# Spatial abundance and human biting rate of *Anopheles arabiensis* and *Anopheles funestus* in savannah and rice agro-ecosystems of Central Tanzania

**Leonard E.G. Mboera,[1] Veneranda M. Bwana,[2] Susan F. Rumisha,[1] Grades Stanley,[1] Patrick K. Tungu,[2] Robert C. Malima[2]**

*[1]National Institute for Medical Research, Dar es Salaam; [2]Amani Medical Research Centre, Muheza, Tanzania*

## Abstract

This study was carried out to determine the spatial variations in malaria mosquito abundance and human biting rate in five villages representing rice-irrigation and savannah ecosystems in Kilosa District, central Tanzania. The study involved five villages namely Tindiga and Malui (wetland/rice irrigation), Twatwatwa and Mbwade (dry savannah) and Kimamba (wet savannah). Indoor mosquitoes were sampled using Centers for Disease Control and Prevention light traps in three houses in each village. *Anopheles gambiae* s.l. molecular identification was carried out using polymerase chain reaction (PCR). A total of 936 female mosquitoes were collected. About half (46.9%) were malaria mosquitoes (*Anopheles gambiae* s.l.=28.6%; *An. funestus*=18.3%). A total of 161 (60.1%) of the morphologically identified *An. gambiae* s.l. (268) and subjected to PCR analysis for speciation were genotyped as *An. arabiensis.* The *An. funestus* complex mosquitoes were composed of *An. funestus funestus* and *An. rivulorum* at the 5:1 ratio. On average, 17.9 *Anopheles* mosquitoes were collected per village per day. Two-thirds (62.8%) of the malaria mosquitoes were collected in Malui (rice agro-ecosystem) and the lowest number (2.3%) in Twatwatwa (dry savannah ecosystem). The biting rate per person per night for *An. arabiensis*+*An. funestus* s.s. was highest in Malui (46.0) and lowest in Twatwatwa (1.67). The parity rate of the *An. funestus* mosquitoes was lower compared to that of *An. arabiensis* and none of the mosquitoes was infected with malaria sporozoites. In conclusion, *An. arabiensis* is the most abundant malaria vector in Kilosa district and its variation is related to the ecological system. The heterogeneity in malaria mosquito abundance and human biting rate could be used to guide selection of locally appropriated control interventions.

Correspondence: Leonard E.G. Mboera, National Institute for Medical Research, 3 Barack Obama Drive, 9653 Dar es Salaam, Tanzania.
Tel: +255.754.314701 - Fax: +255.222.121360.
E-mail: lmboera@nimr.or.tz

Key words: Malaria; *Anopheles arabiensis; Anopheles funestus;* ecosystem; Tanzania.

Ethical considerations: this study received an ethical approval from the Medical Research Coordinating Committee of the National Institute for Medical Research, Tanzania. Permission to enter into houses was obtained from heads of the respective households after explaining the objective of the study.

Conflict of interest: the authors declare no potential conflict of interest.

Contributions: LEGM conceived the study, was the project coordinator and led the writing of this article. SFR led the study design, data analysis and interpretation. VMB, GS, RCK provided technical support during project implementation, and contributed to data collection. PT did mosquito genotyping. All authors contributed to the study design, interpretation, and drafting of the article. All authors read and approved the final version of the manuscript.

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## Introduction

In Tanzania malaria is mainly transmitted by *Anopheles gambiae, An. arabiensis* and *An. funestus* (White, 1974). Other important vectors include *An. merus* (Mnzava, 1991; Kigadye, 2006), *An. rivulorum* and *An. marshallii* (Wilkes *et al*., 1996; Malima, 1999; Magesa *et al*., 1991). Malaria transmission in most areas of Tanzania has been generalized by zone, region or district (Clyde, 1967). However, it has been observed in Sub-Saharan Africa that there are variations in anopheline mosquito composition and malaria transmission locally, *i.e.* within districts (even within villages) and between seasons (Ijumba *et al*., 2002;

Appawu *et al*., 2004; Mboera *et al*., 1997, 2007, 2010). Craig *et al.* (1999) and Hay *et al*. (2000) have demonstrated the existence of important, small-scale, local variations in the malaria transmission and endemicity across Africa as a whole. Differences in micro-ecological and socio-economic factors, including vector density heterogeneity, mosquito survival, vector host contact and their innate feeding preference are likely to have contributed to these variations (Smith *et al*., 1995).

Malaria mosquito abundance varies in space and time, hence displaying species-specific seasonality. Generally, malaria transmission in Tanzania has been described to be higher in rice irrigation ecosystems than in any other ecosystem (Ijumba and Lindsay, 2001; Ijumba *et al*., 2002; Mboera *et al*., 2010). It has already been observed that irrigated cultivation enhances population development of many malaria mosquito species and is associated with high malaria transmission in sub-Saharan Africa (Dossou-Yovo *et al*., 1994). Generally, the biting rate is highest shortly after the mosquito density peak, near breeding sites where adult mosquitoes emerge and around the edges of areas where humans are aggregated (Smith *et al*., 2005). These sources of spatial and temporal heterogeneity in the distribution of mosquito populations are associated with biting rate variability, the proportion of mosquitoes that are infectious and that for human infection (Smith *et al*., 2005). Similar to mosquito density, the annual entomological inoculation rates (EIR) estimates in Tanzania display marked temporal and spatial variation, with the likelihood of communities in irrigation ecosystems experiencing higher EIR throughout the year (Mboera *et al*., 2010).

The variation in mosquito abundance and EIR between ecosystems and land use may be explained by differences in the ecological settings, and more specifically by the availability of favorable breeding sites (Dossou-Yovo *et al*., 1994; Appawu *et al*., 2004; Mboera *et al*., 2010). Malaria transmission is influenced by variations in ecological conditions, which have impact on the biology of the parasite and its mosquito vector. On the other hand, malaria transmission influences daily life and socio-economic conditions, which impact human vulnerability and vector habitats. These variables can lead to conditions and environments conducive to mosquito proliferation, human exposure to biting mosquitoes translating into enhanced malaria transmission (Mboera *et al*., 2011; Lowe *et al*., 2013).

Only a few studies in Tanzania have investigated the variations in malaria mosquito density and EIR in relation to agro-ecosystems or land use (Ijumba and Lindsay, 2001; Ijumba *et al*., 2002; Mboera *et al*., 2011). In northern Tanzania, Ijumba and Lindsay (2001) observed that the potential risk of malaria due to *An. arabiensis* and *An. funestus* was four-fold higher in rice agro-ecosystem than in sugarcane or savannah ecosystems. In East-Central Tanzania, Mboera *et al*. (2011) reported that the mean annual inoculation rate for *An. gambiae* s.l. was significantly higher in traditional flooding irrigation than in other agro-ecosystems. However, there is limited knowledge of the malaria vector species and transmission indices in different ecosystems in central Tanzania.

This study was carried out to determine the spatial variations in mosquito abundance and biting rates in five villages representing different ecosystems and daily activities in this area.

## Materials and Methods

### Study area

The study was carried out in Kilosa District (5°55' -7°53' S; 36°30' - 37°30 E) in central Tanzania. The district has a total surface area of about 14,400 km$^2$, a population of 489,513 people (NBS, 2013) and a tropical climate, characterized by a monomodal rainfall pattern beginning in October with a peak in April-May. The mean annual temperature is 25°C. Agriculture is the main activity and most people are smallholders or work at estate farms. The main crops are maize, rice, sorghum, beans, cassava, sweet potatoes, cotton, sunflower, sesame and sisal. Free-range livestock production is an important type of land use in the district.

The study was conducted in five villages, namely Tindiga and Malui (rice irrigation ecosystem), Twatwatwa, Mbwade (dry savannah ecosystem) and Kimamba (wet savannah ecosystem). The area has been described recently by Rumisha *et al*. (2014). Tindiga and Malui are in the south-eastern part of the district and are characterized by swampy flatland and wetlands belonging to the Kilangali alluvial basin. Most of the communities in Tindiga and Malui are small-scale rice farmers using traditional ground flooding irrigation practice. Mbwade and Twatwatwa are located in the North-Eastern part of the district and are characterized by dry savannah type of vegetation, with most of the areas covered by short grasses, trees and shrubs that provide a wide range of pasture for livestock grazing (Figure 1). The villages are mainly inhabited by Maasai pastoralists keeping cattle, sheep, goats and donkeys. Kimamba, a fast growing township with mixed livelihood routines, which include maize farming and large sisal-producing estates. There are also many sisal factories and grain mills that attract employment.

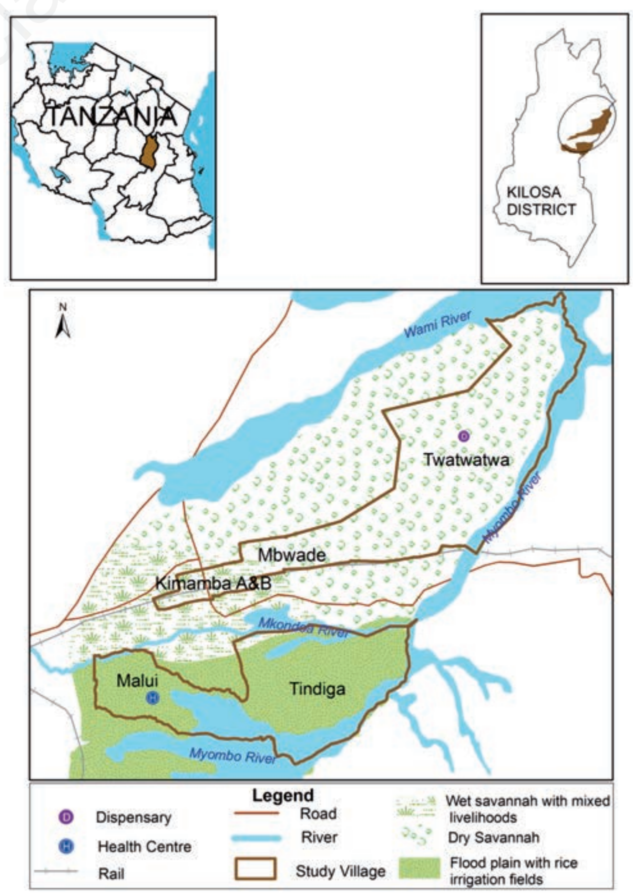


**Figure 1. Map of the study area in Kilosa District in Tanzania.**

## Mosquito collection, identification and processing

Adult mosquitoes were sampled in three houses in each of the five villages for three consecutive days in May 2012. Mosquito collections were done using Centers for Disease Control and Prevention (CDC) light traps (J.W. Hock Ltd, Gainesville, FL, USA). Each light trap was hung at the top of the foot-end of the bed with an adult person sleeping under a untreated mosquito net (Mboera *et al.,* 1998). The light traps were set at 18.00 h and collected the following morning at 06.00 h.

Collected mosquitoes were kept in cool boxes and brought to a field laboratory for identification and further processing. At the field laboratory, mosquitoes were anaesthetised, sorted, counted and identified morphologically with respect to species (Gillies and De Meillon, 1968; Gillies and Coetzee, 1987). Parity of female *An. funestus* and *An. gambiae* s.l. from a sample of unfed mosquitoes was determined using the conventionally used technique described by Detinova (1962). The presence of malaria sporozoites in the salivary glands was determined by examining the salivary glands under microscope. A proportion of each catch of *An. gambiae* s.l. and *An. funestus* s.l. was kept dry on silica gel in 0.5 mL polypropylene tubes for later genotyping using the polymerase chain reaction (PCR) technique. Briefly, genomic DNA was extracted from the whole female mosquito using standard methods. The PCR amplification for *An. gambiae* s.l. and *An. funestus* sibling species molecular identification was carried out using their respective specific diagnostic primers according to the standard PCR method (Scott *et al*., 1993).

## Data analysis

Data were entered in Epi Info database version 6 (CDC, Atlanta, GA, USA) and then transferred to STATA statistical analysis software package version 11 (Stata Statistical Software, College Station, TX, USA). The parity rates were determined as the proportion of *Anopheles* mosquitoes found to be parous. The biting rates were calculated as the number of *Anopheles* biting per person per night using the formula by Lines *et al.* (1991). Maps were created using ArcGIS version 9.3 (ESRI, Redlands, CA, USA).

# Results

A total of 936 female mosquitoes were collected in 15 houses. Some 46.9% were malaria mosquitoes (*An. gambiae*=28.6%; *An. funestus*=18.3%). *Culex quinquefasciatus* accounted for 30.3% of the total mosquito population. Other mosquito species accounted for 22.8%. The largest proportion of the malaria mosquitoes (62.8%) was collected in Malui and the smallest (2.3%) in Twatwatwa (Table 1). *An. funestus* mosquitoes were collected in all villages except in Twatwatwa. Malui accounted for the largest proportion (69.6%) of the *An. funestus* collections. On average, 17.9 *Anopheles* mosquitoes were collected per village per day (Kimamba=3; Malui=26; Tindiga=6; Mbwade= 3; Twatwatwa=1). A total of 161 (60.1%) of the morphologically identified *An. gambiae* s.l. (268) were further subjected to PCR analysis for speciation and all of them were genotyped as *An. arabiensis*. Eighty-one *An. funestus* complex mosquitoes were also subjected to PCR analysis. The *An. funestus funestus* to *An. rivulorum* ratio was 5:1 (Table 2).

The biting rate for *An. gambiae* s.l. (26.8) was higher than that for *An. funestus* (17.1). On average an individual human received 43.9 *Anopheles* bites per night. The biting rate per person per night for the two malaria mosquitoes was highest in Malui (46.0) and lowest in Twatwatwa (1.7) (Figure 2).

The overall parity rates for *An. arabiensis* and *An. funestus* were 72.1 and 42.6%, respectively (Table 3). A total of 62 and 54 *An. arabiensis* and *An. funestus*, respectively, were examined for presence of malaria sporozoites by salivary gland microscopy. None of the mosquitoes was infected.



**Table 1. Number and percentage of mosquito species collected by village and ecosystem.**

| Ecosystem | Village | *Anopheles gambiae* s.l. | *Anopheles funestus* | *Culex quinque fasciatus* | Other species | No. of malaria mosquitoes | Total |
|---|---|---|---|---|---|---|---|
| Wet savannah | Kimamba | 18 | 2 | 99 | 2 | 20 (4.6) | 121 (12.9) |
| Rice irrigation | Malui | 157 | 119 | 16 | 162 | 276 (62.9) | 454 (48.5) |
| Rice irrigation | Tindiga | 58 | 43 | 24 | 43 | 101 (23) | 168 (17.9) |
| Dry savannah | Mbwade | 25 | 7 | 7 | 6 | 32 (7.3) | 45 (4.8) |
| Dry savannah | Twatwatwa | 10 | 0 | 138 | 0 | 10 (2.3) | 148 (15.8) |
| Total (n) | 268 | 171 | 284 | 213 | 439 | 936 | |
| Total (%) | 28.6 | 18.3 | 30.3 | 22.8 | 46.9 | 100.0 | |

Values within brackets are represented as percentage.

**Table 2. Malaria mosquito speciation by polymerase chain reaction by village.**

| Species | Kimamba | Malui | Mbwade | Tindiga | Twatwatwa | Total |
|---|---|---|---|---|---|---|
| *Anopheles arabiensis* | 17 | 95 | 10 | 32 | 7 | 161 (60.98) |
| *An. funestus funestus* | 0 | 59 | 1 | 21 | 0 | 81 (30.68) |
| *Anopheles rivulorum* | 1 | 4 | 2 | 9 | 0 | 16 (6.06) |
| Not amplified/faint | 0 | 4 | 0 | 2 | 0 | 6 (2.28) |
| Total | 18 | 162 | 13 | 64 | 7 | 264 |

Values within brackets are represented as percentage.

## Discussion

Both the abundance and house density of the *Anopheles* mosquitoes collected from the two rice-farming villages were higher than anywhere else. The variation in the abundance of *Anopheles* mosquitoes was observed between villages and between different ecosystems. Malaria mosquito abundance and biting rates vary markedly in space and time. Spatially, the variables can vary over the space of a few kilometres (Kulkarni *et al*., 2010; Mboera *et al*., 2010). This spatial heterogeneity in abundance and biting indicates variation in environmental conditions that affect mosquito distribution (Ernst *et al*., 2006; Kulkarni *et al*., 2010).

All samples of *An. gambiae* s.l. were genotyped as *An. arabiensis,* in accordance with previously reported geographical distribution of *An. gambiae* sibling species across Tanzania (White, 1974; Mnzava and Kilama, 1986). On the other hand, the ongoing climatic changes across Africa favors the environmental variables, which very much support the increased distribution of *An. arabiensis,* which in turn exhibits greater ecological flexibility than other members of the *An. gambiae* complex from a historical perspective (Meyrowitsch *et al*., 2011). *An. arabiensis* and *An. funestus* were the major malaria vectors sampled in our study. *Anopheles rivulorum* has been reported as a vector of malaria in North-Eastern Tanzania (Magesa *et al*., 1991; Wilkes *et al*., 1996; Malima, 1999) and is known to be the next most widespread species in the *An. funestus* group in Africa. Despite its known role in malaria transmission, its impact as a malaria vector has not been fully studied (Awolola *et al*., 2003, 2005; Temu *et al*., 2007; Kweka *et al*., 2008). Previous studies in North-Eastern Tanzania by Gillies and Smith (1960) reported that *An. rivulorum* has the potential to replace *An. funestus s.s.* after indoor residual spraying eliminates the more abundant malaria mosquito species. The sympatric occurrence of *An. funestus* s.s. and *An. rivulorum* as observed in our study has also been reported in coastal Tanzania (Temu *et al*., 2007). To our knowledge, this is the first time *An. rivulorum* is reported from Central Tanzania. On average, an individual human received about 44 bites of malaria mosquitoes each night. The biting rate per person per night for the two malaria mosquitoes was higher among the communities in the rice irrigation ecosystem than in those in the savannah ecosystem. The differences in vector composition between the ecosystems are likely to have impact on the level of malaria transmission in the areas studied. A recent investigation in the same study villages indicates that despite the low number and absence of sporozoite-infected *Anopheles* mosquitoes, malaria infection is prevalent. Malui and Tindinga, which had the largest proportions of malaria mosquitoes, also had the highest prevalence of malaria infection (Mboera *et al*., 2013a, 2013b). Previous studies indicated a higher endemicity of malaria in the district (Eriksen *et al*., 2004; Makundi *et al*., 2006; Uddenfeldt Wort *et al*., 2006).

Despite having high parity rates estimated at over 60%, none of the mosquitoes was found to carry sporozoites. The absence of infected mosquitoes is likely to be attributed to the low number of mosquitoes collected and the method used to examine them for sporozoites. The relatively low mosquito densities and absence of sporozoites in the current study is likely to be a result of a high use of mosquito nets in the district. Over 83% of the households had insecticide-treated mosquito nets during the time of the survey (Shayo *et al*., 2015). A more intensive longitudinal study is recommended to establish malaria transmission intensity in the area.

## Conclusions

*An. arabiensis* is the most abundant malaria vector in the Kilosa district and its variation is related to the ecological system. The mosquito is more abundant in rice irrigation than in savannah ecosystems. The heterogeneity in malaria mosquito abundance and human biting rate observed in this study could be used to guide selection of locally appropriated control interventions. It is therefore important that this variation is considered when designing appropriate malaria intervention and rice farming systems.

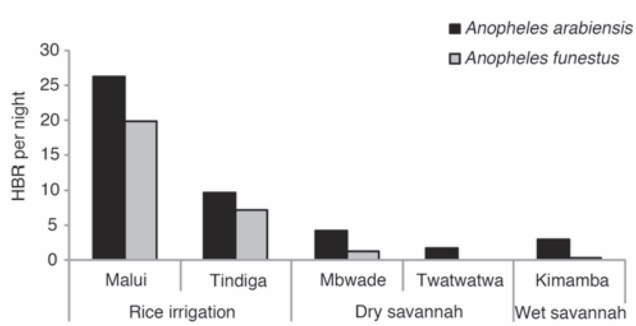


**Figure 2. Malaria mosquito biting rate per person per night.**

**Table 3. Parity rates of *Anopheles arabiensis* and *Anopheles funestus* by village in Kilosa.**

| Village | *Anopheles arabiensis* | | *Anopheles funestus* | |
|---|---|---|---|---|
| | Total dissected | Parity° (%) | Total dissected | Parity (%) |
| Kimamba | 1 | 0.0 | 2 | 100 |
| Malui | 50 | 76.0 | 24 | 58.3 |
| Mbwade | 14 | 71.4 | 5 | 80.0 |
| Tindiga | 16 | 62.5 | 19 | 68.4 |
| Twatwatwa | 5 | 80.0 | 4 | 75.00 |
| Total | 86 | 72.1 | 54 | 42.6 |

°Parity, number parous/total number dissected×100.

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