USAID
FROM THE AMERICAN PEOPLE

U.S. PRESIDENT'S MALARIA INITIATIVE

CDC
CENTERS FOR DISEASE CONTROL AND PREVENTION

U.S. PRESIDENT'S MALARIA INITIATIVE
vectorlink
INTELLIGENT › INNOVATIVE › INTEGRATED

PMI VECTORLINK RWANDA ANNUAL ENTOMOLOGICAL MONITORING REPORT JULY 2019–JUNE 2020

Recommended Citation: U.S. President's Malaria Initiative VectorLink Project. September 2020. *Rwanda: 2020 Entomological Monitoring, July 2019–June 2020*. Final Report. Kigali, Rwanda: VectorLink Project, Abt Associates Inc.

Contract: AID-OAA-I-17-00008

Task Order: AID-OAA-TO-17-00027

Submitted to: United States Agency for International Development/PMI

Submitted on: September 25, 2020

Approved: November 16, 2020

Abt Associates Inc. | 6130 Executive Blvd | Rockville, Maryland 20814
T. 301.347.5000 | F. 301.913.9061
abtassociates.com

PMI VECTORLINK RWANDA ANNUAL ENTOMOLOGICAL MONITORING REPORT

JULY 2019–JUNE 2020

CONTENTS

LIST OF TABLES

LIST OF FIGURES

ACRONYMS

An.	*Anopheles*
BEI	Biodefense and Emerging Infections
CS	Capsule Suspension
ELISA	Enzyme-linked Immunosorbent Assay
HLC	Human Landing Catch
IRS	Indoor Residual Spraying
MOPDD	Malaria and Other Parasitic Diseases Division
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
RBC	Rwanda Biomedical Center
TAE	Tris base, Acetic acid and EDTA
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, especially in the COVID-19 context. Particularly, we thank the Rwanda Ministry of Health through the 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 2019–June 2020, monthly entomological data collection took place in five districts, except in April 2020 due to restrictions related to the COVID-19 pandemic; the five districts were Kirehe, Ngoma, Nyagatare, Kamonyi, and Nyaruguru. Kirehe, Ngoma, and Nyagatare were surveyed as indoor residual spraying (IRS) districts throughout the year (11 months), and Kamonyi and Nyaruguru were used as un-sprayed controls. Data were collected from Kamonyi for two months (July–August 2019), and from Nyaruguru for nine months. Adult mosquitoes were sampled using pyrethrum spray catch (PSC) and human landing catch (HLC) methods, to assess vector species composition, behavior, and seasonal trends in density, human biting rate, parity, and infection. World Health Organization cone bioassays were conducted in the three IRS districts 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 2,174 adult female *Anopheles* mosquitoes were collected, 1,897 using HLC and 277 using PSC. Among the *Anopheles* mosquitoes collected, 82.9% were *An. gambiae* s.l., 12.2% *An. ziemanni*, 3.1% *An. maculipalpis*, and the remaining percentage was shared by *An. pharoensis*, *An. coustani*, *An. funestus* group, and *An. rufipes*. *Anopheles funestus* were collected in only three sites (Gatore, Nyamugali, and Ngera), most from Ngera.

A subsample of *An. gambiae* s.l. was identified using the PCR method; 87.3% were *An. arabiensis* and 12.7% were *An. gambiae* s.s. *Anopheles arabiensis* was dominant in all sprayed sites and in Ngera (Nyaruguru district), one of the control sites. *Anopheles gambiae* s.s. was dominant in Musambira site (Kamonyi control district). The difference in proportion between *An. arabiensis* and *An. gambiae* s.s. in all sites was statistically significant ($p<0.05$). A total of 193 Kisumu strain mosquitoes from the insectary were tested using PCR to determine if the colony had been contaminated, and the results showed that 100% of the tested mosquitoes were *An. gambiae* s.s.

Anopheles 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 of Kirehe district and one site in Ngoma district ($p<0.001$); in other sites the difference was not statistically significant ($p>0.05$). *Anopheles funestus* also showed an exophagic tendency with 30.8% vs 69.2%, but the difference was not statistically significant.

The human biting rate was highest in September–October in all intervention sites except Nyagatare, where two peaks were observed: September–October and February–March. The highest rate at intervention sites was recorded in Rukomo with 9.8 bites/person/night outdoor in September and 8.3 bites/person/night indoor in October.

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 indoors and outdoors.

Although vector density was very low in all surveyed sites over the months of the reporting period, Kirehe district showed a higher average vector density (0.8 *An. gambiae* s.l./house/day) than did the other IRS districts of Nyagatare (0.45 *An. gambiae* s.l./house/day) and Ngoma (0.25 *An. gambiae* s.l./house/day). The control (non-IRS) Kamonyi and Nyaruguru districts also showed low density, 0.46 *An. gambiae* s.l./house/day and 0.5 *An. gambiae* s.l./house/day, respectively.

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

Nyaruguru control site and both sites in Kirehe, Ngoma, and Nyagatare districts. The difference observed in intervention sites could be attributed to the IRS.

The overall sporozoite positivity rate was 0% (n=1,885). Among the tested mosquitoes, *An. gambiae* s.l. represented 80%.

A total of 82 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 83.4%, Ngoma 26.8%, Kirehe 13.5%, Nyagatare 11.5%, and Nyaruguru 4.6%. 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. within 48 hours post exposure, 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 in Kirehe and Nyagatare, and five months post IRS in Ngoma), the mortality rate was over 80% on all surface types one to five days post exposure.

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 September 2019, VectorLink Rwanda sprayed two districts, Kirehe (12 sectors) and Nyagatare (14 sectors) and in January–February 2020 it sprayed one district, Ngoma (14 sectors). Fludora® Fusion (clothianidin/deltamethrin combination) was used for the first time in the country.

This report covers entomological monitoring activities conducted from July 1, 2019, to June 30, 2020. 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
- Assessing the impact of IRS on the 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

2. Data Collection Sites and Methods

2.1 Study Sites

Data collection was conducted on a monthly basis in three IRS districts, Kirehe, Ngoma, and Nyagatare, and one non-IRS (control) district. The original control district Kamonyi was used as control July and August 2019, and was replaced with Nyaruguru district, from September owing to the fact that the Malaria and Other Parasitic Diseases Division (MOPDD) of the Rwanda Biomedical Center (RBC), as Rwanda's national malaria control program is known, decided in September 2019 to spray Kamonyi. In each IRS district, two sites were selected as data collection sites, and in the control district, one site was selected (Figure 1). 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
Kirehe	Gatore, Nyamugali	Sprayed September 2019 using Fludora® Fusion with PMI support
Ngoma	Remera, Zaza	Sprayed March-April 2019 using Actellic 300CS by Government of Rwanda and then sprayed in January–February 2020 using Fludora® Fusion with PMI support
Nyagatare	Nyagatare, Rukomo	Sprayed September 2019 using Fludora® Fusion with PMI support
Nyaruguru (control)	Ngera	Not sprayed
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
Kirehe	Gatore	x	x	x	x	x	x	x	x	x	ND	x	x	11 months of data; because of COVID-19 the collection was not done in April 2020
	Nyamugali	x	x	x	x	x	x	x	x	x	ND	x	x	
Ngoma	Remera	x	x	x	x	x	x	x	x	x	ND	x	x	
	Zaza	x	x	x	x	x	x	x	x	x	ND	x	x	
Nyagatare	Nyagatare	x	x	x	x	x	x	x	x	x	ND	x	x	11 months of data; because of COVID-19 the collection was not done in April 2020
	Rukomo	x	x	x	x	x	x	x	x	x	ND	x	x	
Nyaruguru	Ngera	NA	NA	c	c	c	c	c	c	c	ND	c	c	New control district starting in September 2019; because of COVID-19 the collection was not done in April 2020
Kamonyi	Musambira	c	c	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	

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

2.2 DATA COLLECTION METHODS

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

Spray quality was assessed in six sites (two in each IRS 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, the cone bioassays continued on a monthly basis 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, composed of four people per house per night; two collectors per house, one indoors and another outdoors, collected mosquitoes from 18:00 to 24:00 and two others collected from 24:00 to 06:00. In each site, the collectors switched places (outdoors vs indoors) every hour. Outdoor mosquito collection was carried out about 6 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 (Coetzee 2020).

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 09:00. 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 an aerosol that contains tetramethrin 0.30% w/w, cypermethrin 0.07% w/w, and D-Allethrin 0.12% w/w. Ten minutes after spraying, the mosquitoes that had been knocked down were collected and sorted by species. The abdominal status of all female *Anopheles* was determined, and the 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

A sample of 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. Each head-thorax 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 Biodefense and Emerging Infections (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 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 identified to the species level using 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 SYBR 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 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 hours to five days post exposure. 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 Fludora® Fusion sprayed in houses, 10 female *An. gambiae* s.s. were put in a small wire cage (15 cm x 10 cm) covered with an untreated polyester net. Cages were placed approximately 10 cm from a sprayed wall and about 1 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 and then at 60 minutes. We observed mortality after a 24-hour holding period. A control cage was set up outside under a tree in the shade.

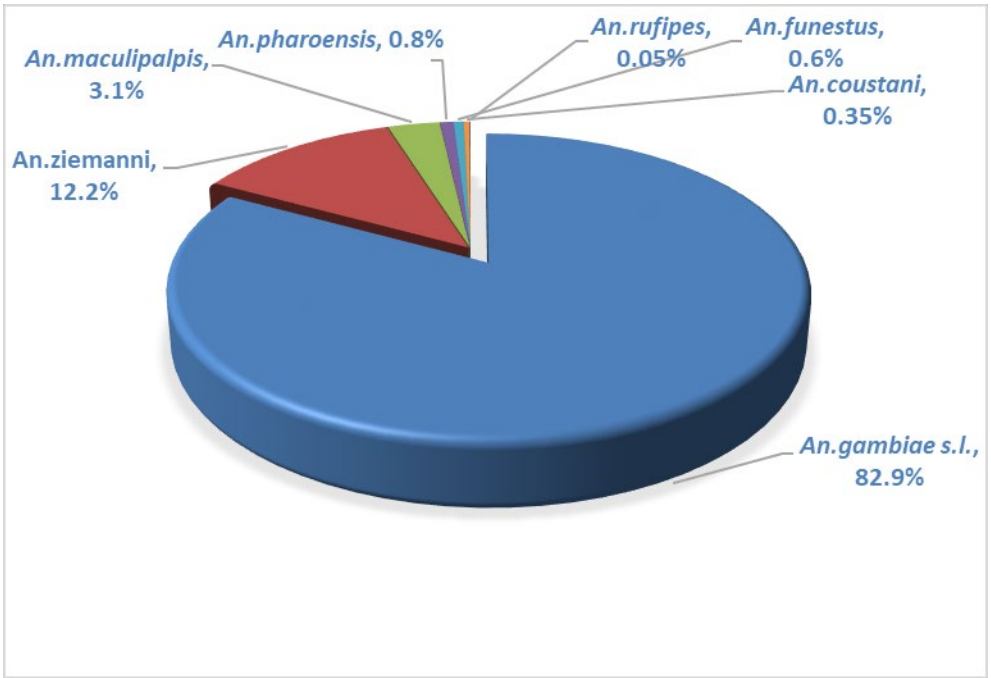
3. Results, Discussion, and Conclusions

3.1 Species Composition and Vector Seasonality

3.1.1 Species Composition

During the reporting period July 2019 to June 2020, a total of 2,174 adult female *Anopheles* mosquitoes were collected; 1,897 were collected using HLC, 277 using PSC. As shown in Figure 2, among the *Anopheles* mosquitoes collected, 82.9% were *An. gambiae* s.l., 12.2% *An. ziemanni*, 3.1% *An. maculipalpis*, and the remaining percentage was shared by *An. pharoensis*, *An. coustani*, *An. funestus* s.l., and *An. rufipes*. All *Anopheles* collected by PSC were *An. gambiae* s.l. In addition, 29,801 non-*Anopheles* mosquitoes were collected. Only *An. gambiae* s.l. and the *An. funestus* s.l. are known as primary vectors of malaria in Rwanda.

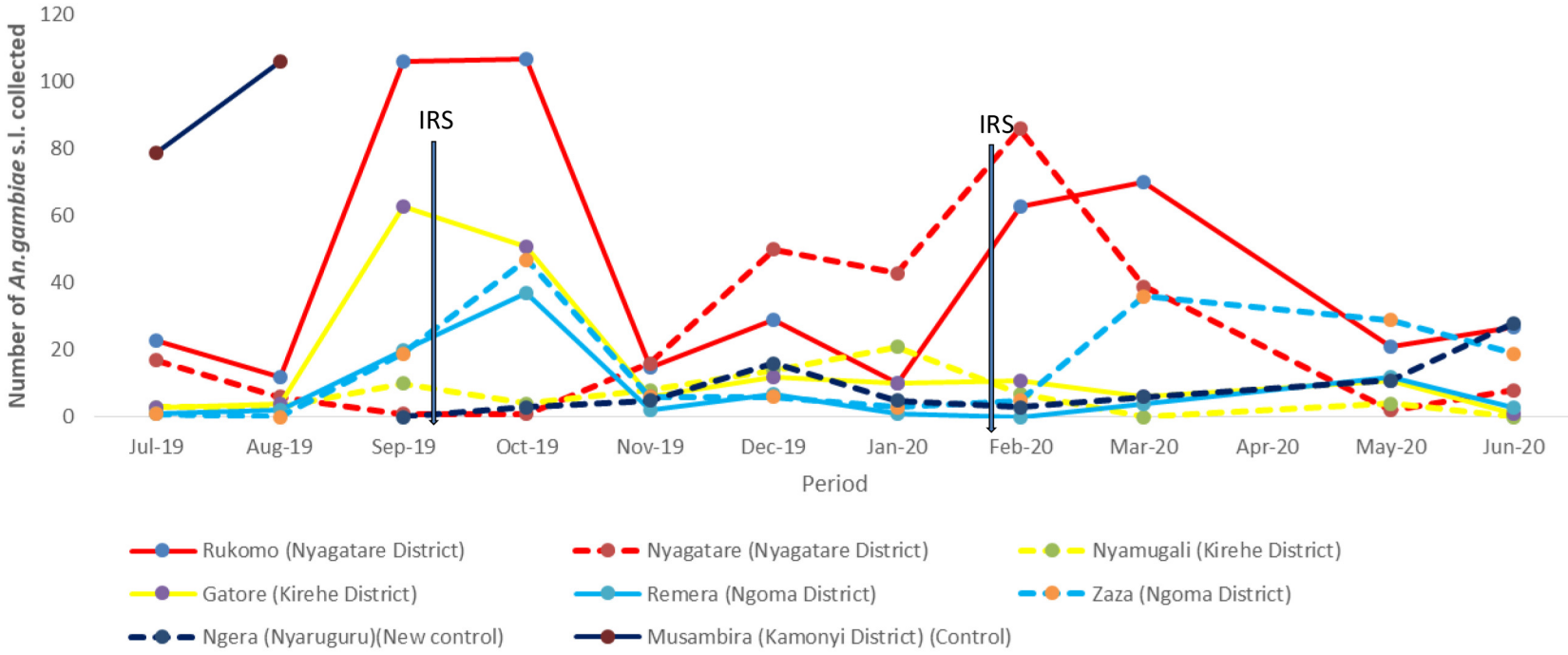
Figure 2: Anopheles Species Composition



3.1.2 VECTOR SEASONALITY

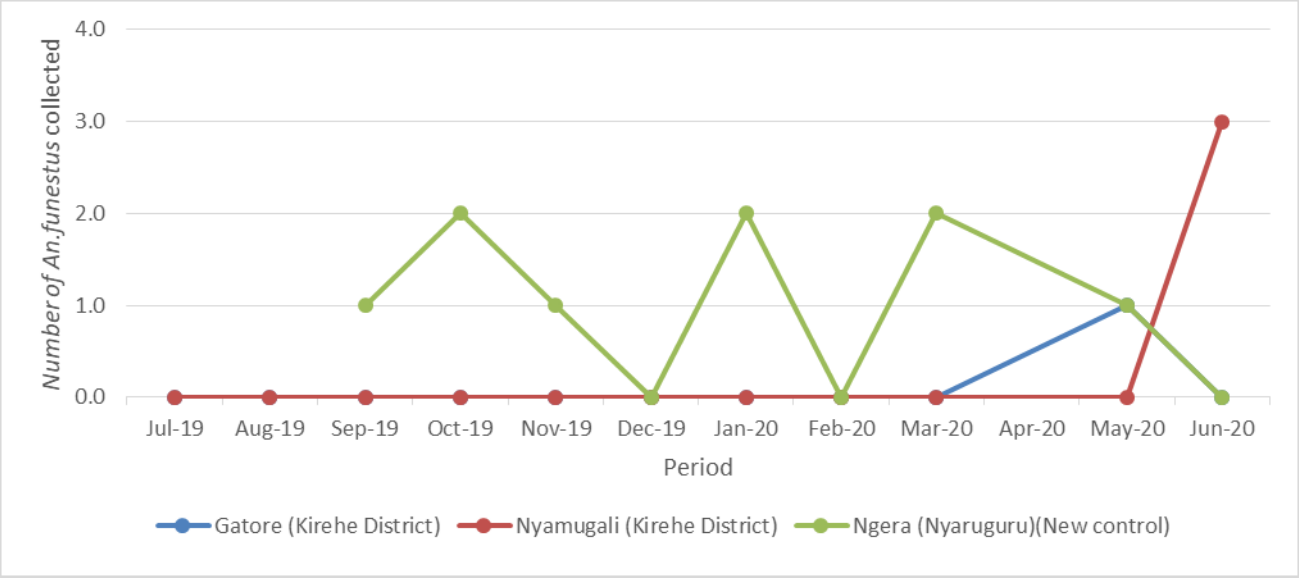
An. gambiae s.l. was the prevalent malaria vector collected by HLC throughout the data collection period in both the intervention and control sites but compared with previous years the number of *An. gambiae* s.l. collected was low. As Figure 3A shows, the number of *An. gambiae* s.l. collected in Gatore site was high in September and October 2019, and then decreased over the following months. The numbers at Nyamugali site (Kirehe district) were low before and after spray. In Nyagatare district, the number of mosquitoes was high in Rukomo site in September and October 2019 and then decreased in the following months, and the rose again in February-March 2020, but in Nyagatare site the number of *An. gambiae* s.l. collected was high from December 2019 to January 2020. In Ngoma district (Remera and Zaza), the numbers were high in September and October 2019 and then started to decrease in December. In the control site Musambira (Kamonyi district), a high number of *An. gambiae* s.l. was collected in July and August; MOPDD decided to spray the district in October 2019 and Musambira was replaced by Ngera (Nyaruguru district), where the number of *An. gambiae* s.l. collected was very low during the survey period. Data were not collected in April 2020 in any site as the country was in lockdown due to COVID-19. *Anopheles funestus* were collected in only three sites (Gatore, Nyamugali, and Ngera); the most *An. funestus* were collected in Ngera (control site) (Figure 3b).

Figure 3a: Number of *An. gambiae* s.l. Collected by Month in All Sites



Nyagatare (Nyagatare and Rukomo) and Kirehe (Gatore and Nyamugali) were sprayed in Sept 2019, whereas Ngoma (Remera and Zaza) was sprayed in Mar 2019 and in Jan-Feb 2020. Musambira site was used as a control up to Sept 2019 and then sprayed in Oct 2019, when it was replaced by Ngera (Nyaruguru district) as control.

Figure 3b: Number of An. funestus Collected by Month



3.2 VECTOR FEEDING TIME AND LOCATION

Anopheles gambiae s.l. showed slightly more exophagic than endophagic tendency in all sites surveyed, except the sites in Kirehe and Zaza site in Ngoma district where the difference was statistically significant. (Table 2). The average district percentage endophagy / exophagy was as follows: Kirehe: 29%/71%, Ngoma: 39.6%/60.4%, Nyagatare: 47.3%/52.7%, Kamonyi: 47%/53%, and Nyaruguru: 40%/60%. A chi-square test showed that the difference between indoor and outdoor collections was statistically significant in both sites of Kirehe district and one site in Ngoma district ($p<0.001$). In other sites the difference was not statistically significant ($p>0.05$). *An. funestus* collected showed exophagic tendency with endophagic vs exophagic behavior of 30.8% vs 69.2% but the difference was not statistically significant and the numbers were too small to make any meaningful comparison.

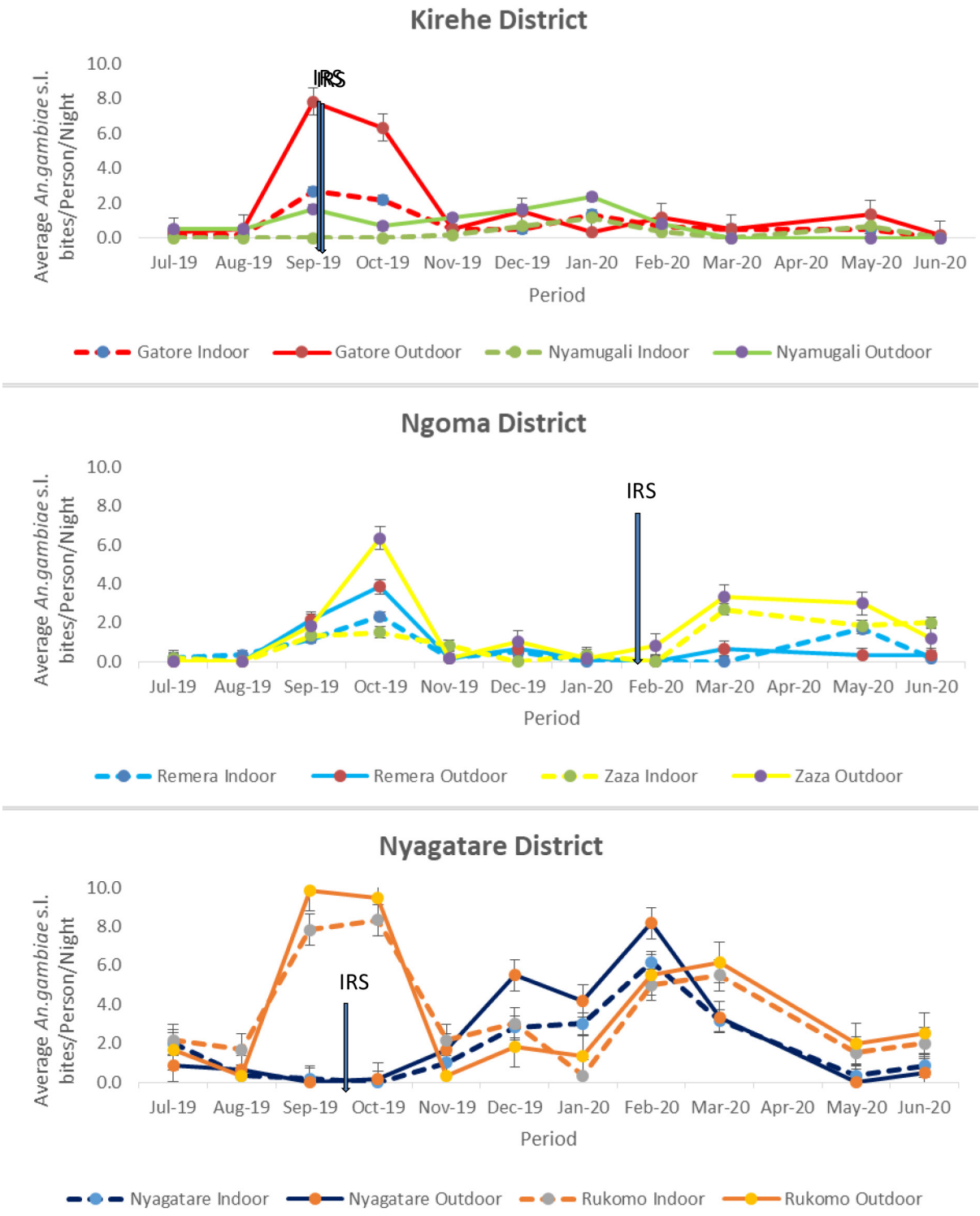
Table 2: Indoor and Outdoor Biting by *An. gambiae* s.l.

District	Site	In	Out	In: Out Ratio	P-value	Result
Kirehe	Gatore	55	123	31:69	p<.001	S
	Nyamugali	18	56	24:76	p<.001	S
Ngoma	Remera	39	50	44:56	0.2436	NS
	Zaza	64	107	37:63	p<.001	S
Nyagatare	Nyagatare	119	150	44:56	0.0587	NS
	Rukomo	237	246	49:51	0.6821	NS
Kamonyi	Musambira	87	98	47:53	0.4186	NS
Nyaruguru	Ngera	31	46	40:60	0.0873	NS

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

As Figure 4 shows, two peak biting seasons, September–October and February–March, were observed in some sites whereas only one peak (September–October) was observed in others. The human biting rates were highest in September–October in all intervention sites except Nyagatare site (Nyagatare district), where the peak was in February–March. The highest bites/person/night in intervention districts was observed in Rukomo site both indoors and outdoors, but the highest overall bites/person/night 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



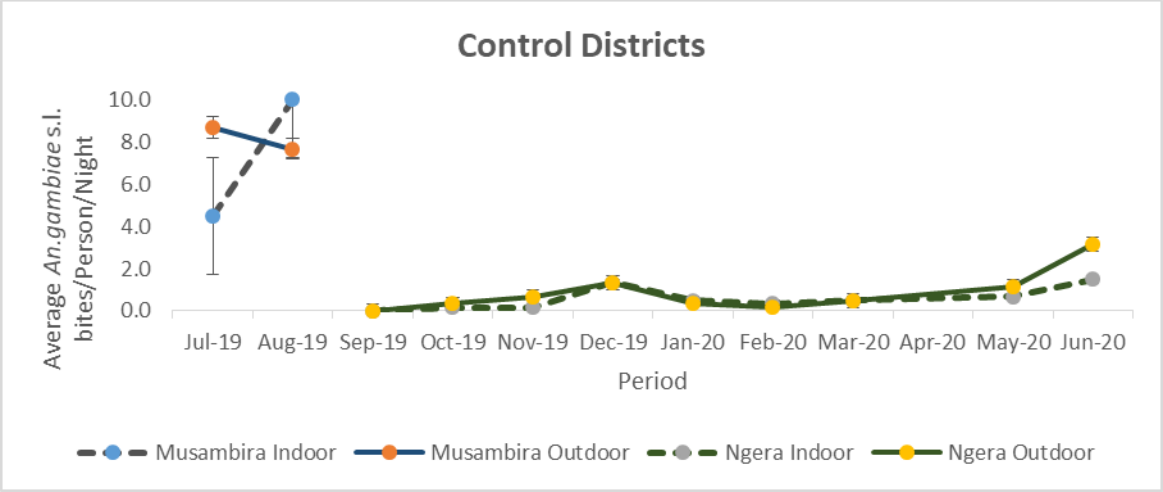
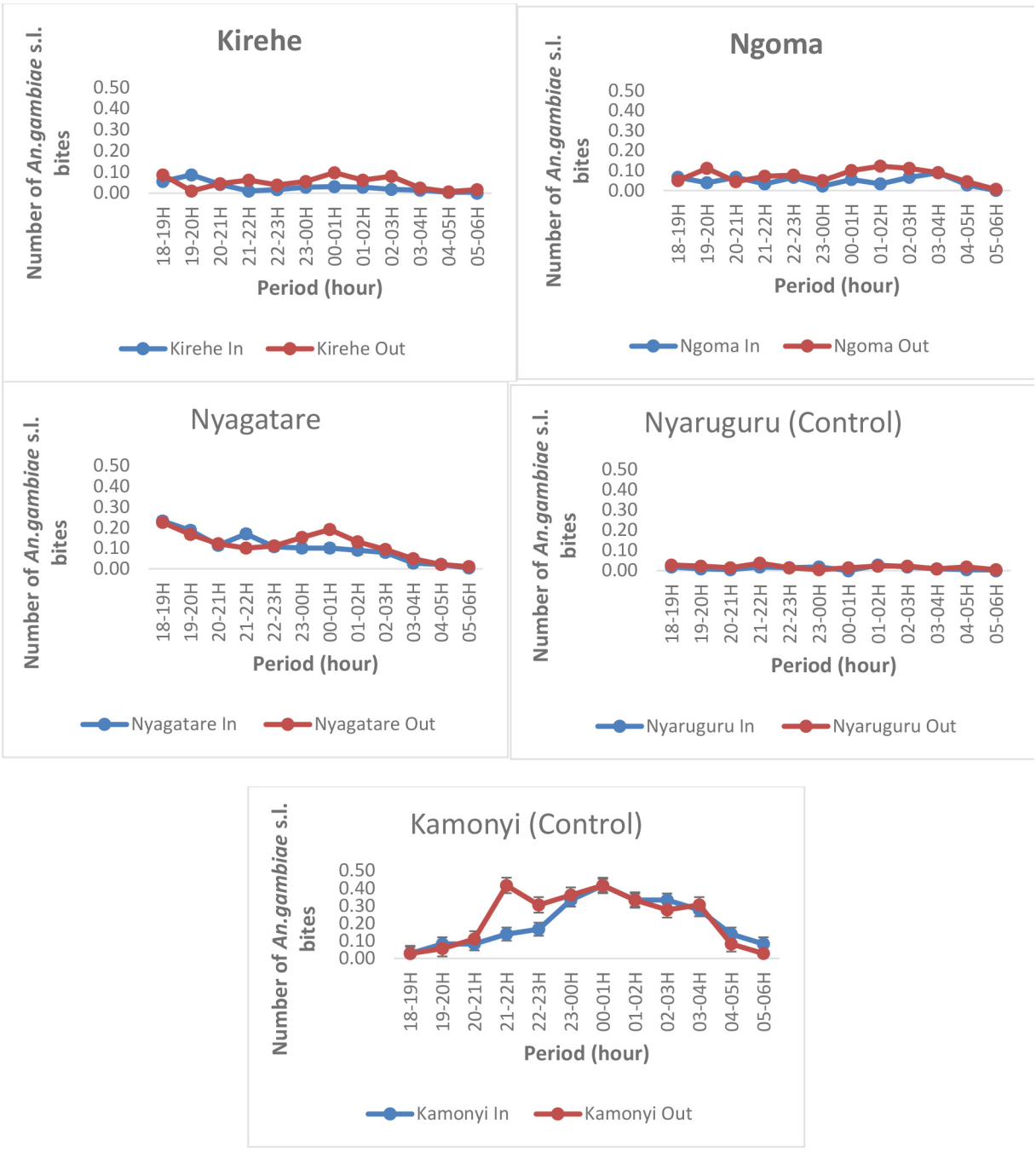


Figure 5 shows average bites per person per hour through the night across the five districts. As the figure shows, more biting took place outdoors. Even though bites per person per hour were very low in all intervention districts, the biting peak was observed very early in the evening (18:00–20:00) both indoors and outdoors in Nyagatare and Kirehe districts. Although it is difficult to make meaningful conclusions given such low biting rates. However, 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.

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



3.3 INDOOR RESTING DENSITY

As noted above, a total of 277 female indoor-resting *An. gambiae* s.l. were collected using PSC in the three IRS districts and the control districts over the July 2019 to June 2020 reporting period. Data were not collected in April 2020. Table 3 shows the disaggregation of the collections and density in the districts.

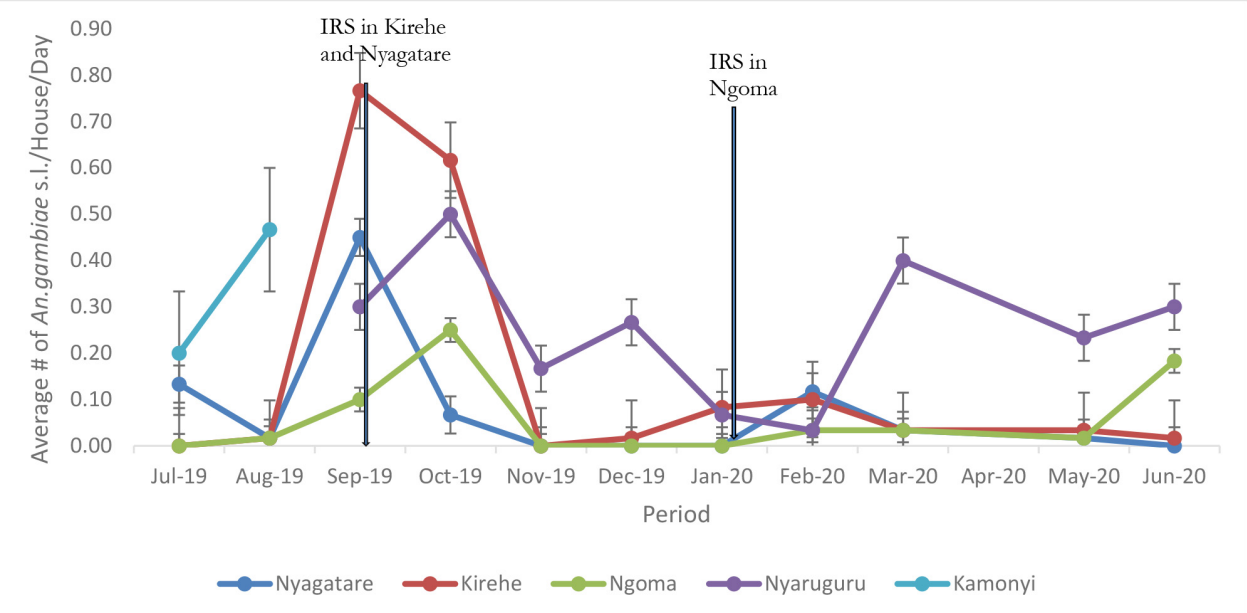
Table 3: *An. gambiae* s.l. Indoor Resting Density from PSC Collections

District	Kirehe		Ngoma		Nyagatare		Nyaruguru (Control)		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-19	0	0	0	0	8		NA	NA	6	0.2
Aug-19	1	0.02	1	0.02	1	0.02	NA	NA	14	0.46
Sep-19	46	0.8	6	0.1	27	0.45	9	0.3	NA	NA
Oct-19	37	0.6	15	0.25	4	0.06	15	0.5	NA	NA
Nov-19	0	0	0	0	0	0	5	0.16	NA	NA
Dec-19	1	0.02	0	0	0	0	8	0.26	NA	NA
Jan-20	5	0.08	0	0	0	0	2	0.06	NA	NA
Feb-20	6	0.1	2	0.03	7	0.11	1	0.04	NA	NA
Mar-20	2	0.03	2	0.03	2	0.03	12	0.4	NA	NA
May-20	2	0.03	1	0.02	1	0.02	7	0.22	NA	NA
Jun-20	1	0.02	11	0.18	0	0	9	0.3	NA	NA
Total	101		38		50		68		20	
September and August collection (%) of the total	82%		55%		62%		35%			
Avg. monthly vector density	9	0.15	3.5	0.05	4.6	0.07	7.6	0.25	10	0.33
P-value	p=0.7311		p=0.2184		p=0.3903		1		NA	NA

*d/h/d: density/house/day
**shading rows: Peak of *An. gambiae* s.l. density
***Kamonyi district was used as a control up to September 2019, when it was replaced by Nyaruguru, so the P-value was calculated between Nyaruguru as control and the other, intervention districts.

Even though the density was very low in all surveyed sites, in general there are two peaks of *An. gambiae* s.l. density, the first one in September–October, and the second one in February–March (Figure 6). The difference between average monthly *An. gambiae* s.l. density in all IRS districts and the control district was not statistically significant ($p>0.05$). Although monthly vector density varied throughout the reporting period, Kirehe district showed the highest average vector density of the IRS districts (0.8 *An. gambiae* s.l./house/day); this was higher than Nyagatare (0.45 *An. gambiae* s.l./house/day) in September and Ngoma (0.25 *An. gambiae* s.l./house/day) in October. The control (non-IRS) district, Nyaruguru, also showed low density, with the highest at 0.5 *An. gambiae* s.l./house/day. Of the total *An. gambiae* s.l. collected resting indoors in the year, 82% in Kirehe, 62% in Nyagatare, and 55% in Ngoma were collected in September and October.

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



All 277 *An. gambiae* s.l. collected in all sites using PSC were classified according to their blood digestion stages: 195 (70%) were unfed, 35 (13%) were fed, 37 (13%) were half-gravid, and 10 (4%) were gravid (Figure 7). The half-gravid and gravid were collected in all districts but in different proportions (Figure 8). The high proportion of unfed mosquitoes collected resting indoors may indicate a high and sustained use of insecticide-treated nets in the communities. Further observation is required.

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

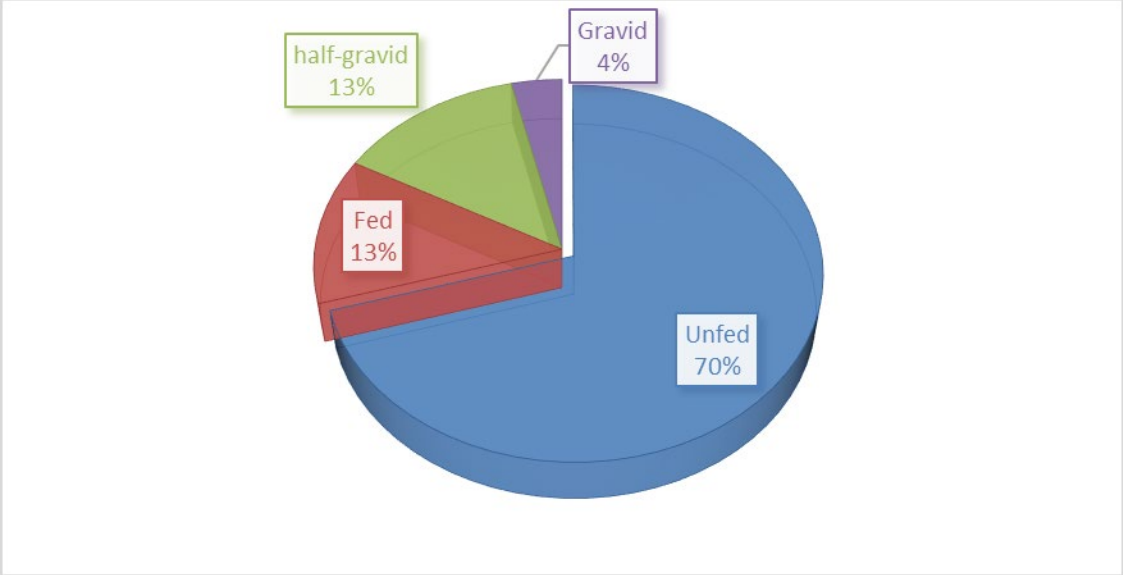
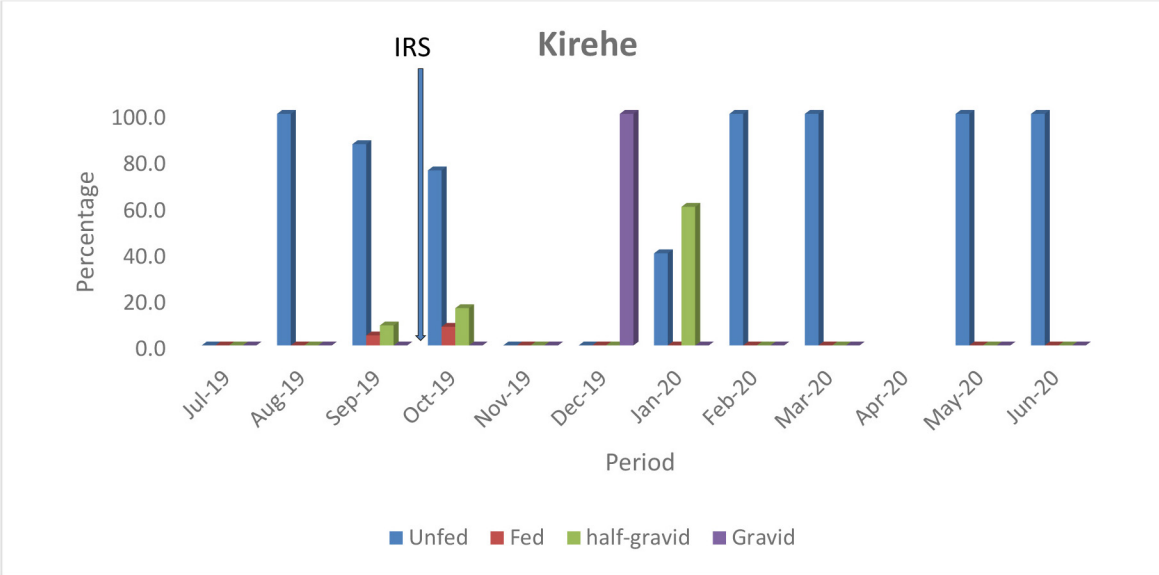
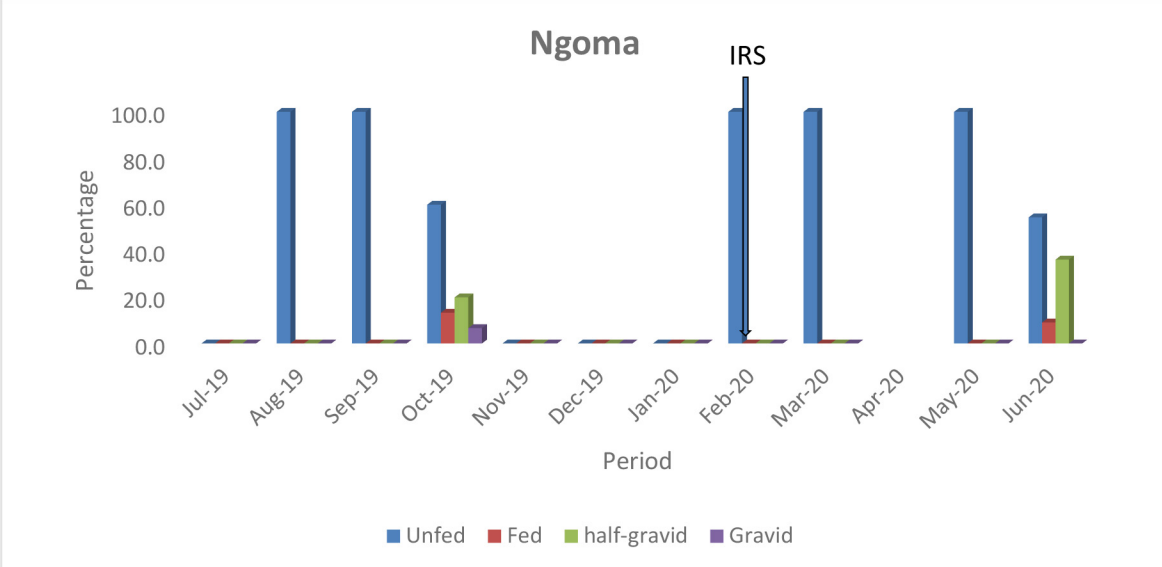
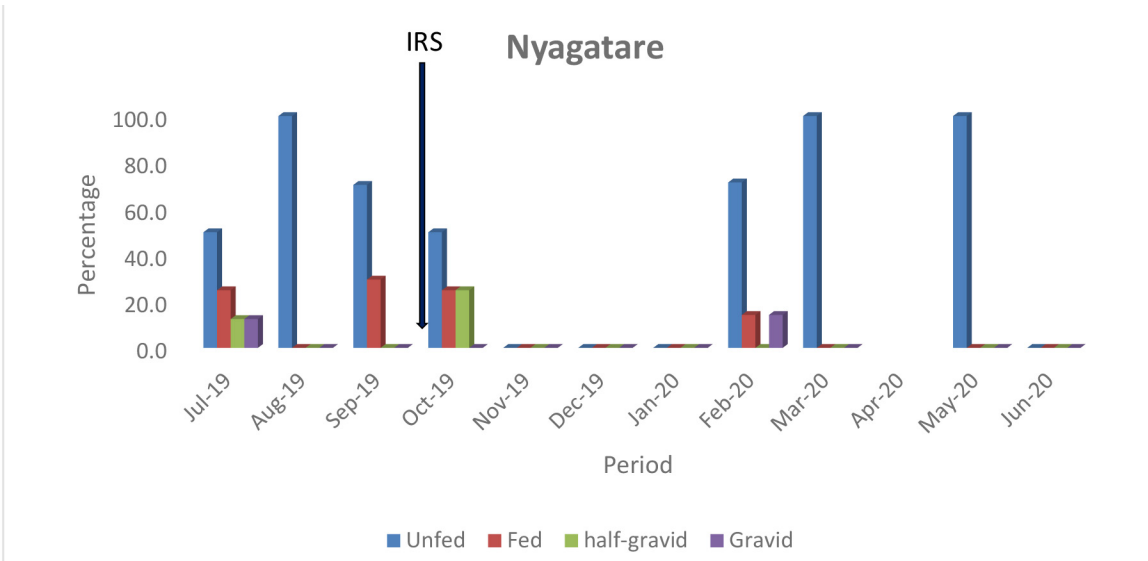
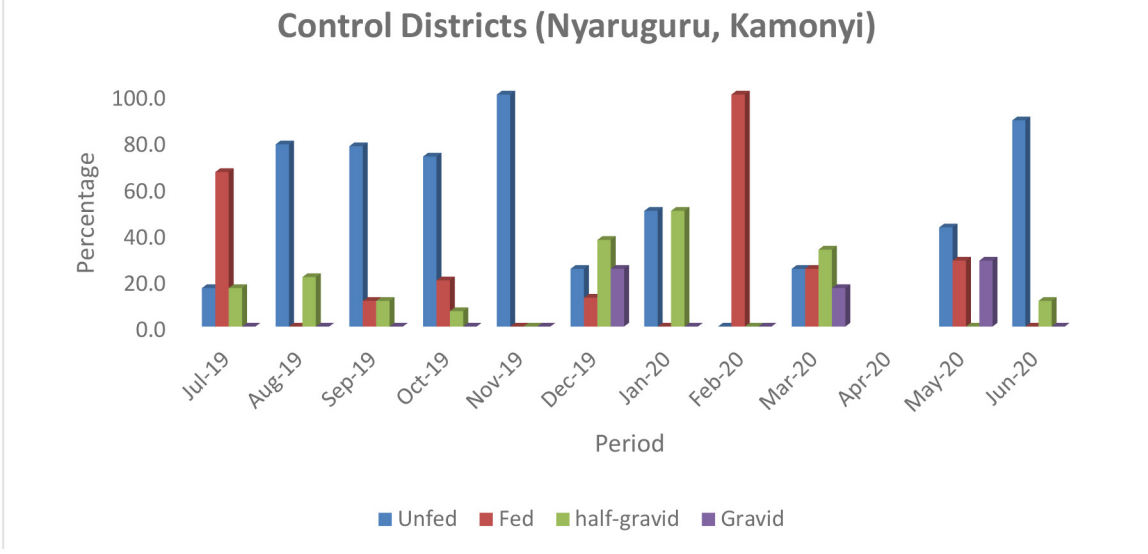


Figure 8: Blood Digestion Stages of An. gambiae s.l. Collected using PSC, by District and Month






IRS
Nyagatare
100.0
80.0
60.0
40.0
20.0
0.0
Percentage
Jul-19
Aug-19
Sep-19
Oct-19
Nov-19
Dec-19
Jan-20
Feb-20
Mar-20
Apr-20
May-20
Jun-20
Period
Unfed
Fed
half-gravid
Gravid


Control Districts (Nyaruguru, Kamonyi)
100.0
80.0
60.0
40.0
20.0
0.0
Percentage
Jul-19
Aug-19
Sep-19
Oct-19
Nov-19
Dec-19
Jan-20
Feb-20
Mar-20
Apr-20
May-20
Jun-20
Period
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 4 shows average percentage parity from July 2019 to June 2020. 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 Nyaruguru control site and in both sites in the Kirehe, Ngoma, and Nyagatare intervention districts. The difference observed in the intervention sites could be attributable to the IRS.

Table 4: Parity

District	Site	Total Collected	Total *An. gambiae* s.l. Dissected	# Parous	% Parity	Confidence Interval	P-value	Result
Kirehe	Gatore	178	102	13	12.7	6.3-19.2	p<.001	S
	Nyamugali	74	37	2	5.4	-1.9-12.7	p<.001	S
Ngoma	Remera	89	41	10	24.4	11.2-37.5	p<.001	S
	Zaza	171	87	16	18.4	10.3-26.5	p<.001	S
Nyagatare	Nyagatare	269	125	21	16.8	10.2-23.4	p<.001	S
	Rukomo	483	200	43	21.5	15.8-27.2	p<.001	S
Nyaruguru	Ngera	77	45	26	57.8	43.3-72.2	1	
Kamonyi	Musambira	185	71	42	59.2	47.7-70.6	NA	NA

Trends in parity rate between the IRS and control districts were compared for the months unfed mosquitoes were collected and dissected. Figure 9 shows while IRS in September seems to have suppressed the proportion of parous females throughout the collection period, parity remained high in the control district. Similarly, the January–February IRS in Ngoma brought parity down to zero in February and kept it low for most of the year, while parity was higher in the control districts in all the months the test was performed (Figure 10).

Figure 9: Parity Rate in September in Two IRS Districts Compared with Control

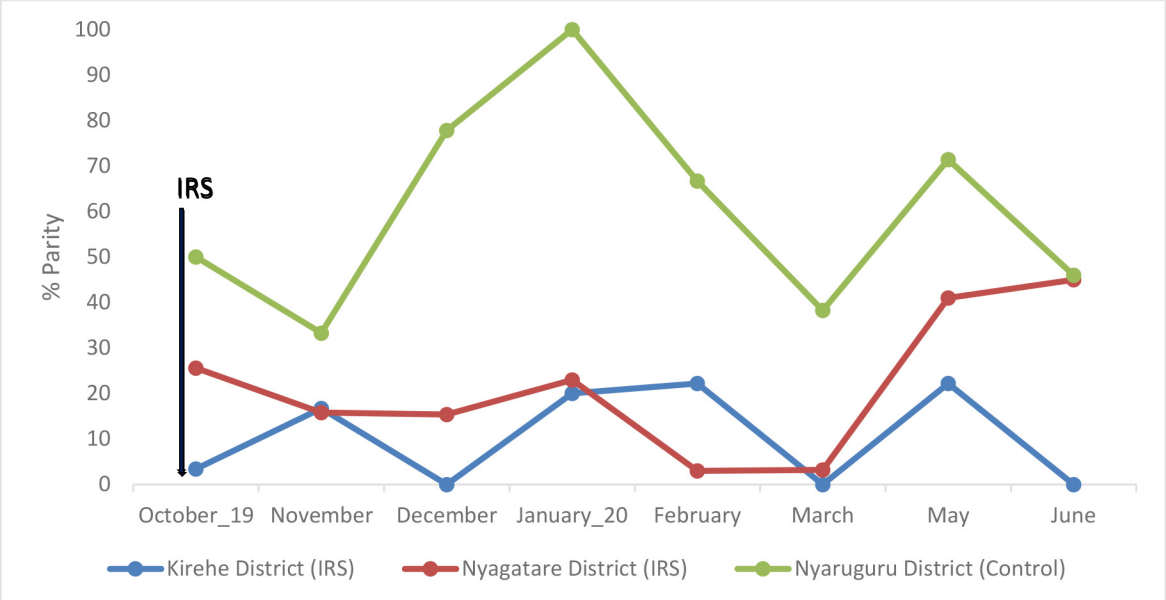
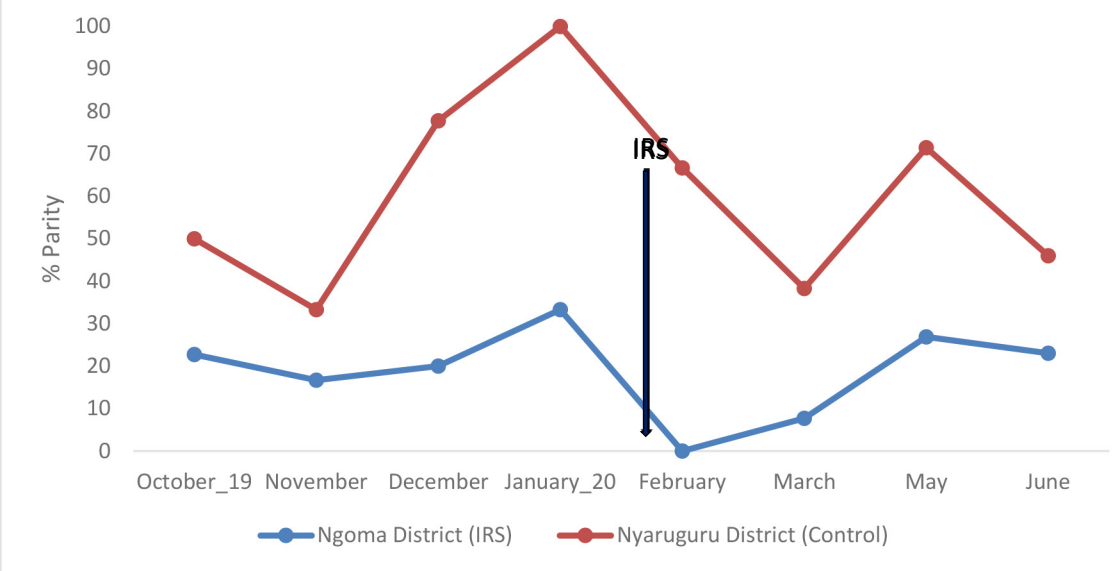


Figure 10: Parity Rate in Ngoma, Sprayed in January–February, Compared with Control



3.5 MOLECULAR SPECIES IDENTIFICATION

A subsample of *An. gambiae* s.l. (n=331) were identified using molecular technique; 87.3% were *An. arabiensis* and the rest were *An. gambiae* s.s. (Table 5).

An. arabiensis was dominant in all sprayed sites and in Ngera (control). *An. gambiae* s.s. was dominant in Musambira site (control). The difference in proportion between *An. arabiensis* and *An. gambiae* s.s. in all sites was statistically significant.

Table 5: *An. gambiae* s.l. Sibling Composition

District	Site	Spray Status/Time	*An. gambiae s.s.*	*An. arabiensis*	P-value	Significance Status
Kirehe	Gatore	Sep 2019	1.8%(1)	98.2%(56)	$p<.001$	S
	Nyamugali	Sep 2019	8.3%(1)	91.7%(11)	$p<.001$	S
Ngoma	Remera	Mar–Apr 2019; Jan–Feb 2020	4.2%(1)	95.8%(23)	$p<.001$	S
	Zaza	Mar–Apr 2019; Jan–Feb 2020	3.1%(1)	96.9%(31)	$p<.001$	S
Nyagatare	Nyagatare	Sep 2019	0%(0)	100%(45)	$p<.001$	S
	Rukomo	Sept 2019	0%(0)	100%(83)	p<.001	S
Kamonyi	Musambira	Control	100%(33)	0%(0)	$p<.001$	S
Nyaruguru	Ngera	Control	11.1%(5)	87.3%(40)	*p<.001*	S

3.6 ENZYME-LINKED IMMUNOSORBENT ASSAY

3.6.1 Sporozoite ELISA

Mosquitoes collected through HLC and PSC were tested for infection using ELISA. A total of 1,885 mosquitoes collected from July 2019 to June 2020 in the districts surveyed were tested for *Plasmodium falciparum* circumsporozoite protein. Different *Anopheles* species were tested; *An. gambiae* s.l. were dominant (80%). All samples tested were negative; Table 6 (i) and (ii) show the numbers of mosquitoes tested, by species and by monitoring site.

Table 2: Numbers Tested for Sporozoite Infection

(i) By Species

Species	Number Tested	Number Positive	% Positive
An. gambiae s.l.	1509	0	0.00
An. ziemanni	249	0	0.00
An. maculipalpis	87	0	0.00
An. pharoensis	19	0	0.00
An. funestus	14	0	0.00
An. coustani	6	0	0.00
An. rufipes	1	0	0.00
Total	1885	0	0.00

(ii) By Site

District	Site	# Tested	Number Positive	% Positive
Kirehe	Gatore	329	0	0
	Nyamugali	79	0	0
Ngoma	Remera	184	0	0
	Zaza	209	0	0
Nyagatare	Nyagatare	254	0	0
	Rukomo	440	0	0
Nyaruguru	Ngera	240	0	0
Kamonyi	Musambira	150	0	0

3.6.2 Blood Meal ELISA

Blood-fed samples from the collections made July 2019 to June 2020 were also assayed to determine the source of the blood meal. A total of 82 *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). Overall only 18.4% of the mosquitoes fed on humans only and an additional 2.4% fed on humans and other animals. A much higher proportion of *An. gambiae* s.l. specimens that fed on human blood only (83.4%) was observed in Kamonyi but the numbers tested were small. In intervention districts *An. gambiae* s.l. fed on bovine blood in high proportions (66.7%–76.6%). The results show that a relatively high proportion of the vectors fed on non-human hosts, especially in IRS districts.

Table 3: Blood Meal Source

Site	Number Tested	Results					
		Human	Bovine	Goat	Human and Other	Goat and Bovine	No Specified Host
Kirehe	22	3(13.5%)	16(72.7%)	0(0%)	1(4.6%)	1(4.6%)	1(4.6%)
Ngoma	15	4(26.6%)	10(66.7%)	0(0%)	0(0%)	1(6.7%)	0(0%)
Nyagatare	17	2(11.7%)	13(76.6%)	0(0%)	0(0%)	2(11.7%)	0(0%)
Nyaruguru	22	1(4.6%)	6(27.3%)	0(0%)	1(4.6%)	6(27.3%)	8(36.2%)
Kamonyi	6	5(83.4%)	0 (0%)	0(0%)	0(0%)	0(0%)	1(16.6%)
Total	82	15(18.4%)	45(54.8%)	0(0%)	2(2.4%)	10(12.2%)	10(12.2%)

3.6.3 Entomological Inoculation Rates

The entomological inoculation rate for *An. gambiae* s.l. was zero, as all *An. gambiae* s.l. tested for sporozoite were negative.

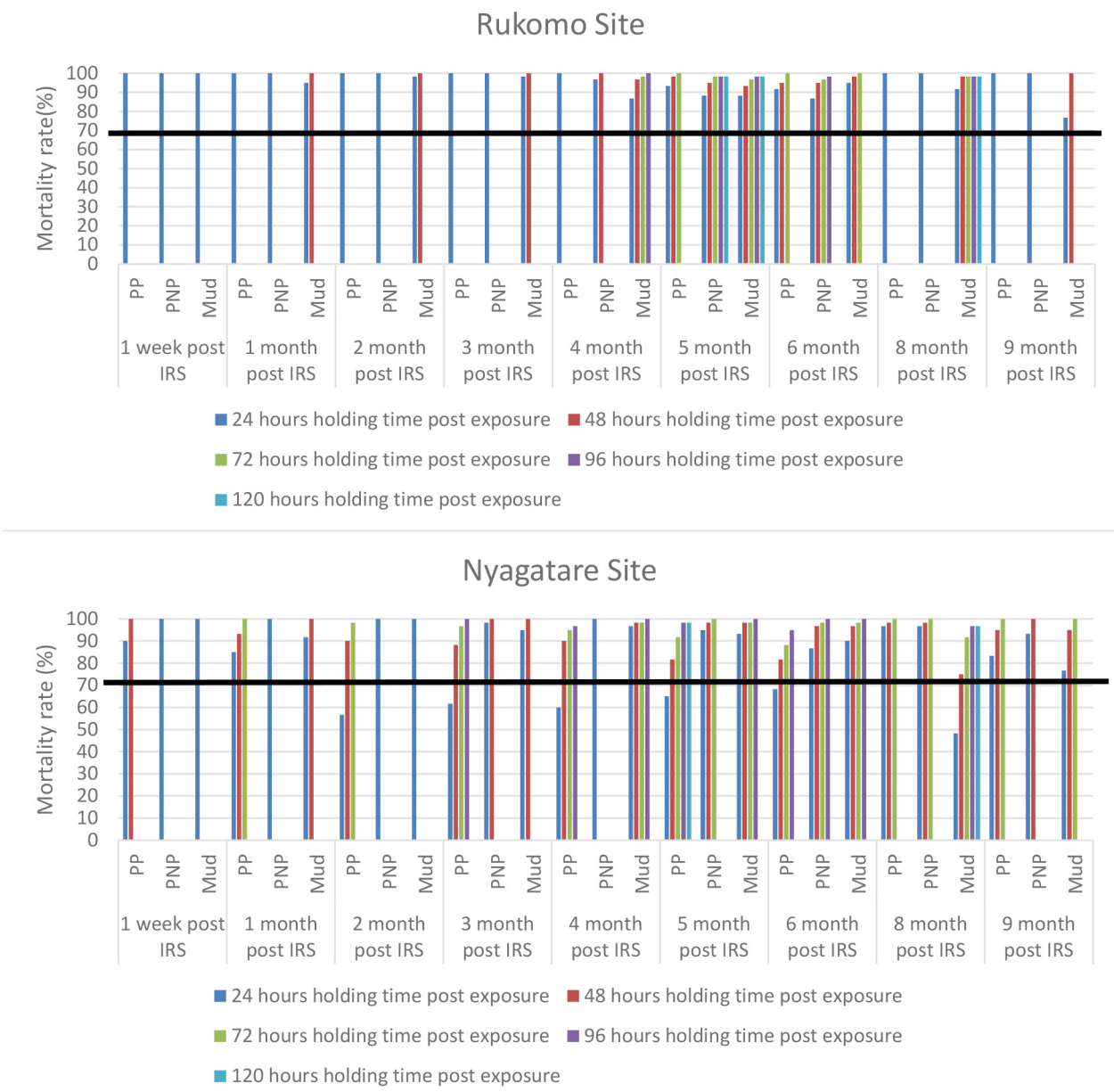
3.7 Quality of Spray, Insecticide Decay Rate, and Fumigant Effect

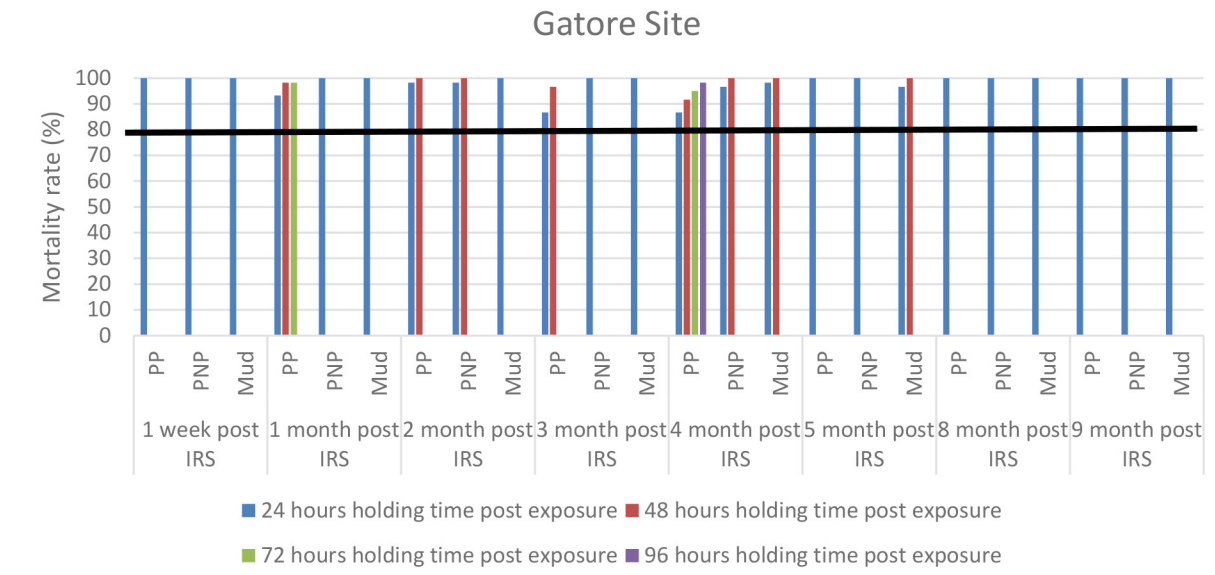
3.7.1 Quality of Spraying and Insecticide Decay Rate

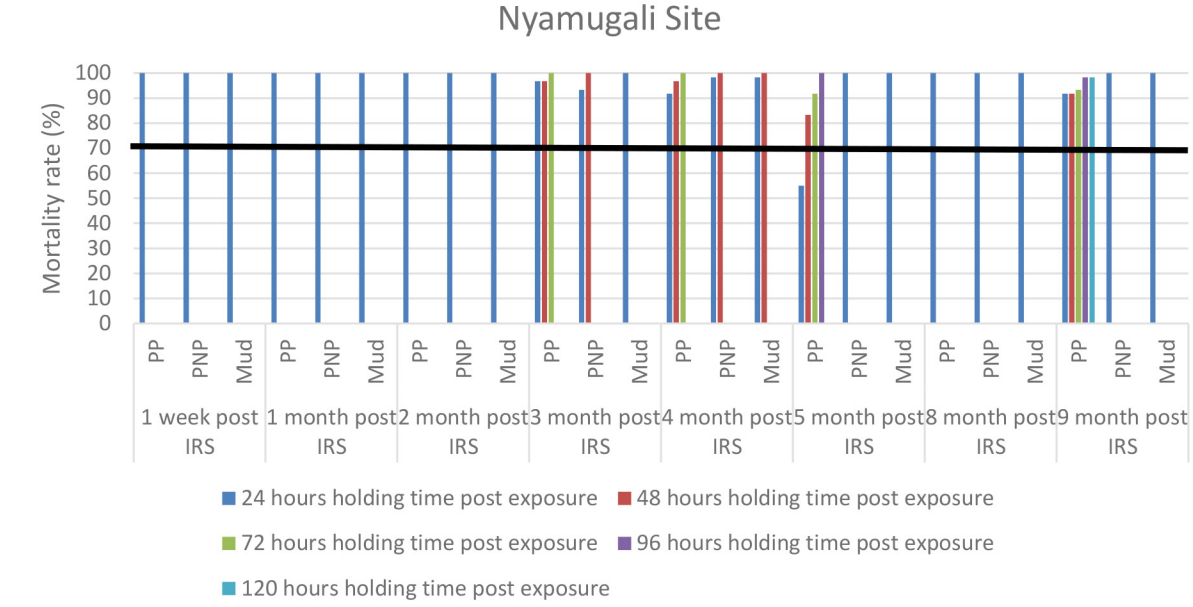
As noted above, VectorLink Rwanda sprayed Kirehe and Nyagatare districts in September 2019, and Ngoma district in January–February 2020, all with Fludora® Fusion. It then carried out WHO cone wall bioassays to assess the quality of spraying. In Kirehe and Nyagatare districts the evaluation of IRS quality was done in September 2019 in 24 sprayed houses, and in Ngoma district it was done in January 2020 in 12 houses. Residual efficacy was then monitored monthly, except in April 2020. 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).

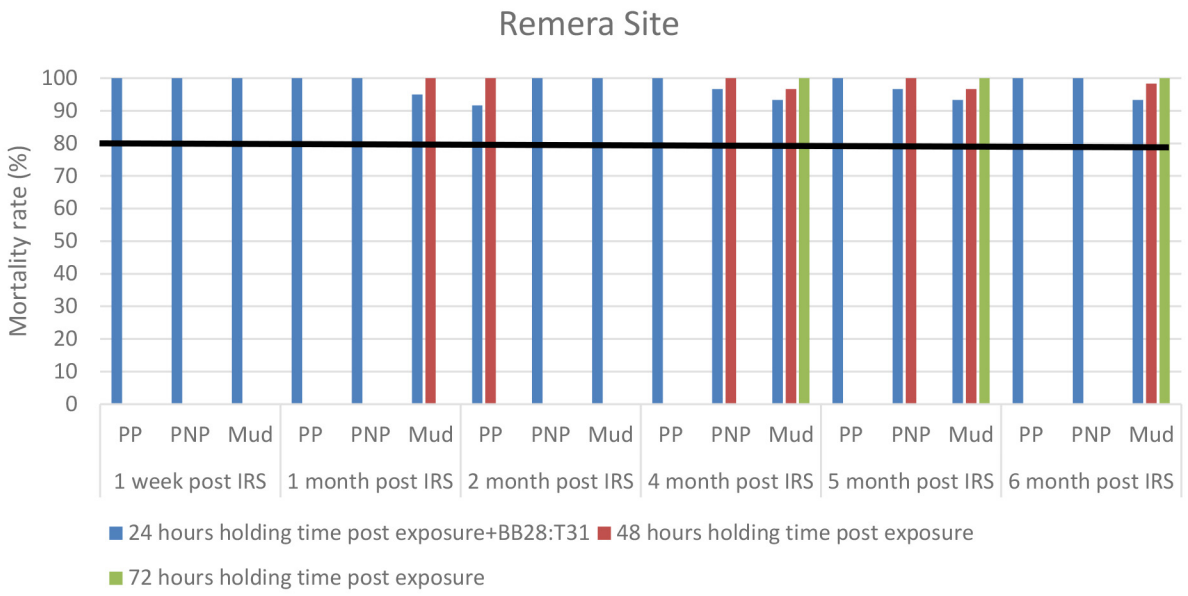
These first cone bioassays showed 100% mortality of susceptible *An. gambiae* s.s., in at least 48 hours post exposure, a proxy measure indicating the spraying was of good quality or that there was no under-dosing. Subsequent bioassays were done each month to monitor the bio-efficacy of the sprayed walls. Through June 2020, the mortality rate was over 80% on all wall surface types after five days post exposure (Figure 11).

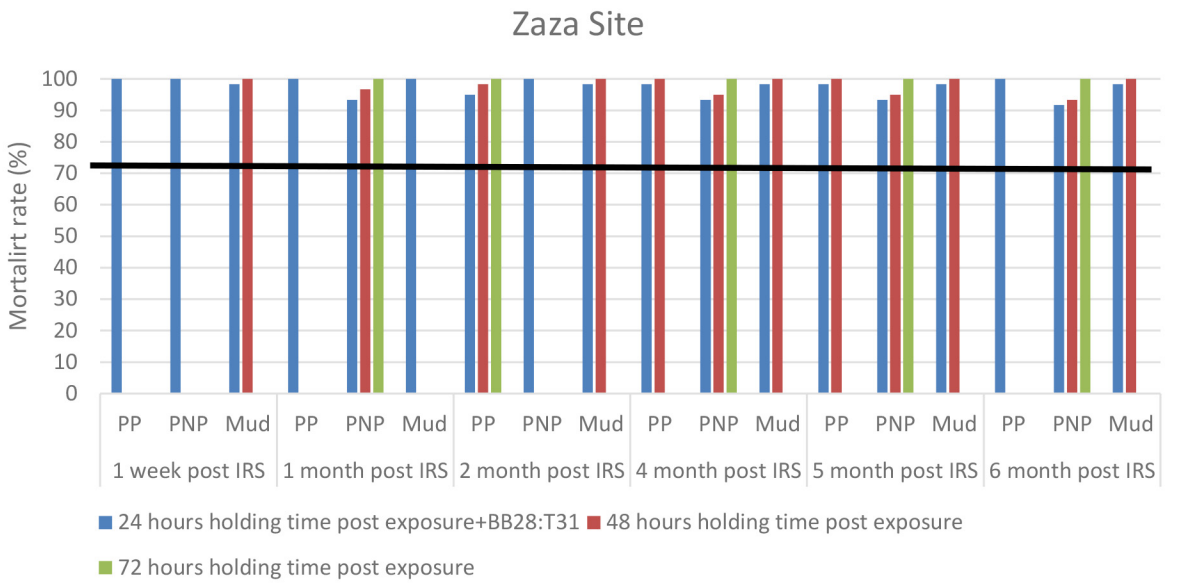
Figure 111: Wall Bioassay Test Results




Gatore Site
Mortality rate (%)
100
90
80
70
60
50
40
30
20
10
0
PP
PNP
Mud
1 week post IRS
1 month post IRS
2 month post IRS
3 month post IRS
4 month post IRS
5 month post IRS
8 month post IRS
9 month post IRS
24 hours holding time post exposure
48 hours holding time post exposure
72 hours holding time post exposure
96 hours holding time post exposure


Nyamugali Site
Mortality rate (%)
100
90
80
70
60
50
40
30
20
10
0
PP
PNP
Mud
1 week post IRS
1 month post IRS
2 month post IRS
3 month post IRS
4 month post IRS
5 month post IRS
8 month post IRS
9 month post IRS
24 hours holding time post exposure
48 hours holding time post exposure
72 hours holding time post exposure
96 hours holding time post exposure
120 hours holding time post exposure





Black line indicates the WHO cone bioassays cut-off (80%).

3.7.2 Fumigant Effect of Fludora® Fusion

The fumigant effect of Fludora® Fusion was tested in the same sprayed houses where the cone wall bioassay was conducted. The results showed that the average mortality rate was high (60–100%) for up to three months but tests were discontinued after that because of high control mortality and shortage of a susceptible colony to repeat the tests.

3.8 CONCLUSIONS

- *Anopheles gambiae* s.l. is the major malaria vector in all surveyed districts and was the prevalent vector throughout the data collection period in both the intervention and control sites. Based on molecular identification, *An. arabiensis* made up the highest percentage (87.3%) of mosquitoes tested. In general the number of mosquitoes collected this year was low compared with previous years. Of the total *An. gambiae* s.l. collected resting indoors, 82% in Kirehe, 62% in Nyagatare, and 55% in Ngoma were collected in September and October. The trend was similar for the HLC collections in all three IRS districts except in Nyagatare, which has another peak in February–March. The data indicate that the timing of IRS in August, just before the peak in September–October, is appropriate.
- The mosquito population during this reporting year is generally low. This could be due to the impact of IRS with the new insecticide, which showed a long residual efficacy. However, numbers were also low in the control district. Other factors, such as climate or the use of insecticide-treated nets could have affected mosquito numbers this year.
- The control site of Musambira (Kamonyi district) showed a high number of *An. gambiae* s.l. during the two months of data collection in July–August. The MOPDD decided to spray Kamonyi in October 2019, and replaced it with Ngera (Nyaruguru district) as a control district. The number of *An. gambiae* s.l. collected from the control was very low during the surveyed period.
- No data were collected in April 2020 in any site because the country was in lockdown due to COVID-19.
- *Anopheles funestus* were collected in only three sites (Gatore, Nyamugali, and Ngera); of the three, the most *An. funestus* were collected in Ngera.
- *Anopheles gambiae* s.l. generally displayed slightly more exophagic than endophagic tendency in all sites surveyed.
- The average bites per person per hour through the night across the five districts showed more biting outdoors than indoors.
- The parity rate was lower in IRS districts than in the control throughout the year. Given the long residual life of the insecticide, IRS seems to have a sustained effect in suppressing parity in the IRS districts.
- *Anopheles* mosquitoes collected through HLC and PSC were tested for sporozoite infection using ELISA. All tested mosquitoes were negative. This could be due to the impact of IRS and other malaria prevention measures. It would be useful to examine the data to see if there was a similar reduction in malaria morbidity.
- A relatively high proportion of the vectors were found to have fed on non-human hosts. This is probably expected given that the dominant vector, *An. arabiensis,* is known to show a zoophilic tendency.
- The insecticide used for IRS, Fludora® Fusion, is still killing more than 80% of exposed mosquitoes nine months after spray; indicating one round of spray can provide protection throughout the year irrespective of when the mosquito population peaks.

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 MOPDD, and the colony reared in the insectary was inspected to determine species specification. A total of 193 Kisumu strain mosquitoes from the insectary were tested using PCR for species identification and the results showed that 100% of tested mosquitoes were *An. gambiae* s.s., indicating that there is no contamination. VectorLink Rwanda also supported the procurement of 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 staff especially in performing laboratory tests on mosquitoes collected in MOPDD's entomology sentinel sites. The project also supported the procurement of insecticide impregnated papers to be used in insecticide resistance testing across the country. The VectorLink Rwanda entomology coordinator participated in the annual conferences of the Pan African Mosquito Control Association (PAMCA) held in Cameroon, where he presented on "*Host preference and feeding patterns of primary malaria vectors, Anopheles arabiensis and Anopheles gambiae s.s. in sites with or without Indoor Residual Spraying in Rwanda.*" Finally the VectorLink Rwanda project supported annual entomology planning and refresher training in MOPDD's sentinel sites.

5. CHALLENGES AND RECOMMENDATIONS

- Because of COVID-19, VectorLink Rwanda did not collect data in April 2020 in any sentinel site.
- The project was not able to do molecular tests for the detection of insecticide resistance mechanism: the plan was to use the Real-Time PCR machine at the Rwanda National Reference Laboratory, but the facility was mobilized in screening of COVID-19.
- All insecticide resistance and related tests are supported by Global Fund and performed by the RBC MOPDD and are not part of this report.

ANNEX A. PARITY

	Kirehe District				Ngoma District				Nyagatare District				Nyaruguru 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-19	6	4	0	0	2	0	0	0	40	30	9	30	NA	NA	NA	NA	79	36	21	58.3
Aug-19	7	4	0	14.3	2	2	0	0	18	8	2	25	NA	NA	NA	NA	106	35	21	60
Sept-19	73	41	6	14.6	39	16	2	12.5	107	32	9	28	0	0	0	0	NA	NA	NA	NA
Oct-19	55	29	1	3.4	84	44	10	22.7	108	39	10	25.6	3	2	1	50	NA	NA	NA	NA
Nov-19	14	6	1	16.7	8	6	1	16.7	31	19	3	15.8	5	3	1	33.3	NA	NA	NA	NA
Dec-19	26	16	0	0	13	5	1	20	79	39	6	15.4	16	9	7	77.8	NA	NA	NA	NA
Jan-20	31	15	3	20	4	3	1	33.3	53	26	6	23	5	2	2	100	NA	NA	NA	NA
Feb-20	18	9	2	22.2	5	0	0	0	149	64	2	3	3	3	2	66.7	NA	NA	NA	NA
Mar-20	6	6	0	0	40	13	1	7.7	109	31	1	3.2	6	6	2	38.3	NA	NA	NA	NA
Apr-20	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
May-20	15	9	2	22.2	41	26	7	26.9	23	17	7	41	11	7	5	71.4	NA	NA	NA	NA
Jun-20	1	0	0	0	22	13	3	23	35	20	9	45	28	13	6	46	NA	NA	NA	NA
Total	252	139	15	10.8	260	128	26	20.3	752	325	64	19.7	77	45	26	57.8	185	71	42	59.2

ANNEX B: SPOROZOITE RATES

	Kirehe			Ngoma			Nyagatare			Nyaruguru			Kamonyi		
	Total tested	# positive	% positive	Total tested	# positive	% positive	Total tested	# positive	% positive	Total tested	# positive	% positive	Total tested	# positive	% positive
Jul-19	9	0	0	9	0	0	56	0	0	NA	NA	NA	70	0	0
Aug-19	10	0	0	6	0	0	20	0	0	NA	NA	NA	80	0	0
Sept-19	119	0	0	55	0	0	93	0	0	14	0	0	NA	NA	NA
Oct-19	84	0	0	104	0	0	118	0	0	20	0	0	NA	NA	NA
Nov-19	33	0	0	22	0	0	35	0	0	11	0	0	NA	NA	NA
Dec-19	32	0	0	44	0	0	78	0	0	22	0	0	NA	NA	NA
Jan-20	54	0	0	15	0	0	59	0	0	10	0	0	NA	NA	NA
Feb-20	18	0	0	11	0	0	130	0	0	5	0	0	NA	NA	NA
Mar-20	11	0	0	12	0	0	23	0	0	9	0	0	NA	NA	NA
Apr-20	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
May-20	22	0	0	65	0	0	30	0	0	38	0	0	NA	NA	NA
Jun-20	15	0	0	51	0	0	52	0	0	111	0	0	NA	NA	NA
Total	407	0	0	394	0	0	694	0	0	240	0	0	150	0	0

ANNEX C. REFERENCES

Beier JC, Koros J. 1991. Visual assessment of sporozoite and bloodmeal ELISA samples in malaria field studies. *J Med Entomol* 28:805-808.

Beier JC, Perkins PV, Wirtz RA, Koros J, Diggs D, Gargan II TP, Koech DK. 1988. Bloodmeal identification by direct enzyme-linked immunosorbent assay (ELISA), tested on *Anopheles* (Diptera: Culicidae) in Kenya. *J Med Entomol* 25:9-16.

Detinova, TS. 1962. Age-Grouping Methods in Diptera of Medical Importance. Geneva: WHO. http://apps.who.int/iris/bitstream/10665/41724/1/WHO_MONO_47_%28part1%29.pdf

Coetzee M. 2020. Key to the females of Afrotropical Anopheles mosquitoes (Diptera: Culicidae). *Malaria journal* 19:20.

Scott JA, Brogdon WG, Collins FH. 1993. Identification of single specimens of the *Anopheles* gambiae complex by the polymerase chain reaction. *Am J Trop Med Hyg* 49:520-529.

Wirtz R, Zavala Charoenvit Y, Campbell GH, Burkot TR, Schneider I, Esser KM, Beaudoin RL, Andre RG. 1987. Comparative testing of Plasmodium falciparum circumsporozoite antibody. *Bull Wld Hlth Org* 65:39-45.

WHO (World Health Organization). 1998. Test procedures for insecticide resistance monitoring in malaria vectors, bio-efficacy and persistence of insecticides on treated surfaces. Document WHO/CDS/MAL/98.12. Geneva.

WHO. 2006. Guidelines for testing mosquito adulticides for indoor residual spraying and treatment of mosquito nets. World Health Organisation. http://apps.who.int/iris/bitstream/10665/69296/1/WHO_CDS_NTD_WHOPES_GCDPP_2006.3_eng.pdf