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Sibling species composition and feeding pattern of malaria vectors in indoor-sprayed and non-sprayed districts of Lira and Kole, northern Uganda

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Abstract

Background Vector control interventions using long-lasting insecticidal nets and indoor residual spraying are common tools deployed for the control of malaria in Uganda. To evaluate the effectiveness of these control tools and understand the prevailing malaria vectors, a study was conducted to determine the species composition, indoor resting population density and biting pattern of malaria vectors in indoor residual spraying (IRS) and non-indoor residual spraying (non-IRS) districts of Lira and Kole, Northern Uganda.

Methods Both indoor and outdoor adult malaria vectors were sampled using Human-Baited Catch and Pyrethrum Spray Catch methods from August to September 2022. Mosquitoes collected were identified to species level using morphological keys and species-specific polymerase chain reaction (PCR) assays. The indoor and outdoor time of biting of the mosquitoes were also recorded.

Results The indoor residual densities (IRD) of anopheles populations in non-IRS sprayed district of Kole did not differ significantly from residual sprayed district of Lira (Man-Whitney U-test, U = 7.0, P = 0.400, N = 6). However, *Anopheles gambiae* sensu stricto (*s.s.*) dominated the vector population (43.1%, 44/102) in the non-IRS district, followed by *An. funestus* (30.4%, 31/102) and *Anopheles arabiensis* (26.5%, 27/102). In the IRS district, *Anopheles funestus* was the predominant species (52.3%, 23/44), followed by *An. gambiae s.s.* (34.1%, 15/44) and *An. arabiensis* (13.61%, 6/44). In IRS district, *An. funestus* and *An. gambiae s.s.* had an indoor biting peak of 03:00–04:00 h and outdoors from 21:00–1:00 h.

Conclusion The findings have important implications for malaria control interventions in areas where IRS is actively used for vector control. However, more longitudinal, ecological, and genetic studies are needed to better understand the entomological impact of indoor residual spraying in northern Uganda.

Keywords Malaria vectors, Species composition, Feeding pattern, Indoor and non-indoor residual spraying, Lira and Kole districts, Northern Uganda

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Background

Malaria remains a leading cause of morbidity and mortality in sub-Saharan Africa, with Uganda among the most affected [1]. The World Health Organization (WHO) estimated that there were approximately 233 million malaria cases and 409,000 deaths in 2022, with the majority of deaths occurring in sub-Saharan Africa [2]. In Uganda, malaria is reported to be the leading cause of morbidity and mortality, accounting for approximately 8–13 million episodes per year; 30–50% of outpatient visits at health facilities, 35% of hospital admissions, and 9–14% of hospital deaths, particularly in children under the age of five [3]. Currently, malaria control in the country is based on case management with artemisinin-based combination therapy and vector control, which involves the use of pyrethroid-treated long-lasting insecticide-treated nets (LLINs) and indoor residual spraying (IRS) with insecticides [4–6]. Long-lasting insecticidal nets (LLINs) are distributed continuously through antenatal and immunization clinics and nationally every three years [7].

In Uganda, IRS was relaunched in 2006 with the support of United States Agency for international Development (USAID), after a long period of no IRS interventions since 1960 s, with a focus on high-transmission and epidemic prone districts in the country's south west [8]. IRS entails spraying insecticide on the interior surfaces of houses to kill mosquitoes that rest indoors, and is conducted annually in selected districts. Between 2007 and 2013, IRS was implemented in 10 highly endemic districts in northern Uganda, initially using bendiocarb for the first three rounds and later switching to a carbamate [9]. The IRS campaign was expanded to 14 historically high burden districts in north-eastern Uganda in 2014. The IRS campaign in the northeast began with a carbamate insecticide (2014–2016), before switching to an organophosphate for the third, fourth, and fifth years (2017–2019). In northern Uganda, following the scale up of IRS, confirmed malaria cases reported from health facilities declined by an average of 10.8% per year between 2013 and 2015, while malaria deaths were reportedly reduced from 59 to 23 deaths per 100,000 between 2010 and 2017 [10–13].

Despite the substantial progress, Uganda was among the four countries accounting for almost half of all malaria cases globally [2, 3, 14]. Several rounds of IRS and widespread use of LLINs, have also led to growing concerns about shifts in malaria vector species composition, resting behaviour and biting patterns, as the disease continues to spread. In some cases, key vector species, such as *Anopheles gambiae* sensu lato (*s. l.*) and *Anopheles funestus* have become resistant to commonly used insecticides classes, including pyrethroids and carbamates [15]. For example, a recent study in Tororo, Eastern Uganda, revealed a shift in the malaria vector from the predominantly *An. gambiae*, which prefers to feed indoors, to *Anopheles arabiensis* following the onset of residual insecticide spraying in 2015. *Anopheles arabiensis* feeds outdoors and can tolerate a wide range of larval conditions habitats, including temporary and permanent man-made habitats, as opposed to *An. gambiae*. [14, 16].

A similar shift in sibling species composition has recently been reported in the Namutumba district, Eastern Uganda [17]. Such changes in species composition may undermine IRS effectiveness because mosquitoes that feed outside are less likely to come into contact with insecticides applied indoors. Furthermore, there is increasing evidence of shifts in vector behaviour, such as feeding outdoors (exophagic behaviour) or on animals (zoophagic behaviour), which allows them avoid control measures [18]. For example, in a study conducted by Padonou et al. [19] in Benin (West Africa), the proportion of mosquitos biting indoors dropped from 67.09 to 42.85% after IRS was implemented. Several studies have also shown that IRS can significantly reduce mosquito populations resting indoors by up to 80% in some cases, depending on coverage and the insecticide used [20, 21]. For example, a recent preliminary study by Sy et al. [21] found that *Anopheles* population the densities were significantly lower in IRS sprayed areas compared to the control. However, areas where IRS is not used tend to maintain higher indoor resting mosquito densities, which could potentially sustain malaria transmission [22]. While these findings provide useful insights, much remains unknown about the specific effects of IRS on the sibling species composition and biting patterns of mosquito vectors, particularly in areas where IRS is implemented. This study aimed to assess the impact of indoor residual spraying with insecticides on the species composition and the indoor and outdoor biting patterns of malaria vectors in northern Uganda, using Lira and Kole districts as model study sites. In Lira district IRS has been in continuous and sustained use (2014–2023), having been launched in 2014 with bendiocarb, a carbamate insecticide, followed by several rounds of other insecticides, such as pirimiphos-methyl (Actellic) and clothianidin (Sumishield). In contrast, the Kole district has received inconsistent IRS applications [3]. It was one of ten districts in Eastern and Northern Uganda that received IRS annually from 2009 to 2014. However, between 2014 and 2016, IRS support shifted to other districts [23]. The difference in IRS use between Lira and Kole districts provides an excellent opportunity to better understand how sustained and continuous IRS use affects malaria vector species composition and resting densities. This knowledge is critical in evaluating the effectiveness

of vector control interventions and tailoring them to the local *Anopheles* populations and their behaviours.

Methods

Study area

The study was conducted in the sub counties of Lira, Agali and Adekokwok in Lira district (IRS implementing area) and in Ayer, Abeli and Okwerodot sub counties in Kole district (non-IRS area) in northern Uganda (Fig. 1). Lira district is located 342 kms (212.81 mi), the capital city of Uganda and coordinates: 02 20 N, 33 06 E (Latitude: 02.3333; Longitude: 33.1000) with a projected population of 242,216 people as of 2024 [24]. Kole district is located at coordinates 02 24 N, 32 48 E and is bordered by Lira district to the east with an estimated projected population of 294,301 [24]. Both districts are located in an area of high malaria transmission intensity in the country and is characterized by numerous temporary and semi-permanent mosquito breeding sites. The two districts experience a bimodal pattern of rainfall with a long rainy season (March–May), which triggers the peak malaria transmission period, short rainy season (September–November), and the hot and dry season is from December to March, which marks a low transmission period. In both districts, the major economic activities include livestock rearing and crop production with rice, millet, maize sorghum, beans, cowpeas and sweet potatoes as the main crops. Malaria vector control interventions in both districts rely on IRS and LLINs, distributed routinely and through mass campaigns in 2013, 2017, 2020, and, most recently in 2023. Indoor residual spraying with bendiocarb was introduced by Government in 14 historically high burden districts in north-eastern Uganda, including Lira district in December 2014 following an upsurge in malaria prevalence [25]. To date, Lira district (hereafter IRS implementing area) has had nine rounds of IRS, with Bendiocarb (December 2014–January 2015, June–July 2015, December 2015, January 2016), three rounds with Actellic (pirimiphos-methyl) (June–July 2016, July–August 2017, June–July 2018), March–April 2019, one round with Sumishield (clothianidin) and thus far, two rounds of Fludora fusion (combination of clothianidin and deltamethrin) conducted in 2020, and the project was expected to expire in May 2023. While IRS in Kole district started in 2009 and came to an end by 2012 [23], hereafter referred to as non-IRS.

Study design

The study was cross-sectional in nature and involved collection of data on species composition, indoor resting population density**,** and biting patterns of malaria vectors from each sampled house once every month in August and September 2022, in both the IRS and non-IRS districts of Lira and Kole, respectively.

Sampling procedure

Using district-based HMIS data, one low, moderate and high malaria burdened subcounty was selected in each district and one village in each of these parishes was randomly selected. In each village, 20 grass-thatched houses with mud walls were randomly selected for PSC. These types of houses provide a favorable, cool, resting place for endophilic and endophagic malaria vectors [26]. Two other houses were randomly selected from each village for human-baited catches, and two nights of mosquito collections were carried out simultaneously in the two districts. Global Positioning System (GPS) coordinates were recorded on the data collection form for the sampled households using a handheld navigational system, to map the vector species composition, indoor resting population densities, and biting pattern of malaria vectors in both districts.

Determination of indoor resting density of malaria vectors and species composition of mosquitoes

Pyrethrum spray catches (PSC)

Houses for PSC were prepared by moving outside all movable items from the house to allow the spreading of the white ground sheets to cover the ground surfaces where mosquitoes found resting indoors would fall dead or knocked down. Two vector control officers sprayed houses with aerosol insecticide, with one spraying from inside a closed door, spraying the eaves and roof while moving in the opposite direction to the one spraying outside eaves to prevent any mosquitoes from escaping. The officer inside then came out and closed the door, timing for 7–10 min to ensure all the mosquitoes present indoors were either killed or knocked down. After 7–10 min, the doors were carefully opened, and the ground sheets that were laid on the ground were systematically folded and brought outside, starting with the one next to the door. All mosquitoes found either dead or knocked down on the ground sheets were carefully picked using forceps and put into a well-labelled petri dish with moist filter paper, and brought to the laboratory for further identification and analysis.

Determination of biting pattern

Mosquito trapping in both IRS and non-IRS districts was conducted using aspirators with trained personnel as bait in a double net for two (2) consecutive nights each month at selected field sites. Both indoor and outdoor biting mosquitoes were collected from 6.00 pm to 6.00 am by a two-person team of trained catchers using bed net traps [1]. The bed net trap was set up by using two

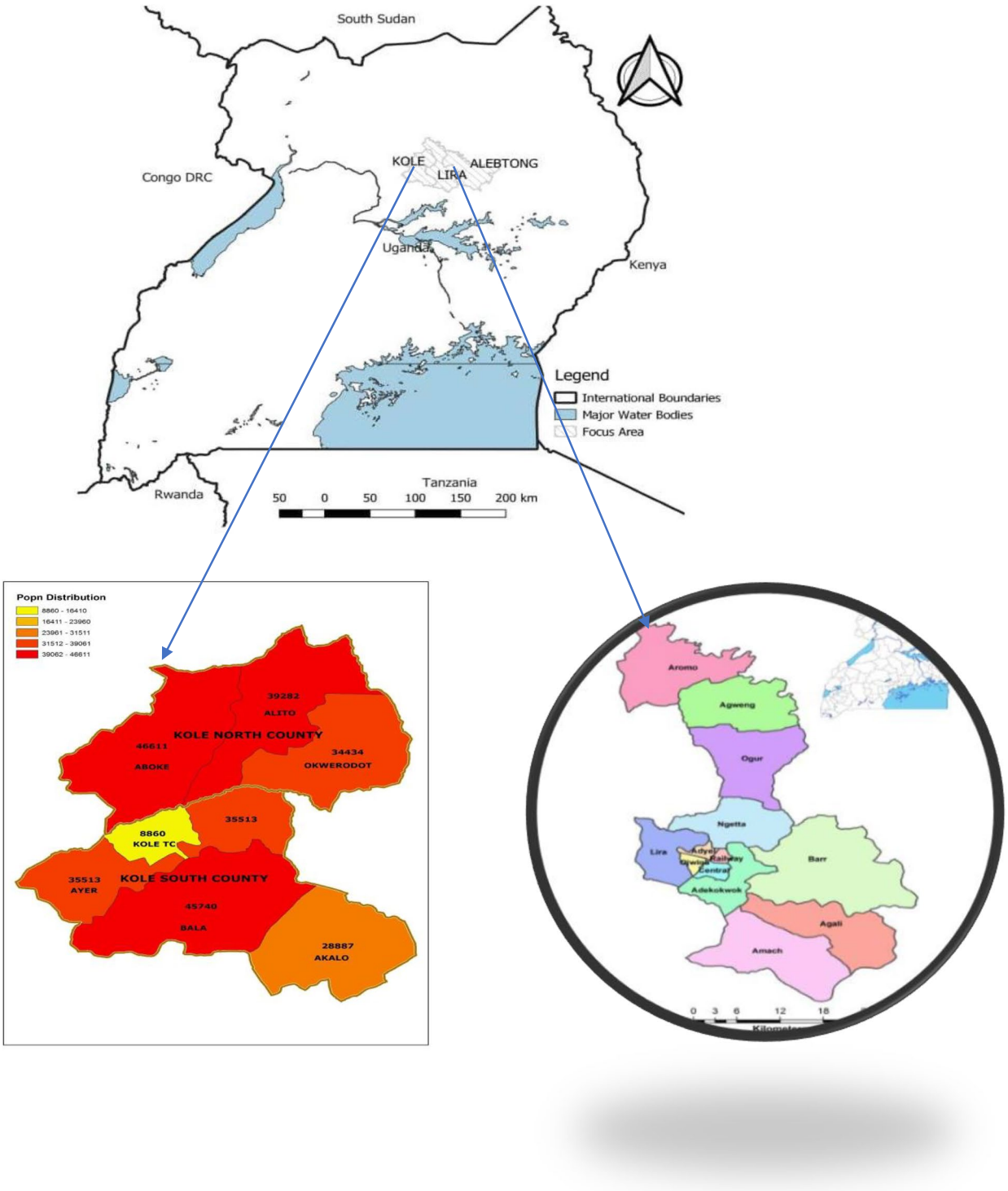

Fig. 1 Map of Uganda showing the location of study sites in both Lira IRS intervention area and Kole non-IRS intervention area. The six study sites in both districts are represented by blue stars in the maps

nets: a smaller inner net in which human volunteers sat and an outer larger net to trap the mosquitoes that was stretched tightly and tied to pegs in the ground leaving 15–20 cm between the ground and the lower edge of the net. People living in a room were each protected with an untreated net. Two catchers sat or laid inside the inner net attracting mosquitoes with their body heat and odor. As mosquitoes persisted in their attempts to look for a blood meal, they were approached, and as they got near the human-baited trap, were caught [27], by the human bait (collector) using an aspirator and a torch [28]. To detect changes in the biting behaviour of indoor-sprayed mosquitoes, an outdoor human-baited net was set up 10 m away from any sleeping house, and both indoor and outdoor catches were conducted concurrently [28]. Six catchers were recruited per house, with each collector working for 4 h while the other rested, and both pairs switched from indoors to outdoors and vice versa after every 4 h. Mosquitoes trapped between the nets were collected after every 10–15 min and aspirated into paper cups corresponding to the hour of collection. Each, hourly catch was separately placed in a paper cup covered with a net and pre-labeled with location, date, and time of capture, and taken to the laboratory for identification. The mosquitoes collected were kept alive by feeding them with a 10% sugar solution through a cotton wick [29].

Identification and molecular characterization of mosquitos

All adult mosquitoes collected were identified morphologically to species level in a field laboratory using morphological keys developed by Coetzee [30] under a high-powered dissecting microscope. Female anopheline mosquitoes were recorded in a form according to their abdominal status as unfed, freshly fed, half gravid, and gravid. Field specimens were individually placed in tubes containing silica gel desiccant and cotton immediately after collection and stored at room temperature until processing. PCR identification of the *An. gambiae s.l.* and *An. funestus* was carried out at Molecular Laboratory of the Vector Borne and Neglected Tropical Diseases Control Division, Ministry of Health. Mosquito DNA was extracted using the Chelex protocol by Musapa et al. [31] and ribosomal DNA amplified following the protocol by Kramer and Coen [32]. Identification of *An. gambiae* complex was performed by PCR amplification following a protocol by Scott et al. [33], while *An. funestus* siblings were identified as performed following the modified protocol described by Koekemoer et al. [34].

Data analysis

The indoor resting density was calculated as the total number of female anopheles mosquitoes collected (by species), divided by the total number of surveyed in that study site. Statistical analyses were performed using SPSS software IBM Statistics 25.0 release (IBM Corporation). The significance of difference in indoor residual densities (IRD) between IRS and Non-IRS was tested using by Mann–Whitney. The level of significance was set at 5% ($P < 0.05$). The human biting rate was calculated as the total number of mosquitoes collected divided by the number of trap nights. Endophagy, exophagy, and nocturnality (the different feeding behaviours exhibited by the proportion of mosquitoes collected between 6:00 pm to 6:00 am), were calculated for each collected mosquito species in both Lira-IRS and Kole- non-IRS districts.

Results

Mosquito species composition

A total of 146 mosquitoes of were collected using pyrethrum spray catch method. Of these, 30.1% (44/146) were captured in the IRS area and 69.9% (102/146) in the non-IRS control areas. Morphological identifications showed that the 92 individuals that were collected belonged to *An. gambiae s.l.* and 54 to *An. funestus* (Fig. 2).

PCR analysis on 146 *Anopheles* samples (Fig. 3a–c), showed that *An. gambiae s.s.* (43.1%, 44/102) was the most prevalent malaria vector sibling species in the non-IRS area, followed by *An. funestus* (30.4%, 31/102) and *An. arabiensis* 27 (26.5%, 27/102) (Table 1). In the IRS district, *An. funestus* was predominant (52.3%, 23/44), followed by An. *gambiae s.s.* (34.1%, 15/44), and *An. arabiensis* (13.6%, 6/44) (Table 1). However, four samples were morphologically unidentifiable and were not analysed. The Bray–Curtis measure of similarity indicated a larger (60.3%) dissimilarity between the IRS and non-IRS districts.

Indoor resting density (IRD) of malaria vectors

Based on the Pyrethrum spray catch (PSC) collections, the Indoor residual density (IRD) of anopheles malaria vectors did not differ significantly between the non-IRS district of Kole the IRS district of Lira (Man-Whitney U-test, $U = 7.0$, $P = 0.400$, $N = 6$, Table 2).

Biting times of malaria vectors

The findings revealed that, *An. funestus* fed after midnight when indoors and was seen in both non-IRS (Kole) and IRS (Lira) districts (Fig. 4), while in the IRS district, *An. funestus* fed late indoors (03:00–04:00 h). In the non-IRS area, *An. funestus* fed rather early (21:00–01:00 h) outdoors but in the IRS district, they were active throughout the night. *Anopheles arabiensis* had two-peak indoor biting times in both non-IRS and IRS districts, however, the IRS district showed a delayed biting pattern. In the case of outdoor biting, *An. arabiensis* had only one

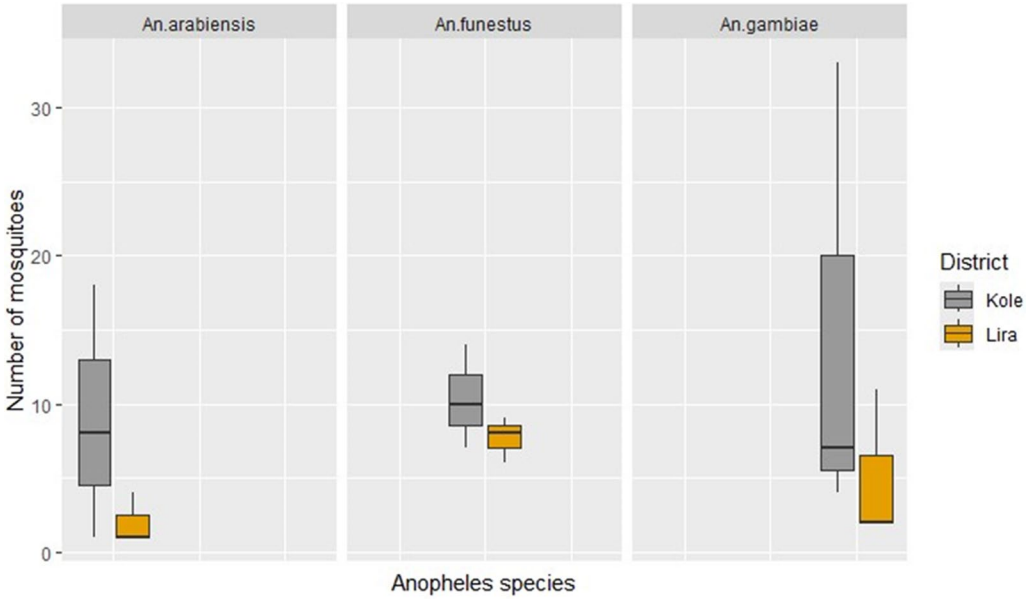

Fig. 2 Species composition of malaria vectors in IRS District (Lira) and non-IRS District (Kole)

peak (21:00–22:00 h) and two peaks (19:00–21:00 h and 04:00–06:00 h) in the non-IRS and IRS districts, respectively. Furthermore, the study indicated that *An. gambiae s.s.* delayed their indoor biting time, and for the IRS district, one peak time (3:00–4:00 h) was observed. In the non-IRS area, two peaks were noted, however, the feeding pattern stopped at 24:00 h. Similar patterns were observed in outdoor biting patterns in both districts. However, in the IRS district, *An. gambiae s.s.* had one early blood meal, contrary to indoor biting behaviour.

Discussion

These results showed no difference in indoor vector density between areas where IRS implementation has been sustained for a longer time compared to that where IRS was discontinued. The results contrasts with the entomological surveillance study conducted in regions bordering Lake Victoria, Tanzania [35], where following IRS, indoor densities were generally low in sprayed sites, at < 3 *An. gambiae s.l.* per trap/night than unsprayed sites. However, it was not possible to rule out the effect of other prevailing vector controlling approaches in the study area other than IRS, including the LLIN.

Nonetheless, a shift in the sibling species composition was observed with continued/sustained implementation of IRS in Lira district. The found that *An. gambiae s.s.* dominated the composition of the vectors in the non-IRS area, whereas *An. funestus* dominated the IRS district. The predominance of *An. funestus* in the IRS area could be attributed to a possible insecticide resistance due to the prolonged indoor residual spraying, while the low numbers of *An. gambiae* may be due to their susceptibility to deployed insecticides. Such shifts in malaria vector species composition have also recently been reported in an earlier study in Namutumba district, Eastern Uganda [17], where, following the upscale of IRS, the predominant sibling malaria species changed to *An. arabiensis*. Similarly, a survey conducted in Tororo, Eastern Uganda also revealed a shift in malaria vector from the predominantly *An. gambiae* to *An. arabiensis* after the implementation of IRS in 2015 [16]. Therefore, the findings of this study, have important implications for malaria control in the two districts.

The findings revealed that *An. funestus* fed after midnight when indoors, and this was clearly shown in both non-IRS and IRS districts. A similar phenomenon in Migori County, western Kenya, where biting by *An. funestus* in the intervention and non-intervention areas, occurred mostly indoors late at night, corresponding to the period when most people were indoors and in bed [36]. However, this study agrees with a study conducted in which *An. funestus* and *An. arabiensis* aggressiveness increased progressively throughout the first half of the night [37].

In this study, *An. arabiensis* had two-peak indoor biting times in both the non-IRS and IRS districts. However, the IRS district exhibited a delayed biting pattern. In the case of outdoors, *An. arabiensis* had one peak only in non-IRS and two peaks in the IRS district. These findings agree with the study [35] where in sprayed sites, the *An. gambiae s.l.* biting rate was higher outdoors than indoors at all times at night. This provides evidence that

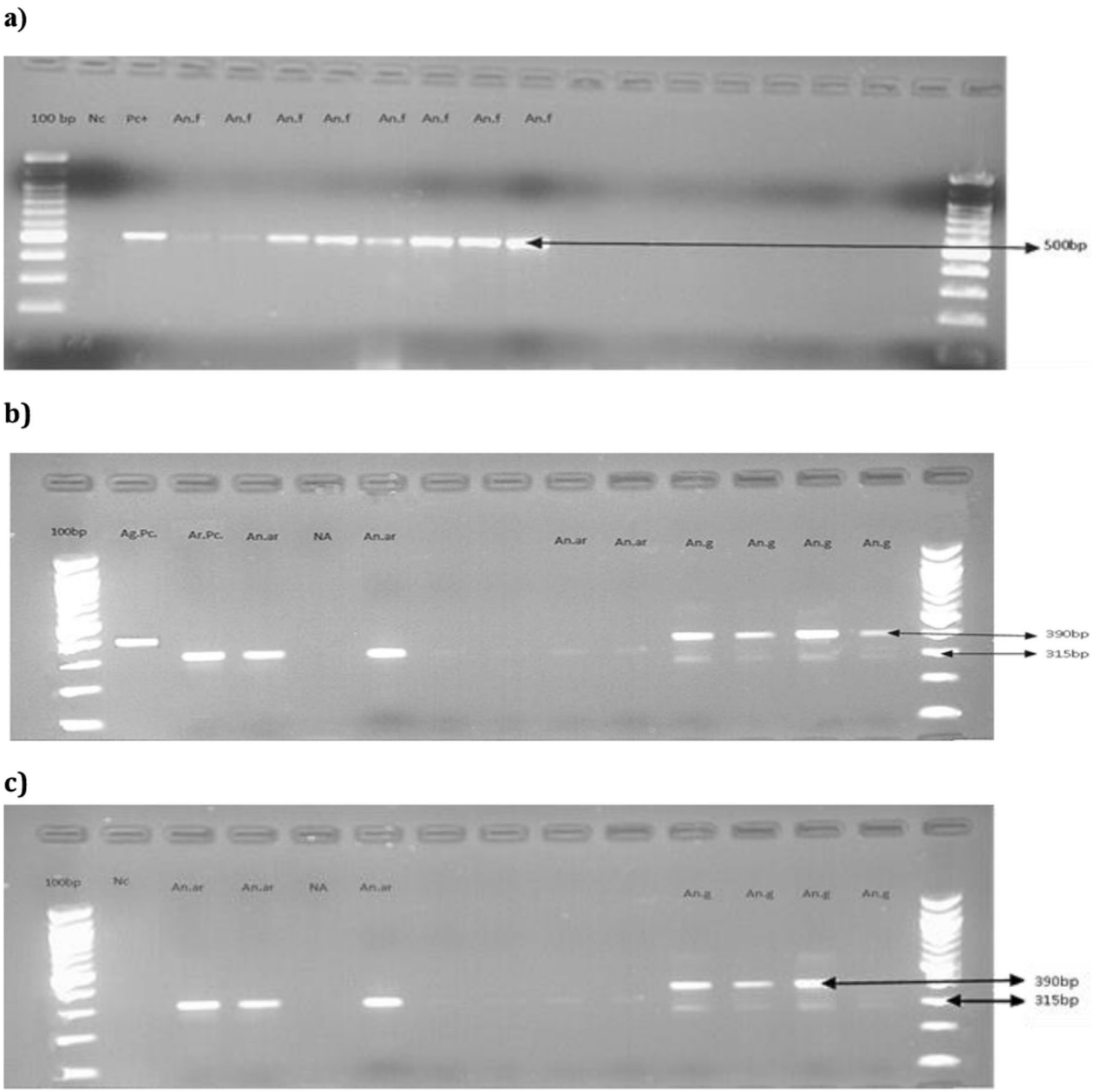

Fig. 3 PCR representative gel picture after electrophoresis on 1.5% agarose gel for **a** *An. funestus* (expected band size of 500 bp), **b** *An. arabiensis* (315 bp), and **c** *An. gambiae s.l.* (390 bp) DNA samples. Lane one is 100 bp-100 base pair ladder, Pc+: Positive control, An. F: *Anopheles funestus* s.s, An. ar: *An. arabiensis* and An. g: *An. gambiae* s.s

An. gambiae s.l. (mostly *An. arabiensis*) may modify their behaviour to avoid contact with insecticide-sprayed walls. However, it should be noted that most *An. gambiae s.l.* in all sites (except in Lira) were collected later in the evening when most people were likely to be indoors and protected by LLINs. Nevertheless, a greater degree of outdoor biting risk was observed early in the evening at the sprayed sites than at the unsprayed sites.

Furthermore, this study indicated that *An. gambiae s.s.* delayed their indoor biting times; for the IRS district, one peak time was observed. In the non-IRS area, two peaks were noted, however, the feeding pattern stopped at 24:00. Similar patterns were shown in outdoor biting patterns in both districts. However, in the IRS district, *An. gambiae s.s.* had one early blood meal, contrary to indoor biting behaviour. Generally, in this

Table 1 Species composition of malaria vectors in IRS and Non-IRS districts of northern Uganda

District	Sub-county	Number of malaria vector		
		An. arabiensis	*An. funestus*	*An. gambiae*
IRS (Lira)	Agali	1	9	2
	Adekokwok	1	6	2
	Lira	4	8	11
Vector's composition in IRS District: N (%)		6(13.6)	23(52.3)	15(34.1)
Non-IRS (Kole)	Ayer	18	14	33
	Abeli	8	10	7
	Okwerodot	1	7	4
Vector's composition in Non-IRS District: N (%)		27(26.5)	31(30.4)	44(43.1)

Table 2 Indoor Resting Density (IRD) of malaria vectors in three sub-counties in Lira and Kole Districts, Northern Uganda

District	Sub-county	No. of Vectors (%)	No. of structure	IRD
Lira	Lira	25 (16.7)	20	1.25
	Adekokwok	9 (6)	20	0.45
	Agali	14 (9.3)	20	0.70
Kole	Ayer	64 (42.7)	20	3.20
	Okwerodot	12 (8)	20	0.60
	Abeli	26 (17.3)	20	1.30

study, the majority of biting by *An. gambiae* and *An. funestus* was in the last third of the night with a peak biting between 24.00 to 02.00 h. This was consistent with the study conducted by Kabale [38] from Kamuli district, where the majority of the biting occurred in the last third of the night with peak biting by *An. gambiae* and *An. funestus* between 23.00 and 05.00 h. This is consistent with the known time of peak biting for the majority of human-biting sporozoites positive *An. gambiae* and *An. funestus* mosquitoes, i.e. between 23.00 and 05.00 h, a period when most people are in bed and under nets if they have them. This indicates that the population could be protected while resting indoors, as the peaks observed during the night correspond to moments when the population is asleep, unlike the peak that occurs during the morning when people are awake and remain unprotected by the LLINs. Thus, in areas planning to eliminate malaria, more attention should be paid to the possible morning biting activity of *Anopheles*, which essentially takes place indoors and throughout the day and could maintain residual levels of *Plasmodium* transmission.

Conclusions

The major and efficient malaria vectors in the study area belonged to *An. gambiae s.s.* and the *An. funestus* complex. The predominant number of malaria vectors of *An. gambiae* in non-IRS district and *An. funestus* in the IRS district predicts a possibility of a malaria upsurge and a very high malaria transmission potential within the study area. In general, the indoor resting density in the non-IRS district was significantly higher than that in the IRS district, although there was no statistically significant difference between the IRD in the two districts. Therefore, behaviour change communication (BCC) and community sensitization on the proper and consistent use of vector control tools in these two districts should be taken as a priority for the control of malaria. These could take care of the morning biting activity of anopheles which essentially takes place indoors and possibly throughout the day maintaining residual levels of malaria transmission. Also, the District Health Office must deploy outdoor vector control interventions, such as Larval Source management, to address the threat of outdoor transmission by *An. arabiensis* mosquito collected from the study area. Additionally, more studies should be conducted to ascertain the preferred blood meal and the role of *An. arabiensis* in the transmission of malaria in the study area, since they were collected, biting from both indoors and outdoors. The predominance of *An. funestus* in the IRS district indicates the possibility of insecticide resistance, and in this study being a cross-sectional study, insecticide susceptibility studies are recommended to confirm the existence and level of resistance. The gap between the numbers of *An. funestus* in IRS districts and non-IRS districts was small. Therefore, there is a need for both districts to map all potential and active breeding sites for the control of young stages of *An. funestus.* In both the IRS and non-IRS districts of Lira and Kole, there is a need to maintain a significant healthcare system that allows rapid

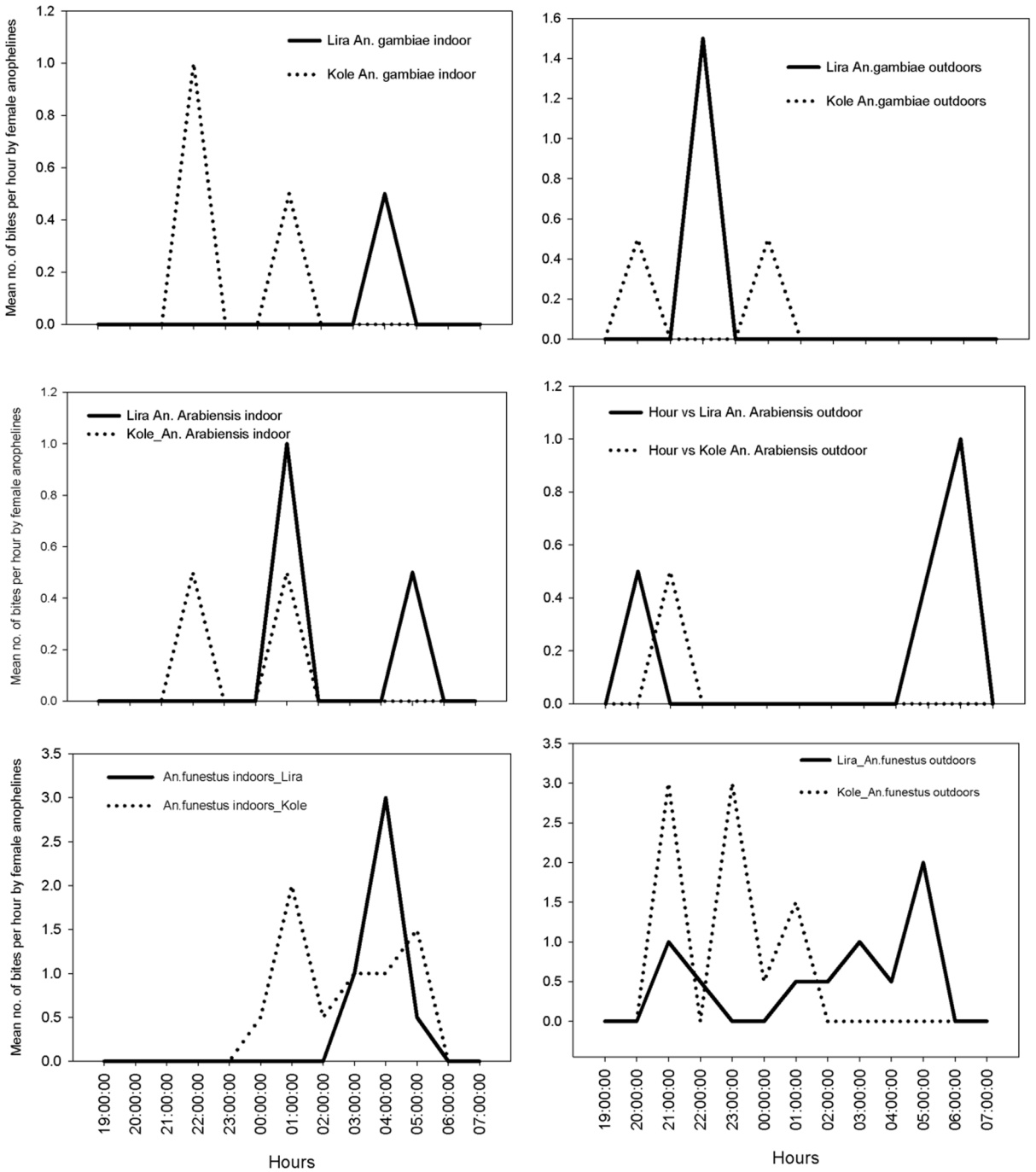

Fig. 4 Biting pattern of the three different species of malaria vectors in IRS district (Lira) and non-IRS district (Kole)

diagnosis and malaria case management combined with close entomological monitoring to detect and prevent outbreaks that could result from low levels of transmission. The high density of *An. funestus* outdoor biting, which was observed in both the IRS and non-IRS districts could sustain the residual transmission of malaria, therefore, the complexity of human exposure to *Anopheles* bites should draw attention to controlling indoor and outdoor residual exposure when IRS or LLINs are in use. Therefore, a more comprehensive understanding of the spatiotemporal dynamic and adaptive responses of *Anopheles* to insecticide-treated tools is required to address some of these issues.

Limitations to the study

This study was limited to a cross-sectional design; therefore, it was not possible to determine if the seasonality influenced the *Anopheles* vector composition, indoor resting density of malaria vectors, abundance, and malaria transmission in both the IRS and non-IRS districts. Therefore, longitudinal studies should be conducted in these two study areas and more sub-counties with a variety of collection methods, including outdoor collections.

Abbreviations

ACT	Artemisinin-based combination therapy
CDC	Centers for Disease Control
CDC-LT	Centers for Disease Control and Prevention-Light Traps
DHIS2	District Health Information System Version two
GUREC	Gulu University Research and Ethics Committee
HBI	Human Blood Index
HLC	Human Landing Catches
IRD	Indoor resting density
IMPs	Intentional Mismatch Primers
IPTp	Intermittent preventive treatment (of malaria) during pregnancy
IRS	Indoor residual spraying
IVM	Integrated Vector Management
LLINs	Long-lasting insecticidal nets
PCR	Polymerase chain reactions
PSC	Pyrethrum spray catch
SNP	Single nucleotide polymorphism

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Author contributions

MR, RO, HA, and SO conceived the study idea, and participated in study design, data acquisition, analysis, and interpretation. MR, RO, HA, SO, JPB, DO, OPB, and GMM participated in manuscript drafting and revision. All authors read and approved the final manuscript for submission.

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Availability of data and materials

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

Before the start of the study, approval was obtained from the Gulu University Research and Ethics Committee (GUREC-2022-336), district authorities, and household owners. Village members were sensitized before the study, and their permission was obtained while the privacy and psychosocial needs of the individual participants and household members were highly protected and confidential. Catchers were selected from the local community to facilitate their acceptance by residents. Informed consent was obtained from each catcher and household head. The catchers were trained to collect landing mosquitoes before blood feeding. They were given antimalarial medicine as prophylaxis to reduce the risk of malaria transmission. The occupants of the participating households were given at least two-bed nets following the study and some disturbance allowances.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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