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Mosquito species composition and distribution dynamics in Kalehe Territory, Democratic Republic of the Congo: A focus on *Anopheles funestus* s.s. and *Anopheles gambiae* s.s

Sévérin N'DO[1-2-3], Bazoma BAYILI[4], Jacques 1er Jumeau KABORÉ[3-5], Janvier BANDIBABONE BALIKUBIRI[6], Bertin ZAWADI MUSAKA[6], Adrien Marie Gaston BELEM[3], and Bantuzeko CHIMANUKA[6-7]

[-1]Laboratoire de Recherche sur les Maladies Infectieuses et Parasitaires, Institut de Recherche en Sciences de la Santé (IRSS, CNRST), Direction Régionale de l'Ouest, 01 BP 545 Bobo-Dioulasso 01, Burkina Faso

[2]Lutte antivectorielle, Département des Maladies Tropicales, Médecins Sans Frontières (MSF) OCBA, Carrer de Zamora 54 Barcelona 08005, Barcelone, Spain

[3]Laboratoire de Recherche en Santé Animale, Université Nazi BONI, 01 BP 1091 Bobo-Dioulasso 01, Burkina Faso

[4]Programme Aménagement et Suivi des Ecosystèmes, Institut de l'Environnement et de Recherches Agricoles (INERA, CNRST), Direction Régionale de Recherche Environnementale et Agricole de l'Ouest, 01 BP 910, Bobo-Dioulasso 01, Burkina Faso

[5]Unité de recherche Maladies à Vecteurs et Biodiversité, Centre International de Recherche-Développement sur l'Elevage en zone Subhumide (CIRDES), 01 BP 454 Bobo-Dioulasso 01, Burkina Faso

[6]Laboratoire d'Entomologie Médicale et Parasitologie, Centre de Recherche en Sciences Naturelles de Lwiro (CRSN, Lwiro), Goma entre de Lwiro, N3, Bukavu, RD Congo

[7]Faculté de Médecine et Pharmacie, Université Officielle de Bukavu, BP 570 Bukavu, RD Congo

ABSTRACT: Malaria remains a major public health issue in the Democratic Republic of the Congo (DRC), with limited entomological data on mosquito species composition in some regions. This study investigates the mosquito species composition in Tushunguti, Kalehe Territory, with a particular focus on *Anopheles funestus s.s.* and *Anopheles gambiae s.s.* Adult mosquitoes were collected using CDC light traps and pyrethrum spray catches. Morphological identification was supplemented by molecular techniques (PCR) to confirm species identities. A total of 245 mosquitoes were collected, with *Culex* species being the most abundant (n=150), followed by *Anopheles funestus* and *Anopheles gambiae*. Molecular analysis identified 49 *Anopheles funestus s.s.* and 20 *Anopheles gambiae s.s.* specimens. The human biting rate (HBR) for *An. funestus* was significantly higher (0.071 bites/person/night) compared to *An. gambiae* (0.028). These results suggest that *An. funestus* plays a dominant role in malaria transmission in the area, particularly in indoor environments. The co-occurrence of both species underlines the need for targeted vector control strategies that address species-specific behavior and distribution. The study emphasizes the importance of continuous entomological surveillance to adapt malaria interventions based on local vector dynamics.

KEYWORDS: *Anopheles funestus, Anopheles gambiae,* Species composition, Mosquito distribution, Democratic Republic of the Congo.

Corresponding Author: Sévérin N'DO

1 INTRODUCTION

Malaria remains one of the leading causes of morbidity and mortality worldwide, disproportionately affecting sub-Saharan Africa, which accounts for over 90% of global cases and deaths [1]. Among the countries most affected, the Democratic Republic of the Congo (DRC) ranks second after Nigeria, contributing significantly to global malaria transmission [2]. The persistence of malaria in the DRC is largely due to the high efficiency of its primary vectors, *Anopheles gambiae* sensu stricto (*An. gambiae* s.s.) and *Anopheles funestus* sensu stricto (*An. funestus* s.s.), both of which exhibit marked anthropophilic and endophilic behaviors, making them particularly effective at transmitting *Plasmodium* parasites [3], [4].

These two vector species differ significantly in their ecological preferences and seasonal dynamics. *An. gambiae* s.s. typically breeds in temporary, sunlit pools and is highly influenced by seasonal rainfall patterns, while *An. funestus* s.s. favors more permanent, vegetated water bodies such as swamps and rice paddies, allowing it to persist year-round [5], [6]. These ecological differences influence their distribution, abundance, and contribution to malaria transmission across varying landscapes and seasons [7].

Understanding the species composition and behavior of malaria vectors is critical for developing and implementing effective vector control strategies, especially in the context of increasing insecticide resistance. Interventions such as long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS) have been cornerstones of malaria control programs. However, their efficacy is compromised by the emergence and spread of resistance mechanisms in *An. gambiae* s.s. and *An. funestus* s.s., as observed in several African countries, including the DRC [4], [8], [9].

Eastern DRC, particularly the Sud-Kivu Province, represents a region of intense malaria transmission but remains underrepresented in entomological research. The local geography, characterized by high rainfall, dense vegetation, and subsistence agriculture, provides ideal breeding conditions for a diversity of mosquito species. However, there is a lack of updated, location-specific entomological data for rural areas such as Tushunguti in Kalehe Territory, hindering targeted, evidence-based vector control interventions [9], [10].

To fill this gap, the present study aimed to characterize the mosquito species composition in Tushunguti, with a particular focus on *An. funestus* s.s. and *An. gambiae* s.s. By integrating morphological identification with molecular techniques, we sought to establish a baseline for vector species diversity and relative abundance. This work provides critical data to support the design of adaptive, ecologically informed malaria control strategies in this high-transmission region of eastern DRC.

2 METHODS

2.1 STUDY SITE

This study was conducted in Tushunguti (1°48'19" S, 28°45'00.5" E), a rural village located in Ziralo Administrative Grouping, Kalehe Territory, Sud-Kivu Province, eastern Democratic Republic of the Congo (DRC). Tushunguti lies within a region characterized by a tropical rainforest climate, with high humidity and abundant rainfall, which provides favorable conditions for mosquito breeding and year-round malaria transmission. The area is situated at an elevation of approximately 1300 m, and is dominated by subsistence agriculture, with households often surrounded by small-scale farms and standing water bodies such as rice paddies, ponds, and slow-flowing streams - ideal habitats for *Anopheles* mosquitoes. The population relies primarily on rainwater and nearby surface water sources, which further contributes to the proliferation of breeding sites. Malaria is endemic in the region, with both *Plasmodium falciparum* (*P. falciparum*) and *P. vivax* reported, though *P. falciparum* is the predominant species [10]. Previous entomological surveys in Sud-Kivu province have identified both *Anopheles funestus* s.s. and *Anopheles gambiae* s.s. as the primary malaria vectors. Tushunguti represents a typical high-transmission setting in eastern DRC, where the effectiveness of vector control interventions is increasingly challenged by insecticide resistance.

2.2 ADULT MOSQUITO COLLECTION TECHNIQUES AND IDENTIFICATION

Mosquito sampling was conducted using two complementary collection methods: Centers for Disease Control and Prevention (CDC) light traps and indoor resting collections via Pyrethrum Spray Catches (PSC).

CDC Light Trap Collections

CDC light traps were deployed in 55 households over a period of 11 nights, with five houses sampled per night. Traps were positioned indoors near sleeping areas where occupants were protected by long-lasting insecticidal nets (LLINs). Due to local

security constraints, traps were operated from 16: 00 to 08: 00. This method was designed to target endophagic, host-seeking mosquitoes during their nocturnal activity period.

Indoor Resting Collections

Indoor resting mosquitoes were collected from 10 additional households using pyrethrum spray catches (PSC). Rooms were prepared by laying white collection sheets on the floor and covering furniture. Aerosol insecticides were sprayed, and knocked-down mosquitoes were collected from the sheets approximately 10-15 minutes after spraying. This method was used to sample blood-fed and resting mosquitoes. All specimens were morphologically identified to genus and species level using the taxonomic key of Gillies and Coetzee [11]. Only *Anopheles* mosquitoes were retained for further analyses. Individual *Anopheles* specimens were stored in 1.5 ml tubes containing silica gel and kept at ambient temperature for subsequent molecular identification.

2.3 Molecular Analysis

Genomic DNA was extracted from individual mosquitoes using the standard cetyltrimethylammonium bromide (CTAB) 2% protocol [12]. Species identification within the *Anopheles funestus* group and the *Anopheles gambiae* complex was performed using PCR-based protocols described by Koekemoer et al. [13] and Santolamazza et al. [14], respectively.

2.4 Data Analysis

Human biting rate (HBR), expressed as the aggressive density (number of bites per person per night), was estimated using CDC light trap collections according to the following formula:

$$HBR = \frac{Total\ number\ of\ unfed\ Anopheles\ females\ collected}{Number\ of\ houses\ \times\ Number\ of\ collection\ nights}$$

To analyze the count data from mosquito collections, a mixed-effects negative binomial regression model was applied using STATA/IC Version 13.1 [15]. Statistical significance was considered at a 95% confidence level ($p < 0.05$).

3 Results

3.1 Mosquito Abundance And Species Composition

A total of 245 mosquitoes were collected using both sampling methods (Table). CDC light traps accounted for the majority of the catch, with 215 mosquitoes collected, while indoor residual collection yielded 30 specimens.

In CDC light traps, *Culex* spp. were the most abundant, representing 69.8% (n = 150) of the total catch, followed by *An. funestus* (20.0%, n = 43), *An. gambiae* (7.9%, n = 17), and *Mansonia* spp. (2.3%, n = 5). The mean number of mosquitoes per household was 3.91, with *Culex* spp. contributing the highest mean density (2.73 mosquitoes/house).

In contrast, Indoor resting mosquitoes collections revealed a different species composition. *An. gambiae* was predominant, accounting for 86.7% (n = 26) of the specimens, followed by *An. funestus* (13.3%, n = 4). No *Culex* or *Mansonia* spp. were recorded with this method. The average mosquito density per household was 3.00, with *An. gambiae* contributing the highest mean (2.60 mosquitoes/house).

Table 1. Comparison of mosquito species composition between CDC light traps and residual indoor collection

Mosquito species	CDC light trap (n= 55 houses)				Residual fauna (n= 10 houses)			
	n	Proportion %	Mean/house	SD	n	Proportion %	Mean/house	SD
Anopheles funestus s.s.	43	20.00	0.78	1.45	4	13.30	0.40	0.70
Anopheles gambiae s.s.	17	7.90	0.31	0.79	26	86.70	2.60	1.43
Culex spp.	150	69.80	2.73	3.27	0	0.00	0.00	0.00
Mansonia spp.	5	2.30	0.09	0.44	0	0.00	0.00	0.00
Total	215	100.00	3.91	-	30	100.00	3.00	-

n = number of mosquitoes; SD = Standard Error

3.2 HUMAN BITING RATE OF ANOPHELES MOSQUITOES

The overall human biting rate (HBR) for *Anopheles* spp., as estimated from CDC light trap collections, was 0.099 bites/person/night. Among the vector species, *Anopheles funestus* exhibited the highest biting rate at 0.071 bites/person/night, followed by *Anopheles gambiae* with a rate of 0.028 bites/person/night (Figure).

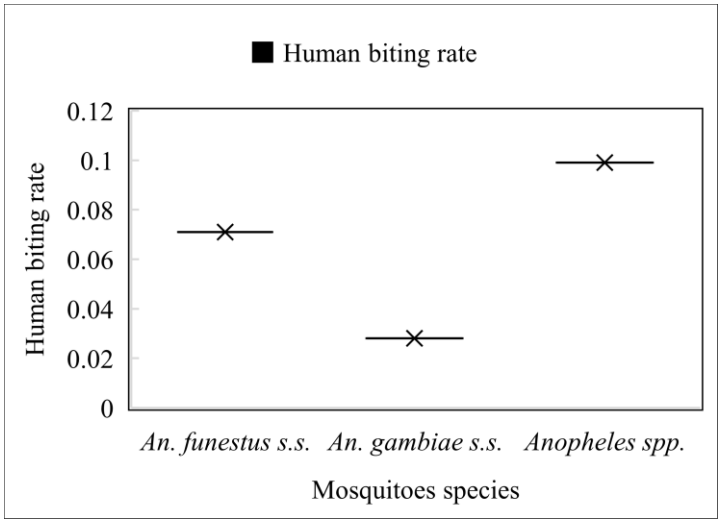

Fig. 1. Indoor human biting rate of Anopheles mosquitoes in Tushunguti in January and February 2018

3.3 MOLECULAR IDENTIFICATION OF MOSQUITO SPECIES

A total of 69 mosquitoes were successfully identified by polymerase chain reaction (PCR) as belonging to the *Anopheles funestus* group and the *Anopheles gambiae* complex. Among them, 49 specimens were identified as *Anopheles funestus* sensu stricto (*An. funestus* s.s.) and 20 as *Anopheles gambiae* sensu stricto (*An. gambiae* s.s.); confirming co-circulation of both major malaria vectors in the area.

4 DISCUSSION

This study provides updated insights into the mosquito fauna composition in Tushunguti, Kalehe Territory, a high-transmission zone in eastern DRC. The co-occurrence of *Anopheles funestus s.s.* and *Anopheles gambiae s.s.* confirms their continued role as the primary malaria vectors in this region, consistent with previous findings from Sud-Kivu and other parts of sub-Saharan Africa [8], [9]. These results are particularly important given the limited and outdated entomological data in this part of the DRC.

Our findings show a higher indoor biting rate and relative abundance of *An. funestus* compared to *An. gambiae,* suggesting a dominant role of *An. funestus* in local malaria transmission. This aligns with recent trends reported across Africa, where *An. funestus* has been resurging as a major malaria vector, especially in areas where environmental conditions support its preferred larval habitats such as permanent water bodies with vegetation [4], [6], [16]. The predominance of *An. funestus* in CDC light trap collections may also reflect its highly endophagic and anthropophilic behavior, which facilitates efficient transmission of *Plasmodium* parasites indoors.

In contrast, the higher proportion of *An. gambiae* in pyrethrum spray catches (PSC) underscores its endophilic resting behavior and its tendency to remain indoors post-feeding. This behavioral distinction between the two species highlights the need for combining multiple sampling methods in entomological surveillance to obtain a comprehensive understanding of vector ecology. It also supports the recommendation that vector control interventions, such as indoor residual spraying (IRS) and long-lasting insecticidal nets (LLINs), must consider species-specific behavioral patterns to maximize impact [7], [17].

The simultaneous presence of both vectors may also complicate malaria control efforts, especially if they display varying levels of insecticide resistance. Previous studies in the Sud-Kivu province have demonstrated resistance to pyrethroids in both *An. gambiae s.s.* and *An. funestus s.s.,* driven by metabolic and target site mechanisms [8], [9]. This resistance may compromise

the efficacy of current LLINs and IRS campaigns, emphasizing the urgent need to implement integrated vector management strategies, including novel tools such as insecticide-treated wall linings or attractive toxic sugar baits [10].

Molecular identification confirmed the species composition determined morphologically, demonstrating the importance of combining these approaches for accurate vector surveillance. Misidentification within the *An. gambiae* complex or *An. funestus* group can lead to incorrect inferences about vector roles, especially when cryptic sibling species are involved [13], [14]. The use of molecular techniques, as done in this study, provides robust evidence of species distribution and strengthens entomological baselines for future monitoring.

These findings have important implications for malaria control in Kalehe Territory and similar high-transmission settings. The local ecology, characterized by rice paddies, permanent streams, and subsistence agriculture, provides year-round breeding sites that favor vector persistence. Therefore, any vector control strategy should be tailored to address both larval and adult stages, ideally combining environmental management, larval source reduction, and adult vector control measures.

Lastly, this study reinforces the critical importance of ongoing entomological surveillance in remote and under-researched areas of the DRC. Vector populations and behaviors are dynamic and may shift in response to environmental changes or control interventions. Sustained monitoring can inform timely adaptations in control programs and prevent the resurgence of transmission in vulnerable communities.

5 CONCLUSION

This study confirms the co-existence of *Anopheles funestus* s.s. and *Anopheles gambiae* s.s. in Tushunguti, Kalehe Territory, with *An. funestus* emerging as the predominant species in indoor biting activity. The observed variation in species composition between sampling methods highlights the ecological and behavioral diversity of malaria vectors in this region. Understanding the relative abundance and biting behavior of these vectors is critical for tailoring effective vector control strategies. In areas like Tushunguti, where environmental conditions favor both species, control measures must consider species-specific differences in habitat use and feeding/resting preferences. These findings provide a valuable entomological baseline for ongoing surveillance and support the integration of ecological and behavioral data into malaria control planning in eastern DRC.

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CONFLICT OF INTEREST

No conflict of interest was declared by any of the authors associated with this work.

ETHICAL APPROVAL

This study protocol received approval from the National Health Ethics Committee of the Democratic Republic of the Congo (Approval No. CNES 001/DPSK/111PM/2017).

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