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Composition and abundance of malaria vectors and demographic risk factors in a high endemic area of rural eastern Tanzania

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Abstract
We assessed composition, abundance and behaviour of malaria vectors, and demographic risk factors in eastern Tanzania. Mosquitoes were collected from 10 households per ward and 10 outdoor points using CDC light traps. Assessment of demographic factors was done in 100 households per ward through interviews and direct observation. Total of 1238 anophelines were collected: *An. gambiae* s.l. (95.48%) and *An. funestus* s.l. (4.52%). Abundance of *An. gambiae* s.l. was 3-fold higher during wet season. Abundance of *An. funestus* s.l. was higher during dry than wet season. Abundance of *An. gambiae* s.l. was 20-fold higher than *An. funestus* s.l. during wet season. Mean abundance per house was 4.35 for *An. gambiae* s.l. and 0.04 for *An. funestus* s.l. in Kiroka, and 1.18 for *An. gambiae* s.l. and 0.92 for *An. funestus* s.l. in Mkuyuni. >95% of households had open-eaves. >76% of households were cooking outdoors. Only 50% of study households owned bednets. Conclusively, vector population in the study area composed of *An. gambiae* s.l followed by *An. funestus* s.l., and abundance was higher indoors during wet than dry season. These along with risk factors like large proportion of open-eaves, low-bednet coverage and outdoor activities suggest high transmission risk in the study-area.

Keywords: Malaria vectors composition, abundance, Kiroka, Mkuyuni

Introduction

Mosquitoes transmit several infectious diseases, which greatly affect the health and socio-economic development of many countries worldwide, particularly in sub-Saharan Africa. These diseases include among other malaria, lymphatic filariasis, dengue and Rift Valley fever. Malaria causes the highest number of cases and deaths [1]. Malaria caused 228 million cases and 405 thousand deaths worldwide in 2019 [1]. African countries accounted for 93% and 94% of the cases and deaths respectively [1]. These figures are lower by more than 50% to what was experienced over the last decade consequent to improved use and coverage of long-lasting insecticidal nets (LLINs), indoor residual spraying (IRS), diagnostics and treatment. However, they are still intolerably high, and thus continued control efforts are needed to reduce malaria burden and achieve elimination.

Tanzania is equally experiencing a high malaria burden, and the population at risk has been increasing over the years. Over 95% of its population is living in areas with high malaria transmission risk [2]. Malaria causes approximately 7.7 million cases [2, 3] and accounts for 33.4‒42.1% of all hospital admissions in the country annually [4, 5]. Under-five children and pregnant women suffer the greatest burden. However, in Tanzania and elsewhere the burden is increasingly shifting to older age categories [6-9]. Like in other endemic areas, primary disease vectors include *Anopheles gambiae* s.s, *Anopheles arabiensis* and *Anopheles funestus* [10, 11, 12]. Within the last 10-15 years, there has been a shift in the composition of these vectors across the country, mainly from *An. gambiae* s.s to *An. arabiensis*. *An. arabiensis* is becoming the dominant malaria vector in sub-Saharan Africa [13-15]. The population and contribution of *An. funestus* in malaria transmission has also increased [16-18]. Notably, *An. funestus* is contributing to >85% of the ongoing malaria transmission events in south-eastern Tanzania despite their lower densities compared to *An. arabiensis* [18]. Moreover, the population of secondary and/or tertiary malaria vectors is increasing in Tanzania and elsewhere in SSA [19-21]. However, the contribution of these vectors in malaria transmission in the country remains speculative. Despite the significant reduction of malaria in the country including eastern Tanzania, foci of high endemicity are purported to remain. This is because malaria is characterized by temporal variability that bestows evolving and new challenges for control strategies [22].

The variability is driven by several factors including the composition, behaviour, and density of mosquito vectors species and/or groups. The same species of mosquitoes in different geographical areas may vary concerning their behaviour, composition, and density [23]. The density of malaria vectors varies among areas within small proximity [22, 24-26]. These go hand in hand with variability in terms of other environmental malaria transmission risk factors such as house types and mosquito entry points, the proximity of settlements to crop fields, livestock keeping, and bed net use. As such, well-targeted efforts that embrace area-specific situations, at least in the remaining high malaria intensity foci, are needed to preserve health gains achieved so far and achieve elimination. Therefore, it compelling to have up-to-date information on the composition and density of malaria vectors to guide the implementation of appropriate vector surveillance and control strategies [27]. Retrospective analysis of malaria cases in eastern Tanzania revealed two potentially high malaria endemic wards, Mkuyuni, and Kiroka, with a prevalence of up to 61% [28]. However, no studies have been conducted to obtain up-to-date information on malaria vector composition, abundance, and behaviour, and demographic/household risk factors. Therefore this study was conducted with an objective to update the malaria vector composition, seasonal abundance, and behaviour, and assess demographic/household transmission risk factors across the wards.

Materials and methods

Study area

The study was conducted in Mkuyuni (latitude 6.57° south and longitude 37.48° east) and Kiroka (latitude 6.83° south and longitude 37.78° east) (Figure 1). These wards are next to each other and are part of Morogoro Rural District, eastern Tanzania. Mkuyuni covers 97.4km^2 with a population of 17,935 people [29]. Kiroka covers 212km^2 with a population of 21,853 people [29]. Agriculture is the major economic activity across the two wards; and the main crops are rice, maize, banana, and coconuts. The long rain season runs from March to August and the short season runs from September to mid-December. The dry season runs from January to end of February.

Study design

This was a repeated cross-sectional entomological survey conducted during the wet and dry season. The survey was done over two months each season, once every month, for five consecutive nights. The survey involved 10 households and outdoor points per ward. The households were selected using a simple random sampling technique. The assessment of potential malaria transmission risk factors was done in 200, 100 households per ward. These households were also selected using a simple random sampling technique across the same villages where the entomological survey was conducted. The assessment was done before the entomological surveys and was done through interviews and direct observations.

Mosquito sampling and identification

Mosquitoes were collected using standard Centre for Disease Control and Prevention light traps (CDC, Atlanta, GA, USA). Mosquito collections were done in 20 households (10 households per) between 1800 and 0600 hrs for 10 nights each season. As such, each ward had a total of 100-night catches per season. One light trap was hung, 30 cm high, at the foot-end of a person sleeping under LLIN; and a second trap was positioned outdoors, 30 cm high and 5 m away from the household. Due to unavoidable circumstances, outdoor collection was only done in Kiroka. The traps were removed in the morning, all cups placed in the cool box and transported to Pest Management Centre for processing and identification. The mosquitoes were identified using taxonomic keys [30] at our laboratory and double-checked by another expert (Mr J. Myamba) with over 30 years experience in mosquito identification, from the National Institute for Medical Research (NIMR), Muheza branch, northern Tanzania.

Assessment of demographic/household transmission risk factors

The potential risk factors assessed included mosquito entry points on different parts of the house, agricultural activities (cultivated crops, the proximity of crop fields to settlements), night outdoor activities (cooking), ownership of bed nets and livestock keeping (the type of animals, number and where they were kept during the night). The household head or anybody older than 18 years was interviewed after verbal consent.

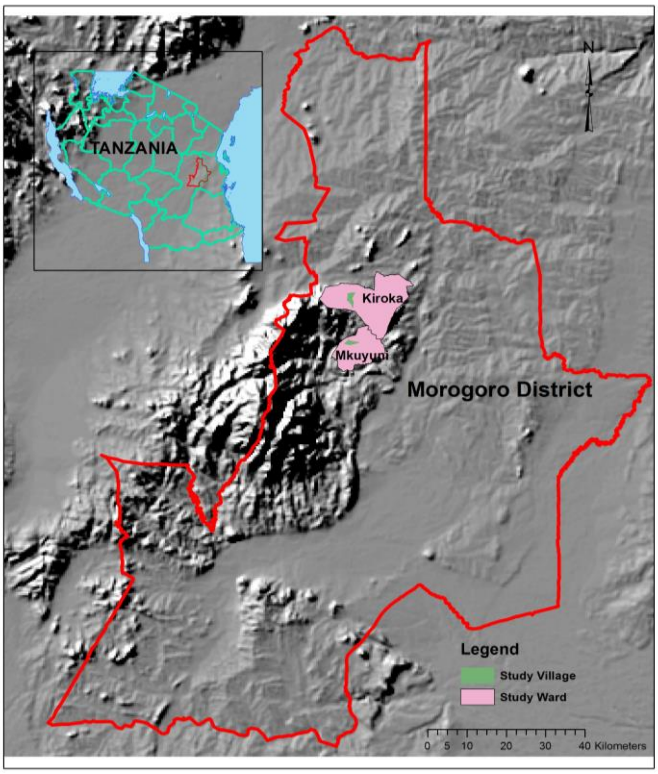

Fig 1: Map of the study area, Morogoro Rural District, Eastern Tanzania.

Statistical analysis

All data were double entered into an Excel spreadsheet and cleaned before they were analyzed in **R** Statistical Software (version 3.6.2). The analysis was only done for malaria vectors, and comparison of mosquito abundance was done across species, season and trap location using Generalized Linear Mixed Model (GLMM). Negative binomial GLMM or quasi-Poisson GLMM was employed dispersed mosquito data. Poisson GLMM was used in undispersed data.

Results and Discussion

Composition and abundance of malaria vectors

A total of 1238 adult anophelines were collected throughout the study period and 67% (n=825) were caught during the wet season and 33% (n=413) during the wet season. These mosquito vectors were composed of *An. gambiae* s.l. (95.48%; n=1182) and *An. funestus* s.l. (4.52%; n=56; Table 1). The abundance of *An. gambiae* s.l. was 3-fold higher during the wet season than the dry season (P < 0.001; Figure 2&3). Contrary, the abundance of *An. funestus* s.l. was significantly higher during the dry season than the wet season (P <0.001). *Anopheles gambiae* s.l. was over 20-fold more abundant than *An. funestus* s.l. (Figure 2). Furthermore, the abundance of mosquitoes indoors was slightly higher than the abundance of mosquitoes outdoors irrespective of the season in Kiroka (P <0.001; Figure 2). The mean abundance per house in Mkuyuni was 1.18 and 0.92 for *An. gambiae* s.l. and *An. funestus* s.l. respectively. The mean abundance per house in Kiroka was 4.35 and 0.04 for *An. gambiae* s.l. and *An. funestus* s.l. respectively (Figure 2&3). Despite the variation in abundance, both anopheline mosquito groups were prevalent across the study wards. The mean abundance of *An. gambiae* s.l. and *An. funestus* s.l. per house was 1.2 and 0.6 times higher in Mkuyuni than Kiroka (P <0.001).

These findings will guide many fundamental aspects for subsequent research notably identification of the next set of questions requiring an immediate attention, study designs and data collection tools. Detailed knowledge of area-specific factors associated with increased risk and burden of malaria is emphasized to specifically tailor and improve interventions targeted particularly against residual malaria [31, 32]. Interestingly, several studies conducted over the past one decade embraced this concept by employing cluster analysis to identify Spatio-temporal malaria transmission hotspots in many parts of Africa [33-36].

The composition of malaria vector population in the study area is consistent with the vector population in Tanzania and elsewhere in the African region [1, 18, 22]. Furthermore, the observed malaria vectors composition was consistent across study sites and the season of the year. The high density of *An. gambiae* s.l arguably implies that species of this complex may be dominating malaria transmission in the study area. However, to re-affirm this, predominant species within each complex, *An. gambiae* s.l. and *An. funestus* s.l., will need to be identified and their relative entomological inoculation rates (EIR) assessed. Depending on transmission efficiency and biting behaviour, certain malaria vector species may dominate transmission despite their low densities. For example, in several parts of Tanzania and elsewhere in the region where *An. funestus s.s.* has somehow increased, the species is dominating malaria transmission despite their density being generally lower than that of *An. arabiensis* [19]. The *An. funestus s.s* and *An. gambiae* s.s. (in the same group as *An. arabiensis*) are more efficient in transmitting malaria parasites compared to *An. arabiensis* and other zoophilic species [37, 38], mainly due to high anthropophilic [39-44] as well as indoor feeding and resting [38, 40, 43]. *An. arabiensis* is often seen as a less efficient vector because of its higher plasticity in blood meal hosts [45].

The comparability of current study areas with other endemic areas in terms of malaria vectors composition and abundance does not always imply comparability in disease transmission intensity. This can be explained by several factors, the most important of which is the intra-species variation across geographical and/or ecological zones. For example sub-populations of anthropophilic and endophagic *An. arabiensis*, known to largely zoophilic and exophilic, have been reported certain parts of Ethiopia and Cameroon [46-50]. Besides, the transmission capacity of similar vectors varies with and/or are influenced by ecological, environmental, demographic (for example mobility and coverage of control interventions) and host factors [51-55]. The sub-populations of early and outdoor feeding *An. gambiae* s.s., largely known to be anthropophagic, are increasingly reported across Africa arguably as a result of the wide coverage of long-lasting insecticidal nets (LLINs) and other indoor based vector control interventions [56-60].

Although the two aforesaid siblings of *An. gambiae* (s.l.) could be composing the vector population in Mkuyuni and Kiroka, both of them are presumably anthropophagic, thus contributing to high malaria prevalence recorded in these areas almost throughout the year [28]. Or else, the relative contribution of *An. funestus* on malaria transmission could be higher than anticipated.

Moreover, the abundance of *An. funestus* was similar during the wet and dry season. *An. funestus* abounds during the dry season and is less rain-dependent than *An. gambiae* s.l., owing to the tendency to breed in permanent or semi-permanent swamps or pools. Because of such characteristics, it is considered as a vector that bridges malaria transmission across the dry season [61]. The current study areas have multiple semi-permanent and permanent breeding sites especially around the interface of low-terrain and hills. This implies a considerably high malaria transmission risk all year round. Our recent retrospective study revealed high malaria prevalence all year round with the peak during the wet season, April and July. This could be attributed by the fact that rice fields, one of the most favourable and large mosquito breeding environments, are exclusively rain-fed and are therefore cultivated during long rains running from March to July. This is consistent with most if not all other studies associating long rains and rice cultivation with high disease prevalence.

Table 1: Number of mosquitoes caught during the wet and dry season in Mkuyuni and Kiroka

Ward	Season	No. of *An. gambiae* s.l.		No. of *An. funestus* s.l.	
		Indoor	Outdoor	Indoor	Outdoor
Mkuyuni	Wet	27*	NT	11	NT
	Dry	8	NT	34*	NT
Kiroka	Wet	786*	330	1	3
	Dry	27*	4	6*	1
Total		848	334	52	4

NT= No trapping was done outdoors; *significance <0.001

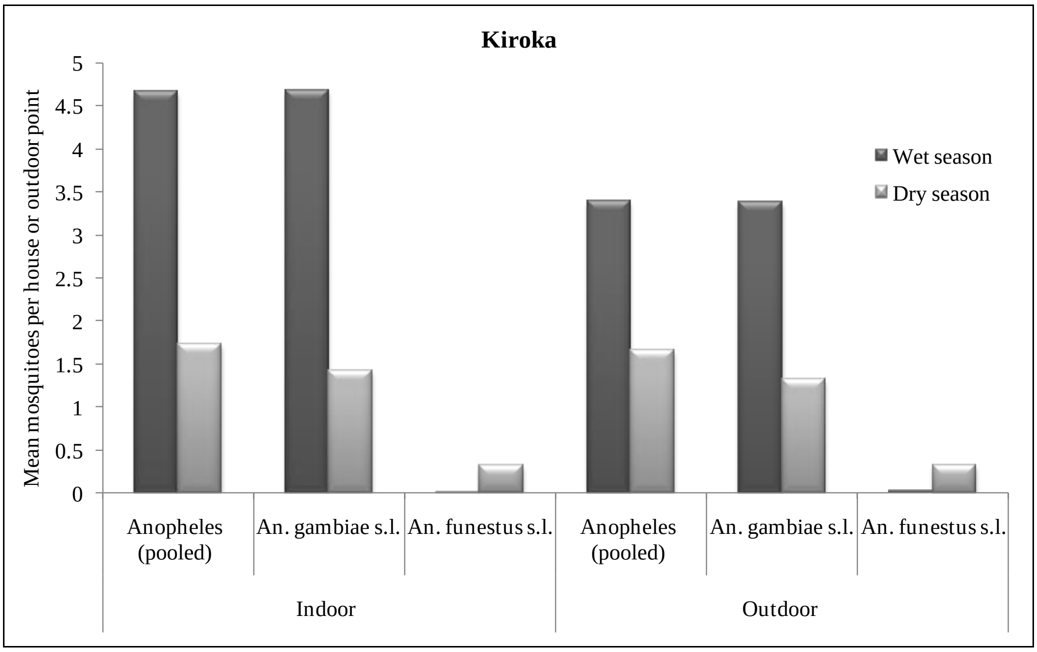

Fig 2: Mean caught numbers of mosquitoes per house during the wet and dry season in Kiroka. Indoor and outdoor catches are presented separately.

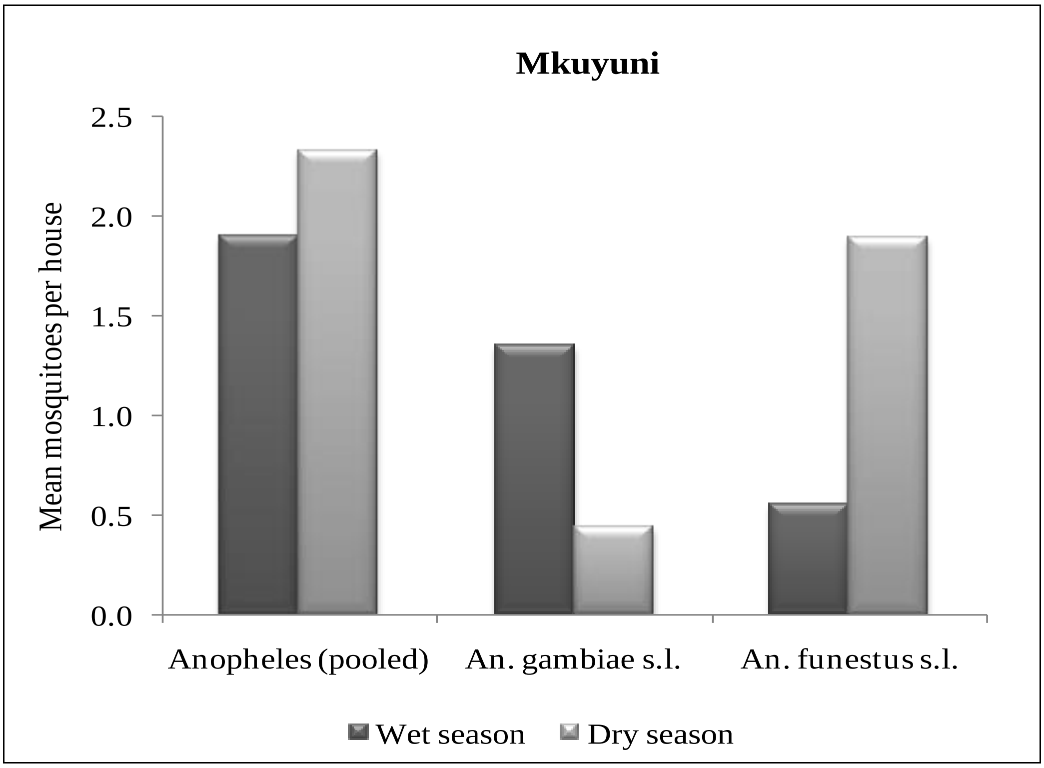

Fig 3: Mean caught numbers of mosquitoes per house during the wet and dry season in Mkuyuni. Only indoor catches are presented.

Demographic/household transmission risk factors

Overall, 100% (n=100) of and 68% (n=34) of the study houses in Mkuyuni and Kiroka respectively had open eaves. More than 95% of the houses in Mkuyuni and Kiroka had multiple openings on walls, windows and doors. About 98% of the respondents in either ward cultivate either or both maize and rice. Both crops are exclusively rain-fed and therefore are cultivated during the long rains between March and July. About 68% (n=34) in Mkuyuni and 24% (n=12) in Kiroka had some of their maize/rice fields within 1 km from their houses. About 76% (n=76) of the respondents in Mkuyuni and 83% (n=83) of the respondents in Kiroka were cooking outside during the night. About 50% of the study households owned bed nets, and the majority (up to 90%) of those had an only one-bed net. Most of the people in either ward were keeping poultry particularly chicken and more than 98% were keeping them inside their houses. The next most common livestock in the area were goats (7%; n=14). Studies have reported disproportionally high proportions of mosquitoes inside houses with open eaves and other alternative openings [62-65]. Assessment of the demographics revealed several risk features which are suggestive high risk of exposure to malaria and other mosquito vectors. More than 95% of the study households had multiple openings on the walls, doors and windows, and up to 100% had open eaves. The high rate of outdoor cooking indicates increasing exposure to mosquito bites and malaria transmission risk [66]. Considering that each household had an average of 3 people, let alone the likelihood that only a few of the bednets were being used regularly and appropriately, most of the study households were at high risk of malaria. Studies have repeatedly reported underutilization of bed nets even in areas with high coverage [67, 68].

Conclusions

This study provides preliminary but equally important, information on malaria vectors composition, seasonal abundance and risk factors in a potentially high endemic area of eastern Tanzania. Malaria vector population in the study areas was mainly composed of *An. gambiae* s.l followed by *An. funestus* s.l., and their abundance is significantly higher indoors particularly during wet season. These along with risk factors like a large number of houses with open eaves and other forms of mosquito entry points, proximity to rice fields, low coverage of bednets and night-time outdoor activities suggest high disease transmission risk in the study area. The findings warrant further research to determine the contribution documented risk factors on malaria burden.

Conflict of interest

The authors declare that they have no competing interests.

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