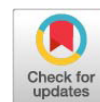


Research Article



Indoor Malaria Vector Species Profile and Their Sporozoite's Rates in Malaria Positive Patients' Households in Endemic Zones of Kisii County, Kenya

PACIFICA CHEPCHUMBA BWOGO^{*}, SAMUEL MONG'ARE¹, RAEL MASAI¹, DR. DAMARIS MATOKE-MUHIA²

¹Kisii University, Kenya; ²Kenya Medical Research Institute, Kenya.

Abstract | Background: *Plasmodium falciparum* is the predominant parasite responsible for approximately 99% of malaria cases in Kenya. Indoor biting by malaria vector plays a significant role in residual malaria transmission; however, the characteristics of the vectors responsible for driving this phenomenon remain unclear. Methods: The present study, investigated the genetically characterized populations and abundance of malaria vectors captured indoors in three chosen endemic sub-counties of Kisii County, Kenya. Results: The research findings revealed a greater abundance of members from the *Anopheles funestus* group (12.47 ± 6.06) in contrast to those belonging to the *Anopheles gambiae* complex (4.20 ± 2.08). The identified common malaria vectors exhibited variations in abundance across the selected sites. Kitutu Chache North showed the highest proportions followed by Bonchari and lastly South Mugirango. The collected vector species did not show any presence of *Plasmodium falciparum* sporozoites. Conclusion: *Anopheles funestus* has been identified as the key vector in malaria transmission, in malaria endemic zones of Kisii County, and it is majorly influenced by human activities. Effective environmental management, particularly through habitat manipulation, has the potential to mitigate malaria transmission in the study area. This is because the expanded coverage of long lasting insecticide nets (LLINs) indoors has caused malaria vectors to have shift in their behaviors.

Keywords | Vector, Transmission, *Anopheles*, Indoor, Endemic, Malaria.

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***Correspondence** | Pacifica Chepchumba Bwogo, Kisii University, Kenya; **Email:** passybwogo@gmail.com/pchepchumba@kisiuniversity.ac.ke

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INTRODUCTION

Malaria is a significant vector-borne disease, impacting hundreds of millions of individuals across the world. The estimated number of malaria deaths reached 627,000 in 2020 worldwide, reflecting an increase of 69,000 deaths compared to the previous year [1]. It persists as a public health concern in Kenya, even with the expansion of LLINs intervention measures [2]. *Plasmodium falciparum* is the predominant parasite responsible for approximately 99% of malaria cases in Kenya. Annually, Kenya records

almost 6.7 million clinical cases of malaria, putting 70% of the population at risk of contracting the disease [3]. Around 4,000 individuals succumb to malaria each year, with a significant portion being children. Additionally, malaria accounts for 13–15% of outpatient consultations [4]. Malaria occurrences in Kenya exhibit regional variations, with the lake endemic zone having the highest prevalence at 27%, followed by the coast endemic zone at 8%, and the highland epidemic zone at 3%. Kisii County, the location of the current study, is situated in the Western highland malarial zone and it borders the lake endemic

zone. Even with these advancements in the use of LLINs as intervention measures, the disease continues to impose a substantial health burden, contributing significantly to public health challenges. This pattern potentially suggests the persistent nature of the disease, influenced by various factors such as the utilization of bed nets or other control measures, house characteristics, vector-related factors as well as human activities and behavior [5]. Nevertheless, entomologic determinants continue to be of paramount importance. For example, alterations in the composition of *Anopheles* vectors may occur as a result of prolonged vector control efforts, impacting their resting and biting behaviors, such as becoming either early feeders or late feeders, particularly in indoor settings. Together, these factors restrict the efficacy of Long-Lasting Insecticidal Nets (LLINs) and Indoor Residual Spraying (IRS), primarily deployed indoors, leading to residual malaria transmission (RMT). RMT is characterized by persistent transmission despite the complete implementation of LLINs and IRS, which are fully effective against local vectors [6]. The focus on outdoor biting has overshadowed indoor biting, primarily due to the presence of vector control measures such as Long-Lasting Insecticidal Nets (LLINs) and Indoor Residual Spraying (IRS). It's important to note that the neglect of indoor biting occurs despite the fact that universal coverage of Insecticide-Treated Nets (ITNs) and IRS has not been fully attained. In a recent study conducted in the Asembo district of western Kenya, it was observed that the majority of malaria vector biting incidents occurred indoors, despite the widespread coverage of Insecticide-Treated Nets (ITNs) in the area [7]. Malaria exhibits meso-endemic in the highlands and holo-endemic patterns in the lowland areas of western Kenya [8]. The transmission dynamics are sustained by *Anopheles arabiensis*, *Anopheles funestus* and *Anopheles gambiae* s.s.. *Anopheles funestus* are recognized as highly anthropophilic and endophilic, while *Anopheles arabiensis* is classified as endophilic and zoophilic [9]. There is a possibility that individuals with anthropophilic and endophilic tendencies could undergo a shift towards zoophilic and exophilic behaviors or experience a reduction, leaving behind zoophilic and exophilic sibling species. This phenomenon could be due to the widespread implementation of Insecticide-Treated Nets (ITNs) and Indoor Residual Spraying (IRS). Therefore, given the growing apprehension surrounding residual malaria transmission in Kenya, there is an urgent demand to deepen our comprehension of the existing vector types and their behaviors. This understanding is crucial for evaluating the potential efficacy of current vector control tools. The primary objective of this study was to acquire insights into malaria persistence in Kisii County, a seasonal epidemic zone in Kenya. This was achieved by examining the availability and composition of malaria vector species and assessing their contribution to indoor malaria transmission.

STUDY SITES

The study took place in malaria endemic sub-counties of Kisii County (latitude 0° 30' and 1° 0' South and longitude 34° 38' and 35° 0' East), Western Kenya. Three sites were selected namely; Bonchari (Kiaruta, Nyamagiri), Kitutu Chache North (Eramba, Sieka) and South Murgirango (Moticho, Suguta). The selection of three sites is based on their classification as malaria endemic zones in Kisii County, as these areas consistently record the highest number of malaria cases annually [10]. The study sites exhibit a bimodal pattern of rainfall, characterized by a long rainy season occurring from April to June, which corresponds to the peak malaria transmission period. Additionally, there is a short rainy season from October to November, during which malaria transmission is minimal. As of the 2019 Kenya Population and Housing Census, the population size is reported to be 1,266,660 persons. The county health system in Kisii consists of both government and private health facilities. In the government sector, there is one teaching and referral hospital (Kisii Teaching and Referral Hospital - KTRH), serving as a regional reference hospital and providing teaching services for Kisii University Medical School. Additionally, there are 14 sub-county hospitals, 84 dispensaries, 28 health centers, and 32 community health units, serving as centers for minor health cases. The county experiences three distinct rain seasons: April–May, August–September, and November–December, with malaria being the primary health concern. Key malaria intervention approaches in the region include proper case management with antimalarial drugs like Artemisinin-based Combination Therapies (ACTs), Intermittent Preventive Treatment in Pregnancy (IPTp), and the use of mosquito nets. The current drug used for treating uncomplicated malaria is Artemether-Lumefantrine (AL). Malaria diagnosis and treatment services are available in all government health facilities and some private facilities. The average temperature range in the county is between 21°C and 30°C (Fig 1).

STUDY DESIGN

The study employed a random sampling method to collect malaria vector species in the households of confirmed malaria-positive patients attending health centers located in the selected three malaria endemic zones.

MOSQUITO COLLECTIONS

Adult mosquito collections were conducted monthly, specifically during the long rainy season in May and June, as well as at the conclusion of the long rainy season in July 2021. Mosquitoes seeking hosts indoors were captured using Centers for Disease Control and Prevention (CDC) light traps (John W. Hock Ltd, Gainesville, FL., USA).

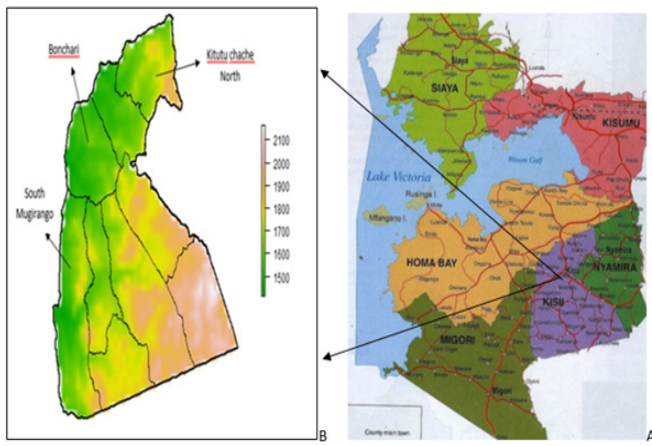


Figure 1: A map showing the study area. **A.** Shows Western region where Kisii County is located and **B.** shows different sub-counties of Kisii County where sampling was conducted (obtained from © 2018 GADM)

The traps were positioned inside houses near the beds where the selected malaria positive patient sleep, placed at a height of 1.5 m, and operated from 18:00 to 06:00 h in households where study participants were found to be microscopically positive for malaria. All the mosquitoes that were collected underwent morphological identification to determine their genus, utilizing established identification keys [11]. Female *Anopheles* mosquitoes were further categorized as unfed, blood-fed, half-gravid, and gravid. Each mosquito was carefully placed in a labeled 1.5 ml Eppendorf tube, which contained silica gel desiccant and cotton wool. The samples were subsequently stored at a temperature of -20 °C in a refrigerator at the Kenya Medical Research Institute (KEMRI) in Nairobi until they could be further processed.

DETERMINATION OF VECTOR SPECIES

At KEMRI in Nairobi, all female *Anopheles* species were identified to their specific species using established identification keys [10]. Additionally, the protocols developed by Koekemoer et al. for *An. funestus* s.l. [12] and Scott et al. for *An. gambiae* s.l. [13] were followed.

IDENTIFICATION OF SPOROZOITE ELISA

The dried head and thorax of the preserved *Anopheles* mosquito specimens were meticulously separated from the abdomen. Subsequently, these separated parts were tested for *P. falciparum* circumsporozoite proteins (CSPs) utilizing the sandwich ELISA method [14].

DATA ANALYSIS

The density of adult *Anopheline* mosquitoes was computed as the count of female mosquitoes per trap/night for each collected location and calculated as proportions and mean $\pm$ standard error (SE). The sporozoite rate was calculated as the ratio of mosquitoes testing positive for *P. falciparum*

CSPs to the overall number tested. The annual entomological inoculation rate (EIR) was computed based on mosquito collections using CDC light traps. The formula utilized was $1.605 \times (\text{number of CSP-positive ELISA results from CDC light traps} / \text{total number of mosquitoes tested}) \times (\text{number of mosquitoes collected from CDC light traps} / \text{number of trap-nights}) \times 365$ [15].

RESULTS

MOSQUITOES COLLECTED

A total of 1305 mosquitoes were collected, of which Eramba, Sieka, Kiaruta, Nyamagiri, Suguta, and Moticho contributed a total mean of 24.06, 17.16, 17.09, 16.78, 14.87, and 10.04, respectively (Fig 2). A notable disparity in the co-occurrence of mosquitoes collected was observed among the study locations ($p < 0.001$). The majority of the *Culex* mosquitoes collected were female across all selected study areas (Fig 3). However, in the case of *Anopheles* mosquitoes, the distribution of females varied depending on the collection area (Fig 4).

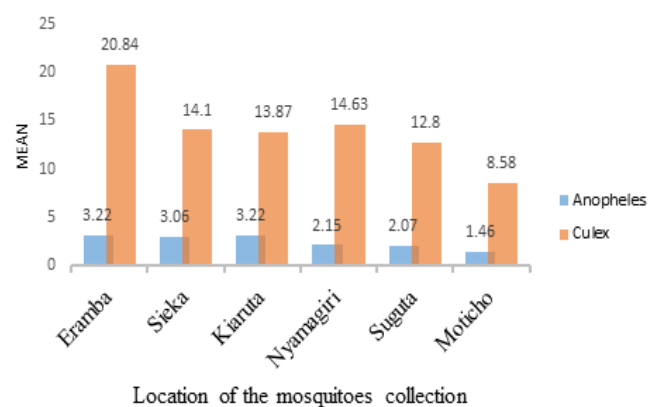


Figure 2: Mean of all mosquitoes collected in different locations in Kisii County

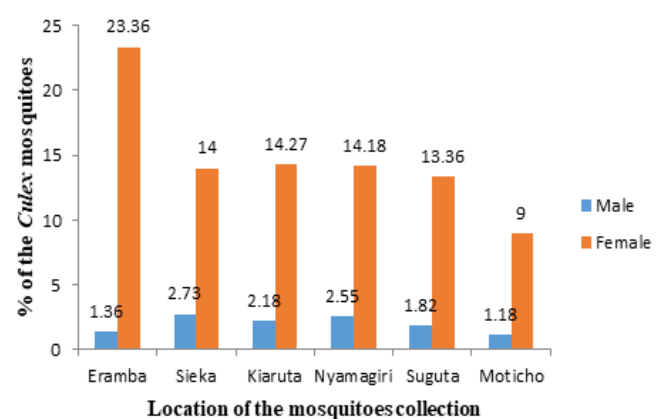


Figure 3: Percentage of the *Culex* mosquitoes collected in different locations in Kisii County

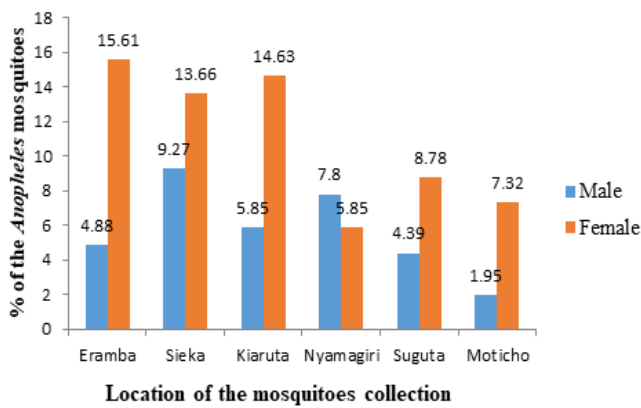


Figure 4: Percentage of the *Anopheles* mosquitoes collected in different locations in Kisii County

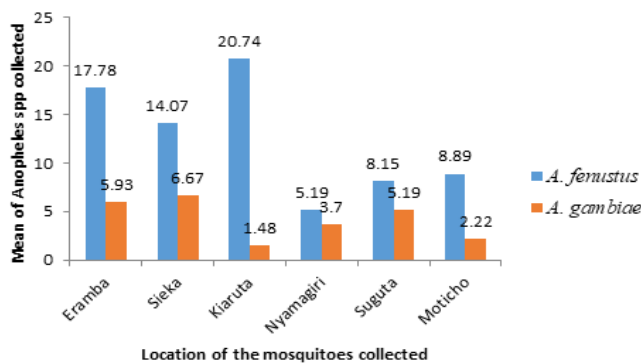


Figure 5: Mean of *Anopheles* species mosquitoes collected in different locations in Kisii County

FEMALE ANOPHELES MOSQUITOS' SPECIATION

Among the total female *Anopheles* mosquitoes collected, *Anopheles funestus* emerged as the predominant species exhibiting a mean of 12.47 ($\pm$ SE of 6.06), whereas *Anopheles gambiae* showed a mean of 4.20 ($\pm$ SE of 2.08) (Fig 5). All collected female *Anopheles* species were examined for *P. falciparum* CSPs. However, none of them tested positive for *P. falciparum* CSPs.

DISCUSSION

The present study showed the dominance of *Culex* mosquitoes in the endemic areas of Kisii County similarly to the previous study in Nigeria [16]. The substantial presence of *Culex* mosquitoes in rural regions is a cause for concern. While this *Culex* mosquitoes has traditionally been linked to urban filariasis, there is a possibility that it could play a role alongside *Anopheles gambiae* in the development of rural bancroftian filariasis. The prevalence of *Culex* mosquitoes in the area under investigation raises the likelihood of coinfections (bancroftian filariasis, malaria) among patients. The present study has revealed that *Anopheles* and *Culex* species coexist in the study area due to differences in niche specialization, feeding and breeding

habits, seasonal activity patterns, and distinct ecological functions. These variations enable both species to thrive in the same geographic location without direct competition for resources. The current research also disclosed that the primary vector influencing disease transmission in the region is *Anopheles funestus*. In this study, *Anopheles funestus* has been identified as the key vector in malaria transmission, attributed to its remarkable ability to adapt to survival strategies during the dry season. This supports the earlier report indicating the resurgence of *Anopheles funestus*, suggesting its potential role in malaria transmission in western Kenya [17]. Artificial ponds, primarily constructed for activities such as brick making, significantly contributed to the prevalence of *Anopheles funestus* abundance in the study areas. Field observations indicate that man-made ponds function as permanent habitats, retaining water for more extended periods compared to other habitat types. This observation suggests that malaria transmission in the study areas is, in part, influenced by human activities. Therefore, effective environmental management, particularly through habitat manipulation, has the potential to mitigate malaria transmission by major vectors of human malaria. This study underscores the significant impact of anthropogenic modifications on ecosystems in shaping the abundance and distribution of malaria vectors. The present study has observed the coexistence of *Anopheles* spp mosquitoes and *Culex* mosquitoes. Similarly, to the present study, a study conducted in Uganda [18] has noted that *Anopheles funestus* coexists in habitats with other *Anopheles* and *Culex* species. This finding suggests that any control program focused on targeting *Anopheles funestus* could have a significant impact on controlling other equally important vectors responsible for human malaria and other mosquito-borne diseases. The current investigation gathered *Anopheles* species mosquitoes indoors that showed no presence of sporozoites. The ongoing proportional rise in the absence of sporozoites in indoor-collected *Anopheles* population could be attributed to the expanded coverage of Long-Lasting Insecticidal Nets (LLINs) and/or the zoophilic, exophagic, or exophilic behavior of this species. Another possibility is a shift in the prevalent mosquito species either being early/late feeders. This indicates a heightened risk of disease transmission despite the nighttime use of bed nets as a preventive measure by the population. The effectiveness of Long-Lasting Insecticidal Nets (LLINs) as control intervention methods has been compromised by these shifts in the behaviors of the malaria parasite vector. Additionally, such shifts in the composition of vector species like this, may compromise the effectiveness of Insecticide-Treated Nets (ITNs), as these interventions may not be designed to target zoophilic and exophilic vector species. This avoidance of the lethal effect of ITNs can perpetuate residual malaria transmission.

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DECLARATION OF INTEREST STATEMENT

The authors have no relevant financial or non-financial interests that are directly or indirectly related to the work submitted for publication.

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AUTHOR CONTRIBUTIONS

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by [Pacifica Chepchumba Bwogo], [Samuel Mong'are], [Rael Masai] and [Dr. Damaris Matoke-Muhia]. The first draft of the manuscript was written by [Pacifica Chepchumba Bwogo] and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

ETHICS APPROVAL

Approval was granted by the Ethics Committee of Kisii County Government County Health service ref no: DTR (4)16.

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