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THE PMI VECTORLINK PROJECT RWANDA

ANNUAL ENTOMOLOGICAL MONITORING REPORT

JULY 2021–JUNE 2022

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THE PMI VECTORLINK PROJECT RWANDA ANNUAL ENTOMOLOGICAL MONITORING REPORT JULY 2021–JUNE 2022

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ACRONYMS

An.	*Anopheles*
EDTA	ethylene diamine tetra acetic acid
EIR	entomological inoculation rate
ELISA	enzyme-linked immunosorbent assay
HBI	human blood index
HBR	human biting rate
HLC	human landing catch
IRS	indoor residual spraying
MOPDC	Malaria and Other Parasitic Diseases Control
PCR	polymerase chain reaction
PMI	U.S. President's Malaria Initiative
PSC	pyrethrum spray catch
RBC	Rwanda Biomedical Center
WHO	World Health Organization

ACKNOWLEDGMENTS

We acknowledge the invaluable contributions and support from many people and institutions that contributed to the collection and analysis of the entomological data reported here, especially in the COVID-19 pandemic context. Particularly, we thank the Rwanda Ministry of Health through the Rwanda Biomedical Center's Malaria and Other Parasitic Diseases Control Division for their fruitful collaboration in conducting the entomology monitoring activities including field data collection, supervision, analysis, and review of this report.

EXECUTIVE SUMMARY

During the year-long reporting period July 2021–June 2022, monthly entomological data collection took place in three indoor residual spraying (IRS) districts, Kirehe (Gatore and Nyamugali sites), Ngoma (Remera and Zaza sites), and Nyagatare (Nyagatare and Rukomo sites), and one non-IRS (control) district. This control district was originally Nyaruguru District (Ngera site), which was then replaced by Gicumbi district (Rwamiko site) in April 2022, after Nyaruguru was sprayed in March 2022.

Adult mosquitoes were sampled using pyrethrum spray catch (PSC) and human landing catch (HLC) methods, to assess vector species composition and behavior, seasonal trends in density, human biting rate (HBR), parity, and infection. World Health Organization (WHO) 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, unpainted cement, and painted cement. Malaria vectors were identified morphologically, and a subsample of *Anopheles (An.) gambiae* s.l. were identified using the polymerase chain reaction (PCR) method.

During the reporting period, a total of 1,327 adult female *Anopheles* mosquitoes were collected, 1,140 using HLC and 187 using PSC. Among the *Anopheles* mosquitoes collected, 76.6% were *An. gambiae* s.l., 12.4% *An. ziemanni*, 4.1% *An. squamosus*, 2.4% *An. maculipalpis*, 2.2% *An. pharoensis,* 2% *An. funestus* s.l., and 0.2% *An. coustani. An. funestus* species were collected in only three sites (Nyagatare, Rwamiko, and Ngera), with most of them collected from Ngera.

A subsample of the *An. gambiae* s.l. were identified using the PCR method; 91.2% were *An. arabiensis* and 8.8% were *An. gambiae* s.s. *An. arabiensis* dominated in all sprayed sites and in Ngera site (control) but not in Rwamiko (also a control site starting April 2022). The difference in proportion between *An. arabiensis* and *An. gambiae* s.s. in all sites except the Rwamiko site was statistically significant ($p<0.05$).

To assess the quality of the insectary colony (Kisumu strain) and confirm the absence of any contamination, a total of 230 Kisumu strain colony mosquitoes from our insectary were identified using molecular techniques in November 2021 and in April 2022 to confirm the species, and 100% were *An. gambiae* s.s. indicating that there is no contamination with the wild mosquitoes.

An. gambiae s.l. showed a slightly more exophagic than endophagic tendency in both sites of Kirehe District (Gatore and Nyamugali), both sites of Ngoma District (Remera and Zaza), one site in Nyagatare District (Nyagatare site), and in the Gicumbi District (Rwamiko site). In one site in Nyagatare District (Rukomo), and in Nyaruguru District, control (Ngera site) there was a slight endophagic tendency.

The difference between indoor and outdoor biting of *An. gambiae* s.l. was statistically significant ($p<0.001$) in both sites of Kirehe District (Gatore and Nyamugali), both sites of Nyagatare District (Nyagatare and Rukomo), and in the Zaza site in Ngoma District.

An. funestus s.l. collected showed an exophagic tendency in the Nyagatare site in Nyagatare District, with endophagic vs. exophagic behavior of 0% vs. 100%, but for the Ngera site there was no difference between feeding location preference. *An. funestus* s.l. in Rwamiko site (control) showed more endophagic than exophagic behavior, 67% vs. 33%, and the difference was not statistically significant. This difference in the preference of biting location for *An. funestus* s.l. should be interpreted cautiously, as the numbers collected and compared were very small.

The *An. gambiae* s.l. HBR rate was highest in September 2021 in all IRS sites except in Nyamugali site (Kirehe District), where the number of *An. gambiae* s.l. collected in September was low. The 2021 IRS campaign started in August, which was just before the peak mosquito population. For Rukomo site, there was another peak in March 2022. The highest bites/person/night rate was observed in the Rukomo site (Nyagatare

District) both indoors and outdoors (9.7 bites/person/night indoors and 6 bites/person/night respectively in September). For *An. funestus* s.l. the highest HBR was less than 0.5 bites/person/night in all sites. In Rwanda there are two rainy seasons, from March to May and September to December.

An. gambiae s.l. biting peaked early in the evening (18:00–20:00), both indoors and outdoors, for Nyagatare District. For other districts, no meaningful trend could be observed due to the low biting rates recorded throughout the year.

Although indoor resting vector density was very low in all surveyed districts over the reporting period, Kirehe District showed a higher average vector density (0.1 *An. gambiae* s.l./house/day) than did the other IRS districts, Ngoma District and Nyagatare District (0.01 and 0.07 *An. gambiae* s.l./house/day respectively). The control (non-IRS) Nyaruguru and Gicumbi Districts showed no difference compared to the IRS districts, at 0.06 and 0.01 *An. gambiae* s.l./house/day, respectively.

An. gambiae s.l. collected in all sites using PSC were classified according to their blood digestion and gravidity stages: 84 (55%) were unfed, 33 (22%) were fed; 17 (11%) were half-gravid and 18 (12%) were gravid. Of the *An. funestus* s.l., 5 (62%) were unfed, 0 were (0%) fed, 1 (13%) was half-gravid, and 2 (25%) were gravid.

Ovaries of the *An. gambiae* s.l. collected using HLC were dissected to determine parity rates. There was a significant difference ($p<0.05$) in the proportion of parous *An. gambiae* s.l. between control districts (Nyaruguru and Gicumbi) and IRS districts (Kirehe, Ngoma, and Nyagatare). The difference observed in the *An. gambiae* s.l. parity rate between intervention and control districts could be attributable to the IRS. For *An. funestus* s.l. the parity rate was 50% in Nyagatare District and the control districts (Nyaruguru and Gicumbi).

The overall sporozoite positivity rate was 0.3% (n=1,303), with *An. gambiae* s.l. representing 77.2% of the tested mosquitoes. For *An. gambiae* s.l. the entomological inoculation rate (EIR) in Nyagatare site (Nyagatare District) was estimated at 5.1 infective bites/person/year, whereas in Zaza site (Ngoma District) the EIR was 2.5 infective bites/person/year.

A total of 27 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 using enzyme-linked immunosorbent assay (ELISA) (human, bovine, and goat). Human blood indices (HBIs) were as follows: Kirehe 37.5%, Ngoma 25.0%, Nyagatare 45.5%, and control districts (Nyaruguru and Gicumbi) 0%. 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 of Actellic® 300CS to assess the quality of spraying showed 100% mortality of susceptible *An. gambiae* s.s. 24 hours post exposure, indicating that the quality of the spray operation was good. Subsequently, bio-efficacy of the sprayed insecticide was monitored monthly. Through July 2022 (10 months post IRS), the mortality rate was over 80% on all surface types 24 hours post exposure.

1. INTRODUCTION

The U.S. President's Malaria Initiative (PMI) has protected millions of people in Africa from malaria through IRS. This intervention kills the mosquitoes that transmit malaria, through 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 24 countries in sub-Saharan Africa as well as Cambodia and Colombia, PMI VectorLink is equipping countries with skills 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 August–September 2021, VectorLink Rwanda sprayed three districts, Kirehe (12 sectors), Nyagatare (14 sectors), and Ngoma (14 sectors). The campaign used Actellic® 300CS.

This report covers entomological monitoring activities conducted from July 1, 2021, to June 30, 2022. The entomological monitoring activities were aimed at:

- Assessing seasonal malaria vector density trends and species composition in intervention and selected control areas
- Understanding vector preference for feeding times and locations and estimating HBRs
- 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 infection rates, blood meal source, and EIRs

2. Data Collection Sites and Methods

2.1 Study Sites

Data collection was conducted monthly in three IRS districts, Kirehe, Ngoma, and Nyagatare, and one non-IRS (control) district. Nyaruguru district was used as control from July 2021 to March 2022 and then replaced with Gicumbi District starting in April 2022, after the Malaria and Other Parasitic Diseases Control (MOPDC) agency decided to spray Nyaruguru in March 2022. Two sites from each of the IRS districts and one site from the control district were selected as sentinel sites for data collection (Figure 1). Table 1 lists the data collection sites and their spray status, and the data collection schedule.

Figure 1: Data Collection Districts

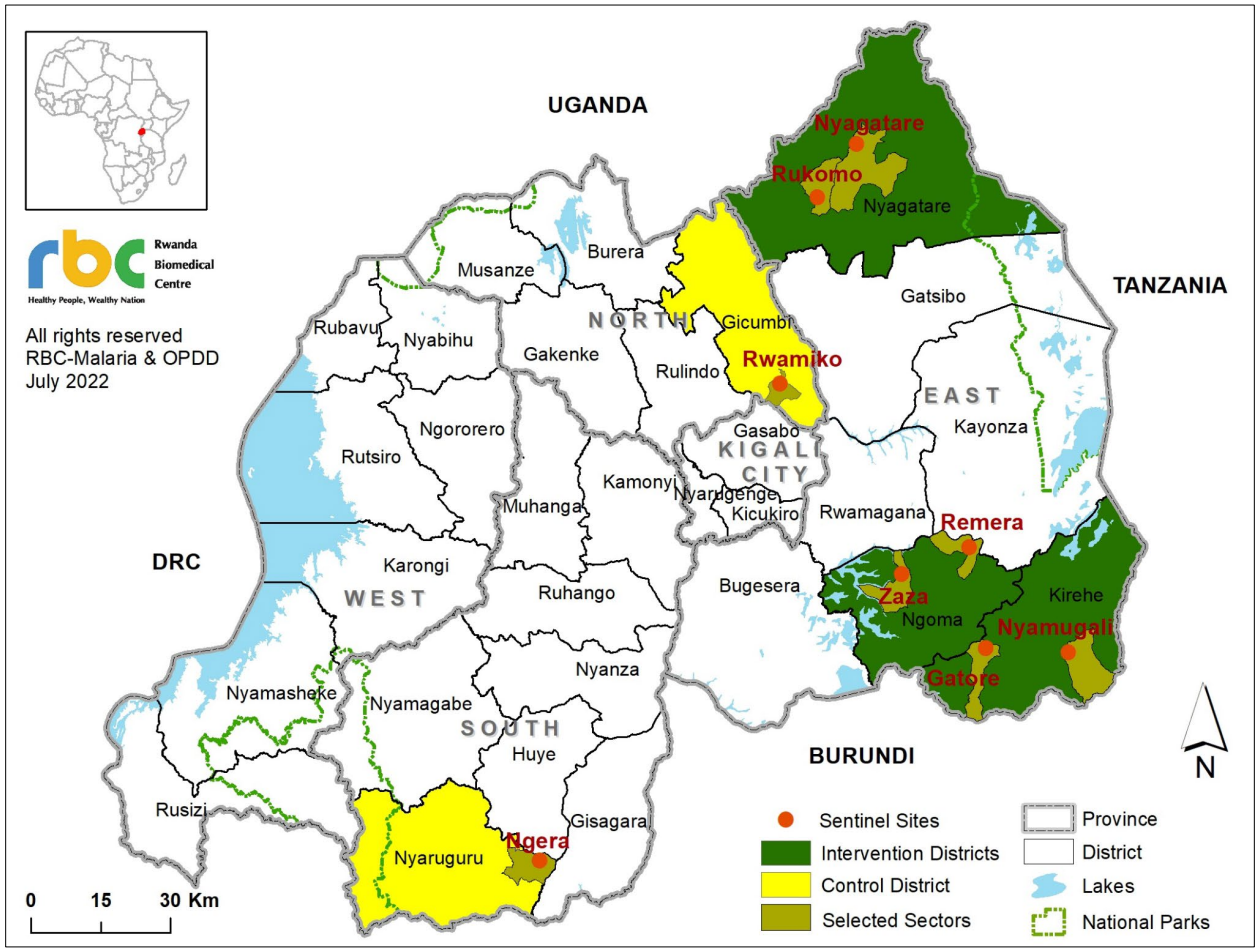


TABLE 1: SPRAY STATUS OF DATA COLLECTION DISTRICTS AND SENTINEL SITES

District	Data Collection Sites	Spray Status	Timeline of Data Collection
Kirehe (IRS)	Gatore, Nyamugali	Sprayed August–September 2021 using Actellic® 300CS with PMI support	July 2021–June 2022
Ngoma (IRS)	Remera, Zaza	Sprayed August–September 2021 using Actellic® 300CS with PMI support	July 2021–June 2022
Nyagatare (IRS)	Nyagatare, Rukomo	Sprayed August–September 2021 using Actellic® 300CS with PMI support	July 2021–June 2022
Nyaruguru (control)	Ngera	Not sprayed	July 2021–March 2022
Gicumbi (control)	Rwamiko	Not sprayed	April–June 2022

2.2 DATA COLLECTION METHODS

Blood-seeking and indoor-resting adult mosquito collections were conducted each month in all sites, using HLC and PSC methods respectively.

Spray quality was assessed in six sites (two in each IRS district) according to the PMI VectorLink SOP009/01 and WHO standard protocol (WHO 2006) for cone/wall bioassays, within one week after the start of the spray campaign. Subsequently, cone bioassays were performed monthly to assess the rate of insecticide decay.

2.2.1 HLC

HLC was done in three households in each site for two consecutive nights per month. The same houses were used for HLCs each month. Each team of collectors consisted of four people per house per night. Two collectors per house, one stationed 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 PSC

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 collections, all occupants were politely asked for their consent to remove foodstuffs and drinking water pots from 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 2.5% natural pyrethrin synergized by PBO 1%. 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 using taxonomic keys (Coetzee 2020), and a subsample of *An. gambiae* s.l. was identified to species level by the conversional PCR method (Scott et al. 1993).

2.4 Determination of Parity

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

2.5 Enzyme-linked Immunosorbent Assay (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 resources (Wirtz et al. 1987). The results were read visually (Beier and Koros 1991). The *Plasmodium* positive samples were confirmed by boiling the aliquot at 100° Celsius for 10 minutes, and then re-tested (Durnez et al. 2011).

2.5.2 ELISA for Blood Meal Source

Wild-caught fully fed to half-gravid 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 was added; the mixture was ground with a pestle, and another 950µl of phosphate buffered solution was added after grinding. Samples diluted (1:50) with phosphate buffered solution 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 *An. 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). The legs and wings were placed in a labeled vial. DNA was extracted by the cetyl trimethyl ammonium bromide method and amplified using universal primer, and Taq polymerase specific to *An. gambiae* s.s., *An. arabiensis*, *An. merus*, and *An. quadriannulatus*. A 1× tris base, acetic acid, and ethylene diamine tetra acetic acid (EDTA) 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 were loaded in gel and subjected to electrophoresis with 1x tris base, acetic acid, and EDTA 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

The quality of spraying, and insecticide decay rates, were assessed using the WHO-approved protocol (WHO 1998) according to the project's Standard Operating Procedure for cone wall bioassays (SOP009/01).

Test cones were placed at three different heights on sprayed wall surfaces, while the control cones were fixed on surfaces known to be free of insecticide. Batches of 10 mosquitoes were introduced into each of the

cones—two- to five-day-old non-blood-fed female *An. gambiae* s.s. (Kisumu strain) reared at the Rwanda Biomedical Center (RBC) insectary. The mosquitoes were left in the cones exposed to the insecticide for 30 minutes, after which they were transferred to paper cups in which they were provided with 10% glucose soaked in cotton.

Knockdown was observed and recorded after 60 minutes of exposure, and mortality was recorded 24 hours post exposure. When mortality in the control cones was between 5% and 20%, results were corrected using Abbot's formula.

2.8 INDICATORS AND DATA ANALYSIS

The following indicators were estimated for *An. gambiae* s.l. and where applicable for the *An. funestus* group, where samples collected were sufficient to allow analysis.

- **HBR:** The number of mosquito bites per person per unit time, reported as bites/person/night or hour indoors or outdoors, was estimated as (SOP002/01):

  Total number of mosquitoes collected by HLC/Number of collectors/Number of nights of collection, or hours

- **Resting density:** Mean monthly indoor and outdoor resting densities per site were calculated as (SOP003/1):

  Number of mosquitoes collected indoors from PSC (by species)/Number of houses /Number of days

- **Endophagic/exophagic index:** The proportion of females of a given species collected by HLC either indoors or outdoors was estimated as (SOP002/01):

  Number of mosquitoes collected by HLC (either indoors or outdoors)/Total number of mosquitoes collected by HLC indoors and outdoors (by species)

- **Parity rates:** Parity rates were estimated for the collection period for each site as (SOP010/01):

  Number of parous females/Total number of females dissected and successfully examined for parity

- **Sporozoite infection rates:** This was estimated monthly for each site as (SOP002/01):

  Number of mosquitoes positive for P. falciparum circumsporozoite proteins/Total number of mosquitoes tested (by species)

- **EIR**: This describes the number of infectious bites an individual in a study area is exposed to in a given time (typically a year), expressed as number of infectious bites/person/unit time. This was estimated as (SOP002/01):

  HBR per unit time x sporozoite rate

- **HBI or animal blood index:** The HBI was estimated from PSC collections across the whole sampling period as (SOP003/01):

  Number of mosquitoes with bloodmeal from humans/Total number of mosquitoes whose bloodmeals were identified

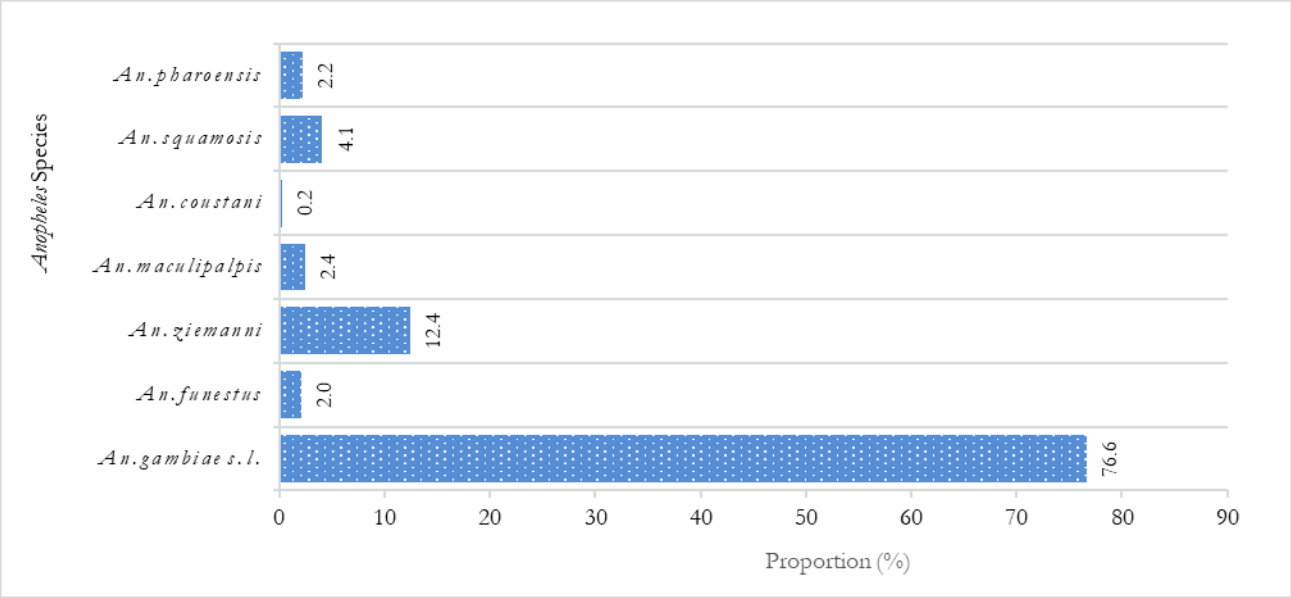
3. Results, Discussion, and Conclusions

3.1 Species Composition and Vector Seasonality

3.1.1 Species Composition

During the reporting period July 2021 through June 2022, a total of 1,327 adult female *Anopheles* mosquitoes were collected; 1,140 were collected using HLC, 187 using PSC. As shown in Figure 2, among the *Anopheles* mosquitoes collected, 76.6% were *An. gambiae* s.l., 12.4% *An. ziemanni*, 4.1% *An. squamosus*., 2.4% *An. maculipalpis*, 2.2% *An. pharoensis,* 2.0% *An. funestus* s.l., and 0.2% *An. coustani* (Figure 2). *An. gambiae* s.l. and *An. funestus* s.l. are generally known as primary vectors of malaria in Rwanda.

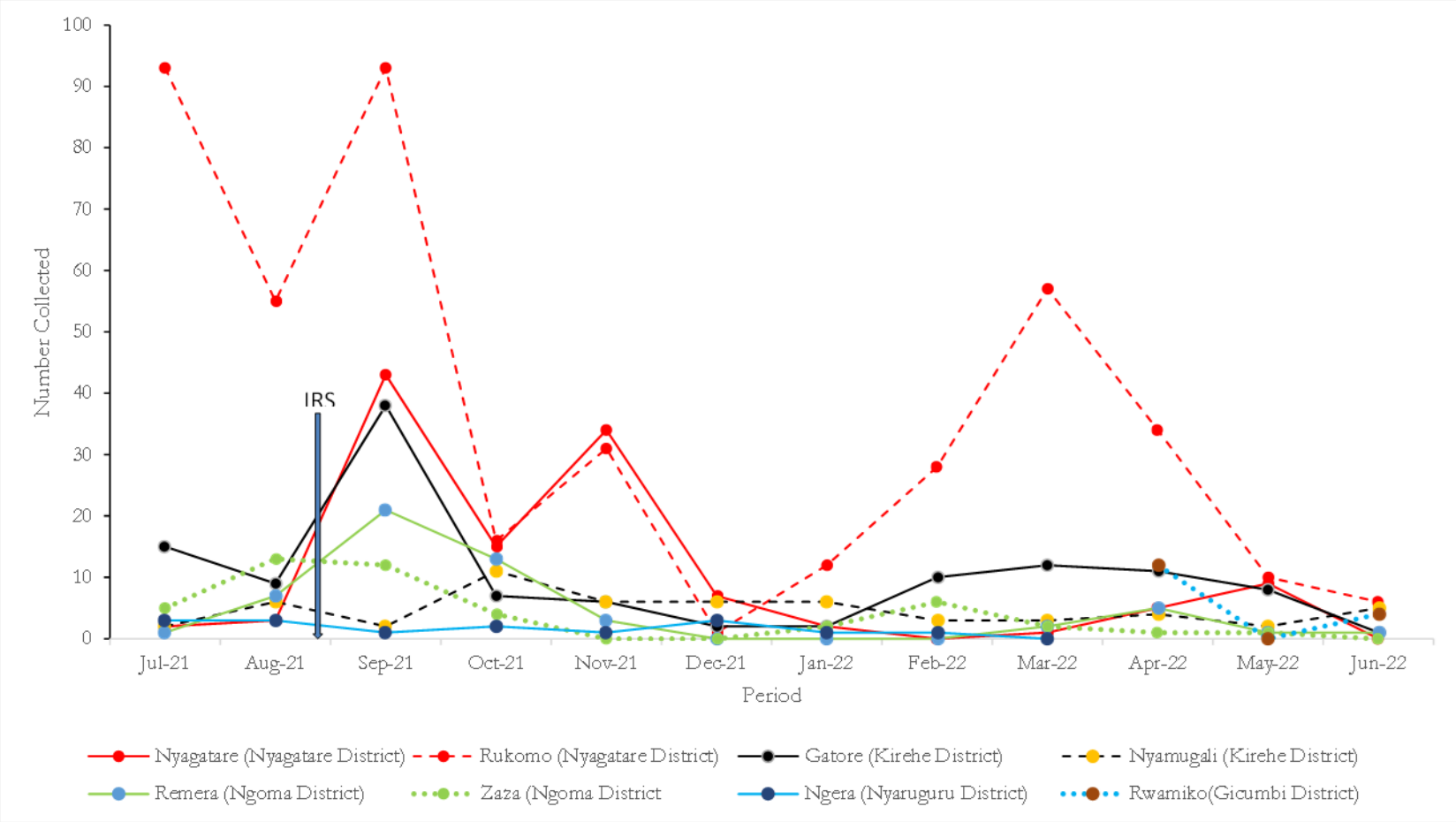
Figure 2: *Anopheles* Species Composition



3.1.2 Vector Seasonality

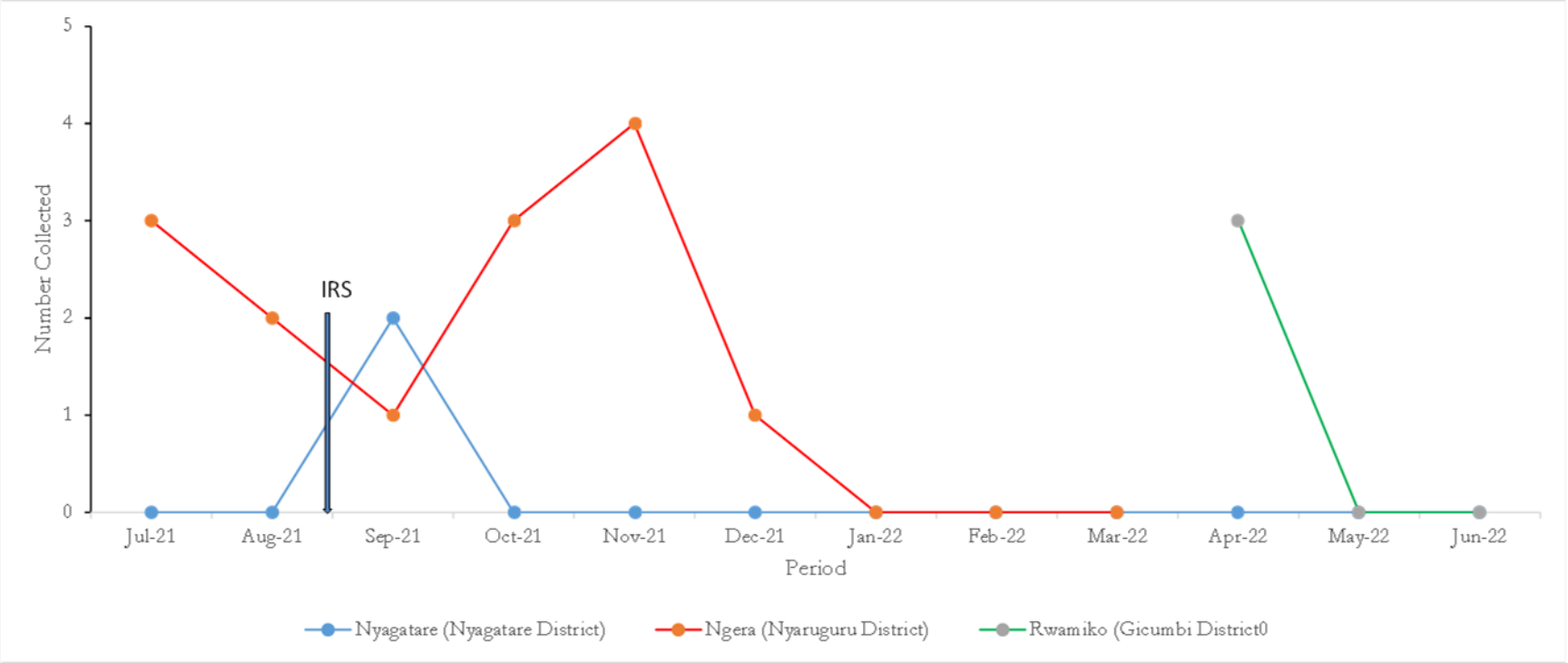
An. gambiae s.l. was the prevalent malaria vector collected by HLC throughout the data collection period in both the intervention and control sites but compared with in previous years the number of *An. gambiae* s.l. collected decreased. As Figure 3 shows, there was one peak (September) of *An. gambiae* s.l. in most of the IRS sites, except in the Nyamugali site, where the number of *An. gambiae* s.l. collected was low. In intervention sites there was a decrease in *An. gambiae* s.l. after the spraying, but in Rukomo intervention site (Nyagatare District) there was another peak in March. In the control sites (Ngera and Rwamiko), the number of *An. gambiae* s.l. collected was very low, making conclusive comparison with intervention sites impossible. During this reporting period, most of the *An. gambiae* s.l. were collected in the Rukomo site. *An. funestus* s.l. were collected in only three sites: Nyagatare site in Nyagatare District, Rwamiko site in Gicumbi District, and Ngera site in Nyaruguru District (Figure 4).

FIGURE 3: TOTAL NUMBER OF *AN. GAMBIAE* S.L. COLLECTED BY MONTH IN ALL SITES



Nyagatare (Nyagatare and Rukomo), Kirehe (Gatore and Nyamugali), and Ngoma (Remera and Zaza) were all sprayed in Aug–Sept 2021.

FIGURE 4: TOTAL NUMBER OF *AN. FUNESTUS* S.L. COLLECTED BY MONTH



3.2 VECTOR FEEDING TIME AND LOCATION

An. gambiae s.l. showed a higher exophagic than endophagic tendency in the Gatore and Nyamugali sites (Kirehe District), the Remera and Zaza sites (Ngoma District), the Nyagatare site (Nyagatare District), and the Rwamiko site (Gicumbi District, the control). *An. gambiae* s.l. showed a more endophagic tendency in Rukomo site (Nyagatare District) and Ngera site (Nyaruguru District, the control). The difference between the number of mosquitoes collected biting indoors and outdoors was highly significant ($p<0.001$) in the two sites of Kirehe District. That difference was also statistically significant in the two sites in Nyagatare District and in the Zaza site in Ngoma District (Table 2). No meaningful analysis of biting behavior can be made for *An. funestus* s.l., as the numbers collected from the two sites were very low (Table 3).

TABLE 2: INDOOR VS. OUTDOOR COLLECTIONS OF *AN. GAMBIAE* S.L. BY HLC

District	Site	In	Out	In: Out Ratio	P-value	Result*
Kirehe	Gatore	38	83	0.31:0.69	*p*<.001	S
	Nyamugali	8	48	0.14:0.86	*p*<.001	S
Ngoma	Remera	23	31	0.43:0.57	0.27630	NS
	Zaza	15	31	0.33:0.67	0.01830	S
Nyagatare	Nyagatare	44	77	0.36:0.64	0.00269	S
	Rukomo	245	191	0.56:0.44	0.00970	S
Nyaruguru	Ngera	8	7	0.53:0.47	0.79625	NS
Gicumbi	Rwamiko	6	10	0.37-0.63	0.31731	NS

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

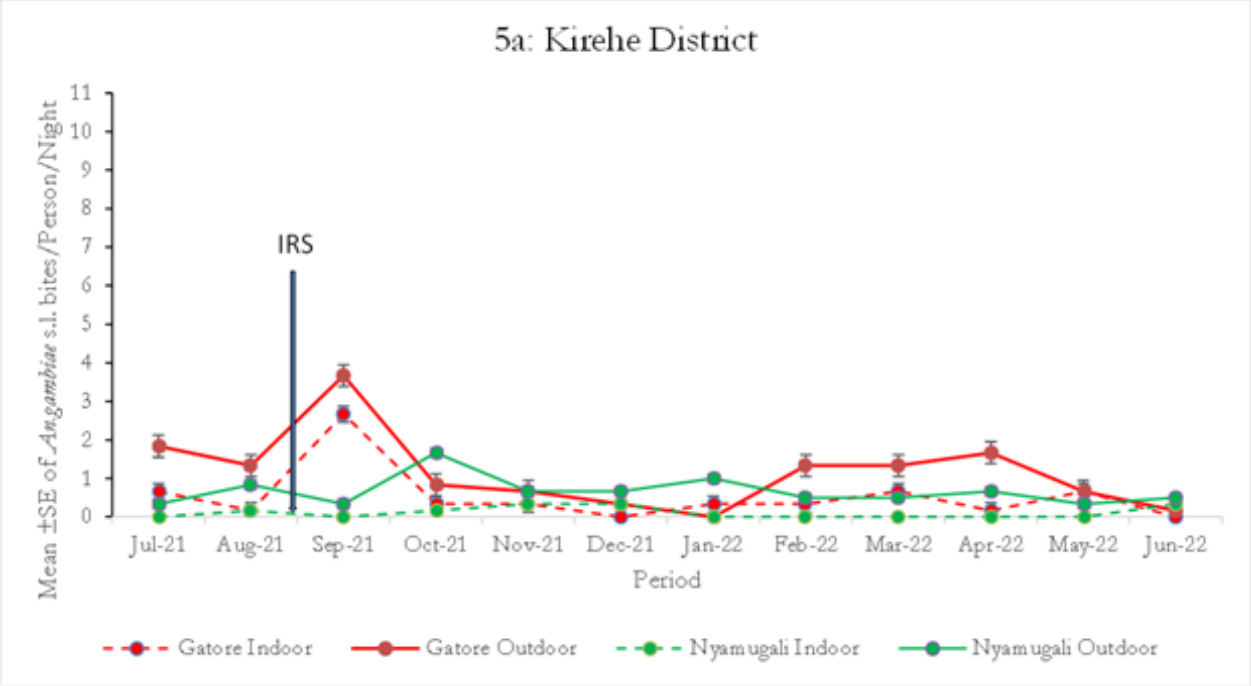
TABLE 3: INDOOR VS. OUTDOOR COLLECTIONS OF *AN. FUNESTUS* S.L. BY HLC

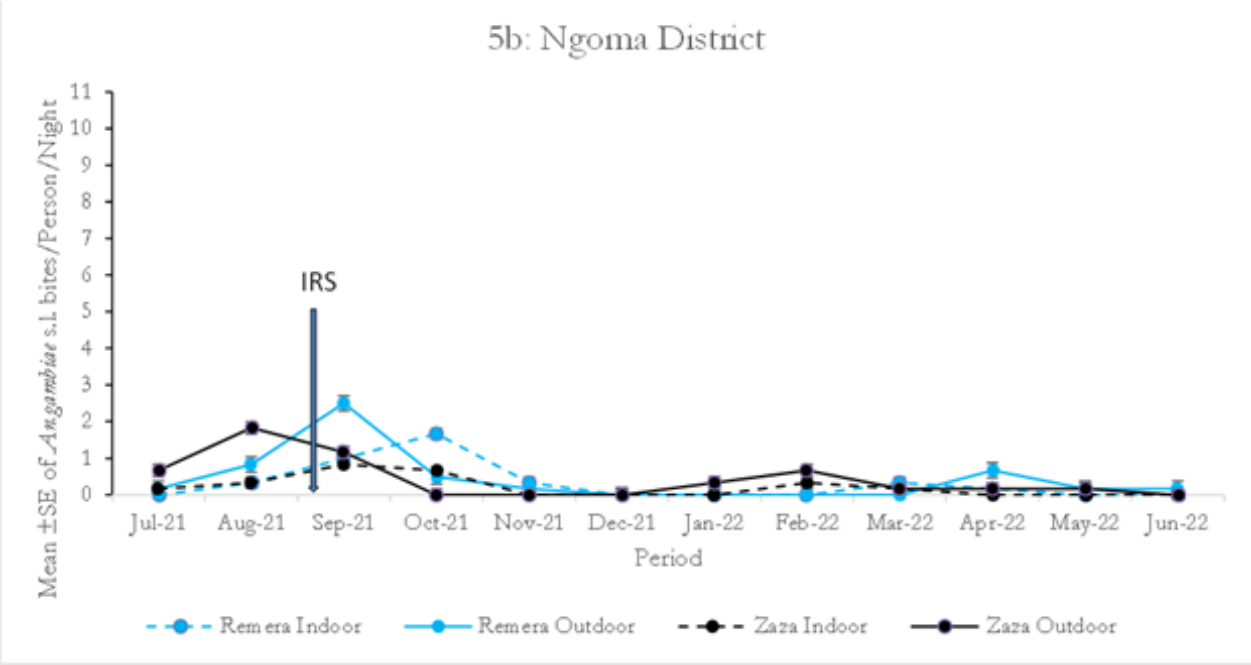
District	Site	In	Out	In: Out Ratio	P-value	Result*
Kirehe	Gatore	0	0	NA	NA	NA
	Nyamugali	0	0	NA	NA	NA
Ngoma	Remera	0	0	NA	NA	NA
	Zaza	0	0	NA	NA	NA
Nyagatare	Nyagatare	0	2	0-100	0.15729	NS
	Rukomo	0	0	NA	NA	NA
Nyaruguru	Ngera	7	7	0.50:0.50	1	NS
Gicumbi	Rwamiko	2	1	0.67–0.33	0.56370	NS

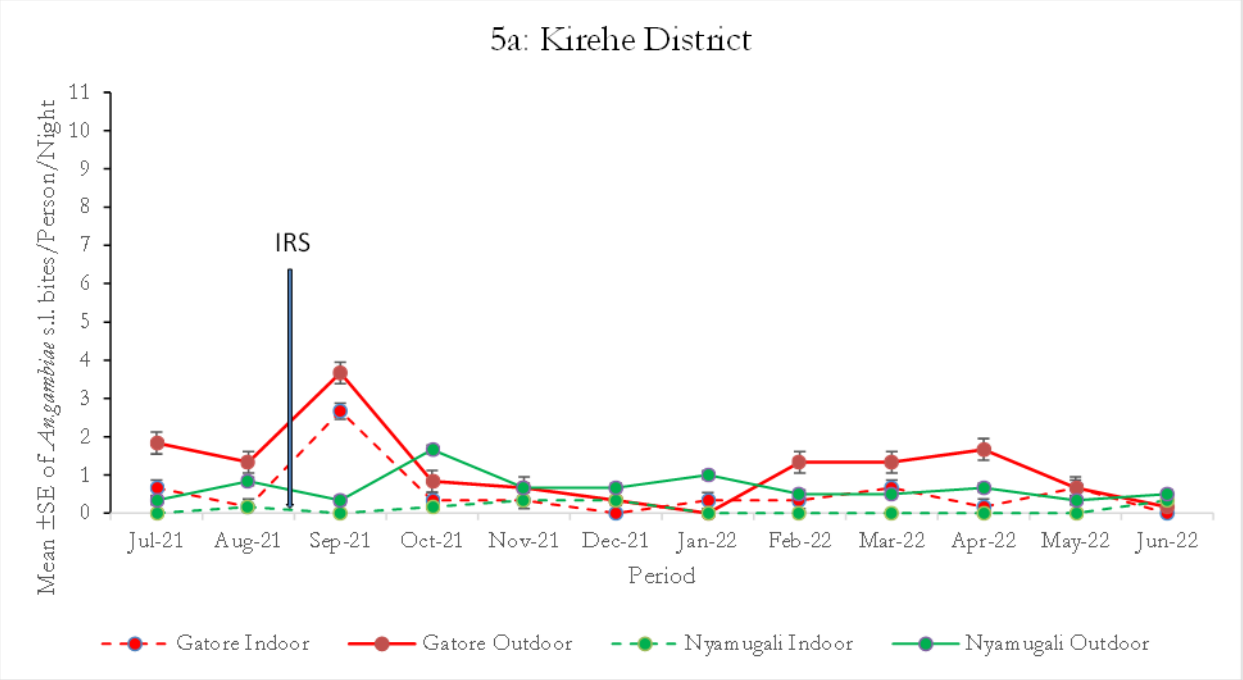
***NS**: not statistically significant, **NA**: not applicable.

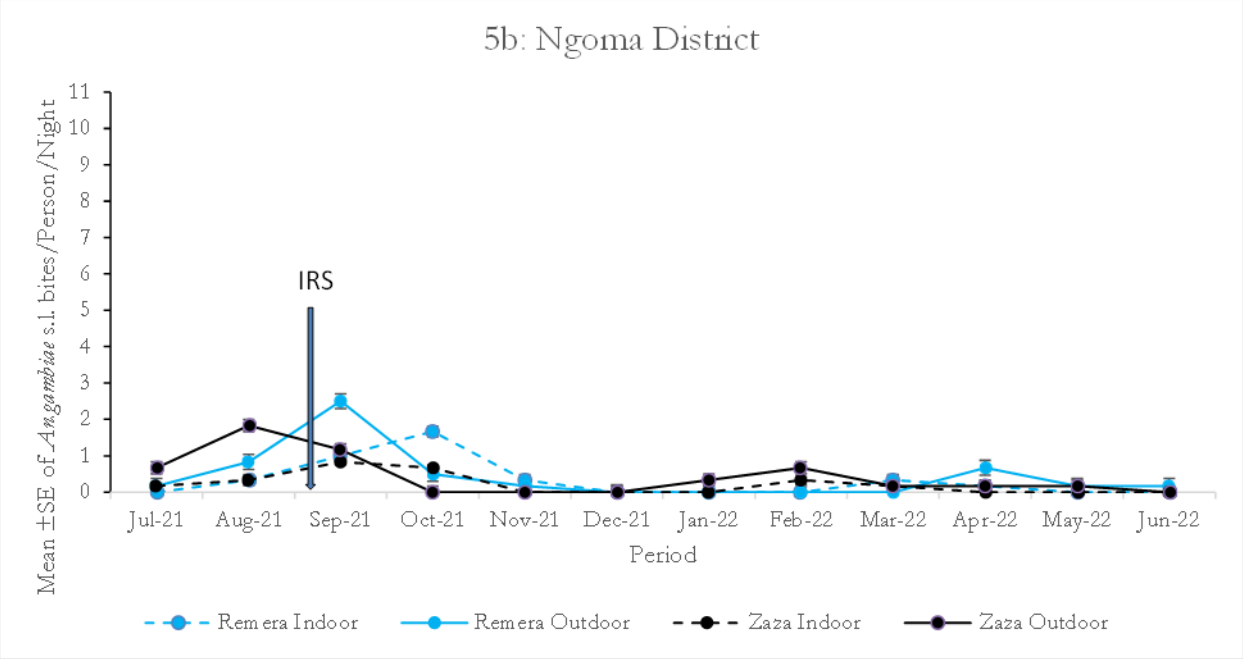
As Figure 5 shows, there is one peak biting season for *An. gambiae* s.l., in September, in most of the sites with IRS, except in the Nyamugali site, where the number of *An. gambiae* s.l. collected was low (5a). For Rukomo site there is another peak in March (5c). In the control sites the HBR was very low compared to in intervention sites (5d). The highest bites/person/night were observed in the Rukomo site, both indoors and outdoors (9.7 vs. 6 bites/person/night respectively in September 2021) (5c).

FIGURE 5: MONTHLY TRENDS IN MEAN HBR (BITES/PERSON/NIGHT) BY *AN. GAMBIAE* S.L.



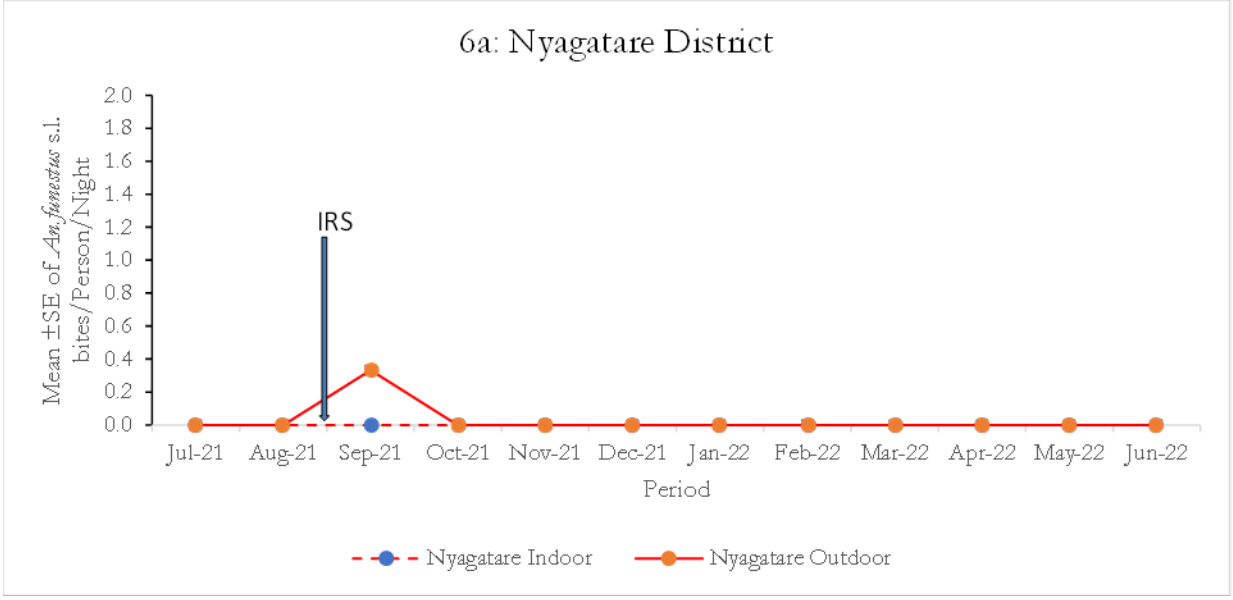






For *An. funestus* s.l., the HBR per night was very low, with fewer than 0.5 bites/person/night in all sites where *An. funestus* was found (Figure 6a and 6b).

FIGURE 6: MONTHLY TRENDS IN MEAN HBR (BITES/PERSON/NIGHT) BY *AN. FUNESTUS* S.L.



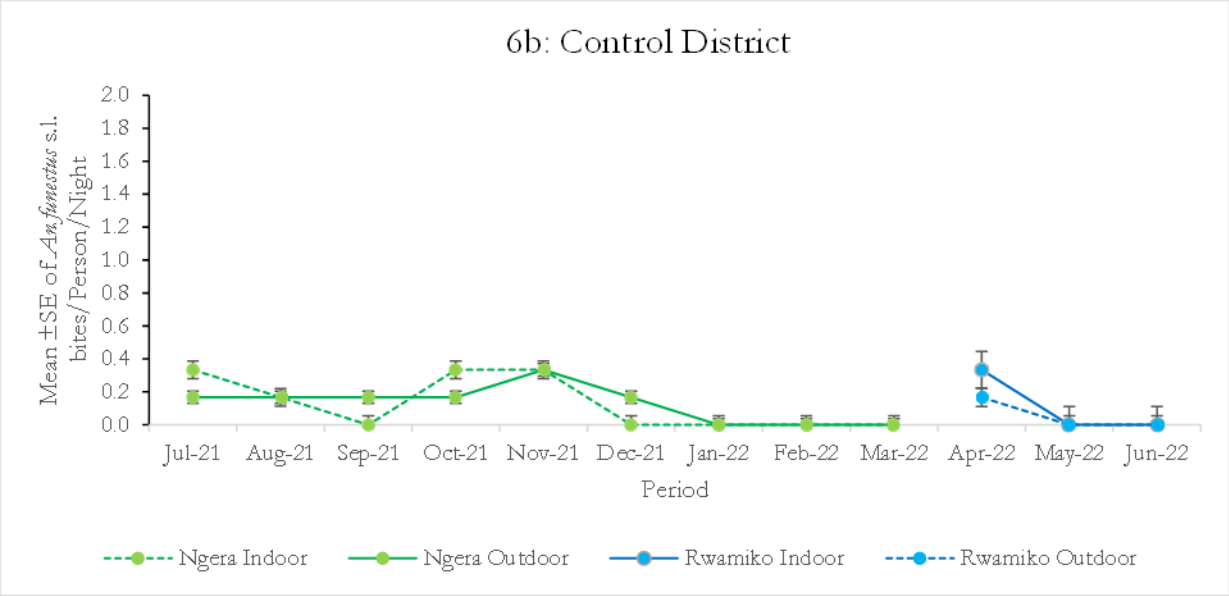
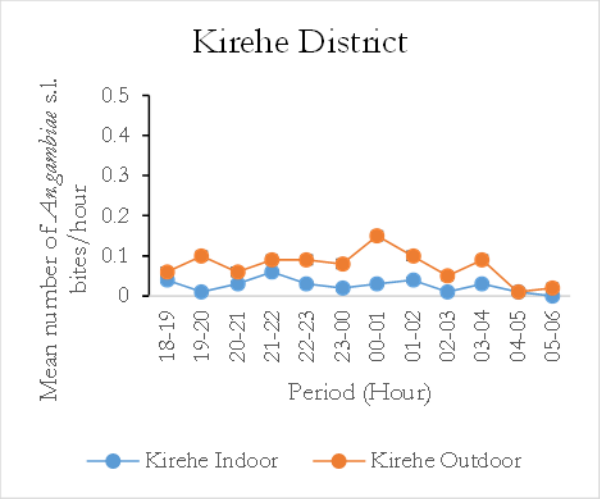
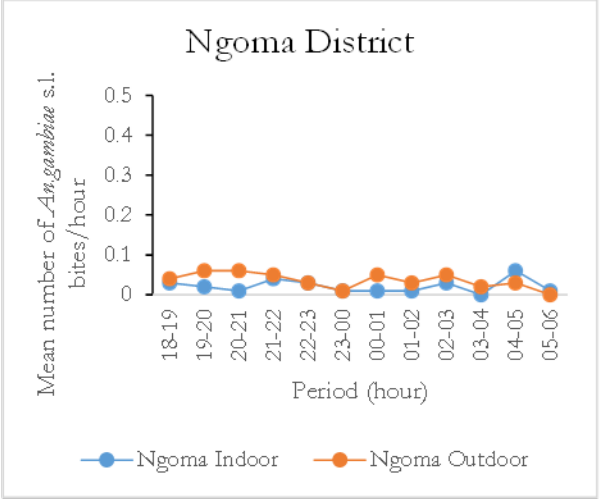
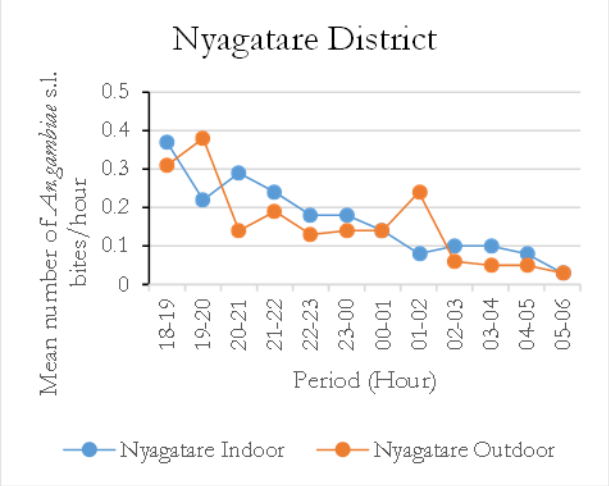


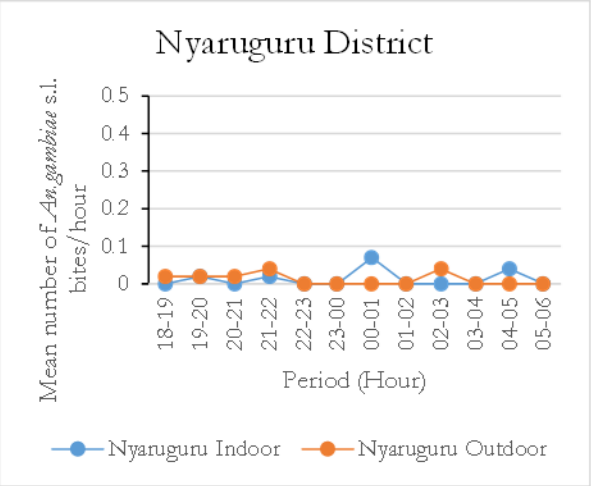
Figure 7 shows average *An. gambiae* s.l. bites per person per hour through the night across the four districts. As the figure shows, more biting took place outdoors in Kirehe and Ngoma District, but in Nyagatare District there is slightly more biting indoors than outdoors. Even though bites per person 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 District. The trend for other districts is not clear as the numbers collected are very low.

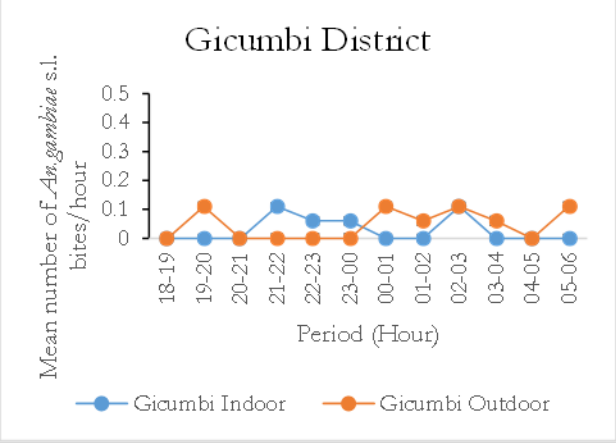
FIGURE 7: INDOOR AND OUTDOOR HOURLY BITING RATE OF *AN. GAMBIAE* S.L.











3.3 INDOOR RESTING DENSITY

Overall, 187 *Anopheles* mosquitoes were collected using PSC in the three IRS and two control districts over the July 2021 through June 2022 collection period. *An. gambiae* s.l. was predominant, at 83.2% of the collections; 9.6% of mosquitoes collected were *An. ziemanni*, 4.3% *An. funestus* s.l., 2.7% *An. squamosus,* and 2.1% *An. maculipalpis*. Table 4 shows the disaggregation of *An. gambiae* s.l. collections and density in the districts. Even though the density was very low in all surveyed sites, in general there were two peaks of *An. gambiae* s.l. population, the first one in August-September, and the second one in March (Table 4). The difference in mean indoor density between Nyaruguru (control) and IRS districts (Kirehe, Ngoma, and Nyagatare) was not statistically significant (p>0.05). However, the indoor resting numbers were very low to make any meaningful comparison. Moreover, mosquito density was very low in the control district throughout the year. Of the total *An. gambiae* s.l. collected resting indoors in the year, 80.5% in Kirehe, 53.8% in Ngoma, 26.5% in Nyagatare, and 17.6% in Nyaruguru were collected in August–September (Table 4).

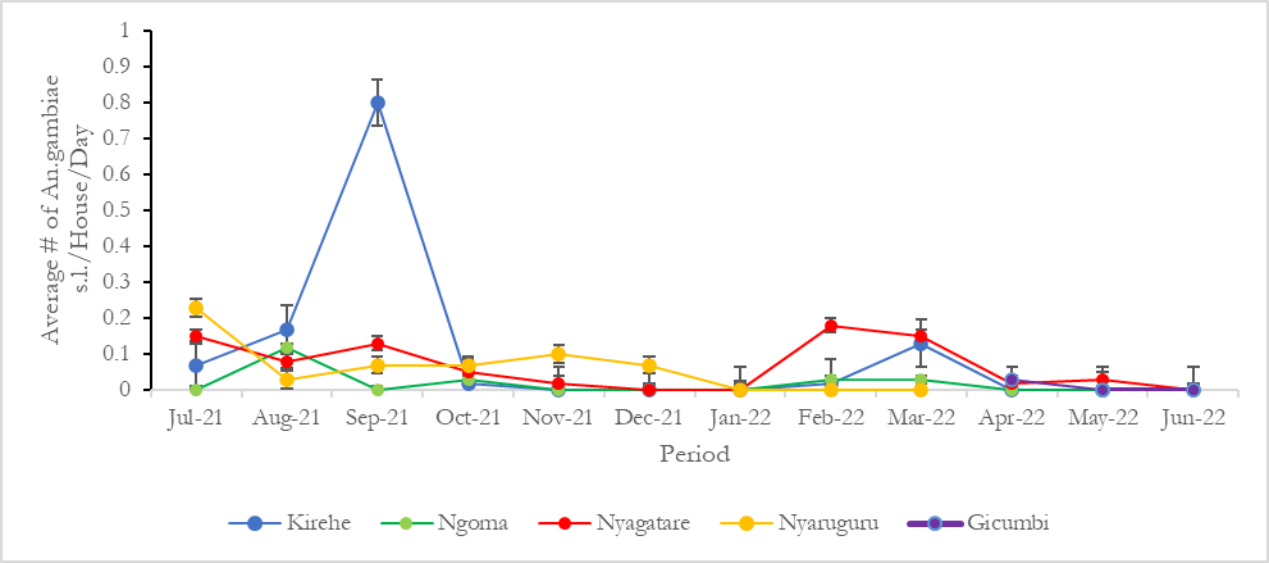
TABLE 4: *AN. GAMBIAE* S.L. INDOOR RESTING DENSITY FROM PSC COLLECTIONS

District	Kirehe		Ngoma		Nyagatare		Nyaruguru (Control)		Gicumbi (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
July 21	4	0.07	0	0	9	0.15	7	0.23	ND	NA
Aug 21	10	0.17	7	0.12	5	0.08	1	0.03	ND	NA
Sep 21	48	0.8	0	0	8	0.13	2	0.07	ND	NA
Oct 21	1	0.02	2	0.03	3	0.05	2	0.07	ND	NA
Nov 21	0	0	0	0	1	0.02	3	0.1	ND	NA
Dec 21	0	0	0	0	0	0	2	0.07	ND	NA
Jan 22	0	0	0	0	0	0	0	0	ND	NA
Feb 22	1	0.02	2	0.03	11	0.18	0	0	ND	NA
Mar 22	8	0.13	2	0.03	9	0.15	0	0	ND	NA
Apr 22	0	0	0	0	1	0.02	ND	NA	1	0.03
May 22	0	0	0	0	2	0.03	ND	NA	0	0
Jun 22	0	0	0	0	0	0	ND	NA	0	0
Total	72	-	13	-	49	-	17	-	1	-
August and September collection (%) of the total	80.5%		53.8%		26.5%		17.6%			
Avg. monthly vector density	6	0.1	1.08	0.01	4.08	0.07	1.9	0.06	0.33	0.01
P-value	*p*=0.3843		*p*=0.4039		*p*=0.1641		1 (ref)			

*d/h/d: density/house/day
**Shaded rows: Peak of *An. gambiae* s.l. density

Although monthly vector density varied throughout the reporting period, Kirehe District showed the highest average vector density, of 0.8 *An. gambiae* s.l./house/day in September 2021 (Figure 8).

FIGURE 8: *AN. GAMBIAE* S.L. INDOOR RESTING DENSITY, BY DISTRICT



All 152 *An. gambiae* s.l. collected in all sites using PSC were classified according to their blood digestion stages: 84 (55%) were unfed, 33 (22%) were fed, 17 (11.2%) were half-gravid, and 18 (11.8%) were gravid (Figure 9). Among eight *An. funestus* s.l., 62% (5) were unfed, 0% (0) fed, 13% (1) half-gravid, and 25% (2) gravid. High proportions of *An. gambiae* s.l. (55%) collected indoors were unfed despite entering the house to take a bloodmeal, and resting inside the houses, which would probably indicate a good amount of use of insecticide-treated nets (ITN) by residents (Figures 9 and 10). The trend was similar with *An. funestus* s.l. though the numbers collected were very small.

FIGURE 9: BLOOD DIGESTION STAGES OF ALL *AN. GAMBIAE* S.L. AND *AN. FUNESTUS* S.L. COLLECTED USING PSC

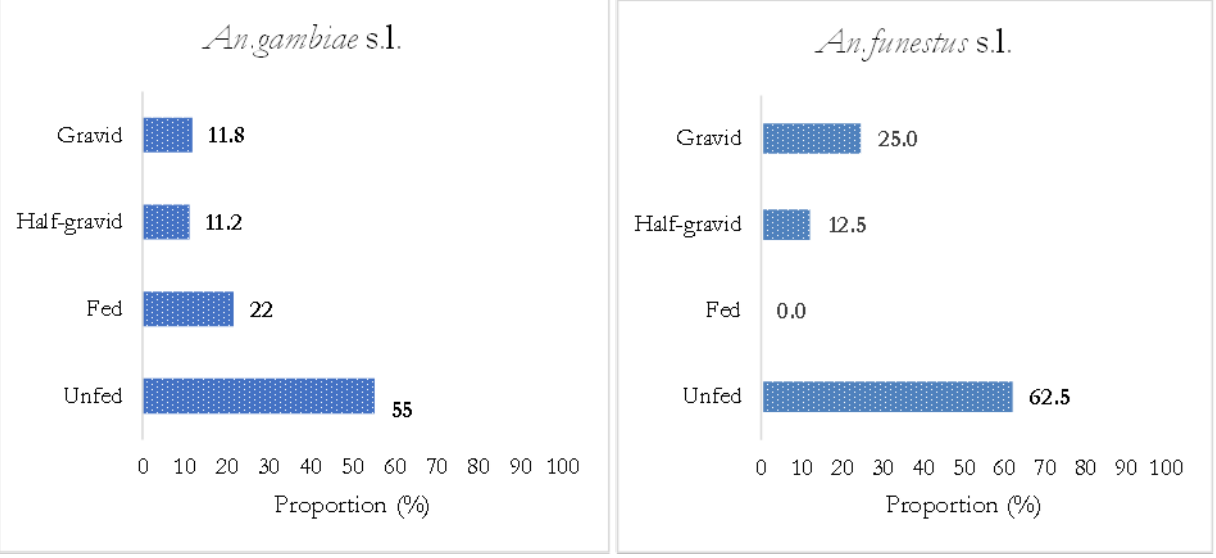
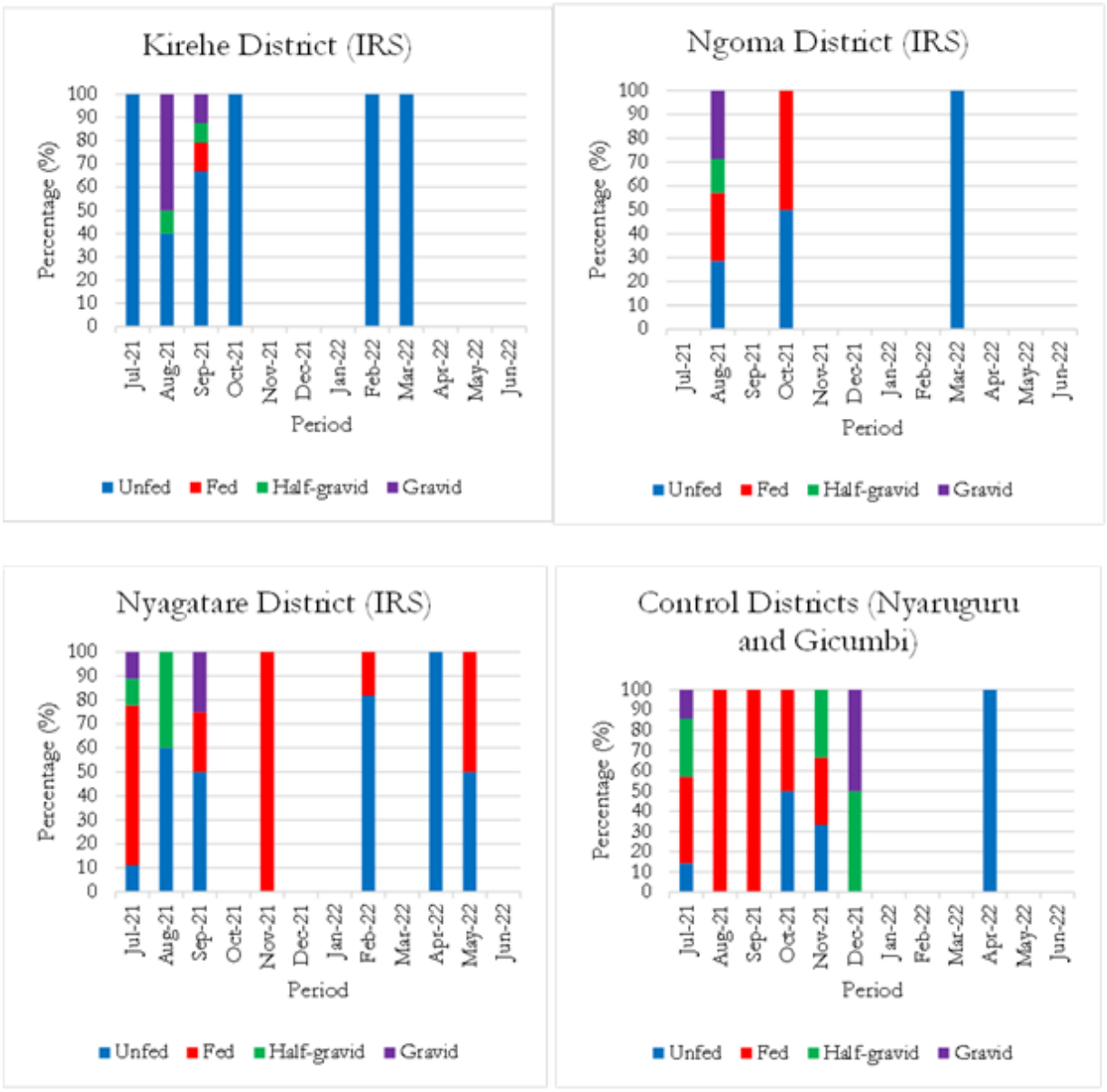


FIGURE 10: BLOOD DIGESTION STAGES OF *AN. GAMBIAE* S.L. COLLECTED USING PSC, BY DISTRICT, AND MONTH



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 the average parity rate from July 2021 through June 2022. A Z-test of proportions showed a significant difference ($p<0.05$) between the proportion of parous *An. gambiae* s.l. in control districts (Nyaruguru and Gicumbi) vs. in Kirehe, Ngoma, and Nyagatare (IRS districts).

For *An. funestus* s.l. the parity rate was 50% in Nyagatare District and 50% in control districts (Nyaruguru and Gicumbi), as shown in Table 6. However, the numbers of mosquitoes dissected were too few to make any meaningful comparison.

The difference observed in the *An. gambiae* s.l. parity rate between intervention and control districts sites could be attributed to the IRS.

TABLE 5: PARITY FOR *AN. GAMBIAE* S.L.

District	Total Collected	Total *An. gambiae* s.l. Dissected	# Parous	% Parity	Confidence Interval	P-value	Result*
Kirehe	177	153	30	19.6	13.3-25.9	$p<.001$	S
Ngoma	100	72	12	16.7	8.1-25.3	$p<.001$	S
Nyagatare	557	317	43	13.6	9.8-17.3	$p<.001$	S
Nyaruguru and Gicumbi (control)	31	31	15	48.4	30.8-66.0	1	

***S**: Statistically significant.

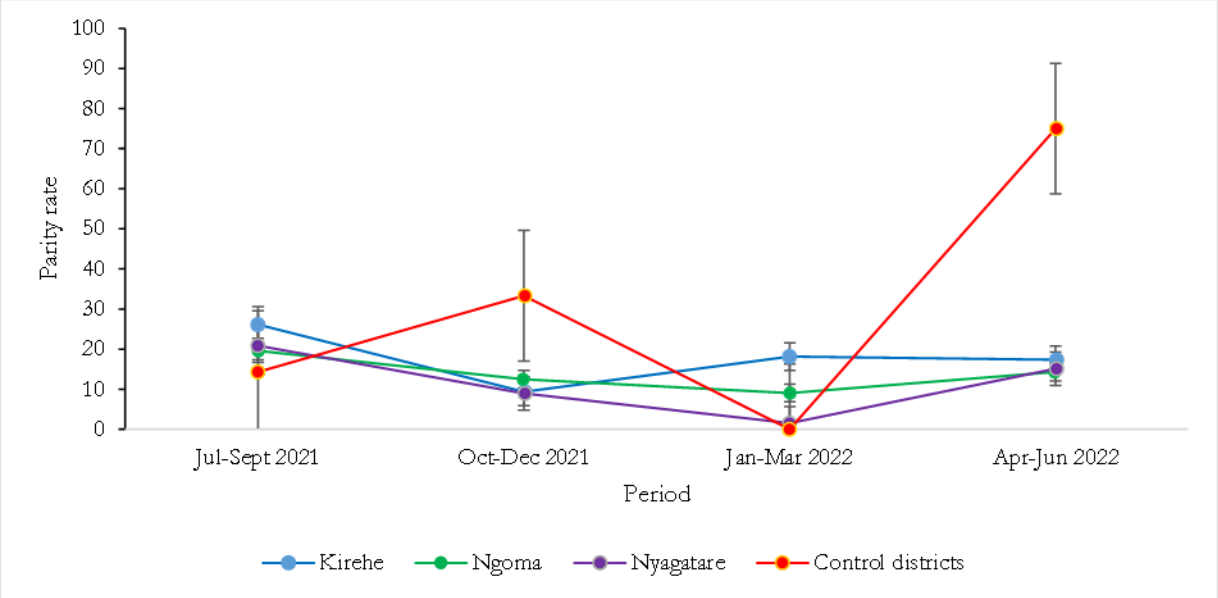
TABLE 6: PARITY FOR *AN. FUNESTUS* S.L.

District	Total Collected	Total *An. funestus* s.l. Dissected	# Parous	% Parity	Confidence Interval	P-value	Result*
Nyagatare	2	2	1	50	NA	1	NS
Nyaruguru and Gicumbi (control))	17	10	5	50	19.0-81.0	1	

*NS: not statistically significant, NA: not applicable.

Quarterly trends in parity rate between the IRS and control districts were compared, and IRS seems to have suppressed the proportion of parous females throughout the collection period. For control districts the trend is not consistent (Figure 11).

FIGURE 11: PARITY RATE IN THREE IRS DISTRICTS COMPARED WITH CONTROL



3.5 MOLECULAR SPECIES IDENTIFICATION

A subsample of *An. gambiae* s.l. (n=797) were identified using molecular techniques; 91.2% were *An. arabiensis* and the remaining samples were *An. gambiae* s.s. (Table 7).

An. arabiensis was dominant in all sprayed sites and in Ngera (control) but not in Rwamiko (control). The difference in proportion between *An. arabiensis* and *An. gambiae* s.s. in all sites except the Rwamiko site was statistically significant (Table 7).

Table 7: *An. gambiae* s.l. Sibling Composition

District	Site	*An. gambiae s.s.*	*An. arabiensis*	P-value	Significance Status*
Kirehe	Gatore	1.2% (2)	98.8% (165)	*p*<.001	S
	Nyamugali	0.0% (0)	100% (44)	*p*<.001	S
Ngoma	Remera	7.1% (3)	92.9% (39)	*p*<.001	S
	Zaza	2.2% (1)	97.8% (44)	*p*<.001	S
Nyagatare	Nyagatare	37.8% (34)	62.2% (56)	*0.020*	S
	Rukomo	5.0% (19)	95.0% (360)	*p*<.001	S
Nyaruguru	Ngera (Control)	26.3% (5)	73.7% (14)	*0.03894*	S
Gicumbi	Rwamiko (Control)	54.5% (6)	45.5% (5)	*0.76302*	NS

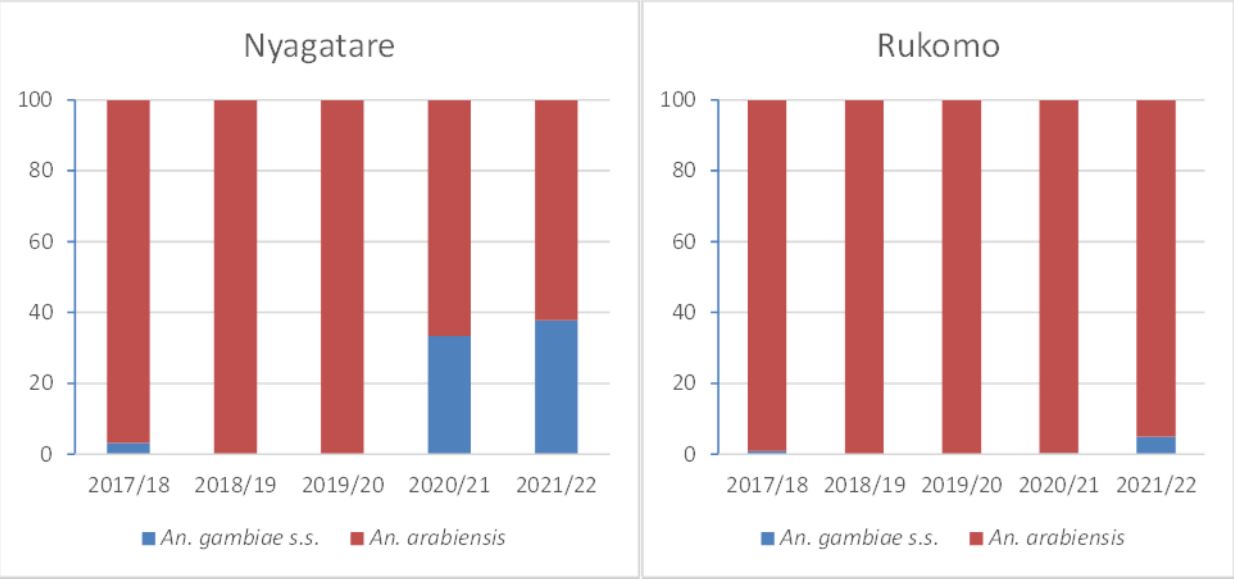
***S**: Statistically significant, **NS**: not statistically significant, **NA**: not applicable.

It also appears the proportion of *An. gambiae* s.s. collected has been increasing in the past two years. None of the *An. gambiae* s.l. collected in 2018–2019 and 2019–2020 were *An. gambiae* s.s. and fewer than 4% of the *An. gambiae* s.l. were *An. gambiae* s.s. in the 2017–2018 collection. The proportion of *An. gambiae* s.s. increased to 37.8% in Nyagatare District and to 5% in the Nyagatare and Rukomo sites, respectively (Table 8 and Figure 12). Moreover, the two mosquitoes found to be positive for *P. falciparum* were *An. gambiae s.s.* Further observation is required to understand the dynamics of change in the proportion of the two sibling species, given that *An. gambiae* s.s. is considered the more efficient malaria vector of the two (Highton et al. 2009).

Table 8: Number and Proportions [N (%)] of *An. arabiensis* and *An. gambiae* s.s. in Nyagatare District 2017–2022

District	Site	2017–2018		2018–2019		2019–2020		2020–2021		2021–2022	
		An. gambiae s.s.	*An. arabiensis*	*An. gambiae* s.s.	*An. arabiensis*	*An. gambiae* s.s.	*An. arabiensis*	*An. gambiae* s.s.	*An. arabiensis*	*An. gambiae* s.s.	*An. arabiensis*
Nyagatare	Nyagatare	2 (3.1)	62 (96.9)	0 (0)	57 (100)	0 (0)	45 (100)	7 (33.3)	14 (66.7)	34 (37.8)	56 (62.2)
	Rukomo	1 (0.9)	106 (99.1)	0 (0)	118 (100)	0 (0)	83 (100)	1 (0.2)	413 (99.8)	19 (5.0)	360 (95.0)

FIGURE 12: TRENDS IN *AN. ARABIENSIS* AND *AN. GAMBIAE* S.S. PROPORTION: 2017–2022



A subsample of the 230 Kisumu colony mosquitoes from the insectary were identified using molecular techniques to confirm the species and ensure that there is no contamination with other species; 100% of them were identified as *An. gambiae* s.s. Further testing for resistance markers for quality assurance of the Kisumu colony will be performed when the qPCR set-up for *kdr* and *ace- 1* detection is established at the MOPDC laboratory.

3.6 ELISA

3.6.1 SPOROZOITE INFECTION RATE

Mosquitoes collected using HLC and PSC were tested for infection using ELISA. A total of 1,303 mosquitoes collected from July 2021 through June 2022 in the districts surveyed were tested for *Plasmodium falciparum* circumsporozoite protein. Different *Anopheles* species were tested, with *An. gambiae* s.l. making up 77.2% of the total screened for infection. Five samples had tested positive on the first ELISA, and one became negative on the confirmation ELISA. Four samples tested were positive for *P. falciparum* infection; Table 9a and 9b show the numbers of mosquitoes tested, by species and by monitoring site. Among the *An. gambiae* s.l. tested positive for *Plasmodium falciparum* circumsporozoite protein, two were *An. gambiae* s.s. in Nyagatare site and one *An. arabiensis* in Zaza site. This was also the first time to find *Anopheles ziemanni* testing positive for *P. falciparum*, which means that this mosquito could also be a secondary malaria vector in the project sites and other parts of Rwanda.

TABLE 9: NUMBERS TESTED FOR SPOROZOITE INFECTION

9a: By Species

Species	Number Tested	Number Positive	% Positive
An. gambiae s.l.	1,006	3	0.30
An. ziemanni	162	1	0.62
An. squamosus	53	0	0.00
An. pharoensis	28	0	0.00
An. maculipalpis	28	0	0.00
An. funestus	23	0	0.00
An. coustani	3	0	0.00
Total	1,303	4	0.30

9b: By Site

District	Site	# Tested	Number Positive	% Positive
Kirehe	Gatore	248	0	0
	Nyamugali	58	0	0
Ngoma	Remera	75	0	0
	Zaza	112	1	0.90
Nyagatare	Nyagatare	126	2	1.60
	Rukomo	482	0	0
Nyaruguru	Ngera	172	0	0
Gicumbi	Rwamiko	30	1	3.33

3.6.2 BLOOD MEAL ELISA

Blood-fed samples from the PSC collections from July 2021 through June 2022 were also assayed to determine the source of the blood meal. A total of 27 *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 10). Overall, 25.9% of the mosquitoes fed on humans only, and an additional 7.4% fed on humans and other animals. The results show that a relatively high proportion of the vectors fed on non-human hosts.

TABLE 10: BLOOD MEAL SOURCE

Site	Number Tested	Results						HBI
		Human	Bovine	Goat	Human and Other	Goat and Bovine	No Specified Host	
Kirehe	8	1 (12.5%)	4 (50.0%)	0 (0.0%)	2 (25.0%)	0 (0.0%)	1 (12.5%)	37.5%
Ngoma	4	1 (25.0%)	2 (50.0%)	0 (0.0%)	0 (0.0%)	0 (0%)	1 (25.0%)	25.0%
Nyagatare	11	5 (45.5%)	4 (36.4%)	0 (0.0%)	0 (0.0%)	1 (9.1%)	1 (9.1%)	45.5%
Control (Nyaruguru and Gicumbi)	4	0 (0.0%)	4 (100.0%)	0 (0.0%)	0 (7.7%)	3 (11.6%)	0 (0.0%)	0.0%
Total	27	7 (25.9%)	14 (51.9%)	0 (0.0%)	2 (7.4%)	1 (3.7%)	3 (11.1%)	33.3%

3.6.3 EIRs

Based on the ELISA results, the EIR for *An. gambiae* s.l. species was calculated in the sites where *Anopheles* mosquitoes were tested positive for sporozoite infection. For *An. gambiae* s.l. the EIR in Nyagatare site (Nyagatare District) was five infective bites/person/year, whereas in Zaza site (Ngoma District) the EIR was two infective bites/person/year.

TABLE 11: ANNUAL EIR OF *ANOPHELES GAMBIAE* S.L.

Month	Nyagatare Site (Nyagatare District)					Zaza Site (Ngoma District)				
	Total *An. gambiae* s.l. Collected	Bites/ Person/ Night	SPZ Rate	Nightly EIR	Monthly EIR	Total *An. gambiae* s.l. Collected	Bites/ Person/ Night	SPZ Rate	Nightly EIR	Monthly EIR
July 21	3	0.25	0.33	0.08	2.58	7	0.58	0.00	0.00	0.00
Aug 21	4	0.33	0.00	0.00	0.00	17	1.42	0.00	0.00	0.00
Sep 21	41	3.42	0.02	0.08	2.49	12	1.00	0.08	0.08	2.50
Oct 21	20	1.67	0.00	0.00	0.00	5	0.42	0.00	0.00	0.00
Nov 21	32	2.67	0.00	0.00	0.00	0	0.00	0.00	0.00	0.00
Dec 21	6	0.50	0.00	0.00	0.00	0	0.00	0.00	0.00	0.00
Jan 22	2	0.17	0.00	0.00	0.00	1	0.08	0.00	0.00	0.00
Feb 22	0	0.00	0.00	0.00	0.00	8	0.67	0.00	0.00	0.00
Mar 22	1	0.08	0.00	0.00	0.00	4	0.33	0.00	0.00	0.00
Apr 22	5	0.42	0.00	0.00	0.00	1	0.08	0.00	0.00	0.00
May 22	6	0.50	0.00	0.00	0.00	0	0.00	0.00	0.00	0.00
Jun 22	0	0.00	0.00	0.00	0.00	0	0.00	0.00	0.00	0.00
Annual EIR					5.07					2.50

3.7 QUALITY OF SPRAYING, INSECTICIDE DECAY RATE

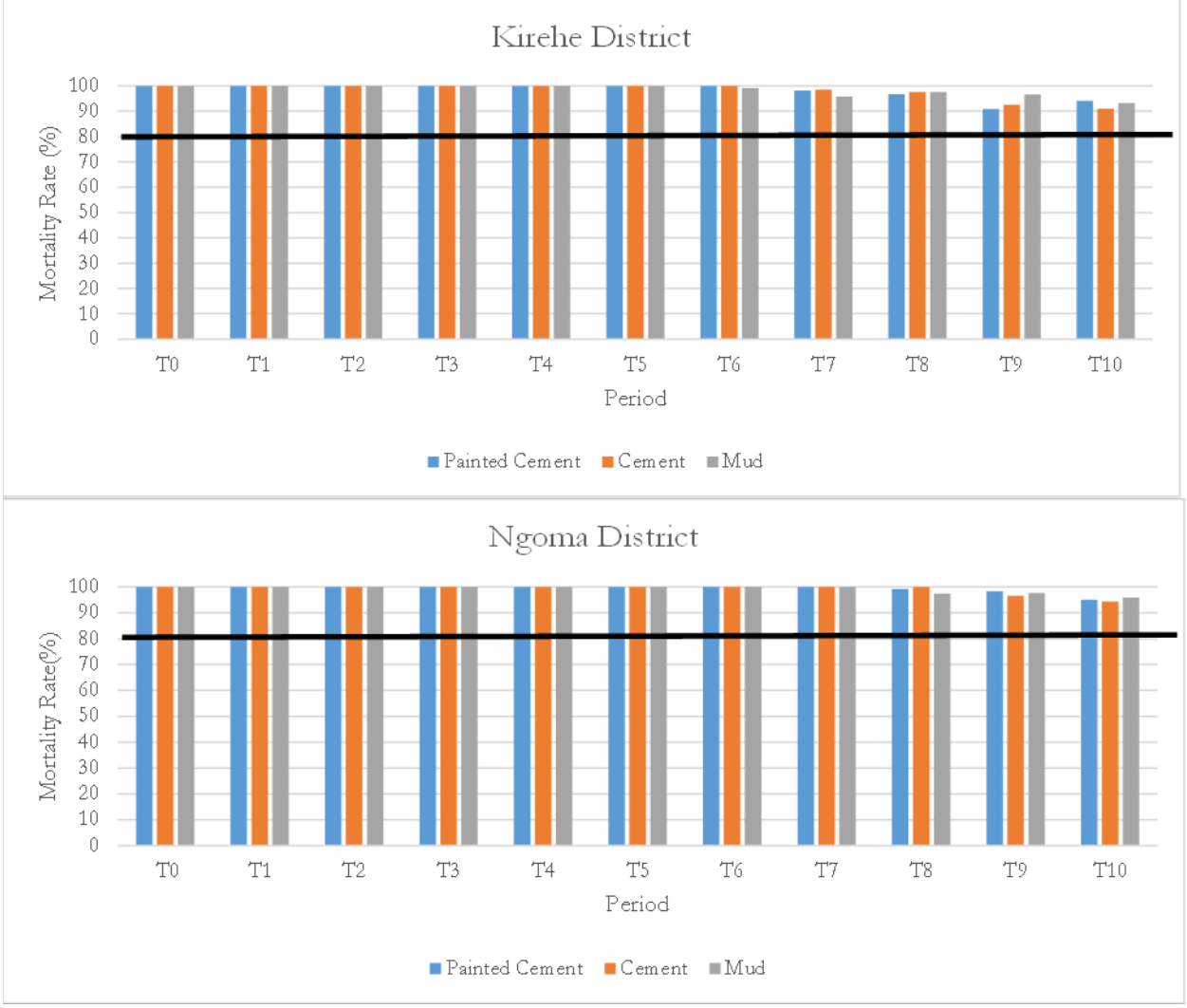
3.7.1 QUALITY OF SPRAYING AND INSECTICIDE DECAY RATE

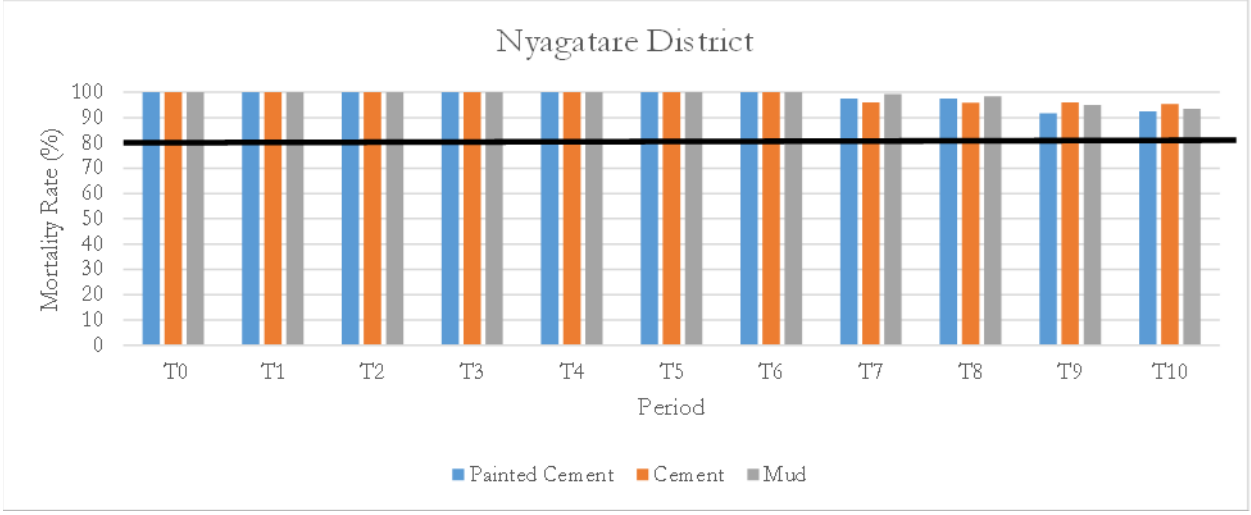
As noted above, VectorLink Rwanda sprayed Kirehe, Ngoma, and Nyagatare Districts in August–September 2021 with Actellic® 300CS. WHO cone wall bioassays were carried out to assess the quality of spraying. The evaluation of IRS quality was done in August 2021 in 12 sprayed houses in each district. Residual efficacy was then monitored monthly in two sites from each district. In each site, six structures were sampled, two each of different wall surface types (mud, cement, and painted cement). 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. (Kisumu) within 24 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 bioefficacy of the sprayed walls. The mortality rate of exposed mosquitoes was above 80% on all wall surface types 24 hours after exposure in the wall assays for 10 months from September to June (Figure 13), which covers until the start of the next IRS campaign in August.

FIGURE 13: RESIDUAL EFFICACY OF ACTELLIC® 300CS FROM WALL BIOASSAY USING KISUMU COLONY





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

3.8 Annual Trends in Vector Bionomics

Data from two districts (Nyagatare and Kirehe) that received IRS for five consecutive years, and from Ngoma, which had been a control district in 2017–2019 but received IRS for the next three years, is shown in Figures 14–15 and Table 12. This simple descriptive analysis shows trends in change of vector bionomics indicators in the three IRS districts over the five-year period. This preliminary analysis indicates a consistent decrease in vector abundance and infection rate over the five-year period in the three IRS districts.

From the PSC collections, the overall reduction in indoor resting density in the three districts is consistent over the five-year period of monitoring. When disaggregated by district, the reduction is more dominant in Ngoma (the former control district) and Nyagatare Districts than in Kirehe. Ngoma district was non-sprayed control in 2017/18 and 2018/19. The fall was sharp after the spray campaigns in 2019/20, 2020/21 and 2021/22. The numbers from PSC collection, however, are small, and the data should be interpreted cautiously.

From the HLC collections, in most cases the biting numbers decreased over the years, and the highest reduction was in the last two years, 2020–2021 and 2021–2022, except in Nyagatare site, where there was a small increase this year. Compared with in the PSC collections, the reduction in the HLC collections in Kirehe was more consistent over the years. The trend in the infection rate is similar. Overall mosquito infection rates in the project districts were lower (0.30%) in 2018–2019 compared to in 2017–2018 (0.59%). No infected mosquitoes were detected in 2019–2020 and 2020–2021, but in 2021–2022 the overall mosquito infection rate in the project districts was 0.20%. However, these results need to be cautiously interpreted given the overall low infection rates of vectors in the project districts. In addition, the number of samples processed was small, so the results may not indicate that transmission was interrupted in the last two years and coming back in 2021/22. The finding of a *P. falciparum*-infected *An. ziemanni* is also of a concern. This mosquito as a suspected or secondary vector may also contribute to malaria transmission.

Trends in vector abundance and infection rates between IRS and non-IRS districts over five years could not be compared, as there was no permanent control district over the five-year period; control districts were either sprayed after one or two years or dropped from being control without being sprayed.

These are preliminary observations comparing entomological indicators from summary data over the five years of available data for these districts. However, a more in-depth statistical analysis of the data that includes case counts could be necessary if the change and trends observed in the entomological parameters of malaria transmission are statistically significant, as the trends in the reduction of the entomological indicators could also have an impact on case burden.

FIGURE 14: TRENDS IN *AN. GAMBIAE* S.L. ABUNDANCE FROM PSC COLLECTIONS OVER FIVE YEARS (2017/18– 2021/22) IN THREE IRS DISTRICTS: A=ALL SITES; B=BY DISTRICT

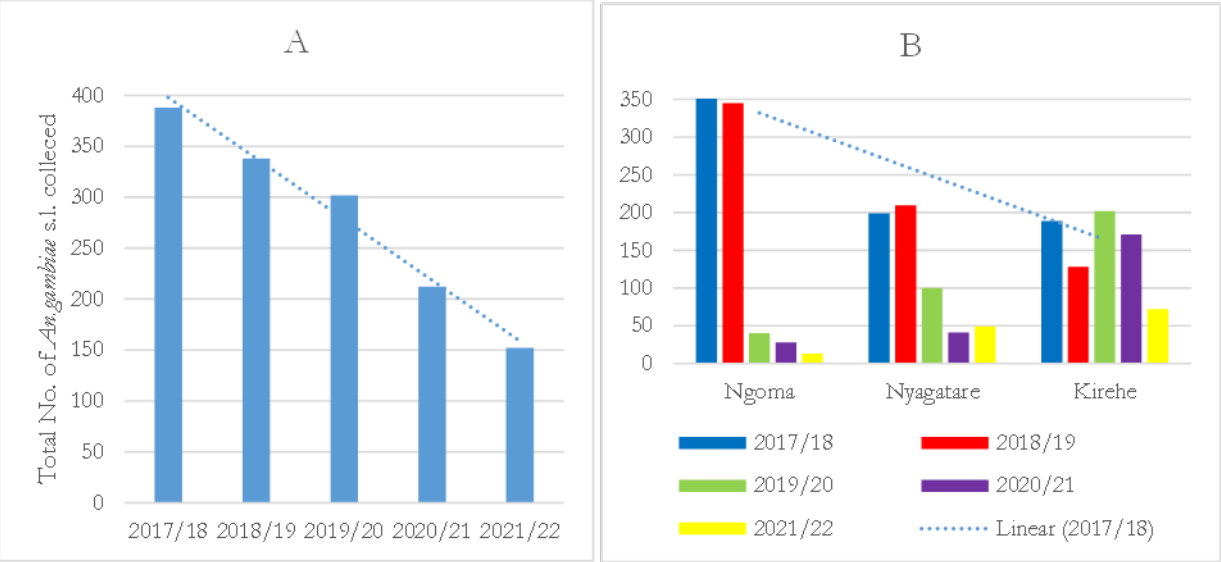
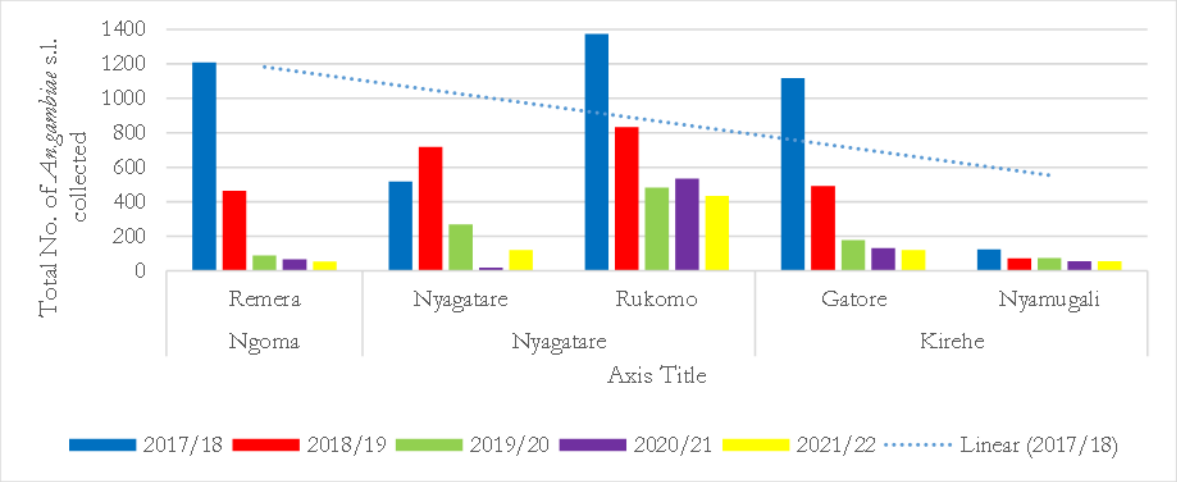


FIGURE 15: TRENDS IN *AN. GAMBIAE* S.L. ABUNDANCE FROM HLC* COLLECTIONS BY DISTRICT OVER FIVE YEARS (2017/18– 2021/22) IN THREE IRS DISTRICTS



*Total numbers instead of HBR are used, as the numbers of houses, collectors, and nights were the same across all sites and years. Not adjusted for one month (April) of missing data in 2019–2020 due to COVID-19.

TABLE 12: TRENDS IN SPOROZOITE INFECTION RATE OVER FIVE YEARS (2017–2022) IN THREE IRS DISTRICTS

District	Site	2017/18			2018/19			2019/20			2020/21			2021/22		
		# Tested	No. Positive	% Positive	# Tested	No. Positive	% Positive	# Tested	No. Positive	% Positive	# Tested	No. Positive	% Positive	# Tested	No. Positive	% Positive
Nyagatare	Nyagatare	356	3	0.84	361	2	0.55	254	0	0	62	0	0	126	2	1.6
	Rukomo	640	3	0.47	529	1	0.19	440	0	0	541	0	0	482	0	0
Kirehe	Gatore	579	2	0.34	526	2	0.38	329	0	0	386	0	0	248	0	0
	Nyamugali	159	0	0	77	0	0	79	0	0	110	0	0	58	0	0
Ngoma	Remera	788	7	0.89	504	1	0.2	184	0	0	126	0	0	75	0	0
Total		2,522	15	0.59	1,997	6	0.3	1,286	0	0	1,225		0	989	2	0.20

4. SUPPORT FOR RWANDA BIOMEDICAL CENTER

4.1 INSECTARY MAINTENANCE AND ASSOCIATED VECTOR CONTROL LABORATORY SUPPORT

VectorLink Rwanda supports the maintenance of the insectary and associated vector laboratory at the RBC MOPDC, and the colony reared in the insectary was inspected to determine species purity. A total of 230 Kisumu strain mosquitoes from the insectary were tested using conventional PCR for species identification, and the results showed that 100% of the 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 (ELISA and PCR) and other materials (for field and laboratory) and equipment (ELISA reader, ELISA washer machine, centrifuge, electronic balance and DNA extractor machine) for entomology monitoring and general laboratory activities. VectorLink Rwanda also provides technical support to the entomology laboratory staff in performing laboratory tests on mosquitoes collected from the MOPDC's entomology sentinel sites. The project also supported the procurement of insecticide-impregnated papers to be used in insecticide resistance testing across the country. VectorLink Rwanda supported durability monitoring of long-lasting insecticidal nets for 12 months and 24 months post distribution. VectorLink Rwanda supported the rehabilitation of the entomology laboratory and insectary. Finally, VectorLink Rwanda supported annual entomology planning and refresher training in the MOPDC's sentinel sites and 66 participants attended this training.

5. Discussion and Conclusions

- *An. 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 (91.2%) of the *An. gambiae* s.l. tested. In general, the number of mosquitoes collected this year was low compared with in previous years. Of the total *An. gambiae* s.l. collected resting indoors, 80.5% in Kirehe, 53.8% in Ngoma, and 26.5% in Nyagatare were collected in August through September. The data indicate that the timing of IRS in August, just before the peak in September, is appropriate. The distribution of *An. gambiae* s.s., the more efficient malaria vector of the two, was uniform across the sites, most collected from Nyagatare and the two control districts.
- *An. funestus* were collected in only three sites (Nyagatare, Rwamiko, and Ngera); of the three, the most *An. funestus* were collected in the control site of Ngera.
- *An. gambiae* s.l. displayed a more exophagic than endophagic tendency in most of the districts and sites.
- The highest biting rate by *An. gambiae* s.l. occurred in September in most of the IRS sites, except in the Nyamugali site; for Rukomo site there is another peak in March. In the control sites (Ngera and Rwamiko) the HBR was very low compared to in intervention sites. The highest bites/person/night rate was observed in the Rukomo site, both indoors and outdoors.
- No meaningful analysis of biting behavior can be made for *An. funestus* s.l. as the numbers collected from the two sites were very low. The *An. funestus* s.l. mean biting rate was very low (the highest was less than 0.5 bites/person/night).
- The mean number of bites per person per hour for *An. gambiae* s.l. through the night across the five districts showed more biting outdoors than indoors except in Nyagatare District, which is consistent with the overall more exophagic behavior of the vector.
- The parity rate was lower in IRS districts than in the control district 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. The sporozoite rate was 1.6% in Nyagatare site, 0.90% in Zaza site, and 3.33% in Rwamiko site. For An. gambiae s.l. the EIR in Nyagatare site (Nyagatare District) was five infective bites/person/year, whereas in Zaza site (Ngoma District) the EIR was two infective bites/person/year. It would be useful to examine the data to see whether there was a similar reduction in malaria morbidity.
- The finding of a *P. falciparum*-infected *An. ziemanni* is also a concern, as this mosquito is also a suspected secondary vector that may contribute to malaria transmission.
- 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.
- A high proportion of unfed *An. gambiae* s.l. (55%) were collected by PSC resting indoors. The fact that these mosquitoes entered the house but were not able to take a bloodmeal could indicate a good rate of ITN use among residents.
- The insecticide used for IRS, Actellic® 300CS, is still killing more than 80% of exposed mosquitoes 10 months after spray, indicating that one round of spraying can provide protection throughout the year irrespective of when the mosquito population peaks.

ANNEX A: SPOROZOITE RATES

Month and Date	Nyagatare District						Ngoma District					
	Nyagatare Site			Rukomo Site			Remera Site			Zaza Site		
	Total Tested	# Positive	% Positive	Total Tested	# Positive	% Positive	Total Tested	# Positive	% Positive	Total Tested	# Positive	% Positive
July 21	3	1	33.3	99	0	0	1	0	0	7	0	0
Aug 21	4	0	0	54	0	0	9	0	0	18	0	0
Sep 21	41	1	2.4	100	0	0	25	0	0	12	1	8.3
Oct 21	20	0	0	20	0	0	14	0	0	8	0	0
Nov 21	32	0	0	32	0	0	4	0	0	17	0	0
Dec 21	6	0	0	1	0	0	5	0	0	6	0	0
Jan 22	4	0	0	12	0	0	0	0	0	8	0	0
Feb 22	3	0	0	38	0	0	0	0	0	10	0	0
Mar 22	2	0	0	75	0	0	1	0	0	11	0	0
Apr 22	5	0	0	35	0	0	5	0	0	5	0	0
May 22	6	0	0	10	0	0	3	0	0	7	0	0
Jun 22	0	0	0	6	0	0	8	0	0	3	0	0

Month	Kirehe District						Control Districts					
	Gatore Site			Nyamugali Site			Ngera Site (Nyaruguru District)			Rwamiko Site (Gicumbi District)		
	Total Tested	# Positive	% Positive	Total Tested	# Positive	% Positive	Total Tested	# Positive	% Positive	Total Tested	# Positive	% Positive
July 21	21	0	0	11	0	0	60	0	0			
Aug 21	18	0	0	0	0	0	27	0	0			
Sep 21	91	0	0	0	0	0	16	0	0			
Oct 21	8	0	0	12	0	0	13	0	0			
Nov 21	13	0	0	6	0	0	18	0	0			
Dec 21	14	0	0	5	0	0	13	0	0			
Jan 22	12	0	0	5	0	0	8	0	0			
Feb 22	14	0	0	3	0	0	13	0	0			
Mar 22	20	0	0	3	0	0	4	0	0			
Apr 22	13	0	0	5	0	0				20	0	0
May 22	14	0	0	2	0	0				4	1	25
Jun 22	10	0	0	6	0	0				6	0	0

ANNEX B. PARITY

	Kirehe District				Ngoma District				Nyagatare District				Control (Nyaruguru and Gicumbi 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
July 21	17	17	5	29.4	6	6	1	16.7	95	44	5	11.4	3	3	0	0
Aug 21	15	14	4	28.5	20	16	4	25	58	32	4	12.5	3	3	1	33.3
Sept 21	40	34	8	23.5	33	24	4	16.7	136	58	19	32.8	1	1	0	0
Oct 21	18	14	2	14.3	17	5	1	20	31	27	3	11	2	2	0	0
Nov 21	12	10	0	0	3	3	0	0	65	33	2	6	1	1	0	0
Dec 21	8	8	1	12.5	0	0	0	0	8	7	1	14	3	3	2	66.7
Jan 22	8	8	3	37.5	2	2	0	0	14	9	1	11	1	1	0	0
Feb 22	13	12	0	0	6	6	0	0	28	22	0	0	1	1	0	0
Mar 22	15	13	3	23	4	3	1	33.3	58	32	0	0	0	0	0	0
Apr 22	15	11	2	18	6	5	1	20	39	28	4	14	12	12	10	83.3
May 22	10	6	1	16.7	2	1	0	0	19	19	3	15.8	0	0	0	0
Jun 22	6	6	1	16.7	1	1	0	0	6	6	1	16.7	4	4	2	50
Total	177	153	30	19.6	100	72	12	16.7	557	317	43	13.6	31	31	15	48.4

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