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**Research Article**

# Assessment of Malaria Vectors in Three ecological zones of Kigali City

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## Abstract

**Background:** Vectors, biologically defined as insects that transmit pathogens to humans, play a critical role in disease transmission. Among them, mosquitoes are considered an important class of vectors. Malaria remains a major public health concern, and understanding vector ecology is essential for effective control. This study aimed to assess malaria vectors in three ecological zones of Kigali city: Kabuye, Niboye, and Vuganyana. **Methods:** A cross-sectional study was conducted in the three sites. Malaria vectors were collected using the Human Landing Catch (HLC) mosquito sampling method. Identification of *Anopheles* species was performed at the Kicukiro sentinel site entomology laboratory using entomological microscopy and morphological identification keys. ELISA tests were conducted at the National Entomological Laboratory of the Rwanda Biomedical Center to determine sporozoite infectivity. **Results:** The results of the study revealed clear variations across the three ecological zones. At the Kabuye site, *An. gambiae s.l.* was the dominant species, with a density of 7.1 b/p/n, accounting for 72% of the total collections, and showing a sporozoite rate of 0.55%. Other vectors identified included *An. coustani* (2.26%) and *An. ziemanni* (1.69%). At the Niboye site, *An. gambiae s.l.* had a density of 1.33 b/p/n, representing 13.53% of the total collections, but with zero sporozoite rate; additional species included *An. coustani* (3.17%) and *An. ziemanni* (2.74%). The Vuganyana site recorded the lowest density of *An. gambiae s.l.* at 0.16 b/p/n, representing 1.69% of the total collections, also with zero sporozoite rate, alongside *An. coustani* (1.69%) and *An. ziemanni* (2.11%). Overall, *An. gambiae s.l.* emerged as the major malaria vector, particularly dominant in Kabuye, an ecosystem characterized by rice cultivation. The Entomological Inoculation Rate (EIR) was calculated at 14, underscoring the high transmission potential in this area. **Conclusion:** This study demonstrates that *An. gambiae s.l.* is the primary malaria vector in Kigali's ecological zones, with significant infectivity observed in Kabuye. Other species, including *An. coustani* and *An. ziemanni*, were present but showed no sporozoite infectivity. Given the high transmission risk in marshy and rice cultivation areas, effective malaria control strategies should include mosquito nets, indoor residual spraying, environmental management, repellents (coils, vaporizers), and larvicides. Future research should further investigate the ecology and infectivity potential of secondary vectors to strengthen malaria prevention efforts in Rwanda.

**Keywords:** *Anopheles gambiae s.l.*; Malaria transmission; Entomological Inoculation Rate (EIR); Kigali ecological zones.

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## 1. INTRODUCTION

Entomology is the study of insects and their relationship to humans, the environment, and other organisms. In many urban settings of East and West Africa, these hydro-agricultural lands serve mostly for the cultivation of rice. The impact of rice paddies on mosquito fauna has been assessed in many towns, their influence on malaria transmission being variable. On the reverse, in most Central African urban or less urbanized settings as is the case in Mali, hydro-agricultural lands are mostly occupied by vegetable crops. Depending on the level of urbanization, the development of hydro-agricultural lands may cause important ecological changes with possible effects on malaria vectors and malaria transmission dynamics (*Sissoko et al., 2004*). The risk of malaria infection is partly dependent on the proportion of mosquito vectors that contain the infectious sporozoite stages of *Plasmodium* species. Sporozoites can only be found in female mosquitoes which have lived long enough for the parasites to complete their sporogonic development and migrate from oocysts on the mild wall to the salivary glands for onward transmission. When an infectious female bites a human host and starts probing, sporozoites are injected with saliva before ingestion of blood *(Hafalla, Silvie, & Matuschewski, 2011)*. Irrigation development projects have been associated with negative impacts on human health, particularly concerning vector-borne diseases. There is evidence for a direct relationship between irrigation development and increased malaria transmission. Rice fields have proved to be particularly well suited as larval breeding sites for *Anopheles gambiae s.l* which is the main malaria vector in sub-Saharan Africa. Malaria entomology is the study of the biology and ecology of the mosquitoes that transmit malaria (*Mutero et al., 2000*). This study involves understanding the relationship between the vector, its ecology and behavior, the parasite, and, the host to develop and implement effective vector control strategies. Briefly, this study was focusing on the identification of malaria vectors in three ecological zones of Kigali city according to their cultivation; it was comparing the malaria vectors which were collected from where they cultivate rice, where they cultivate legumes, and where they found the legumes and the sugar cane.

## 2. MATERIALS AND METHODS

### *2.1 Study setting*

It was a cross-sectional study conducted from April to July 2022 in three ecological zones of Kigali city including Niboye, Kabuye, and Vuganyana located in Kicukiro, Gasabo and Nyarugenge districts respectively. The collection site involves the village rounded by a marshy with legumes cultivation, rounded with rice cultivation and rounded by a marshy with legume and sugar cane cultivation respectively. Malaria vectors were collected using Human Landing Catches method and identification was realized with entomological microscopy and morphological features. Malaria vectors identified were 472 of which 413 were *An.gambiae* s.l, 31 were *An.ziemani,* and 28 of *An.coustani*

### *2.2. Sample collection and processing*

The mosquitoes collected from three sites were selected and from each site, three houses were randomly selected for mosquito collection during two consecutive nights per month. Thus, a total of three houses were sampled in each site per month. For All houses, mosquitoes were collected using a hand-held global position system. Mosquito biting behavior was assessed by collecting mosquitoes inside and outside each house between 6 pm to 6 am. Two trained volunteers sat with a torch and an aspirator, one inside the house (at the house entrance) and the

other approximately 5–8m away from the house aspirated mosquitoes that landed on their exposed legs. At each house, the door was usually left open before the sleeping period and still opens until morning. In collection for differential mosquito attractiveness between the collections and to avoid fatigue and sleeping, there were two supervisors which control time-to-time activities, and, the collectors took a 10 min break every hour and switched positions at midnight. Mosquitoes collected at each hourly interval were placed in separate paper cups pre-labeled with the date, time, and location of the catch and transported from the field to the laboratory for morphological identification.

### *2.3. Malaria vector identification.*

All mosquitoes collected by Human Landing Catches were transported from the collection field to the Entomological laboratory at Kicukiro Sentinel Site for morphological identification of malaria vectors. Identification was done using a specific microscope designed for entomological examination. Morphological features key according to the figure 1 and 2, identification was done morphologically.

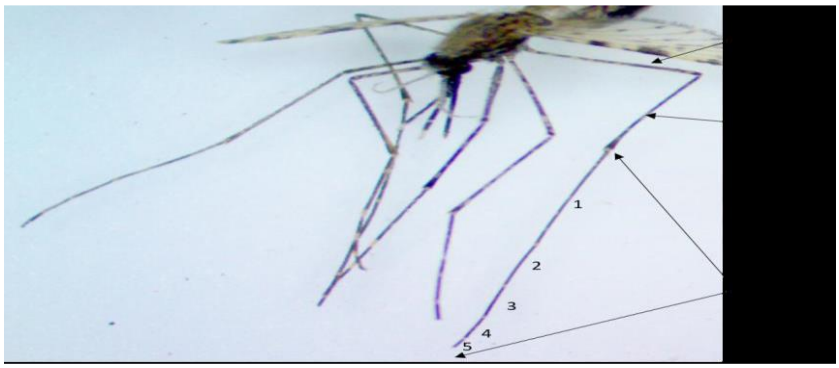



Figure 1: *An.gambiae* s.l legs.

Briefly, Fig. 1 shows a large anopheles with five points; from one point to five points was the Tarsus, those tarsus segments 1-5 pale at tips other point Tibia has a narrow pale ring at tip, and the femur which held on the thorax. On the legs, in general, there are irregularly speckled, those are some features which were based on an identification of *An.gambiae* s.l for *An.ziemanie,* and *An.coustani* the palps were dark and very shaggy usually with four pale rings including one

at the tip, on the legs front and middle is dark but usually with pale rings at tips of most segments, the difference between *An.coustani* and *An.ziemani is* found on the tibia where for *An.coustani* has pale on apex streak which is broadly apical pale band than on *An.ziemani*. The identification continued by looking at the leg's characteristics (Tarsus, Tibia, and femur), and the pulps, every species has it characteristically different from another.




Figure 2: Head of *An.gambiae* s.l.

### *2.4. Malaria vector infectivity tested with ELISA test*

The heads and thoraxes of the individual Anopheles in percentage (59.3%) were tested for CircumSporozoite Antigen using monoclonal antibody according to standard procedure of the national entomology laboratory. Peroxidase conjugated antibodies were used in this Sandwich ELISA method according to the standard protocol. One negative control (male anopheles) and positive control (the appropriate synthetic peptide) were included in each plate. Samples were read with beer Lambert low where the light which traverses the sample is proportional to a concentration in the solution. The authorization to conduct this study was obtained from the National Entomological Laboratory of Rwanda Biomedical Center.

## 3. RESULTS

### *3.1. Species Compositions*

Mosquitoes were collected in three different sites using Human Landing Catches sampling method. They were identified using a morphological identification key published by *Gillies et al. 1987* under entomology microscopy at the entomology laboratory of Kicukiro S.S. Table 1 shows the total number by month, by species, and by study site with their corresponding percentages.

### *3.2. Biting behaviors (Indoor & Outdoor).*

*An.gambiae* s.l were collected in different sites and were identified using entomology microscopic as described here below in table 2 which shows the total number per month, the collection inside and outside by site. Table 3 describes Anopheles species according to the collection sites and months. Anopheles were collected in different sites using Human Landing Catches method and identified with entomology microscopic. Anopheles densities according to the collection site and month. The number of anopheles which was collected, identified, and presented in table number 3 was used in the calculation of densities as presented in table 4.

**Table 1:** Species compositions

| Site / Species | Kabuye | | Niboye | | Vuganyana | |
|---|---|---|---|---|---|---|
| *An.gambiaes.l* | 341 | 9.97% | 64 | 3.0% | 8 | 0.32% |
| *An.ziemani* | 8 | 0.23% | 13 | 0.60% | 10 | 0.40% |
| *An.coustani* | 6 | 0.17% | 15 | 0.70% | 8 | 0.32% |
| *Culexspp* | 3057 | 89.41% | 2035 | 95.67% | 2383 | 96.8% |
| *Aedesspp* | 7 | 0.20% | 0 | 0% | 51 | 2.0% |
| Total | 3419 | | 2127 | | 2460 | |

Table 2: Biting behaviors

| Species | An.gambiaes.l | | | | | |
|---|---|---|---|---|---|---|
| Sites | Kabuye | | Niboye | | Vuganyana | |
| Resting areas | Inside | Out | Inside | Out | Inside | Out |
| Months | | | | | | |
| April | 41 | 121 | 16 | 18 | 0 | 2 |
| May | 34 | 48 | 5 | 2 | 0 | 0 |
| June | 18 | 26 | 11 | 8 | 3 | 0 |
| July | 13 | 40 | 2 | 2 | 0 | 1 |
| Total and % | 106(31%) | 235(69%) | 34(53%) | 30(46.8%) | 3(50%) | 3 (50%) |

Table 3: Anopheles composition by month and collection site

| Species | *An.gambiae* | | | *An.ziemani* | | | *An.coustani* | | |
|---|---|---|---|---|---|---|---|---|---|
| Site / Month | K | N | V | K | N | V | K | N | V |
| April | 162 | 34 | 4 | 3 | 5 | 0 | 0 | 2 | 0 |
| May | 82 | 7 | 0 | 2 | 2 | 0 | 4 | 11 | 2 |
| June | 44 | 19 | 3 | 3 | 5 | 5 | 2 | 2 | 4 |
| July | 53 | 4 | 1 | 0 | 1 | 5 | 0 | 0 | 2 |
| % | 341(72%) | 64(13.5%) | 8(1.7%) | 8(1.7%) | 13(2.7% | 10(2.11%) | 6(1.3%) | 15(3.2%) | 8(1.7%) |

K: Kabuye, N: Niboyi, V: Vuganyana

Table 4: Densities of Anopheles by month and by collection site

| Species | *An.gambiae* | | | *An.ziemani* | | | *An.coustani* | | |
|---|---|---|---|---|---|---|---|---|---|
| | K | N | V | K | N | V | K | N | V |
| April | 13.5 | 2.8 | 0.33 | 0.25 | 0.41 | 0 | 0 | 0.16 | 0 |
| May | 6.8 | 0.58 | 0 | 0.16 | 0.16 | 0 | 0.33 | 0.91 | 0.161 |
| June | 3.6 | 1.58 | 0.25 | 0.25 | 0.41 | 0.41 | 0.16 | 0.16 | 0.33 |
| July | 2.75 | 0.33 | 0.08 | 0 | 0.08 | 0.41 | 0 | 0 | 0.16 |
| Total | 7.1 | 1.33 | 0.16 | 0.16 | 0.27 | 0.20 | 0.12 | 0.29 | 0.16 |

### *3.3. Hourly biting of An.gambiae s.l by site and by species.*

The number of anopheles collected, identified and presented in figure 3 displayed per hour from 6 pm moreover, the hourly biting per village per month and site on *An.gambaie.* s.l also has been analyzed at Kabuye. The hourly biting analysis of *An.gambiae s.l* found that the peak was from mid-night with 49 (14.3%) of A*n.gambiae* s.l and culminates at one hour 01 am with the highest level of 52(15.2%) of *An.gambiae s.l.* At the Niboye site, the peak was found from midnight to one hour 01 am with 14 (21.8%) of *An.gambiae* s.l and at 02 am with 13 (20.3%) of *An.gambiae* s.l. At the Vuganyana site the peak was found at one hour 01 am with (2 *An.gambiae s.l.*) those are well presented in Fig 3

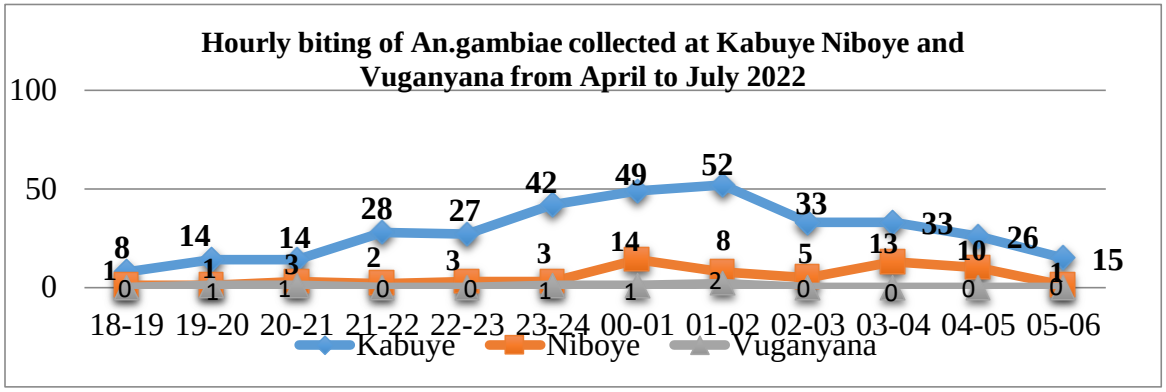



Figure 3: Hourly biting of anopheles collected at Kabuye, Niboye, and Vuganyana.

### *3.4. Infectivity of malaria vectors*

The infectivity of sporozoites was studied using the ELISA technique as the results are shown in Table 5.

Table 5: Summary of ELISA test

| Site / Species | Kabuye | | | | Niboye | | | | Vuganyana | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | TC | TT | TP | TN | TC | TT | TP | TN | TC | TT | TP | TN |
| *An. gambaie* | 341 | 182 | 1 | 181 | 64 | 40 | 0 | 40 | 8 | 6 | 0 | 6 |
| *An. ziemani* | 8 | 7 | 0 | 7 | 13 | 13 | 0 | 13 | 7 | 7 | 0 | 7 |
| *An.coustani* | 6 | 6 | 0 | 6 | 14 | 14 | 0 | 14 | 8 | 5 | 0 | 5 |
| *Sporozoite rate* | 0.55 | | | | 0 | | | | 0 | | | |

TC: Total Collected, TT: Total Tested, TP: Total Positive, TN: Total Negative

### *3.5. Sporozoite rate and Entomology Inoculation Rate of An.gambiae s.l. collected from Kabuye*

The Sporozoite rate is the number of Anopheles containing the sporozoite times a hundred and divided by the total Anopheles tested. The Entomology Inoculation Rate represents the number of infected mosquito bites per person per night or calculated for a period of a month or per year.

Table 6: Sporozoite rate and Entomology Inoculation Rate of *An.gambiae* s.l. Collected from Kabuye.

| Species | TC | TT | TP | % | Sporozoite Rate in % | EIR/year (b/p/year) |
|---|---|---|---|---|---|---|
| *An.gambiae*s.l | 341 | 182 | 1 | 53.3 | 0.55 | 14 |

## 4. DISCUSSION

Table 1 indicated that, *An. gambiae s.l* with 341(9.97%) *was* commonly found at Kabuye where there was more rice agriculture than in other sites. At Niboye, 64 An. *gambiae s.l* has been collected and represented 3.0% of the total catches while other genera were found at the highest level. The genus of *Culex was* found at Vuganyana with 96.8% (n = 2383), at Niboye with 95.6% (n= 2035) and Kabuye at 89.4% (n = 3057), and other species were found in small number. Table 2 presented *An.gambiae* s.l collected inside and outside in which the Kabuye site presented 69% (n = 235) of all *An.gambiae* s.l collected from outside and more than inside. At Vuganyana the collections were 50% from inside and outside. The above collections show how it is complicated to prevent malaria parasites in that area because many populations use the Long Lasting Insecticidal Net inside their houses but the gap remains in control measures to prevent acquiring infection outside their house houses. The letters in table 3 stand for the names of regions where K stands for Kabuye, N for Niboye, and V for Vuganyana. As shown in table 3, *An. gambiae s.l* with 72.0 % (n = 341) was commonly found at the Kabuye site where there was more rice agriculture than at other sites. Other species were found in small quantities as presented in table 3. This study showed similar results as described with another study carried out by Hakizimana, *et al*. 2018. The latter study showed that in Mashesha, Western Province with two annual cycles of irrigated rice, it was recorded there the highest number of *An. gambiae s.l.* with 62,018 (73.7%) of the total *An. gambiae* s.l collected in 7 sites and *An. gambiae* s.l was the dominant species in areas with rice production. Table 4 presented the densities of all anopheles collected with Human Landing Catches where *An.gambiae* s.l was the only dominant Anopheles species. A high number of *An.gambiae* s.l was collected at Kabuye, an area dominated by rice cultivation. The high density was collected there in April corresponding to the highest rain and rice cultivation period. These findings corroborate the results from another study done by Hakizimana, *et al*. 2018, in Rwanda which showed that *An. gambiae* s.l is a dominant species in areas with rice production with high densities of mosquitoes throughout the year. The above site of Mashesha also recorded the highest estimated number of malaria infective bites. Figure 3 shows the hourly biting behavior of *An.gambiae. s.l* collected by Human Landing Catches from 6 pm to 6 am at the Kabuye site, Niboye, and Vuganyana in April, May, June, and July 2022. It showed the period when the search for blood meal by a mosquito happened and the corresponding peaks as it is described. Similar findings of the study carried out by Hakizimana, *et al*. 2018 at Kigali where showed that *An. gambiae* s.l collected with HLC, the average peak in biting activity was recorded during the second half of the night from 1 am to 2 am for each year from 2010 to 2013 of the survey conducted from 36 sites. From 6 pm to midnight, 36.9% of mosquitoes were collected while the remainder (63.1%) was collected during the second half of the night.

Table 5 shows that 182 (53.3% ) of total *An.gmbiae. s*.l collected from Kabuye was tested by ELSA with infectivity of sporozoite and showed sporozoite infectivity of 0.55. At Niboye, *An. gambiae* s.l tested was 40 (62.5%) of total collected and showed zero infection rate while at Vuganyana 6 (76.5%) of *An.gambiae s.l* tested also showed zero infectivity. For other anopheles

species, Both *An.coustani* and *An.ziemani* were collected from all sites and showed zero infectivity. After that analysis, we could understand the reason to say that *An.gambaie s.*l was the only major vector of malaria in comparison with *An. ziemani* and *An.coustani* because *An.gambaie s.l* was the only species shown 0.55 of infectivity, comparing the villages without rice cultivation and the villages with rice cultivation, Kabuye had vectors of malaria parasite-infected by sporozoite in comparison with other villages cultivated vegetables and sugar cane cultivation. The results in the current study were compared with the study done by Orondo, *et al.* 2021, in Kenya where the annual infectivity rate of 2.21% for this species of *An.gambaie* s.l from non-irrigated or in the village with rice was significantly higher than 0.08 for the planned rice village.

Table 6 here presented shown the infectivity of *An.gambiae* s.l collected from Kabuye, and the Entomology Inoculation Rate was calculated to know what degree of malaria transmission risk at this site, where it was found a high level of mosquito production and more infectivity than in other areas. According to other related studies, the annual entomological inoculation rates (EIRs) must be reduced to less than one to substantially reduce the prevalence of malaria infection and achieve elimination (Shaukat, 2010). This study has shown EIR of 14 infected bites per person and per year and that result indicates that using Long Lasting Insecticidal Net methods alone in Malaria prevention cannot ultimately eradicates malaria parasite transmissions because no indoor intervention sustained an annual EIR of less than one. While researchers develop new tools such as repellents products (coils, vaporizers, spatial repellents), indoor residual spraying, and environment management, the strategies allied with integrated vector management which is based on the following pillars: Evidence-based decision-making; integrated approaches; collaboration within the health sector and other sectors; advocacy, social mobilization, legislation; and capacity-building, and these strategies may make the greatest impact on malaria elimination at the Kabuye site specifically and generally in Rwanda.

## 5. CONCLUSION

The research on the assessment of malaria vectors in three ecological zones of Kigali city revealed that the dominant malaria vector which was identified was *An.gambiae. s.l* with 7.1 of density and representing 72% of total collections in the Kabuye site with 0.55 of infectivity of sporozoite rate. Other vectors are *An.ziemani* 1.69% at Kabuye, 2.74% at Niboye, and 2.11% at Vuganyana and the least vector was *An.coustani* with 1.26% at Kabuye; 3, 17. % at Niboye and 1.69% at Vuganyana and that entire anopheles have zero infectivity of sporozoite rate.

Due to the obtained results, the objectives of this study have been achieved; among two hypotheses: the malaria vectors were identified and the infectivity of sporozoite has been tested. This study indicates that using Long Lasting Insecticidal Net methods alone at the Kabuye site, in malaria prevention could not ultimately eliminate the malaria parasite because the Entomology Inoculation Rate of 14 b/p/year seemed to be high than the threshold of one b/p/year of Entomology Inoculation Rate. A policy should be set involving an increased sensitization about using the tools of fighting malaria transmissions such as Long Lasting Insecticidal Net, indoor residual spraying, and environmental management, with larvicidal products and repellents products (vaporizers, coils, spatial sprays) applied through an integrated vector management approach. Those strategies may make the greatest impact on malaria elimination specifically at the Kabuye site, and thus in Rwanda.

*Data availability*

The raw data used to support the findings of this study are available from the corresponding author upon request.

*Disclosure*

The research was conducted with the contribution of authors working at INES-Ruhengeri; at the University of Rwanda and Rwanda Biomedical Center.

*Conflict of interest*

The authors declare that they have no conflicts of interest.

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