MALARIA VECTORS COMPOSITION, ABUNDANCE AND PREVALENCE OF MALARIA IN POTENTIALLY HIGH ENDEMIC AREA OF MOROGORO RURAL DISTRICT, EASTERN TANZANIA

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A DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN PARASITOLOGY OF SOKOINE UNIVERSITY OF AGRICULTURE. MOROGORO, TANZANIA.

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EXTENDED ABSTRACT

This dissertation was prepared based on “publishable manuscripts” format of the Sokoine University of Agriculture. The dissertation discusses the composition and seasonal abundance of malaria vector species and disease prevalence in potentially high endemic foci in Morogoro region. Well targeted efforts that embrace area-specific situations, at least in high malaria endemic foci, are needed to preserve realized health gains and achieve elimination. This is because malaria is increasingly characterized by temporal variability that bestows evolving and new challenges for malaria control programs. Morogoro region, eastern Tanzania is a typical reflection of such phenomenon because of its appreciable fine-scale variability in ecology and topography. Therefore, it is likely that we are missing certain salient foci with unprecedented malaria transmission intensity. It was therefore critical to have up-to-date information on the species composition and abundance of malaria vectors; and disease prevalence in order to design and/or implement appropriate surveillance and control strategies. Mkuyuni and Kiroka, adjacent wards within Rural Morogoro District, are purported to form such foci and were therefore the focus of this study. The determination of malaria vector species composition and seasonal abundance was achieved through a repeated cross-sectional survey conducted during the wet and dry season. It involved collection of adult mosquitoes inside 10 randomly selected households and adjacent outdoor points using CDC light traps. This was accompanied by the assessment of environmental risk factors which could be potentiating malaria transmission risk. The prevalence of malaria in the study area was determined through a retrospective analysis of six-year (2014 - 2019) data on malaria cases. This study indicated that malaria vector population in study areas is largely composed of *An. gambiae* s.l followed by *An. funestus* s.l.; and their abundance is equally concerning across seasons. The study also revealed high malaria intensity in the study areas, with prevalence rate as high as ~61%. The mosquito species composition and equally concerning seasonal abundance all year round along with risk factors like open eaves, proximity to rice fields and low usage of bed nets could be among the factors that underline high malaria transmission in the study areas. These preliminary findings warrant more comprehensive longitudinal study in these and other high endemic foci in Tanzania in order to inform future course of action in terms of disease surveillance and control.

Keywords: Malaria, mosquito composition, abundance, malaria prevalence, Mkuyuni, Kiroka

DECLARATION

I, **AIKAMBE JOSEPH NICHOLAUS,** do hereby declare to the Senate of the Sokoine University of Agriculture that this dissertation is my own original work and it has neither been nor is it being concurrently submitted for a higher degree award in any other institution.

Joseph N. Aikambe Date

(MSc. Candidate)

The above declaration confirmed by;

Ladslaus L. Mnyone, PhD Date

(Supervisor)

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DEDICATION

This research work is dedicated to my adorable father the late Nicholaus Lucas Nyallu and my lovely mother Ponsiana S. Mlay for their heartfelt love, care and encouragements throughout my studies.

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LIST OF ABBREVIATIONS

ALU	Artemether-Lumefantrine
CDC	Centre for Disease Control
DDT	Dichloro Diphenyl Trichloroethane
GLMM	Generalized Linear Mixed Model
IRS	Indoor Residual Spraying
LLTNs	Long Lasting Insecticidal Nets
MIS	Malaria Indicators Survey
MoHSW	Ministry of Health and Social Welfare
mRDT	Malaria Rapid Diagnostic Test
NBS	National Bureau of Statistics
RCPs	Representative Concentration Pathways
SSA	sub-Saharan Africa
TDHS	Tanzania Demographic and Health Survey
URT	United Republic of Tanzania
WHO	World Health Organization

CHAPTER ONE

1.0 GENERAL INTRODUCTION

1.1 Background

Malaria is a mosquito-borne disease which affects many people worldwide. The burden of this disease has significantly decreased over the last one decade; however, current number of cases and deaths is still intolerably high. According to World Health Organization (WHO) African countries account for 93% of the cases and 94% of the deaths (WHO, 2018). Tanzania is among the top ten countries with high malaria transmission in the World (Winskill *et al.,* 2011; WHO, 2019). Malaria is endemic almost throughout the country and over 95% of its population is at risk of contracting the disease. Malaria causes approximately 7.7 million confirmed and clinical cases in the country annually (WHO, 2011; TDHS-MIS, 2016). There are some parts of the country which are identified as malaria hotspot including southern part of the country, northern western part surrounding lake Victoria as well as western part of Tanzanian mainland (Hagenlocher *et al.,* 2015).

1.2 Malaria Vectors

Over 460 species of *Anopheles* mosquitoes have been described to date, however only 70 species have been identified to transmit malaria (White, 1974). In Africa, the *Anopheles gambiae* sensu lato (s.l.) complex and *An. funestus* s.l form the most efficient groups of malaria vectors. The *An. gambiae* complex consists of eight sibling species and these include *An. gambiae* s.s, *An. coluzzii*, *An. arabiensis*, *An. melas*, *An. merus*, *An. bwambae*, *An. quadriannulatus* (White, 1974, Coetzee *et al.,* 2000, Coetzee, 2004 and Coetzee *et al*., 2013) and *An. amharicus* (Hunt *et al.,* 1998 and Coetzee *et al.,* 2013). While *An. gambiae s.s.* prefers to bite humans indoors, *An. arabiensis* has a less restricted behavior and may feed indoors or outdoors and bite humans and other mammalian hosts (Gillies *et al*., 1987; Sinka *et al*., 2010 and Kileen *et al*., 2013). The *An. funestus* complex comprises of nine sibling species of which *An. funestus s.s*. is the most anthropophilic and predominant, both in numbers and geographical distribution (Gillies *et al*., 1987; Chanda, 2011). The other sibling species under this group include *An. funestus s.s*, *An. funestus aremi*, *An. funestus fuscivenosus*, *An. vanadeni*, *An. funestus parensis*, *An. funestus confuses*, *An. funestus leesoni*, *An. funestus rivolorum* and *An. funestus brucei* (Gillies and Coetzee, 1987).

The developmental rate of these malaria vectors is highly affected by temperature. The most favored temperature for the three malaria vectors that is *An. gambie*, An. *arabiensis* and *An. funestus* ranges from 15ºC to 32ºC. Developmental rate start to decline tremendously starting from 32ºC and once it reaches 35ºC it has been noticed that no development rate (Lyons *et al*., 2013). The dispersal of *An. gambie* has been traced to be about 579 meters. This is almost a half kilometer in radius (Saddler *et al.*, 2019).

There have been considerable changes in terms of mosquito behavior like increasing outdoors biting, shifts in the mosquito population between different malaria vector species. For example, *An. arabiensis* is outweighing *An. gambiae* in terms of population and role in malaria transmission throughout the malaria endemic countries in sub-Saharan Africa. These and other changes could be contributed by different factors, the most important of which are environmental changes and control interventions (Kileen *et al.*, 2013 and Kitau *et al*., 2012). Notably, different malaria vector species are characterized by seasonal and geographical patterns and land use (Coluzzi *et al.*, 1979; Coluzzi *et al.*, 1985; Toure *et al.*, 1994; Toure *et al.*, 1996; Lindsay *et al.*, 1998; Bayoh *et al.*, 2001 and Minakawa *et al.*, 2002). At relatively finer scales (e.g. within a village), high incidence of malaria has been associated with housing conditions such as open eaves, grass-thatched roofs, nearby irrigated land and tethering livestock inside houses. (Lindsay *et al.,* 1995; Ghebreyesus *et al.*, 2000; Lindsay *et al.*, 2002; Yé *et al.*, 2006; Mutuku *et al.,* 2011; Temu *et al.,* 2012 and Animut *et al.,* 2013). Similarly, the habit of *An. gambiae* and *An. funestus* to rest inside human dwellings enhances their efficiency in transmitting malaria parasites (Beier, 1996; Costantini *et al.,* 1999 and Takken and Knols, 1999; Antonio-Nkondjio *et al.,* 2002; Wanji *et al.,* 2003; Cano *et al.*, 2004 and Sinka *et al.,* 2010).

1.3 Global Malaria Burden and Distribution

Malaria is caused by protozoan single cell organisms of the genus *Plasmodium*. Five important species have been described so far: *P. falciparum, P. vivax*, *P. ovale, P. malariae* and *P. knowlesi.* Of these however, *P. falciparum* is involved in most of the cases globally (Nicodem, 2010). The disease is globally distributed and causes 219 million cases and 435 000 related deaths annually (WHO, 2019). Over 90% of these cases and deaths occur in Africa. About 7% occurs in south East Asia and 2% from Mediterranean region. In Africa, the largest share of malaria cases is contributed by Nigeria 27% and followed by Democratic Republic of Congo 10%. Tanzania contributes 3% of the malaria cases recorded in sub-Saharan Africa (WHO, 2016).

Financially, it is estimated that African countries spend about US 12 billion dollars per year in fighting against malaria disease. The average per house hold is about 25% of their total income for treatment and control (Breman *et al.*, 2004). In Tanzania the average cost incurred for each malaria case ranges from US 5.2 to 137.74 (Sicuri *et al.*, 2013).

1.4 Malaria Situation in Tanzania

Like in other endemic countries, malaria remains a big health and socio-economic problem in Tanzania. Currently, about 95% of the country's population is at risk of malaria (Chacky *et al.*, 2018). The disease prevalence in the country varies considerably, however the average national prevalence rate is around 9%. The prevalence ranges from <1% in the highland to about 41% at the shore of lake Victoria. The number of regions which have malaria prevalence less than one percent cumulatively have increased from six to seven regions while regions with more than 25% have decreased from four regions to three regions (TMIS, 2017). Of 6.5 million malaria cases reported in 2016, 2.7 million cases occurred in children (PMI, 2019). People living in the rural areas where agricultural activities are conducted are proportionately more affected (Leornard *et al.*, 2013). However, the overall disease burden has decreased significantly over the years due to improved use of LLINs and IRS as well as improved diagnosis and treatment. *Plasmodium falciparum* is the major causative agent of malaria in Tanzania; *P. malariae* has been observed in certain areas, but at very low levels (NMSP, 2014-2020). There has been an exchange of drugs but Artmether - lumefantrine (ALU) is first line drug of choice against malaria in the country (URT, 2014).

1.5 Malaria Control

The control of malaria mostly relies on the prevention of disease mosquito vectors. In some cases, antimalarial drugs are also used to provide prophylaxis (WHO, 2016). Malaria vector control remains the cornerstone of malaria control. The most powerful and wide spread vector control measures to date include long lasting insecticidal Nets (LLINs) and indoor residual spraying (IRS) (WHO, 2019). These contemporary control methods coupled with early diagnosis and treatment have halved malaria burden in most of the disease endemic countries particularly in the African region from the year 2000 to

2008 (O'Meara, 2010). However, the sustainability and effectiveness of these control measures are constrained by different challenges which should be addressed timely and adequately in order to preserve health control gains achieved so far and advance towards elimination. The major challenges include among others, the rapid development and spread of insecticide resistance in major malaria vector species. Majority of the major malaria vectors have developed resistance to virtually all insecticide classes (Kisinza *et al*., 2017). In the year 2016, of the 73 countries with ongoing malaria transmission which shared data, 60 countries reported resistance to at least one class of insecticides, while 50 reported resistance to two or more insecticide classes (WHO, 2017). It is therefore of the utmost importance that novel insecticides and new control tools be designed to help manage and mitigate the impact of resistance. In view of that there are already several on-going global efforts to address this problem including scaling up of other rarely used control measures such as use of repellents and larval source management (LSM) (Finda *et al*., 2020).

1.6 Problem Statement and Justification

Despite considerable reduction of the burden of malaria in most endemic countries, the prevailing number of cases and deaths is still intolerably high. In the year 2017 malaria caused 216 million cases and 445 000 deaths worldwide; particularly in children less than five years of age (WHO, 2017). There are some health gain obtained so far due to the effort and strategies to eliminate the disease globally. Among the health gained benefit obtained are, the control tools like LLINs and IRS as well as rapid diagnostic tests have increasingly become available and accessible to the affected population in malaria endemic countries (Dhiman, 2019). In order to safeguard the health benefits achieved so far and advance towards malaria elimination, the challenges constraining existing vector control methods, particularly LLINs and IRS, need to be addressed. Such challenges include among others the changing malaria mosquito vectors species composition and biting behavior. Indeed, mosquito species composition, biting behavior and pattern dictate the malaria transmission rate (Loaiza *et al.*, 2008). Therefore, understanding the trend of such behaviors at local level is essential for effectively applying effective vector control methods. Also less is known about the *Anopheles* mosquito species in Kiroka and Mkuyuni in Morogoro. Last but not least no single study has been conducted in the area to obtain the basic information which could shed light for more detailed studies in future. In view of this, our study aimed at understanding the *Anopheles* mosquito species composition, seasonal abundance and consequently their role in malaria prevalence in potentially high disease endemic areas, at Kiroka and Mkuyuni wards within Morogoro Rural District, Eastern Tanzania.

1.7 Objectives

1.7.1 Main objective

To understand the malaria vector species composition, season abundance, environmental risk factors and their role in malaria prevalence in potentially high endemic area of Morogoro Rural District, Eastern Tanzania.

1.7.2 Specific objectives

i. To determine environmental risk factors and species composition of malaria vectors in Kiroka and Mkuyuni wards;
ii. To determine seasonal variation in abundance of malaria vector species in Kiroka and Mkuyuni wards;
iii. To conduct retrospective analysis of six-year malaria cases data in Kiroka and Mkuyuni wards.

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CHAPTER TWO

Composition and abundance of malaria vectors in a potentially high endemic area of Morogoro Rural District, 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-bed net coverage and outdoor activities suggest high transmission risk in the study-area.

Keywords: malaria vector composition, abundance, Demographic risk factors, Kiroka, Mkuyuni

2.1 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 and 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.

2.2 Materials and Methods

2.2.1 Study area

The study was conducted in Mkuyuni (latitude 6.57^{o} south and longitude 37.48^{o} east) and Kiroka (latitude 6.83^{o} south and longitude 37.78^{o} east) (Figure 2.1). 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.

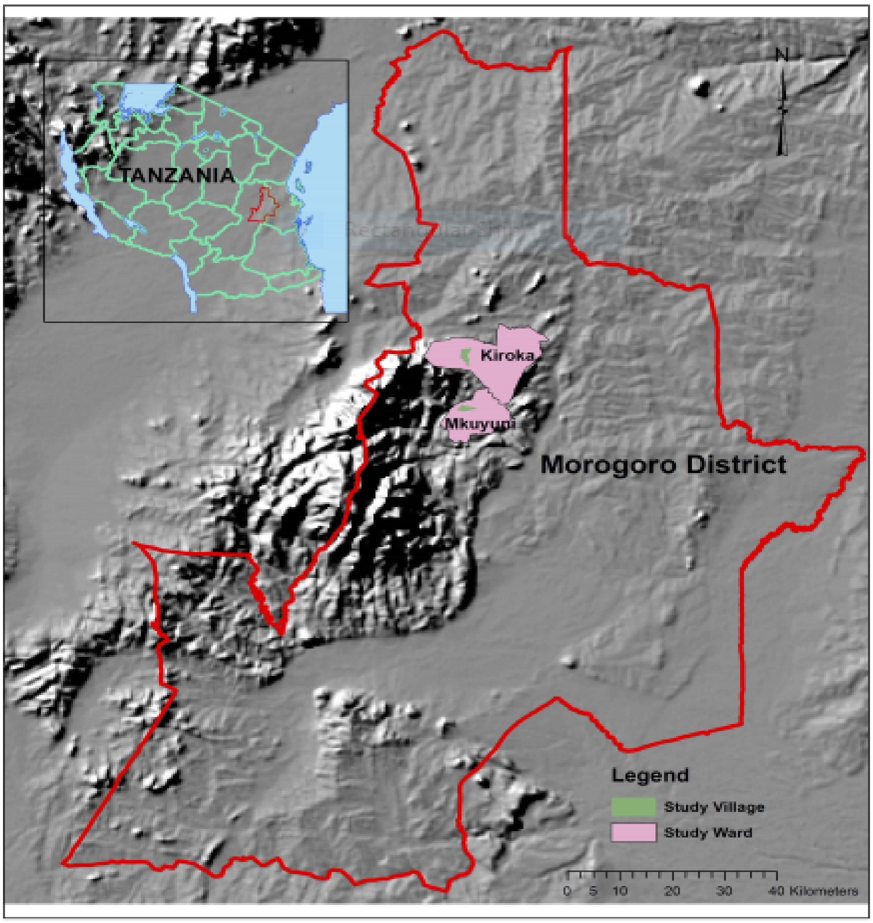

Figure 2.1: Map of the study area, Morogoro Rural District, Eastern Tanzania

2.2.2 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.

2.2.3 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.

2.2.4 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.

2.2.5 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.

2.3 Results and Discussion

2.3.1 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 2.1). The abundance of *An. gambiae* s.l. was 3-fold higher during the wet season than the dry season ($P < 0.001$; Figure 2.2 and 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 2.2). 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 2.3). 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 2.2 and 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 2.1: Number of mosquitoes caught during the wet and dry season in Mkuyuni and Kiroka

Ward	Season	No. of *An. gambiae* s.l. Indoor	Outdoor	No. of *An. funestus* s.l. 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**

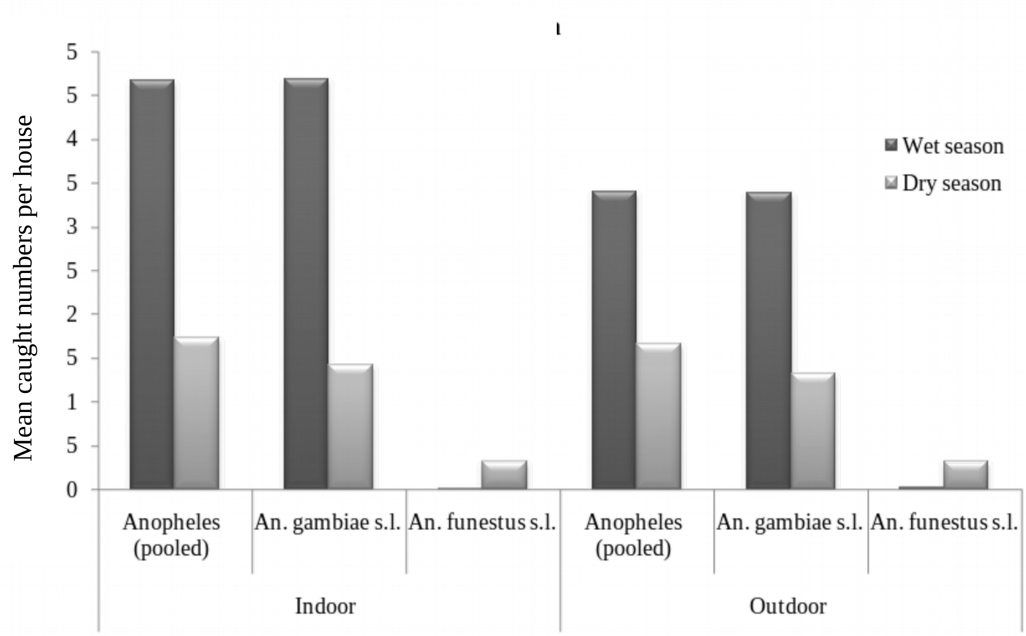

Figure 2.2: Mean caught numbers of mosquitoes per house during the wet and dry season in Kiroka. Indoor and outdoor catches are presented separately

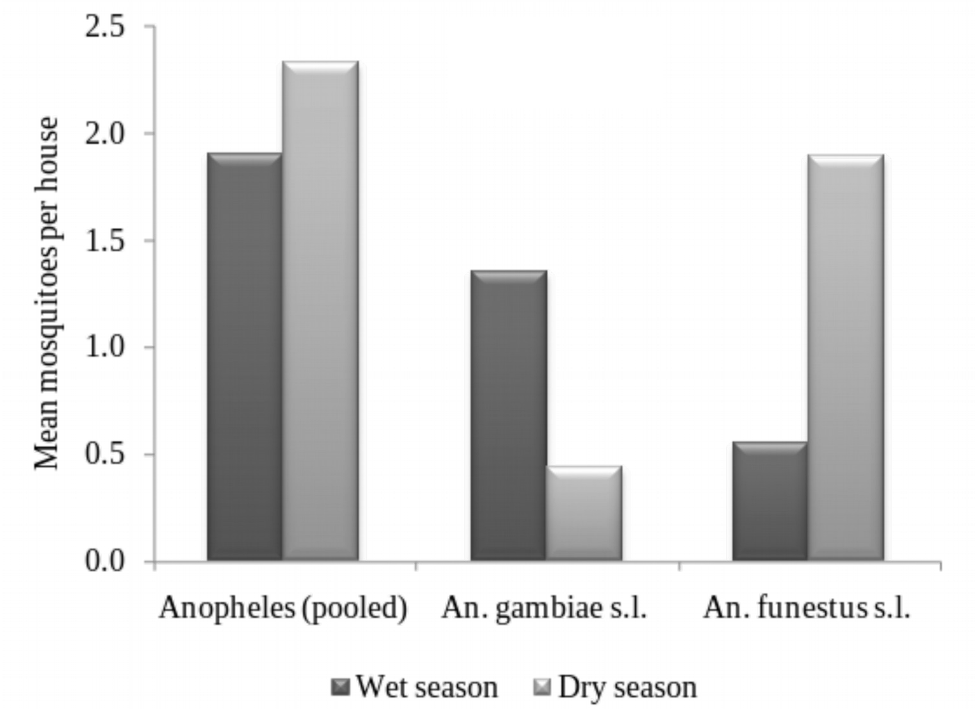

Figure 2.3: Mean caught numbers of mosquitoes per house during the wet and dry season in Mkuyuni. Only indoor catches are presented.

2.3.2 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].

2.4 Conclusion

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.

Acknowledgement

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CHAPTER THREE

Retrospective analysis of malaria cases in a potentially high endemic area in Morogoro Rural District, Eastern Tanzania

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Abstract

Background: Malaria is increasingly characterized by appreciable fine-scale variability in ecology and topography and it is likely that we are missing some salient foci with unprecedented malaria transmission intensity in different parts of Tanzania. Therefore, efforts aimed at identifying area-specific malaria situation and intervening are needed to preserve the realized health gains and achieve elimination. Mkuyuni and Kiroka, adjacent wards within Morogoro Rural District, are purported to form one of such foci.

Patients and Methods: A retrospective study was conducted to determine six-year (2014– 2019) malaria prevalence rates based on outpatients and laboratory registers obtained from two health facilities, one per ward, carrying out diagnosis of malaria either through microscopy or malaria rapid diagnostic test (mRDT). These data were checked for completeness before carrying out statistical analysis.

Results: Overall, 35 386 (46.19%) out of 76 604 patients were positive for malaria. The average proportion of malaria cases was significantly higher in Mkuyuni (51.23%; n= 19 438) than Kiroka (41.21%; n = 15 938) ($P < 0.001$). Females were more affected than males ($P < 0.001$); and irrespective of the sex, most malaria cases were recorded in children < 5 years of age ($P < 0.001$) except at Mkuyuni. Malaria was recorded virtually all year round; however, the highest proportion of cases was recorded in April and July ($P < 0.001$).

Conclusion: This study revealed high malaria endemicity in Mkuyuni and Kiroka, with prevalence rate as high as 60.98%, which is far higher than the overall national average prevalence of 9%. More studies are needed in these and other putatively high endemic foci in Tanzania in order to inform the future course of action in disease surveillance and control.

Keywords: malaria, retrospective analysis, high endemic, Mkuyuni and Kiroka wards

3.1 Introduction

Like many other countries, Tanzania has reduced malaria burden by >50% over the last decade[1]. This has been achieved primarily through improved access and use of vector control interventions, diagnostics and treatment. Yet, the current disease burden is still unacceptably high; with an overall prevalence of around 9% in mainland Tanzania.[2] People living in resource poor and marginalized areas suffer most; much so the under-five children and pregnant women. These groups are severely affected because they lack acquired and/or have suppressed immunity, respectively.[3]

Well-targeted efforts that embrace area-specific situations, at least in high disease endemic foci, are needed to preserve the realized health gains and advance towards elimination. This is because malaria is increasingly characterized by temporal variability that bestows evolving and new challenges to malaria control programs.[4] Morogoro region, eastern Tanzania is a typical reflection of such a phenomenon because of its appreciable fine-scale variability in ecology and topography. Therefore, it is likely that we are missing salient foci with unprecedented malaria transmission intensity. Kiroka and Mkuyuni, adjacent wards within Morogoro Rural District, are purported to form kind of such foci. Health workers in these areas assert that they receive many cases of severe malaria (Pers. comm.). To preliminarily confirm such assertion and guide the future course of research and action, we analyzed recorded data of malaria cases at the catchment health centres from 2014 to 2019. Retrospective records provide an excellent resource for estimating area- or region-specific disease burden, thus informing prioritization and/or improvement of surveillance and control strategies. Through this study we obtained useful insights on (i) characteristics of patients (age, sex); (ii) variation of malaria cases with sex and age; (iii) months with high malaria cases and (iv) trend of malaria cases over the years.

3.2 Patients and Methods

3.2.1 Study area

The retrospective malaria cases data were obtained from Kiroka (latitude 6.8316° south and longitude 37.7889° east) and Mkuyuni (latitude 6.57° south and longitude 37.48° east) (Figure 3.1). These wards are next to each other and are part of Morogoro Rural District, Eastern Tanzania. Kiroka covers 212km^2 with a population of approximately 21 853 people.[5] Mkuyuni covers 97.4km^2 with a population of approximately 17 935 people.[5] Agriculture is the main economic activity and the main crops include rice, maize, banana and coconut. 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. Mkuyuni is mountainous and adjacent to several natural forests, thus at times it experiences orographic rainfall. The landscape is bestowed with temporal, semi-permanent and permanent mosquito breeding habitats, particularly in and around the agricultural fields. Despite the asserted malaria transmission risk and intensity, these areas are understudied, if at all.

3.2.2 Study design

The retrospective study was conducted to determine six-year (2014–2019) malaria prevalence based on outpatients and laboratory registers.

3.2.3 Collection of malaria cases data

The six-year data on malaria cases were obtained from Mkuyuni and Kiroka health centres from 2014 to 2019. We used two health centers, one in each ward. These were the only health centres where malaria diagnosis with either microscopy and/or malaria diagnostic test (mRDT) is done. We only considered malaria cases data which were diagnosed with either microscopy or mRDT. The required sets of information were extracted from patients' register books, and these included reporting date, sex, age and lab results. These data were checked for completeness before being analyzed; and this was done with close assistance from the laboratory personnel in the two health centres. We found a negligible number of incomplete records; and these were excluded from the analysis. Personal information of individual patients was excluded from the final dataset.

3.3 Statistical Analysis

The data set was firstly aggregated by wards, years, age and sex. Based on the age, the data were grouped into two age categories, <5 years and >5 years. A simple linear regression model in R Statistical Software was used to determine how the number of malaria cases varied with years, season, age and sex. Data are also presented with appropriate Tables and Figures.

3.4 Results

3.4.1 Demographic characteristics

A total of 76 604 patients, 12 767 per year, were screened for malaria at Kiroka (n = 38 698 50.52%) and Mkuyuni (n = 37 906 49.48%) over 6 year period (2014−2019). Of these, 36 952 were ≤5 years of age (Kiroka = 17 844, Mkuyuni = 19 108) and 39 652 were >5 years of age (Kiroka = 20 854, Mkuyuni = 18 798). There were 45 767 females (Kiroka = 22 587, Mkuyuni = 23 180) and 30 837 males (Kiroka = 16 111, Mkuyuni = 14 726).

Year		Sex	Screened	Malaria +ve	Prevalence (%) (95% CI)
2014	Sex	Male	508	341	67.13 (63.04 - 71.21)
		Female	879	486	55.29 (52.00 - 58.58)
	Age	<5 years	582	379	65.12 (62.61 - 67.63)
		>5 years	805	448	55.65 (53.04 - 58.27)
		Overall	1387	827	59.63 (57.04 - 62.21)
2015	Sex	Male	991	353	35.62 (32.64 - 38.60)
		Female	1512	528	34.92 (32.52 - 37.32)
	Age	<5 years	1193	411	34.45 (32.59 - 36.31)
		>5 years	1310	470	35.88 (34.00 - 37.76)
		Overall	2503	881	35.20 (33.33 - 37.07)
2016	Sex	Male	1389	514	37.01 (34.47 - 39.54)
		Female	1902	664	34.91 (32.77 - 37.05)
	Age	<5 years	1560	652	41.79 (40.11 - 43.48)
		>5 years	1731	526	30.39 (28.82 - 31.96)
		Overall	3291	1178	35.79 (34.16 - 37.43)
2017	Sex	Male	3567	2102	58.93 (57.31 - 60.54)
		Female	5393	2847	52.79 (51.46 - 54.12)
	Age	<5 years	4768	2781	58.33 (57.31 - 59.35)
		>5 years	4192	2168	51.72 (50.68 - 52.75)
		Overall	8960	4949	55.23 (54.20 - 56.26)
2018	Sex	Male	5174	3161	61.09 (59.77 - 62.42)
		Female	8331	4249	51.00 (49.93 - 52.08)
	Age	<5 years	6727	4183	62.18 (61.36 - 63.00)
		>5 years	6778	3227	47.61 (46.77 - 48.45)
		Overall	13505	7410	54.87 (54.03 - 54.03)
2019	Sex	Male	3097	1615	52.15 (50.39 - 53.91)
		Female	5163	3422	66.28 (64.99 - 67.57)
	Age	<5 years	4278	2765	64.63 (63.60 - 65.66)
		>5 years	3982	2272	57.06 (55.99 - 58.12)
		Overall	8260	5037	60.98 (59.93 - 62.03)

Table 3.2: Malaria prevalence distributed according to sex and age at Kiroka ward from 2014 –2019

Year		Sex	Screened	Malaria +ve	Prevalence (%) (95% CI)
2014	Sex	Male	2174	675	31.05 (29.10 - 32.99)
		Female	2930	713	24.33 (22.78 - 25.89)
	Age	<5 years	2373	651	27.43 (26.21 - 28.66)
		>5 years	2731	737	26.99 (25.77 - 28.20)
		Overall	5104	1388	27.19 (24.85 - 29.54)
2015	Sex	Male	3032	1532	50.53 (48.75 - 52.31)
		Female	4655	1868	40.13 (38.72 - 41.54
	Age	<5 years	3326	1634	49.13 (48.01 - 50.25)
		>5 years	4361	1766	40.50 (39.40 - 41.59)
		Overall	7687	3400	44.23 (42.56 - 45.90)
2016	Sex	Male	2183	684	31.33 (29.39 - 33.28)
		Female	3005	818	27.22 (25.63 - 28.81)
	Age	<5 years	2406	714	29.68 (28.43 - 30.92)
		>5 years	2782	788	28.32 (27.10 - 29.55)
		Overall	5188	1502	28.95 (26.66 - 31.25)
2017	Sex	Male	1847	816	44.18 (41.91 - 46.44)
		Female	2298	985	42.86 (40.84 - 44.89)
	Age	<5 years	1897	812	42.80 (41.30 - 44.31)
		>5 years	2248	989	43.99 (42.48 - 45.51)
		Overall	4145	1801	43.45 (41.16 - 45.74)
2018	Sex	Male	4223	2400	56.83 (55.34 - 58.33)
		Female	5868	2919	49.74 (48.47 - 51.02)
	Age	<5 years	4577	2568	56.11 (55.14 - 57.07)
		>5 years	5514	2751	49.89 (48.92 - 50.87)
		Overall	10091	5319	52.71 (51.37 - 54.05)
2019	Sex	Male	2652	1008	38.01 (36.16 - 39.86)
		Female	3831	1530	39.94 (38.39 - 41.49)
	Age	<5 years	3265	1281	39.23 (38.05 - 40.42)
		>5 years	3218	1257	39.06 (37.87 - 40.25)
		Overall	6483	2538	39.15 (37.25 - 41.05)

3.5 Discussion

This study was done retrospectively using malaria confirmed hospital malaria data collected over a six-year period from 2014 to 2019; with the aim of providing an immediate and readily available resource for estimating area-specific malaria incidences. Based on this retrospective analysis, we have putatively affirmed the assertion that Kiroka and Mkuyuni wards are among the local areas in the Morogoro region that still experience proportionally high malaria incidences. Over the six-year period, both wards recorded 76 604 patients whose malaria infection status was examined by either microscopy or mRDTs. Nearly half (n=35 386 46.19%) of these patients were malaria positive. The recorded number of patients might be lower than it should be because self-medication without confirmatory diagnosis is still a common practice in Tanzania.[6–12] Self-medication in Tanzania and most of the SSA is driven by several factors including distance to health facility, cost of medication and services, shortages of medicines, waiting times for receiving services and attitudes toward patient displayed by health care workers.[10,11,13] Furthermore, since this study only considered malaria cases confirmed by microscopy or mRDTs, many malaria cases could have been missed during stockouts of reagents and/or mRDTs in the study health facilities. Health workers in the study health centers affirmed to have experienced the stockouts or reagents and/or mRDTs, sometimes for several months.

Despite the fluctuation in malaria cases across the years and study areas, there was a general increase in disease prevalence rates from 2017 to 2019. Overall, the highest malaria prevalence rates (up to 60.98%) were recorded across the study sites from 2017 to 2019. These prevalence rates were relatively higher than the national average. The average malaria prevalence in mainland Tanzania stands at 9%.[1] However, the prevalence varies considerably between and within regions across the country.

Notably, malaria prevalence varies from <1% in the highlands of Arusha to as high as 41% along the Lake Victoria shores.[1] The general increase in the proportion of patients and confirmed malaria cases across the study sites from 2017−2019 is unlikely due to increased malaria transmissions, but rather due to improved community awareness and availability of diagnostics mainly mRDTs. Such an association has been emphasized elsewhere in SSA.[14] Before the introduction and improved access to diagnostics like mRDTs particularly in infrastructure and resource-challenged rural settings; malaria diagnosis was done overwhelmingly based on clinical presentation. As such, many people felt contempt that they could diagnose and treat themselves.

Moreover, other factors which could have been responsible to the fluctuation of malaria cases observed over the six-year period of this study include ecologic and environmental factors, host and vector behavioral characteristics, population immunity to malaria, efficiency and/or coverage of mosquito control interventions as well as the economic status of reference communities.[15] Our follow-up studies in the present sites will explore the status and dynamics of these and other relevant factors.

Males across all age groups were more affected compared to females; and this corroborates with the findings of many other studies carried out in Tanzania and elsewhere.[4,15-20] The study done in selected areas of Mvomero district, Tanzania revealed 16% higher odds of having malaria in males relative to females. This could be attributed to the lifestyle and occupation of males. Males are usually involved in agricultural, day labor and hunting in environments that are suitable for mosquito breeding. Besides, most males spend much time outdoors and/or go to bed late compared to females, thus increasing their exposure to mosquito bites.

All age groups were affected by malaria. However, children below 5 years of age were disproportionately affected. This is consistent with many other studies.[1,19,21] Globally, children under 5 years of age suffer the greatest malaria burden, as they are yet to develop immunity to malaria.[22–24] Indeed, this group accounts for approximately 61% of all malaria-related deaths worldwide.[25] However, studies are emphasizing the shift of malaria incidences to older age categories.[26–28] The study done in Gwanda district, Zimbabwe showed that malaria incidences are higher (95%) in the >5 age category.[31] A similar shift has been reported in Tanzania,[29–31] Botswana,[32] Ghana[33] and Gambia.[34] Results of the present study showed no variation of malaria cases between the younger and older age categories at Mkuyuni ward. Presumably, this could be an indication of the shift of malaria to older age categories.

This study depicted malaria cases virtually all year round, with peak in April and July. These months coincide with the beginning and end of long rain season which runs from March to August each year. Many parts of the Morogoro region and Tanzania in general experience high malaria prevalence rates more or less during these months.

Although the prevalence of malaria in both wards is equally concerning, the highest proportion of malaria cases were recorded in Mkuyuni. This variation could be explained by a higher proportion of semi-permanent and permanent breeding sites at Mkuyuni relative to Kiroka. Mkuyuni has a relatively higher proportion of watersheds. Studies conducted in Mvomero district, Morogoro region associated watersheds with high malaria prevalence.[4] High watersheds ensure temperature and humidity conditions that are conducive to the distribution and survival of malaria vectors.[4] Moreover, Mkuyuni is far from Urban Morogoro relative to Kiroka and as such majority of its population depends on smallholder farms hence they are somehow economically disadvantaged.

As such, their use of control interventions such as LLINs could be comparatively low. Unfortunately, this proposition was beyond the scope of this study; thus, it remains rather speculative; therefore, our future studies will aim to confirm it among other factors.

The coverage of LLINs in certain rural areas of the Morogoro region, presumably Mkuyuni and Kiroka wards, is regrettably still low; and even worse, majority of the people who own LLINs do not deploy them appropriately.[35]

Like any other retrospective study relying on existing records, this study had some limitations. There was a possibility of missing data and/or wrong entry in some of the records. Health facility data has a potential for under-reporting malaria cases as a considerable proportion of people may not have presented at the health facilities due to different factors. Besides, a considerable proportion of malaria cases must have been missed during stockouts of reagents and/or mRDTs. Equally important, several other factors may have confounded the observed results, for example, impact of malaria control activities as well as host- and mosquito-related ecological and environmental factors.

3.6 Conclusion

Although the overall morbidity and mortality of malaria have decreased in Tanzania, some high endemic foci, like Mkuyuni and Kiroka, may still be available in different parts of the country. The present study revealed malaria prevalence rate of up to 60.98%, which is far higher than the national average. Therefore, further research to understand and/or estimate malaria transmission risk and incidences in putatively high endemic foci are desirable in view of informing rational and well-targeted surveillance and control efforts.

Data Sharing Statement

The datasets generated and analysed during this study are not publicly available but can be obtained from the corresponding author on reasonable request.

Ethics and Consent Statement

Ethical approval was granted by the Ethics Review Committee of Sokoine University of Agriculture (SUA), Morogoro, Tanzania. The malaria cases data were provided upon request to the Health Officers in charge. All patient data complied with applicable data protection regulations.

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Author Contributions

Both authors made substantial contributions to the conception and design, data collection or analysis and interpretation of data; took part in drafting the article and revising it; and gave final approval of the version to be published and agree to be accountable to all aspects of the work.

Disclosure

The authors declare that they have no competing interests.

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CHAPTER FOUR

4.0 SUMMARIZING DISCUSSION, CONCLUSION AND RECOMMENDATION

4.1 Summarizing Discussion

The present study was aimed at assessing the composition and seasonal abundance of malaria vector species as well as disease prevalence in potentially high endemic area, Kiroka and Mkuyuni wards, of Morogoro Rural District, Tanzania. Its findings have answered a set of questions requiring immediate attention in order to comprehensively understand different factors and their interactions that underlay high malaria transmission intensity in the study area. Eventually, such information will lead to the improvement of Malaria disease surveillance and control strategies.

The present study revealed that malaria vector population across the study area is largely composed of *An. gambiae* s.l. followed by *An. funestus* s.l. and their abundance is equally concerning across the wet and dry seasons. These along with other risk factors like large numbers of houses with open eaves and other forms of mosquito entry points and proximity to rice fields could be underlying the on-going high malaria transmission intensity in the study areas. The retrospective analysis of malaria cases data revealed of up ~61% which is far higher than the national average of around 9% (THMIS, 2017). This malaria transmission intensity is high almost all year round, with peak in April and July. This is noticeably consistent with most if not all other studies associating long rains and rice cultivation with high disease prevalence (Mboera *et al*., 2010). The high malaria prevalence during those months of the year could be explained by increased proportion of breeding sites during the months of April and July which coincides with long rains. Indeed, the abundance of mosquitoes, particularly the *Anopheles gambiae* s.l. was relatively high during the wet season, both indoors and outdoors (Chapter Two). The malaria prevalence in the study area is also unacceptably high during the dry season and this could be explained by several factors including presence of semi-permanent and permanent larval habitats, which maintain favorable breeding sites for the mosquitoes. This is supported by our observations which revealed concerning abundance of malaria vectors, particularly *An. gambiae* s.l. during the dry season as well. These variations could also be attributed by other ecological and environmental factors, host and vector behavioral characteristics, population immunity to malaria, efficiency and/or coverage of mosquito control interventions as well as the economic status of reference communities (Alemu *et al*., 2012).

4.2 Conclusions

Malaria vector population in study areas is largely composed of *An. gambiae* s.l. followed by *An. funestus* s.l. and their abundance is equally concerning across seasons. These along with risk factors like open eaves, proximity to rice fields and low usage of bed nets underline high malaria transmission risk. This study revealed malaria prevalence rate of up to 60.98%, which is far higher than the national average.

4.3 Recommendations

The findings of this study warrant more comprehensive longitudinal studies before making strong and decisive recommendations in view of informing rational and well-targeted surveillance and control efforts in the study areas. The subsequent studies will address several limitations of the current study including molecular identification of mosquitoes to species level, assessing larval abundance, employing multiple entomological surveillance tools. Furthermore, such studies need to comprehensively assess different ecologic and environmental factors, host and vector behavioral characteristics, population immunity to malaria, efficiency and/or coverage of mosquito control interventions as well as the economic status of reference communities; all of which will strengthen findings of the current study and decisively inform the course of action in improving disease control strategies in the study areas.

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