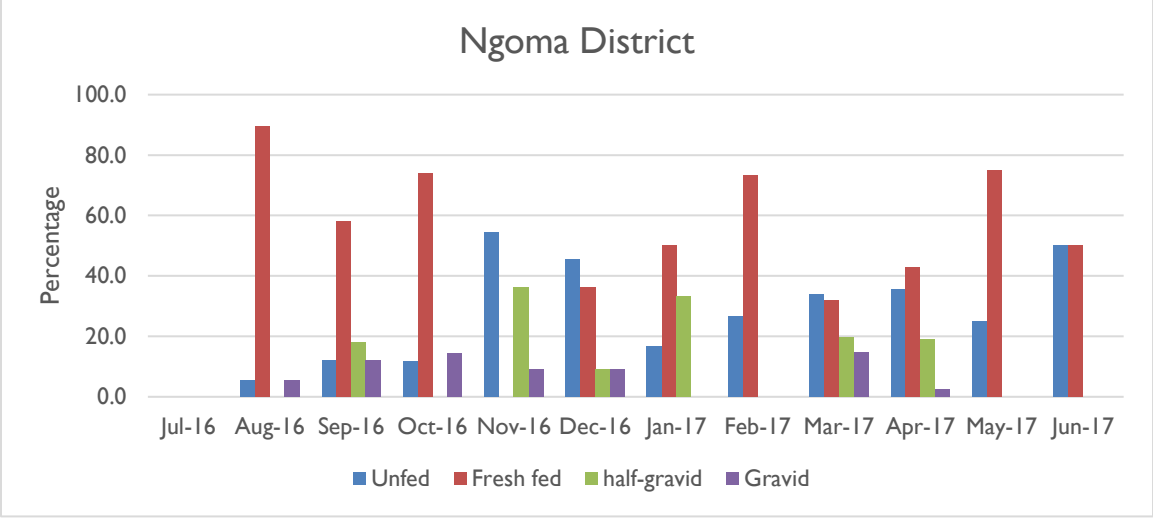

Bugesera District
Number of An. gambiae s.l.
100
80
60
40
20
0
Jul-16
Aug-16
Sep-16
Oct-16
Nov-16
Dec-16
Jan-17
Feb-17
Mar-17
Apr-17
May-17
Jun-17
Month
Unfed
Fresh fed
half-gravid
Gravid


Ngoma district
Number of An.gambiae s.l
210
180
150
120
90
60
30
0
Jul-16
Aug-16
Sep-16
Oct-16
Nov-16
Dec-16
Jan-17
Feb-17
Mar-17
Apr-17
May-17
Jun-17
Month
Unfed
Fresh fed
half-gravid
Gravid


Nyagatare District
Percentage
120.0
100.0
80.0
60.0
40.0
20.0
0.0
Jul-16
Aug-16
Sep-16
Oct-16
Nov-16
Dec-16
Jan-17
Feb-17
Mar-17
Apr-17
May-17
Jun-17
Unfed
Fresh fed
half-gravid
Gravid


Kirehe District
Percentage
120.0
100.0
80.0
60.0
40.0
20.0
0.0
Jul-16
Aug-16
Sep-16
Oct-16
Nov-16
Dec-16
Jan-17
Feb-17
Mar-17
Apr-17
May-17
Jun-17
Unfed
Fresh fed
half-gravid
Gravid


Gugesera District
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
Percentage
Jul-16
Aug-16
Sep-16
Oct-16
Nov-16
Dec-16
Jan-17
Feb-17
Mar-17
Apr-17
May-17
Jun-17
Unfed
Fresh fed
half-gravid
Gravid


Ngoma District
100.0
80.0
60.0
40.0
20.0
0.0
Percentage
Jul-16
Aug-16
Sep-16
Oct-16
Nov-16
Dec-16
Jan-17
Feb-17
Mar-17
Apr-17
May-17
Jun-17
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 in the year July 2016–June 2017. There was a significant difference ($p<0.05$) between the average number of parous An. gambiae s.l. in the Ngoma control site and Nyagatare and Kirehe intervention sites but the difference was not statistically significant in the Bugesera district intervention sites (Table 4). The difference observed in Nyagatare and Kirehe could be attributed to spraying with Actellic 300CS. The similar parity rates in Bugesera and Ngoma (control) could be because Bugesera was sprayed later, in November 2016, with bendiocarb, which showed a high insecticide decay rate.

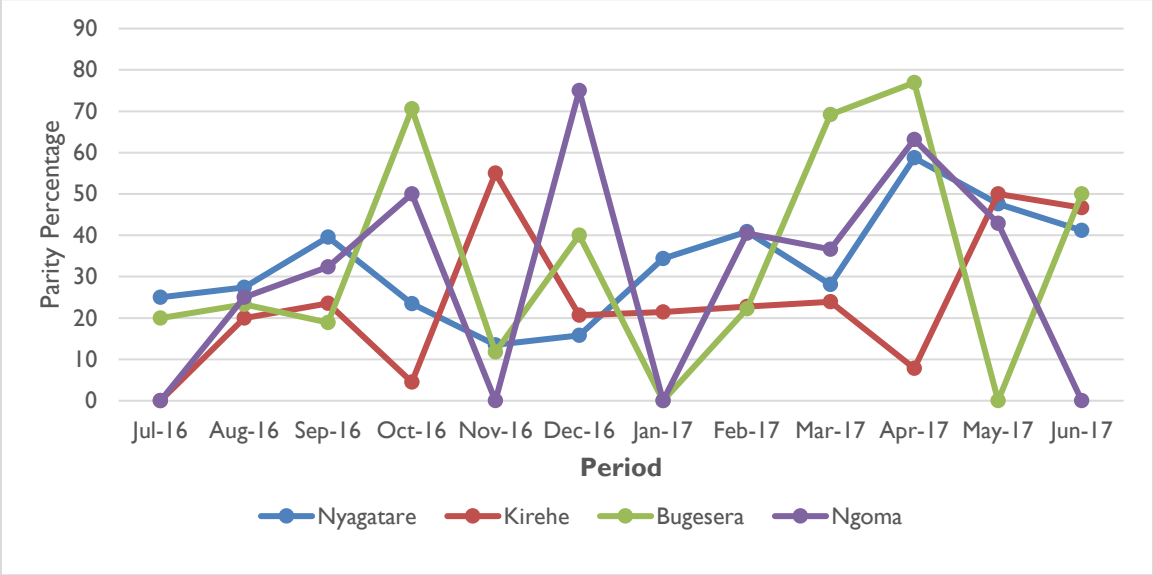
TABLE 4. PARITY

District	Sector	Total Collected	Total *An. gambiae* s.l. Dissected	# Parous	% Parity	P value	Result
Bugesera	Nyarugenge	538	174	80	46.0	0.57	NS
	Musenyi	129	47	21	44.7	0.85	NS
Kirehe	Gatore	1183	290	61	21.0	0.01	S
	Nyamugali	311	102	27	26.5	0.003	S
Nyagatare	Nyagatare	1206	267	89	33.3	0.021	S
	Rukomo	2532	487	171	35.1	0.033	S
Ngoma	Remera	1486	243	105	43.2	1	

Note: NS=not significant, S=significant

Parity remained generally high with variations through the months although there was no definitive trend observed in monthly percentage parity in the intervention sites or the control site. The effect of IRS on parity is not clear from these data and may be a result of the inconsistent numbers of mosquitoes dissected during the period in all sites (see Figure 9 and Annex B).

FIGURE 9. PARITY

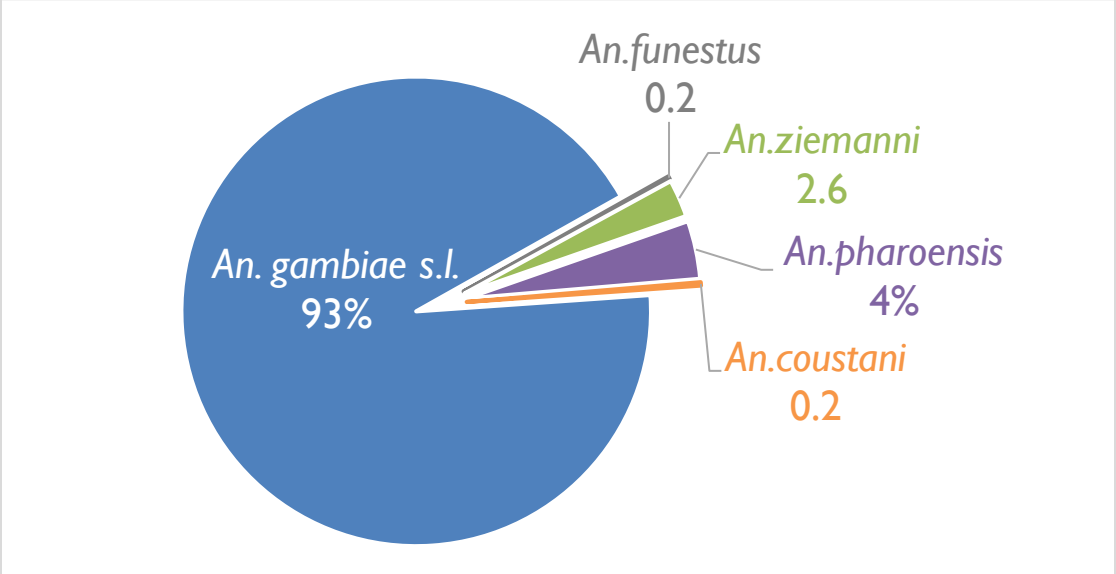


3.5 Enzyme-Linked Immunosorbent Assay (ELISA)

3.5.1 Sporozoite ELISA

Mosquitoes collected through HLC and PSC were used for the Enzyme-Linked Immunosorbent Assay (ELISA). A total of 3,504 mosquitoes collected from July 2016 to June 2017 from the four districts were tested for the Plasmodium circumsporozoite protein. Different Anopheles species were tested; An. gambiae s.l. were dominant (93%) (Figure 10).

FIGURE 10. *ANOPHELES* MOSQUITOES TESTED, BY SPECIES



Pre-IRS (July 2016–September 2016), a total of 617 mosquitoes collected in Nyagatare, Kirehe, and Ngoma districts were tested for *Plasmodium* circumsporozoite proteins, and only 17 were found positive (positivity rate of 2.8%): 13 out of 408 (3.2%) samples tested from Nyagatare district, two of 116 (1.7%) from Kirehe district, and two of 93 (2.2%) from Ngoma (control) district. Bugesera district was sprayed in November 2016 so its pre-IRS is different from other intervention districts; a total of 305 samples collected in this district (July 2016–Nov 2016) were tested and only three (0.98%) were positive for *Plasmodium* circumsporozoite proteins.

Post-IRS (Oct 2016–June 2017), a total of 2,151 mosquitoes were collected in Nyagatare, Kirehe, and Ngoma districts and tested for *Plasmodium* circumsporozoite proteins, and only 15 were found positive (positivity rate of 0.51%): seven out of 1,050 (0.67%) samples tested from Nyagatare district, six out of 737 (0.81%) tested from Kirehe district, and two of 364 (0.55%) from Ngoma (control) district. The post-spray period in Bugesera district was conducted from December 2016 to June 2017; in this period, 419 samples collected in this district were tested and eight (1.9%) were positive. The *Plasmodium*-infected anopheline mosquitoes were observed to decrease when you compare pre-spraying and post-sprayingwith time in both Nyagatare and Kirehe 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 5 (i)). There was also no significant 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 (Table 5 (ii)). There was a significance difference between the positivity obtained in pre-IRS and post-IRS in Nyamugari sector (Kirehe district), Rukomo and Nyagatare sectors (Nyagatare district). there was no significant difference in positivity obtained in pre-IRS and post-IRS in Gatore sector (Kirehe district) (table 5 iii). 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 (Table 5 (iv)).

TABLE 5. SPOROZOITE RATES IN ALL SURVEYED SITES

(I)

District	Sector	Total Tested	Number Positive	% positive	P value	Result
Bugesera	Nyarugenge	465	5	1.07	0.7574	NS
	Musenyi	271	6	2.2	0.13356	NS
Kirehe	Gatore	580	6	1.03	0.79450	NS
	Nyamugali	273	2	0.73	0.83633	NS
Nyagatare	Nyagatare	528	6	1.13	0.68353	NS
	Rukomo	930	14	0.97	0.32977	NS
Ngoma	Remera	457	4	0.9	1	

(ii)

District	Sector	Pre spray (Jul Sep 2016)			Post spray (Oct 2016 June 2017)				
		Total Tested	Number Positive	% Positive	Total # Tested	Total # Positive	% Positive	P value*	Result
Kirehe	Gatore	99	1	1.01	481	5	1.04	0.43641	NS
	Nyamugali	17	1	5.88	256	1	0.4	0.77901	NS
Nyagatare	Nyagatare	48	2	4.16	480	4	0.83	0.62685	NS
	Rukomo	360	11	3.05	570	3	0.52	0.96231	NS
Ngoma	Remera	93	2	2.15	364	2	0.55	1	

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

(iii)

District	Sector	Pre spray (Jul Sep 2016)			Post spray (Oct 2016 June 2017)				
		Total Tested	Number Positive	% Positive	Tota. # Tested	Total # Positive	% Positive	P value*	Result
Kirehe	Gatore	99	1	1.01	481	5	1.04	0.97899	NS
	Nyamugali	17	1	5.88	256	1	0.4	0.01013	S
Nyagatare	Nyagatare	48	2	4.16	480	4	0.83	0.03776	S
	Rukomo	360	11	3.05	570	3	0.52	0.00203	S

*Calculated by using the monthly averages of the pre-IRS collections vs monthly averages of the post-IRS collections. Comparison is between pre- and post-IRS in each site.

(iv)

Sites	Indoor Collection			Outdoor Collection			P value*	Results
	Total Tested	# Positive	% Positive	Total Tested	# Positive	% Positive		
Rukomo	498	4	0.80	430	10	2.33	0.0578	NS
Nyagatare	239	3	1.26	289	3	1.04	0.8147	NS
Gatore	281	0	0.00	310	6	1.94	0.0188	S
Nyamugali	122	1	0.82	151	1	0.66	0.8794	NS
Musenyi	124	2	1.61	116	4	3.45	0.3627	NS
Nyarugenge	363	2	0.55	102	3	2.94	0.0386	S
Remera	284	3	1.06	159	1	0.63	0.6462	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 2016 to June 2017 were also assayed to determine the source of the blood meal. A total of 251 *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 two of the sites (Nyagatare and Ngoma) was positive for a human blood meal, at 65.1% and 39.4%, respectively; in Kirehe, the human blood index was lower than the bovine blood index, at 25.6%% vs 46.2%, respectively. The results show that a relatively high proportion of the vectors also fed on non-human hosts (Table 6).

TABLE 6. BLOOD MEAL SOURCE

Site	Number Tested	Results				
		Human	Bovine	Goat	Human and Bovine	No Specified Host
Nyagatare	63	65.1% (41)	14.3%(9)	0%(0)	12.7%(8)	7.9% (5)
Ngoma	71	39.4% (28)	35.2%(25)	0%(0)	7%(5)	18.3% (13)
Kirehe	39	25.6%(10)	46.2%(18)	0%(0)	5.1%(2)	23.1% (9)
Bugesera	78	21.8% (17)	27%(21)	1.3%(1)	18%(14)	32% (25)
Total	251	38.2% (96)	29.1% (73)	0.4% (1)	11.6% (29)	20.7% (52)

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 2016 to June 2017. Nyagatare showed more months (August, September, and December 2016) of highest infective biting relative to the other intervention districts and control district. Although the period of highest infectivity is not the same in the four districts, there seems to be some general trend in the months of August and September in all districts. The EIR was higher pre-IRS than post-IRS in all intervention districts (see Figure 11 and Annex D). The EIR of *An. gambiae* s.l. based on collection location showed that, in Nyagatare and Kirehe districts, the outdoor EIR is higher than the indoor EIR (Figure 12).

FIGURE 11. ENTOMOLOGICAL INOCULATION RATES

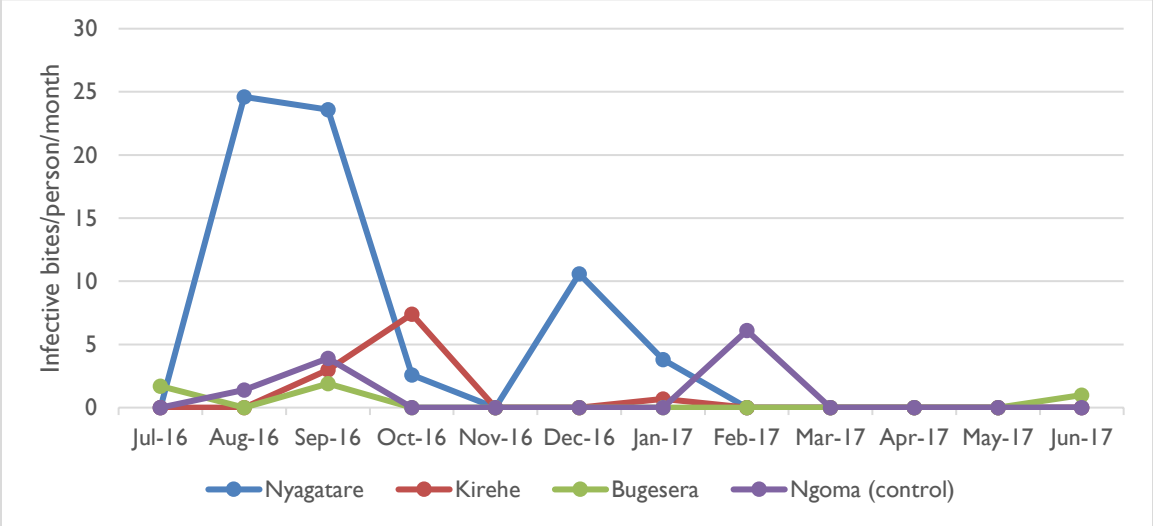
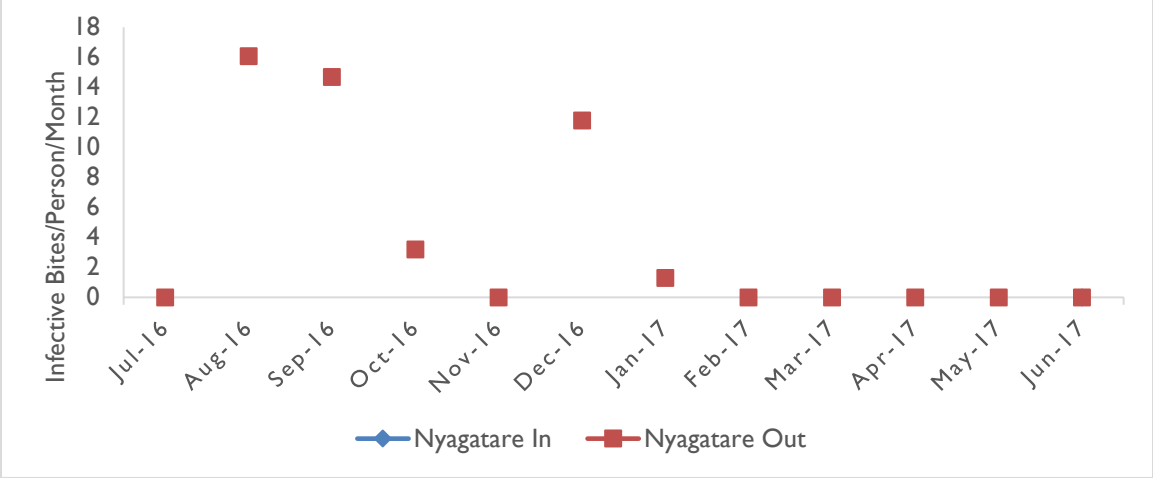
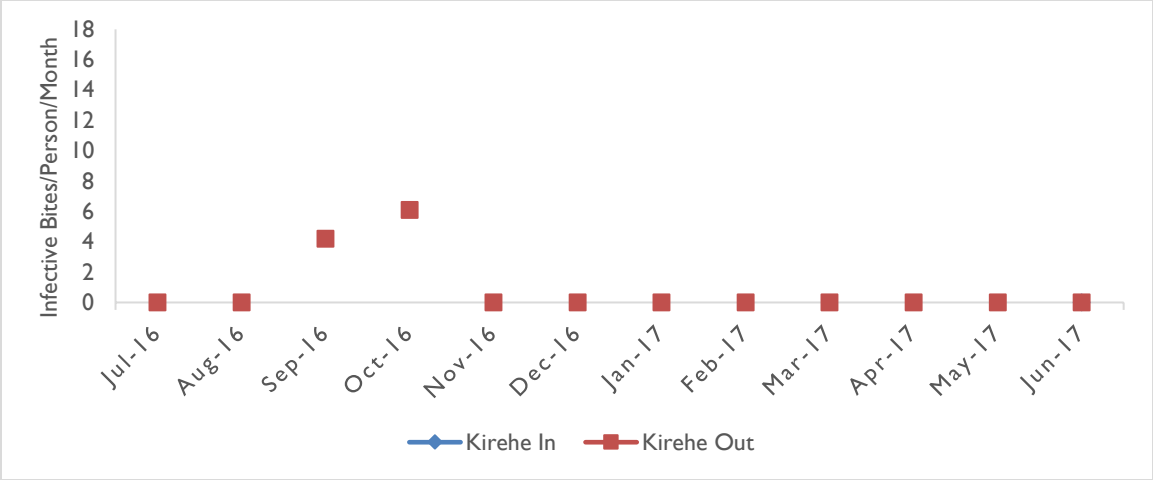
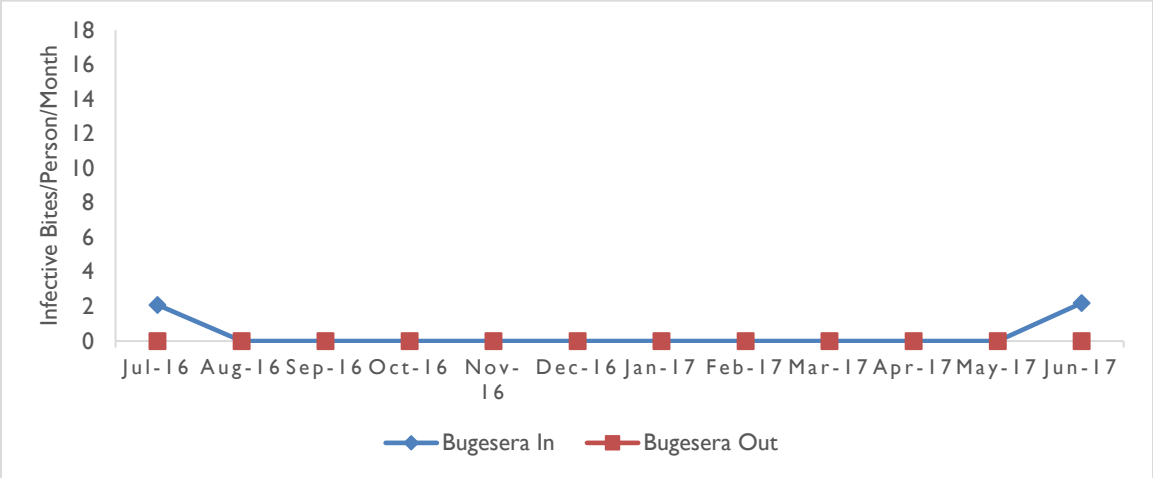
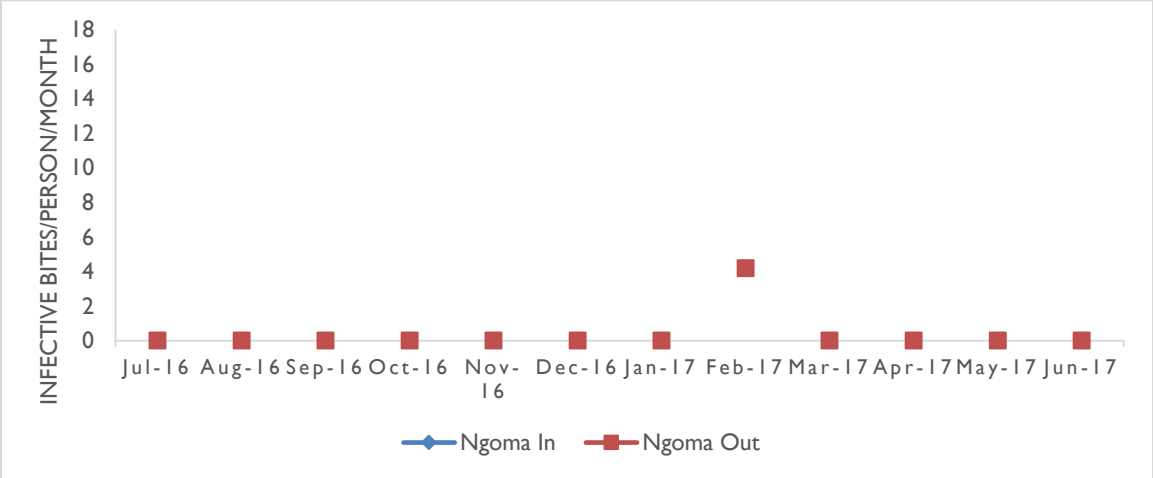


FIGURE 12. INDOOR VS OUTDOOR ENTOMOLOGICAL INOCULATION RATES




Infective Bites/Person/Month
18
16
14
12
10
8
6
4
2
0
Jul-16
Aug-16
Sep-16
Oct-16
Nov-16
Dec-16
Jan-17
Feb-17
Mar-17
Apr-17
May-17
Jun-17
Kirehe In
Kirehe Out


Infective Bites/Person/Month
18
16
14
12
10
8
6
4
2
0
Jul-16
Aug-16
Sep-16
Oct-16
Nov-16
Dec-16
Jan-17
Feb-17
Mar-17
Apr-17
May-17
Jun-17
Bugesera In
Bugesera Out

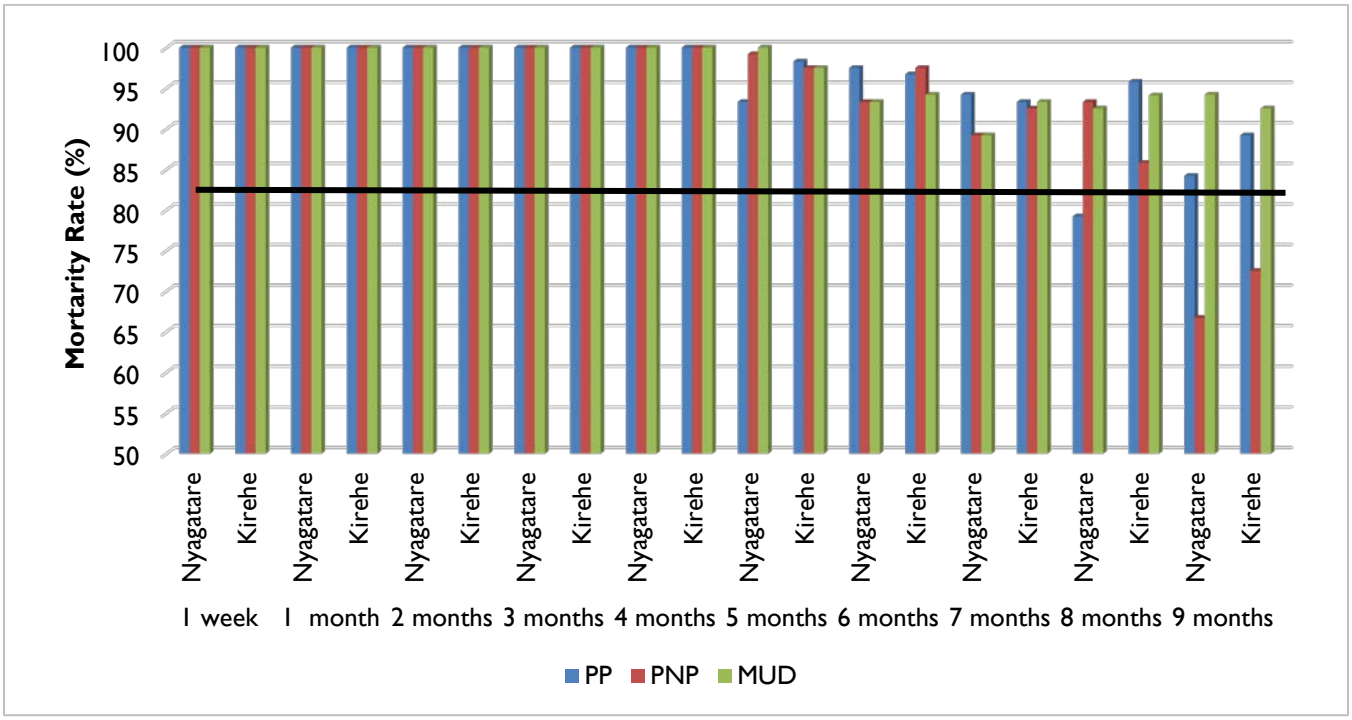

INFECTIVE BITES/PERSON/MONTH
18
16
14
12
10
8
6
4
2
0
Jul-16
Aug-16
Sep-16
Oct-16
Nov-16
Dec-16
Jan-17
Feb-17
Mar-17
Apr-17
May-17
Jun-17
Ngoma In
Ngoma Out

3.6 QUALITY OF SPRAY AND INSECTICIDE DECAY RATE

After AIRS Rwanda sprayed in September 2016 using pirimiphos-methyl (Actellic 300CS) in two districts (Kirehe and Nyagatare), it carried out WHO cone bioassays in 24 of the sprayed structures. It tested three different wall surfaces (mud, plastered not painted (PNP), and plastered and painted (PP)) in both districts. In each district, two different sectors were sampled, and in each sector six structures were sampled. Out of the six structures in each sector, two 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 2017 (nine months post-spraying), the mortality rate was over 80% on mud and PP surfaces. Test mortality dropped below 80% in both districts in houses with PNP surfaces (Figure 13).

FIGURE 13. WALL BIOASSAY TEST RESULTS (SEPTEMBER 2016–JUNE 2017)



3.7 CONCLUSIONS

- *An. gambiae* s.l. is the major malaria vector in Bugesera, Kirehe, Nyagatare, and Ngoma districts. The number of collected *An. gambiae* s.l. was high in the pre-IRS period (July–mid-September 2016) and dropped just after the September–October 2016 spray campaign.
- *An. gambiae* s.l. showed more exophagic than endophagic behavior in the four data collection districts. The difference was statistically significant in Kirehe and Ngoma districts.
- Hourly biting rates per person varied across the four districts; they were highest in Nyagatare followed by Ngoma (control district) and Kirehe; Bugesera showed the lowest mean rate. The higher rates in Nyagatare and Kirehe 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 Nyagatare district.
- In the Nyagatare site, indoor biting was highest at 00:00–01:00, as was the outdoor biting rate. The biting rate was constant between 18:00 and 23:00, then increased to peak at 00:00–00:01 before dropping to its lowest at 5:00–6:00. In Kirehe district, the peak for indoor biting was 18:00–19:00, and the peak for outdoor biting was 00:00–01:00. In Bugesera district, the peak for indoor biting and outdoor biting was 00:00–01:00. In Ngoma district, the peak for indoor biting was 22:00–23:00 and for outdoor biting was 02:00–03:00.
- Community members should be given information on and encouraged to stay indoors in the early hours of the night and to use ITNs while in bed.
- There was a significant difference in average monthly vector PSC collections in all intervention districts and the control district ($p<0.05$). Although vector density varied through the reporting months, Bugesera district showed the highest average vector density among IRS districts (0.55 *An. gambiae* s.l./house/day), compared with Kirehe (0.43 *An. gambiae* s.l./house/day) and Nyagatare (0.33 *An. gambiae* s.l./house/day). The control (non-IRS) district showed the highest density of one *An. gambiae* s.l./house/day relative to the intervention districts.
- There was no clear trend observed for parity in the IRS districts relative to the control district.
- Biting rates were higher in Nyagatare than in the other districts (including the control district). Infectivity was also observed to be higher in Nyagatare, with 24.6 infective bites per month in August 2016. EIR was higher outdoors than indoors in Nyagatare and Kirehe districts. General comparison of positivity between the intervention and control sites showed no significant difference ($p>0.05$) between positivity in the control site and the six intervention sites.
- There was also no significant difference in the infection rates between the samples tested from the control site and all six intervention sites on the post-spray period collections.
- There was a significance difference between the positivity obtained in pre-IRS and post-IRS in Nyamugari sector (Kirehe district), Rukomo and Nyagatare sectors (Nyagatare district). there was no significant difference in positivity obtained in pre-IRS and post-IRS in Gatore sector (Kirehe district)

- *An. gambiae* s.l. showed opportunistic feeding behavior, that is, they fed on all three blood sources tested. A higher proportion of *An. gambiae* s.l. specimens in two of the sites (Nyagatare and Ngoma) showed a higher anthropophilic behavior than in other intervention and control sites.
- Cone bioassays conducted in Kirehe and Nyagatare districts within one week of spraying to assess the quality of spraying showed 100% mortality of susceptible *An. gambiae* s.l., indicating quality spraying took place. Subsequently, bio-efficacy of the sprayed insecticide was monitored monthly. Through June 2017 (nine months after spraying), the mortality rate was over 80% on mud and PP surfaces. In houses with PNP surfaces, test mortality dropped below 80% in both districts.

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

4.1 Insectary Maintenance and Associated Vector Control Laboratory Support

Through the support of USAID/PMI, AIRS Rwanda 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.

4.2 Entomology Sentinel Sites Support

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

A three-day refresher training session were held at Gashora Palise hotel on February 21–24, 2017. The refresher training strengthened the skills of the sentinel site technicians on mosquito identification using taxonomic keys, mosquito ovary dissection, and entomological data reporting. The trainees were drawn from the 12 entomology sentinel sites and the facilitators were from the Ministry of Health's Malaria and Other Parasitic Diseases Division and AIRS Rwanda.

5. Challenges and Recommendations

- Molecular tests, especially PCR for species identification, were not done because of a delay in delivery of reagents and consumables.
- The results will be shared once they are available.

ANNEX A. 2017 IRS AND CONTROL DISTRICTS' INSECTICIDE RESISTANCE DATA

#	District	Sites	Period	Deltamethrin 0.05%	Permethrin 0.75%	Lambda cyhalothrin 0.05%	Pyrimiphos methyl 0.25%	Bendiocarb 0.1%	Fenitro thion 1%	DDT 4%
1	Nyagatare	Nyagatare	Mar-17	80	80	58	100	100	100	50
2	Ngoma	Remera	Mar-17	82	64	67	100	90	100	79
3	Kirehe	Bukora	May-17	98	97	97	100	100	100	100
4	Nyagatare	Mimuli	Jul-17	98	98	85	100	100	98	100
						Mimuli+PBO: 100				

ANNEX B. 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-16	253	60	15	25	3	0	0	0	8	5	1	20	2	0	0	0
Aug-16	289	62	17	27.4	10	5	1	20	85	30	7	23.3	9	4	1	25
Sept-16	629	96	38	39.6	219	51	12	23.5	139	37	7	18.9	110	34	11	32.4
Oct-16	1023	132	31	23.5	294	67	3	4.5	44	17	12	70.6	85	32	16	50
Nov-16	67	37	5	13.5	40	20	11	55	41	17	2	11.8	2	0	0	0
Dec-16	126	38	6	15.8	85	29	6	20.7	5	5	2	40	8	4	3	75
Jan-17	66	32	11	34.4	29	14	3	21.4	11	4	0	0	0	0	0	0
Feb-17	98	44	18	40.9	74	22	5	22.7	36	9	2	22.2	128	42	17	40.5
Mar-17	287	64	18	28.1	150	46	11	23.9	219	65	45	69.2	1027	82	30	36.6
Apr-17	592	109	64	58.7	454	77	6	7.8	53	26	20	76.9	102	38	24	63.2
May-17	269	63	30	47.6	106	46	23	50	4	0	0	0	13	7	3	42.9
Jun-17	39	17	7	41.2	30	15	7	46.7	12	6	3	50	0	0	0	0
Total	3738	754	260	34.48	1494	392	88	22.4	657	221	101	45.7	1486	243	105	43.2

ANNEX C. SPOROZOITE RATES

	Nyagatare			Kirehe			Bugesera			Ngoma		
	Total tested	# positive	% positive	Total tested	# positive	% positive	Total tested	# positive	% positive	Total tested	# positive	% positive
Jul-16	99	0	0	7	1	14.3	15	1	6.7	2	0	0
Aug-16	103	7	6.8	11	0	0	91	1	1.1	17	1	5.9
Sept-16	206	6	2.9	98	1	1.02	93	1	1.08	74	1	1.4
Oct-16	428	1	0.23	154	3	1.94	68	0	0	33	0	0
Nov-16	32	0	0	24	0	0	38	0	0	4	0	0
Dec-16	45	3	6.7	40	0	0	16	2	12.5	15	0	0
Jan-17	65	3	4.6	56	1	2.2	29	0	0	6	0	0
Feb-17	71	0	0	99	0	0	50	0	0	107	2	1.9
Mar-17	110	0	0	88	2	2.3	158	4	2.53	152	0	0
Apr-17	142	0	0	124	0	0	83	0	0	28	0	0
May-17	116	0	0	106	0	0	56	0	0	17	0	0
Jun-17	41	0	0	46	0	0	39	2	5.12	2	0	0
Total	1458	20	1.37	853	8	0.94	736	11	1.5	457	4	0.9

ANNEX D. ENTOMOLOGICAL INOCULATION RATES

	Nyagatare					Kirehe				
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-16	253	10.5	0	0	0	3	0.1	14.3	0.014	0.42
Aug-16	289	12	6.8	0.82	24.57	10	0.4	0	0	0
Sept-16	629	26.2	3	0.79	23.59	219	9.1	1.02	0.092	2.76
Oct-16	1023	42.6	0.2	0.09	2.56	294	12.3	1.94	0.238	7.14
Nov-16	67	2.8	0	0	0	40	1.7		0	0
Dec-16	126	5.3	6.7	0.35	10.55	85	3.5		0	0
Jan-17	66	2.8	4.6	0.13	3.8	29	1.2	2.2	0.0264	0.793
Feb-17	98	4.1	0	0	0	74	3.1		0	0
Mar-17	287	12	0	0	0	150	6.3	2.3	0.1449	4.347
Apr-17	592	24.7	0	0	0	454	18.9		0	0
May-17	269	11.2	0	0	0	106	4.4		0	0
Jun-17	39	1.6	0	0	0	30	1.3		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-16	8	0.3	6.7	0.02	0.6	2	0.2	0	0	0
Aug-16	85	3.5	1.1	0	0	9	0.8	5.9	0.05	1.42
Sept-16	139	5.8	1.08	0.06	1.88	110	9.2	1.4	0.13	3.86
Oct-16	44	1.8	0	0	0	85	7.1	0	0	0
Nov-16	41	1.7	0	0	0	2	0.2	0	0	0
Dec-16	5	0.2	12.5	0.025	0.75	8	0.7	0	0	0
Jan-17	11	0.5	0	0	0	0	0	0	0	0
Feb-17	36	1.5	0	0	0	128	10.7	1.9	0.20	6.1
Mar-17	219	9.1	2.53	0.23	6.9	1027	85.6	0	0	0
Apr-17	53	2.2	0	0	0	102	8.5	0	0	0
May-17	4	0.2	0	0	0	13	1.1	0	0	0
June-17	12	0.5	5.12	0.0256	0.768	0	0	0	0	0

ANNEX E. REFERENCES

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