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Moustapha Nikiema
[1] Centre de Recherche en Santé de Nouna (CRSN) BP: 02 Nouna Rue Namory Keita Burkina Faso
[2] Université Joseph Ki-Zerbo 03 BP 7021 Avenue Charles De Gaulle Ouagadougou, Burkina Faso

Awa Gneme
Université Joseph Ki-Zerbo 03 BP 7021 Avenue Charles De Gaulle Ouagadougou, Burkina Faso

Peter Dambach
Institute of Global Health University Hospital Heidelberg Im Neuenheimer Feld 36569120, Heidelberg, Germany

Aboubakar Sanon
Université Joseph Ki-Zerbo 03 BP 7021 Avenue Charles De Gaulle Ouagadougou, Burkina Faso

Boubacar Coulibaly
Centre de Recherche en Santé de Nouna (CRSN) BP: 02 Nouna Rue Namory Keita Burkina Faso

Justine Kabore
Université Joseph Ki-Zerbo 03 BP 7021 Avenue Charles De Gaulle Ouagadougou, Burkina Faso

Moussa W Guelbeogo
Centre National de Recherche et de Formation sur le Paludisme (CNRFP) Rue 1847 Avenue Kumda Yonré, 01 BP 2208. Ouagadougou, Burkina Faso

Athanase Badolo
Université Joseph Ki-Zerbo 03 BP 7021 Avenue Charles De Gaulle Ouagadougou, Burkina Faso

Ali Sie
Centre de Recherche en Santé de Nouna (CRSN) BP: 02 Nouna Rue Namory Keita Burkina Faso

Gustave B Kabre
Université Joseph Ki-Zerbo 03 BP 7021 Avenue Charles De Gaulle Ouagadougou, Burkina Faso

Corresponding Author:
Moustapha Nikiema
[1] Centre de Recherche en Santé de Nouna (CRSN) BP: 02 Nouna Rue Namory Keita Burkina Faso
[2] Université Joseph Ki-Zerbo 03 BP 7021 Avenue Charles De Gaulle Ouagadougou, Burkina Faso

Species diversity, blood meal source and infection rate of malaria vectors in the village of Kodougou, Northwestern Burkina Faso

Moustapha Nikiema, Awa Gneme, Peter Dambach, Aboubakar Sanon, Boubacar Coulibaly, Justine Kabore, Moussa W Guelbeogo, Athanase Badolo, Ali Sie and Gustave B Kabre

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Abstract
Background and Objectives: *Anopheles gambiae* members are the main malaria vectors in Burkina Faso seconded by *Anopheles funestus*. However, other anophelines species are found in particular environments such as forest and localities bordering the rivers. The aim of this study, was to assess the species diversity, the blood feeding preferences and the infection rate of all anophelines species found in Kodougou, a village situated near the Mouhoun River.
Methods: Adult mosquitoes were collected from September to December 2018 using insecticide spraying catch in 160 randomly selected houses. DNA extracted from wing/legs, head/thorax, abdomens were used respectively for mosquitoes species molecular identification, infection detection and blood meal source identification from female anophelines. The entomological inoculation (EIR) rate was to estimate malaria transmission intensity in the study area.
Results: A total of 1528 anophelines were collected consisting of 1392 (91.1%) *Anopheles gambiae*, 115 (7.5%) *Anopheles nili*, 8 (0.5%) *Anopheles funestus*, *13 (0.9%) Anopheles pharoensis*. The most abundant specie was *Anopheles coluzzii* representing for 87% of total of *Anopheles gambiae* s.l. Blood meal source was mostly human host (88%), followed by cattle (11%) and pigs (1%). The overall EIR was 0.1 infective bite per human during the study period. The highest EIR with 0.08 infective bite per human (i.b/h) for *Anopheles coluzzii*. Likely, minor vectors *Anopheles nili* and *Anopheles pharoensis* presented an EIR of 0.02 i.b/h and 0.04 i.b/h respectively.
Conclusion: This study shown that in addition to *Anopheles gambiae*, minor vectors like *Anopheles nili* and *Anopheles pharoensis* contribute to malaria transmission in the northern part of Burkina Faso. So, control measures should take into account these species for effective result.

Keywords: Malaria vectors, blood meal source, Infection rate, entomological inoculation rate, Kodougou, Burkina Faso

1. Introduction
Malaria remains the world's most prevalent human parasitic disease despite the colossal and diverse means available for it control and elimination [1]. More than 200 million reported cases worldwide and nearly 400,000 deaths, with about 95% of cases are reported from the African region alone by 2020 [2]. In Burkina Faso, malaria is endemic and accounted for 3.4% of cases globally with about 12.2 million clinical cases reported in 2021, a total of 4,350 deaths occurred, of which 2,900 were among children under five [3]. Three species of Plasmodium are involved in malaria transmission in the country, including *Plasmodium falciparum* with over 98% of cases, *Plasmodium malariae* and *Plasmodium ovale* which share the remaining 2% [4].
The major malaria vectors in Burkina Faso are members of the *Anopheles gambiae* complex which are abundant in the rainy season i.e. July to October while *Anopheles funestus* is prevalent during the cold season i.e. November to February [5]. This temporal dynamic of the vectors explains the continuity of malaria transmission throughout the year [6]. In addition to these major vectors, other species of anopheles of local importance are found in particular environments such as forests, swamps and in localities bordering rivers [7].

These vectors include *Anopheles nili* with a large distribution along rivers, the forest and *Anopheles pharoensis* which is most abundant in the soudano sahelian climate and savannah areas [12, 11].

Determining malaria transmission intensity is crucial for the adapted selection a control strategy and can be assessed by the use of entomological, parasitological and clinical data [10]. Entomological surveys provide data on the composition of the anopheline fauna, blood meal source and entomological inoculation rate (EIR) [11]. Previous studies have shown a high diversity in anopheles species composition especially in the localities bordering the Mouhoun River [14, 15, 16]. The current study reports on the blood feeding preferences and the contribution of anopheles mosquitoes to malaria transmission in Kodougou in Western Burkina Faso with the involvement of *Anopheles pharoensis* as malaria vector.

2. Material and Method

2.1 Study area

The study was carried out in Kodougou (N 12°82; 3°60 E) (Fig 1). This locality is part of the Demographic and Health Surveillance System (SSDS) of the Nouna Health Research Center (CRSN). Kodougou is located about 250 km Northwest from Ouagadougou, the political capital of Burkina Faso. The village is bordering the Mouhoun River, a permanent stream allowing fishing activities, rice growing and gardening all year round. The houses are for the most part made of mud with straw roofs. The climate is of soudano-sahelian type with a wet season (June to October) and a dry season (November to May). The period of high malaria transmission follows the rainy season with few weeks of lagged onset. Kodougou has a microclimate characterized by the permanent presence of vegetation, water in certain places (rice fields).

2.2 Study design and mosquito collection

Indoor-resting mosquitoes were collected weekly from September to December 2018 by using pyrethrum spray (PSC). Each week, 10 randomly selected houses were visited. Houses characteristics, impregnated bed-net use, number of person in the house and presence of animals were recorded.

Before mosquitoes collection, informed consent was obtained from the head of the household.

2.3 Laboratory processing

After morphological identification, mosquitoes were sorted according to the physiological statut as fed, unfed and gravid. The blood from fed mosquitoes was collected on filter paper. The spot was dried at room temperature and then individually packaged with silica gel and kept at -20°C until molecular processing. The mosquitoes were stored individually in tubes containing silica gel and kept at room temperature until PCR analyses.

2.3.1 Anophelines species molecular identification

A sub-sampling of 397 *Anopheles* was done randomly considering their density collected per species and per month. Only the specimens of *Anopheles gambiae* s.l (n=261) and *Anopheles nili* s.l (n=115) were used for molecular identification. Mosquitoes were dissected and wings and legs were analysed by PCR diagnostic assays described by Scott *et al*., 1993 for *Anopheles gambiae* sl species identification and the protocol of Kengne *et al*., 2003 for *Anopheles nili* group identification [12, 13]. Former molecular form M (*Anopheles coluzzii*) and S (*Anopheles gambiae*) detection was carried out using the protocol of Favia *et al*., 2001 [19].

2.3.2 Blood meal source identification

DNA was extracted from blood spots using Qiagen mini kits (Qiagen Germany) and was processed as described by Kent and Norris., 2005 [20]. Five primers corresponding to mammalians potentially present in visited houses (human, cattle, goat, porcine, dog) were used for DNA amplification.

2.3.3 Plasmodium detection

DNA extracted from heads/thoraxes of all collected mosquitoes species were tested for *Plasmodium infection* by PCR diagnostic assays as described by Snounou *et al*.,1993 [21]. The amplified DNA product was electrophoresed in TBE buffer on 2% agarose gel (3:1 SeaKem Agarose:NuSieve Agarose) and visualized under ultraviolet transilluminator after staining with ethidium bromide.

2.4 Data analysis

All data were analysed using version 3.6.0 of R Software package. Infection rate (IR), Human Biting rate (HBR) and Entomological inoculation rate (EIR) were calculated. IR is the ratio of the number of mosquito detected positive for *Plasmodium falciparum* to the total number of mosquitoes tested. Human blood index (HBI) represents the proportion of female mosquito with human blood in their abdomen [22]. Human biting rate (HBR), refers to the number of mosquito per house per person multiplied by the human blood index (HBI). EIR is the product of the HBR and the IR. The ratio fed/gravid mosquitoes was calculated to determine the endophilic and exophilic behavior. The proportions were compared using the Chi-squared test. The Kruskal–Wallis test was used to assess the difference in the mosquito abundance according to the species and habitat characteristics. A P-value of 0.05 was considered indicative of a statistically significant difference.

3. Results

Tables and Figures

Table 1: Physiological status of female mosquitoes and Fed/Gravid ratio

Anophelines species	Fed	Half-gravid	Gravid	Unfed	Ratio Fed/Gravid
Anopheles coluzzii	62	103	44	18	1.4
Anopheles gambiae s.s	03	18	04	05	0.75
Anopheles arabiensis	01	02	01	00	01
Anopheles nili	54	08	12	41	4.50
Anopheles pharoensis	04	03	02	04	02
Anopheles funestus	05	00	01	02	5
Total	129 (32.5%)	134(33.8%)	64(16.1%)	70(17.6%)	1.6

Table 2: Blood meal source of collected anopheline species

Species	Human	Cattle	Pig	HBI
Anopheles arabiensis	01	0	0	100%
Anopheles coluzzii	50	11	01	80.6%
Anopheles gambiae s.s	03	0	0	100%
Anopheles nili	31	0	0	100%
Anopheles pharoensis	04	0	0	100%
Total	89	11	01	83.2%

Table 3: Infection rate (IR), Human biting rate and Entomological inoculation rate of mosquitos

Anophelines species	Proportion of mosquitos tested	IR	HBR	EIR
Anopheles arabiensis	1.5%	00	0.05	00
Anopheles coluzzii	87%	2.7%	3.0	0.08
Anopheles gambiae ss	11.5%	6.7%	0.4	0.02
Anopheles nili	100%	1.7%	1.2	0.02
Anopheles pharoensis	100%	15.4%	0.3	0.04
Anopheles funestus	100%	00	2.1	00

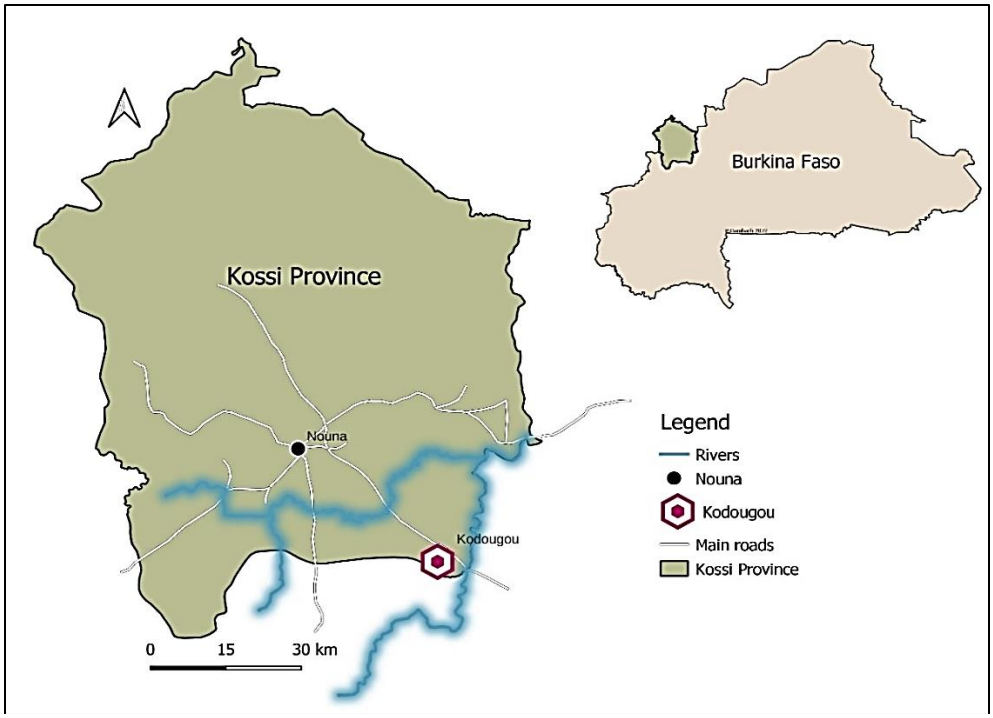

Fig 1: Map of the study site showing it proximity to the Mouhoun River

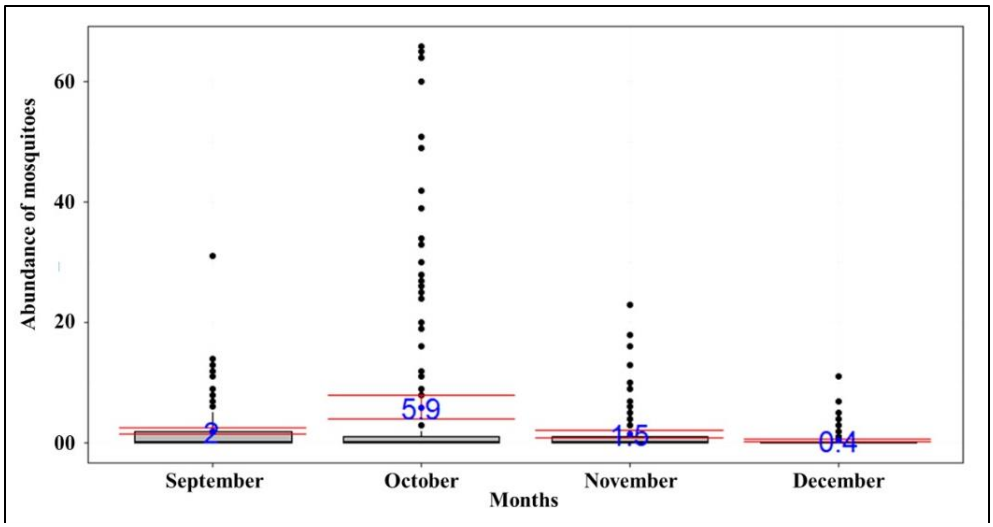

Fig 2: Graphic representing the monthly variation of mean number with confidence intervals of anophelines collected with insecticide spray

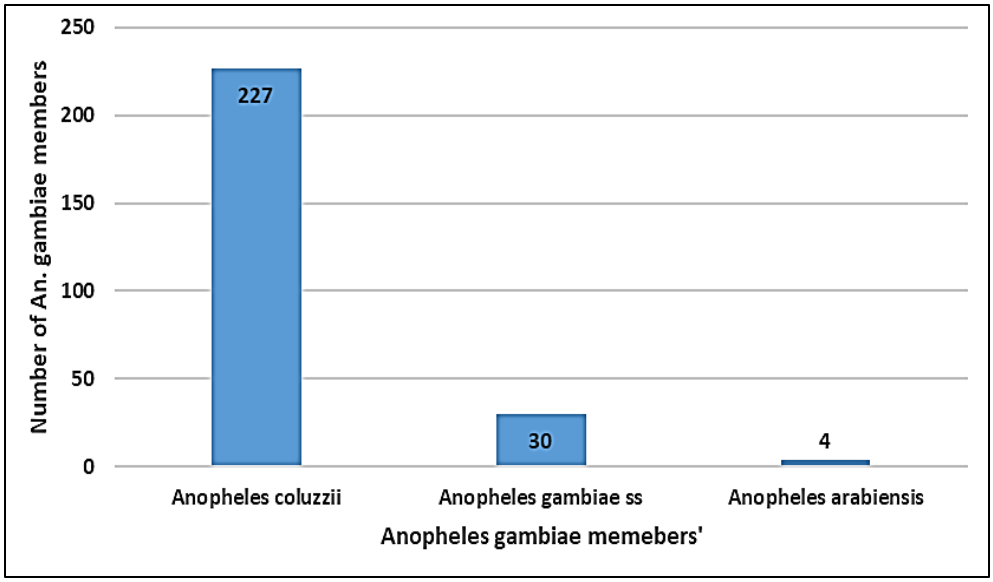

Fig 3: Composition and abundance of members of the *Anopheles gambiae* sl complex after molecular analysis of 261 specimens randomly selected.

3.1 Habitat characteristics

A total of 160 houses were visited during the study. Eighty six (53.75%) were traditional, 60 (40%) were mix (traditional and modern) and 10 (6.25%) were modern. The average house occupancy rate was 4.45 individuals. The majority (94.5%) of concessions had animals including pets. One hundred and forty-five (90.62%) of the houses surveyed were using insecticides treated bed-nets. In 33.3% of houses insecticides were regularly used by the inhabitants. The mean number of mosquitoes collected in each type of house was 2.8 (95% CI 2.1-3.5) for the mix houses, 2.9 (95% CL 1.6-4.1) for the modern and 2.0 (95% CL 1.6-2.5) for the traditional.

3.2 Diversity and abundance of mosquitoes

A total of 1,528 anophelines were collected during this study including 91.1% (n=1392) of *Anopheles gambiae* s.l, 7.5% (n=115) of *Anopheles nili*, 0.85% (n=13) of *Anopheles pharoensis* and 0.52% (08) of *Anopheles funestus*. The mean number of anophelines fluctuated according to the months of collection (Fig 2). In September, this mean was 2.0 (95% CL 1.5-2.40), October recorded the high mean of mosquitos collected, 5.9 (95% CL 5.0-6.8), in November the mean was 1.52 (95% CL 1.0-2.0) and 0.4 (95% CL 0.1-0.7) for the month of December. Analysis of the median showed a significant difference in the distribution of mosquitoes by collection period (Kruskal-Wallis test = 37.3; dl= 3, p =3.810^{-8}). Out of 261 *Anopheles gambiae* s.l, analysed by PCR for specie identification, 87% (n=227) were *Anopheles coluzzi*, 11.5% (n=30) were *Anopheles gambiae*, 1.5% (n=04) were *Anopheles arabiensis (*Fig 3). All the specimen of *Anopheles nili* s.l analyzed by molecular identification belonged to the same species, *Anopheles nili* s.s.

3.3 Mosquito feeding behavior

Observation of the physiological state of the female mosquito abdomens showed that 17.6% of *Anopheles* were unfed, 32.5% were fed, 33.8% semi gravid and 16.1% gravid. The ratio fed/gravid of *Anopheles gambiae* sl was 1.13 and 4.5 for *Anopheles nili* (Table 1).

3.4 Blood meal source

Molecular analysis for the blood meal source of 101 abdominal contents showed that the majority of anopheles that were caught during the survey fed on human hosts (Table 2), as evidenced by the human blood index (HBI), which was 83.2%. *Anopheles coluzzii* fed on three hosts, humans, cattle and pigs. No blood meal was taken from goats and dogs. All specimens of *Anopheles nili* and *Anopheles pharoensis* analysed had taken their blood meal on humans. Mixed meals have not been observed.

3.5 Human biting rate

The mean human biting rate (HBR) was 3.2 (95% CL 2.8-3.53) bites per person during the study period. According to the collection period, the HBR was 1.5 (95% CL 0.68-1.92)) bites per person during September, 7.7(95% CL 6.7-8.6) during October, 1.7 (95% CL 1.4-1.9) during November and during December, it was 1.0 (95% 0.9-1.1). According to the species the mean HBR was 3.5 (95% CL 3.2-3.73) bites person for *Anopheles gambiae* s.l, 1.2 (95% CL 1.4-1.8) bite per person for *Anopheles nili*, 0.3 (95% CL 0.3-0.35) for *Anopheles pharoensis* and this rate was 0.4 (95% CL 0.1-0.7) for *Anopheles funestus*.

3.6 Infection rate

Out of 397 mosquitoes tested for *Plasmodium falciparum* infection, the overall infection rate was 3.5%. This rate fluctuated according to the *Anopheles* species, it was 2.7% for *Anopheles coluzzii*, 6.7% for *Anopheles gambiae* s.s, 1.7% for *Anopheles nili* and 15.4% for *Anopheles pharoensis*. All the specimens of *Anopheles funestus* were tested negative to plasmodium infection.

3.7 Entomological inoculation rate (EIR)

The overall EIR was 0.1 infective bite per person during the study period. The highest EIR was observed with *Anopheles coluzzii*, with 0.08 infective bite per person (Table 3). For *Anopheles nili* and *Anopheles pharoensis*, the EIR was 0.02 and 0.04 infective bites per person during the four months respectively (Table 3).

4. Discussion

Malaria vectors species diversity and transmission dynamic were assessed in Kodougou (North-western Burkina Faso) during the high malaria transmission period. A total of six anopheles species were caught during this study mainly composed of *Anopheles coluzzii*, *Anopheles gambiae* s.s *Anopheles arabiensis*, and to a much fewer amount of *Anopheles nili*, *Anopheles funestus*, and *Anopheles pharoensis* which was it first report as malaria vector in Burkina Faso. Out of six anopheline species, four were positive for *Plasmodium falciparum*. The majority of anopheline species were anthropophilic.

The coexistence of several species of anopheles in the village of Kodougou had already been reported during an entomological survey in 2012 [13]. The high diversity of anopheline species recorded in this village could be explained by its proximity to the Mouhoun River, which offers supportive ecological conditions for the development of most anophelines species [23]. Near the river, agricultural activities such as rice cultivation and vegetable gardening are being developed. These activities lead to the creation of permanent larval breeding sites where anopheles proliferate [23]. Also, the larvae of certain species such as *Anopheles nili* and *Anopheles funestus* develop preferentially in river water [24]. The density and diversity of mosquitos have fluctuated depending on the collection time period. High densities were observed during the months of September and October while the lowest densities were observed in November and December. These observations are related to rainfall which is linked to the malaria transmission intensity. The low numbers of *Anopheles funestus* and *Anopheles pharoensis* obtained during this study can be explained by the method used for mosquito collection which was only specific to endophilic mosquitos. Also the high sensitivity of those mosquitos to insecticides used for impregnation of bed-nets and in agriculture [25]. The high prevalence of *Anopheles coluzzii* is in line with the studies of Ilboudo-Sanogo *et al*., 2010 [26] and Traoré *et al*., 2019 [27] who observed that *Anopheles coluzzii* is the dominant vector among members of the *Anopheles gambiae* complex in rural Burkina Faso. All the specimens of *Anopheles nili* complex analysed by PCR were *Anopheles nili* s.s. This result is similar to that reported by Adja *et al*., 2011 [29] who concluded that *Anopheles nili* s.s could be the only species of the complex present in West Africa while the others members were observed in Equatorial Africa [30].

The overall human blood index was relatively high (83.2%). This result is questionable due to the very low number of fed female mosquitos of species other than *Anopheles gambiae*. However, previous studies had already noted theirs preferences for human blood [28, 24]. Members of the *Anopheles gambiae* complex diversified their hosts in terms of trophic preference, particularly in *Anopheles coluzzii* where, in addition to humans, feeds on cattle and pigs. This diversity in host selection among members of this complex has already been reported in numerous studies including those of Robert *et al*. (1998) [31] in Niakkar, Senegal and Doannio and Diarrassouba (1998) [29] in Bouaké, Côte d'Ivoire. In *Anopheles funestus* and *Anopheles coustani* specimens, we did not obtain any blood fed females, therefore we could not perform the blood meal analysis. The Fed/Gravid ratio is globally above 1, which means that anopheles have a very high tendency to exophilia.

In our study, only *Plasmodium falciparum* species was screened because it is the main malaria pathogen with a contribution to malaria contribution of more than 98% of cases in Burkina Faso [32]. The results of the molecular analysis for the infection detection from heads-thoraxes revealed that *Anopheles coluzzi*, *Anopheles gambiae*, *Anopheles nili* and *Anopheles pharoensis* were positive for *Plasmodium falciparum*. The overall infection rate was 3.5%. This rate is comparable to that reported by Atangana *et al*. (2010) [33] in a similar environment in Cameroon, where the rate was 3.3%. Infection rates varied from one species to another. For example among the members of *Anopheles gambiae* s.l, *Anopheles coluzzii* had a significantly lower infection rate than *Anopheles gambiae* s.s. This observation is in line with the results obtained by Ndiath *et al*. (2008) [6] in Senegal who demonstrated that *Anopheles gambiae* was more susceptible to infection than *Anopheles coluzzi*. As for *Anopheles arabiensis* and *Anopheles funestus* the search for *Plasmodium falciparum* was negative on all tested specimens. However, *Anopheles funestus* has been reported in other studies to be a carrier of infesting forms of plasmodium [33, 6], and is even reported to be the second major vector in Sub-Saharan Africa countries [5]. These results likely to be related to the high sensibility of this species to insecticides used for impregnating bed nets and in agriculture [35]. For *Anopheles nili*, the infection rate was 1.7%. This result is similar to a study conducted by Adja *et al*., (2011) [28] in Gansé in Côte d'Ivoire, which obtained 1.7% as infection rate. *Anopheles pharoensis* had an infection rate of 15.4%, which is much higher compared to that obtained by Atangana *et al*. (2010) [33] who found the infection rate to be 3.57%. These differences could be due to the fact that the density of this species obtained in our study is low and also by the ecological conditions of the two localities which are almost different. These are the first reported *Anopheles pharoensis* specimens infected by *Plasmodium falciparum* in Burkina Faso. In fact, the analyses for the search of plasmodium performed by Carnevale *et al*. (2009) [36] on 4000 specimens in the Valley of Kou, in Burkina Faso gave a negative result [31]. The reasons for this observation could be that the PCR method we used for the detection of plasmodium infection is more specific than the ELISA method used by Carneval *et al*. The overall EIR was 0.1 infective bite per person (i.b/p) during the whole period of the study. The highest transmission intensities were recorded with *Anopheles coluzzii* (0.08 infected bites per person during the study period). For *Anopheles nili* and *Anopheles pharoensis* these rates were higher at 0.02 i.b/p during the study period and 0.04 i.b/p during the study period respectively. This observation makes *Anopheles pharoensis* a potential vector in malaria transmission.

Limitations: The number of mosquitos obtained was low considering the number of houses and months taken for collection, especially with less prominent mosquito species. Also, we used the PSC for the calculation of the EIR, which remains an approximate method compared to the human landing catches (HLC) which are the reference for the calculation of the EIR.

5. Conclusion

This study showed that *Anopheles coluzzii* was the major vector in malaria transmission in the village of Kodougou due to its abundance and entomological inoculation rate. This work also confirmed the presence and local vectorial role of *Anopheles nili* and *Anopheles pharoensis* in malaria transmission in the village of Kodougou. However further

investigations using different capture methods are needed to further determine the contribution of these minor vectors to malaria transmission in the locality.

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7. Potential reviewers
1. Djogbenou Luc: ldjogbenou22002@yahoo.fr
2. Toe K Hyacinthe: thyacinthe@gmail.com
3. Sombié Aboubacar: aboubacarsombie@yahoo.fr

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