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Impact of interventions on mosquitoes resting behaviour and species composition in Lugeye village in Magu district, Northwestern Tanzania

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Abstract

Background: Understanding the impact of intervention tools on vector behaviour, host preference, resting, and infectivity rates is paramount in malaria control planning. Magu district was one of the districts in lake zone regions in northwestern Tanzania covered with indoor residual spray and long-lasting insecticidal nets in the main malaria control campaign. After interventions, this study evaluated the mosquito's host preference and resting behaviour in Lugeye village in rainy and dry seasons.

Methods: Mosquitoes were collected both indoors and outdoors using the rest pots. The collection was done in both rainy and dry seasons. The samples were collected bi-weekly for three months each season.

Results: A total of 254 mosquitoes were collected in both dry and rainy seasons, indoors and outdoors. The most abundant species were *Anopheles funestus* s.s. and *An.arabiensis*. Most blood meals from bovines rested outdoors, while human blood meal sources rested outdoors. Sporozoite-positive mosquitoes were found only during the dry season.

Conclusion: This study's findings have shown that implementing IRS and LLIN interventions has led to a species shift from An.gambiae s.s. to An.funestus and An.arabiensis. The inclusion of vector insecticide resistance information can be of paramount importance in appropriate intervention tool selection.

Keywords: Bloodmeals, *An.funestus, An.arabiensis,* outdoor, indoor

Introduction

Malaria is still one of the public health challenges in sub-Saharan Africa(WHO, 2023). In sub -Saharan Africa, four countries have been reported contributing the highest malaria cases globally within the continent (Nigeria (26.8%), the Democratic Republic of the Congo (12.3%), Uganda (5.1%) and Mozambique (4.2%)) and other four countries contributing more than half of malaria death globally are, Nigeria (31.1%), the Democratic Republic of the Congo (11.6%), Niger (5.6%) and the United Republic of Tanzania (4.4%)(WHO, 2023). The malaria-related mortality has been decreasing from 25 in 2000 to 10 in 2019 deaths per 100,000 populations at risk (WHO, 2020). All these achievements have been attained due to the implementation of sensitive malaria diagnostic tools, the prescription of appropriate anti-malarial drugs and effective vector control tools (WHO, 2020). The effective vector control has been implemented widely using long-lasting insecticidal nets (LLINs), Indoor residual spray (IRS) and in very limited application of Larval Source Management (LSM)(Derua et al., 2019; Diouf et al., 2020; Tusting et al., 2013; Zhou et al., 2020).

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Tanzania's main efficient malaria vectors are the sibling species of *Anopheles gambiae* and *An. funestus* group (Kabula et al., 2011; Kweka et al., 2008a; Kweka et al., 2020). Among the *An. gambiae* sibling species, *An. arabiensis*, *An. gambiae* s.s. and *An. merus* have been reported to be vectors in Tanzania (Kweka et al., 2008a; Kweka et al., 2020; Kweka et al., 2008b). In *An. funestus* group, Tanzania's most efficient recorded vectors are *An. funestus* s.s (Kweka et al., 2008a; Kweka et al., 2020), *An. parensis*(Kweka et al., 2008a), *An. rivulorum* (Kweka et al., 2008a; Kweka et al., 2020) and *An. leesoni* (Kweka et al., 2020).

Since the wide community coverage with LLINs in 2000, the vector population has been shrinking across the country, and vector species have shifted. (Bayoh et al., 2010; Kitau et al., 2012)Due to the wide coverage of indoor surfaces with insecticides, either LLINs or IRS, mosquitoes have opted to forfeit the benefits of LLINs and IRS by feeding and resting outdoors. (Russell et al., 2011) or developing insecticide resistance for progeny survivorship (Kreppel et al., 2020; Kulkarni et al., 2006; Mahande et al., 2012; Mbepera et al., 2017; Nnko et al., 2017). The increased proportion of outdoor vectors leading to outdoor residual malaria transmission has been witnessed in different areas with high malaria transmission (Russell et al., 2011). Insecticide resistance in vector populations has been found to exist in all classes of Insecticides used for public health vector control. (Kabula et al., 2014; Matowo et al., 2014).

In Lake Zone regions, including the study areas, Magu District, with 91.8% coverage between 2015 to2017 were sprayed with Actellic 300CS (Primiphos methyl) (Kakilla et al., 2020; Mashauri et al., 2017). Insecticide resistance has been reported to occur in this region among vector species (Kakilla et al., 2020; Kisinza et al., 2017; Philbert et al., 2017)The occurrence of insecticide resistance threatens the use of tools with insecticides. This study assessed the impact of the intervention tools implemented in the study area on species composition, feeding and resting behavior, and infective rates among vector populations in rainy and dry seasons.

Material and Methods

Study Site

This study was conducted in the Lugeye village (02.332159S, 33.150529E) in Magu District, Mwanza Region. Mwanza is among the Lake Zone regions with high malaria prevalence in Tanzania. They are highly inhabited by peasants who produce maize, paddy, cotton, and vegetables in small-scale farming. The study area has two rain seasons: the long rain season starts from October to December, while the short rain season starts from February to April. This district had full coverage of IRS using Primiphos methyl insecticides and LLINs (Kakilla et al., 2020; Mashauri et al., 2017) .

Mosquitoes Collection and Identification

Mosquitoes were collected using two methods during the study. Firstly, the Center for Disease Control (CDC) light trap (model 512, John W. Hock Company, Gainesville, FL) was operated as elaborated by a previous study for the collection of indoor host-seeking mosquitoes (Lines et al., 1991; Shiff et al., 1995). Secondly, outdoor resting mosquitoes were collected using pots previously utilized for the purpose (Odiere et al., 2007; van den Bijllaardt et al., 2009). Twenty houses were used, each house trapping mosquitoes both indoors and outdoors over the same night. The collection was done for one month in the rainy and dry seasons. The mosquitoes collected were identified morphologically in the field using the key developed by Gillies and Coetzee (Gillies and Coetzee, 1987). The collected *An. gambie* s.l. mosquitoes were identified at species level using the method developed by Scot and others (Scott, Brogdon and Collins, 1993), while the *An. funestus* sibling species were identified using a method developed by Koekemoer and others (Koekemoer et al., 2002). Mosquitoes were separated by seasons collected.

Host blood meal identification.

The blood-fed mosquitoes collected indoors and outdoors were prepared by smearing the abdomen in the Whatman filter paper No.1(Bray, Gill and Killick-Kendrick, 1984) .They were labelled by place and date of collection. The blood meal source host was identified using the Enzymes-linked immunosorbent assay (ELISA) protocol. (Beier et al., 1988). The study tested four hosts as possible blood meal sources: bovine, goat, dog, and human.

Sporozoite rates

The collected mosquitoes of both *An. gambiae* s.l. and *An. funestus* group, the head and thorax were taken and subjected to the ELISA protocol developed by Wirtz et al. 1987 (Wirtz et al., 1987). The specimen was considered positive when the cut-off value was similar to or above the positive control.

Data Analysis

The data analysis was done using 1BM SPSS Version 26 (IMB Corp., Armonk, NY, USA). The proportion of mosquitoes collected outdoors and indoors was compared using the Chi-square test. The comparison by seasons was done using the Chi-square test. The comparison was regarded to have significance when the P-value was less than 5%.

Results

Mosquitoes collection and species identification

A total of 254 mosquitoes were collected indoors and outdoors for both seasons. In the rainy season, eight (8) (100%) mosquitoes were sampled, and all were identified as *An. leesoni*. In dry season 246 mosquitoes were collected, 96 (39.02) were *An. arabiensis*, 129 (52.44%). *An. funestus s.s.*, 1 (0.41%) *An. constani* and 20(8.13%) specimens were not identified (Figure 1). The *An. funestus s.s.* abundance was statistically significantly higher in the dry season than in the rainy season ($C^2 = 68.59$, P<0.001, Figure 1). The abundance of *An. arabiensis* was statistically abounding in the dry season and then in the rainy season ($C^2 = 45.13$, *P*<0.001, Figure 1). *An. leesoni* population was higher in the rainy season than in the dry season, which was found to be statistically significant (C^2=192.08, P<0.001, Figure 1).

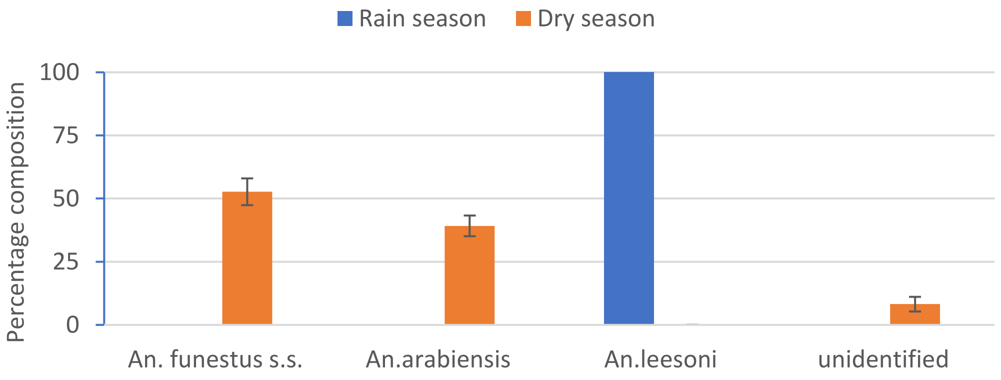

Figure 1: The species composition of mosquitoes collected in all seasons.

Blood meal host identification

A total of 115 blood meal samples were analysed. Twelve (12) samples were collected in dry season while one hundred and three (103) were sampled in dry season. In rainy season, 6(50%) were from Bovine, 5(41.67%) from Human, 1(8.33) from dog while none was found from goats (Figure 2**).** In dry season 103 samples of blood were collected. Twelve (12) (11.65%) were unidentified, 24(23.30%) were from bovine, 1(0.97%) was from Dog, 5(4.85%) were from Goat while 61(59.22%) were from Human **(**Figure 2**).** The comparison of blood meals source by season was found to be statistically significant for three host species. Bovine caught most outdoor(x^2=15.73, P<0.001), Human caught most indoor (C^2=5.78 P=0.016), Dog (C^2=5.70 P=0.017) and Goad (C^2=2.75, P=0.097) (Figure 2). The comparison of blood meal by site of mosquitoes collection (outdoor/indoor) found that, for the three host species, there was statistically significant different results between Indoor and outdoor. In Bovine (C^2=70.19, P<0.001), Human (C^2=85.62, P<0.001), Dog (C^2=7.79, P<0.005) and Goat (C^2=0.12, P<0.733) (Figure 3**).**

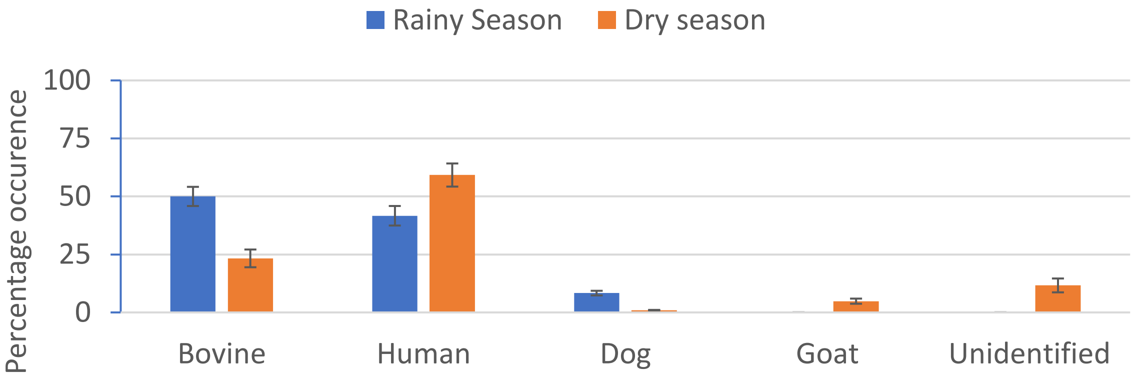

Figure 2: The bloodmeal analysis in seasonality from different hosts

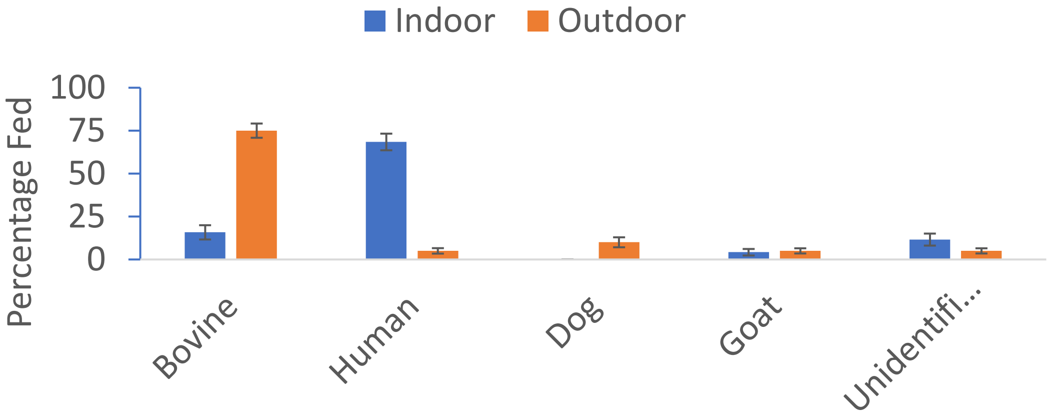

Figure 3: The bloodmeal analysis by resting position of vectors from different hosts.

Sporozoite rates

In the dry season, all eight (8) mosquitoes collected were found to be circumsporozoite protein-negative. In the rainy season, among 246 mosquitoes tested, 10 (4.07%) were found to be circumsporozoite protein positive. Among those tested positive, 6(60%) were An. arabiensis, and 4 (40%) were An. funestus s.s. All sporozoite-positive mosquitoes were collected in the dry season.

Discussion

The findings of this study have shown that malaria vector abundance has been influenced by seasonality in the population of *An. funestus* s.s. dominating during dry season while and *An. arabiensis* dominating the rainy season. The similar species composition was revealed by previous study conducted in a similar area (Kakilla et al., 2020). Also, in other areas of Tanzania, there has been predominance of *An. arabiensis* and *An. funestus* sibling species in the recent past (Kweka et al., 2008a; Kweka et al., 2020; Kweka et al., 2008b; Lwetoijera et al., 2014).

This confirms that, by far, *An. arabiensis* and *An.funestus* are the major malaria vectors in mainland Tanzania (Kweka et al., 2008a; Kweka et al., 2020; Kweka et al., 2008b; Lwetoijera et al., 2014). In this study site, there is a high population shift of the malaria vectors; in a study conducted in the district before the mass intervention of IRS and LLINs, *An. gambiae* s.s. had an upper hand over *An. arabiensis* (Kisinza et al., 2017)*This study revealed that the population of An. gambiae s.s. has been diminished and replaced by An. arabiensis and An. funestus s.s.*

These vectors recently have shown high tolerance to different classes of insecticides used in LLINs and IRS (Kabula et al., 2014; Kakilla et al., 2020; Kisinza et al., 2017; Matowo et al., 2014; Mbepera et al., 2017; Nnko et al., 2017). Different vector species have shown to have different mechanisms to tolerate insecticides toxicity such as biochemical and behavioral resistance (Kulkarni et al., 2006; Kweka et al., 2020; Matowo et al., 2014; Yewhalaw and Kweka, 2016)These mechanisms have enhanced the survivorship of the vector population and transmitted malaria across different ecological areas despite intensive interventions.

The vector abundance was found to be higher in the dry season than the rainy season; this agrees with the ecological studies of the species, which found that in rainy season habitat washing is higher in habitats with shallow water and stable in large water bodies such as in swamps were *An.funestus* and *An. gambiae* s.l. breed most (Kweka et al., 2012). In this study, the *An. arabiensis* and *An. funestus* were abundant in the dry season than in the rainy season. That was found to agree with the ecological conditions of the vector habitats which gain temperature to enhance larval growth and shorten the life cycle in the dry season (Mala et al., 2011). The study site has stable habitats, which become more productive in the dry season than in the rainy season.

A similar scenario has been found by previous studies in different area across Africa for having more vectors in the dry season than in rainy season (Fillinger et al., 2009; Kweka et al., 2011; Kweka et al., 2012). The results of the current study of having high population of vectors in dry season is contrary to what was found in other malaria endemic countries where the population was bottlenecked during the season (Dao et al., 2014) while in Brazil a study indicated reduced adult survivorship when the temperature is increased in the field (Chu et al., 2020). It's also known that, during the rainy season the movements of mosquitoes are restricted due to rain (Roiz et al., 2010). This might have contributed to the observed scenario in this study.

The findings of this study also have shown that bovines and humans were highly preferred hosts while dogs and goats were the least. According to highly abundant species *An. arabiensis* (zoophilic species) and *An. funestus s.s.* (anthropophilic species) which prefers bovine and humans, respectively, most similar preference was observed in previous studies (Kibret et al., 2017).

In both seasons, the higher blood meal was found to be from humans and animals due to vector preference of vectors and accessibility of the hosts (Mahande et al., 2007). The high access to human blood by vectors was worrying as the community had high coverage IRS and LLINs interventions. This high blood meal access from human might be attributed by the insecticide tolerance level within the vector population (Kakilla et al., 2020; Kisinza et al., 2017). Similar has been found that insecticide tolerance enhances the vectors to access human bloodmeals regardless of intervention covering the population (Glunt et al., 2018).

The findings on sporozoite rates have shown that in this study, the infected mosquitoes were found during the dry season, while in the rainy season, none contained sporozoite protein. The dry season has been found to have no shelters outdoors to hide during the day and, therefore, resting mostly indoors. Resting indoors increases the human-vector contact risks. In a previous study it was revealed that adult mosquitoes cannot tolerate high temperatures, therefore during the day they have to hide under the shaded area which include human shelters and cowsheds (Faye et al., 1997; Magombedze, Ferguson and Ghani, 2018; Mayagaya et al., 2015)The increase in sporozoite rates in An. funestus and An. arabiensis characterizes the malaria transmission efficiency played by the two species in the lake zone.

Conclusion

The findings of this study have shown that *An. arabiensis* and *An. funestus* are the main malaria vectors in the study site, with high abundance and infectivity in the dry season. Assessing their insecticide resistance can generate complementary information for designing effective control programmes.

Declarations

Ethics approval and consent to participate: The Catholic University of Health and Allied Sciences gave the ethical approval.

Consent for publication: Not applicable

Availability of data and materials: All data used in this study will be available upon request from the corresponding author

Competing interests: Authors declared to have no competing interests

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Authors' contributions: EJK conceptualized and designed the study, and HDM coordinated field activities. EJK and HDM also conducted data analysis and manuscript writing. Both have endorsed the submission of this manuscript.

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