Mosquito (Diptera: Culicidae) diversity and malaria prevalence in Kovié, prefecture of Zio, Togo

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Abstract
Vector-borne diseases are diseases caused by parasites and viruses transmitted by vectors. Africa is home to a rich diversity of vector species which can transmit various diseases including malaria. Human adaptation strategies to climate change such as irrigation and the development of hydro-agricultural areas create a favorable environment that reinforces the development and proliferation of vectors, particularly Culicidae. To determine the composition of mosquito fauna and the malaria prevalence, series of larval surveys and adult mosquito collections (by Spray catch and Human Landin catch) were conducted in Kovié, a peri-urban locality located in the River Zio basin characterized with irrigated rice production. Results showed that the mosquito fauna at Kovié is primarily made up of Anophelineae (78%) and Culicinae (22%), belonging to 4 genera: Mansonia, Aedes, Culex, and Anopheles. The identification of 813 specimens of Anopheles gambiae s.l. using PCR revealed that Anopheles coluzzii was the only species collected. Epidemiological surveys in Kovié medical center showed that malaria was the only mosquito-borne disease recorded in the area. Malaria prevalence in the locality was more or less high among children under 5 years old and pregnant women.

Keywords: Kovié, mosquito fauna, PCR, malaria prevalence

Introduction
Vector-borne diseases are infectious diseases transmitted by vectors which are mostly hematophagous or non-hematophagous arthropods that transmit pathogens, from one vertebrate to the other [1]. These arthropods are mainly insects and mites that transmit parasitic diseases such as malaria, Chagas disease, plague, dengue, chikungunya, Rift Valley fever, and West Nile disease. There is a diversity of vector species in Africa. These species can redistribute in new habitats created by microclimates which are generated by climate change [2]. This redistribution could lead to a new disease mapping. The most common vector-borne disease is malaria, which is caused by a blood parasite of the genus Plasmodium transmitted by mosquitoes of Anopheles genus. Globally, in 2019, malaria cases and deaths were approximately 229 million and 409,000, respectively [3]. Five Plasmodium species including Plasmodium falciparum, P. malariae, P. vivax, P. ovale, and P. knowlesi are present on all the continents. In 2014, reports indicated that in mosquito-infested areas, approximately 120 million people were infected with filaria, 50 million with arboviruses, and 198 million with plasmodium [4,5]. In many African countries, the food self-sufficiency ambition led to the development of hydro-agriculture including irrigated crops, especially rice [6]. Togo has many irrigated areas of which the most important is the irrigated rice-growing area of the Valley of Zio [7]. This zone includes four villages: Mission-Tové, Kovié, Assomé, and Ziovonou with a total surface area of 660 ha [8]. However, only 373 ha are developed for rice production which favors mosquito proliferation. This study is to investigate mosquito diversity and the diseases transmitted in rice-growing environment. The specific objectives are to list the different mosquito of medical importance, to identify malaria vectors, and to establish a link between the presence of malaria vectors and malaria prevalence.
Materials and Methods

Study site
The study was conducted in four villages of Kovié (Fig. 1), located at 30 km northwest of Lomé in the prefecture of Zio. Kovié (06°34′38″N – 01°11′47″E) is a peri-urban area is located in the valley of Zio in Togo. Geographically, this area presents a favorable ecosystem for the development of insect vector species such as Anopheles gambiae s.l. and Aedes sp. Kovié enjoys a tropical climate with two dry seasons and two rainy seasons. The main dry season is from December to March and the short dry season from July to August. The main rainy season runs from late March to June and the short rainy season from mid-September to late October [8]. Irrigated rice cultivation is the main activity in this area [9].

Mosquito collection and identification
Pre-imaginal mosquitoes were collected in gutters, rice fields, car tire pits, containers, and puddles in the neighborhoods of Kovié and especially in the rice-growing areas. Larvae and pupae were collected by dipping technique [10] in positive breeding sites. Collection was carried out twice a month for 4 months (June to September 2020). The larvae and pupae collected were sorted by genus and reared (T = 27±2°C, RH = 80±5%) to adulthood using the method of Desfontaine et al. [11] at the insectary of the Laboratory of Ecology and Ecotoxicology, University of Lomé. Adult mosquitoes were also collected using the methods of Human Landing Catch and Spray-catch, from September 14 to 17, 2020 in habitats where people sleep under mosquito nets in the study areas. Samples were then conditioned in Eppendorf tubes containing silicagel and kept at −4 °C and identified using the keys of Gillies & De Meillon [12], Gillies & Coetzee [13] for Anophelinae, and Edwards [14] for Culicinae. Sibling species of Anopheles gambiae s.l. were identified by Polymerase Chain Reaction-Restriction according to the protocol of Scott et al. [14]. DNA extraction was performed using the method of Collins et al. [15], resuspended in sterile deionized water and kept at −80 °C. The following steps were undertaken to run the PCR according to the protocol of Favia et al. [17]: an initial step of 10 min at 94 °C was followed by 25 cycles with denaturation at 94 °C for 30 s, annealing at 63 °C for 30 s, and extension at 72 °C for 30 s; the products were extended for 7 min at 72 °C, after the final cycle.

Epidemiological data collection
Epidemiological data were collected at the health center of Kovié. To identify the vector-borne diseases which were the primary cause of consultation in the local population, retrospective data from 2018 to 2020 were considered. We focused only on the data of thick blood smears results as reported in the medical registry of the health center.

Statistical Analysis
The prevalence rate was calculated as the number of positive blood smears over the total number of blood smears. Data were processed with Excel 2010 (Microsoft Corp) spreadsheet and the analyses of variances (ANOVA) were performed with the PAST software version 3.23. The significance level was 5%.

Results
Mosquito diversity
A total of 1,038 mosquitoes belonging to two sub-families: Anophelinae, 78% and Culicinae, 22% (Fig. 2) were collected at Kovié. The two subfamilies are divided into four genera: Anopheles, Culex, Aedes, and Mansonia (Fig. 3). Only Mansonia was collected at adult stage and Aedes was at larval
stage. Anopheles and Culex were caught at both larval and adult stages.

![Distribution of mosquito sub-families identified](image1)

**Fig 2:** Distribution of mosquito sub-families identified

Culicinae sub-family (5 species) was more species-rich than Anophelinae (1 species). Culex, Aedes, and Anopheles were the main genera recorded. (Table 1). The Anopheles (n = 813, 78.32%) of Kovié was the most represented with the main species being An. coluzzii, followed by Culex (n = 172, 17%), Mansonia and Aedes which were less represented.

![Distribution of mosquito genera identified](image2)

**Fig 3:** Distribution of mosquito genera identified

| Sub-families | Genera  | Species         | Number (%)   |
|--------------|---------|-----------------|--------------|
| Culicinae    | Aedes   | Ae. aegypti     | 49 (4.7)     |
|              | Culex   | Cx. pipiens     | 45 (4.33)    |
|              | Culex   | Cx. sp          | 33 (3.2)     |
|              | Culex   | Cx. poicilipes  | 94 (9.05)    |
|              | Mansonia| Ma. uniformis   | 4 (0.4)      |
| Anophelinae  | Anopheles| An. coluzzii   | 813 (78.32)  |

**Table 1: Mosquito species composition at Kovié**

Resting behavior

A total of 483 female mosquitoes from 5 different species were caught in 20 bedrooms (Table 2). The average resting density was 4.87 females per bedroom per day (FBD). The highest resting density was observed in An. coluzzii (20.6 FBD) and followed distantly by Cx. Pipiens (2.25 FBD). Low resting densities were observed in Cx. poicilipes (0.95 FBD), Culex sp (0.15 FBD), and Ma. uniformis (0.4 FBD) (Table 2).

**Table 2: Resting density of mosquito species at Kovié**

| Species          | Number collected | Resting density (FBD) |
|------------------|------------------|-----------------------|
| Cx. pipiens      | 45               | 2.25                  |
| Culex sp         | 3                | 0.15                  |
| Cx. poicilipes   | 19               | 0.95                  |
| Ma. uniformis    | 4                | 0.4                   |
| An. coluzzii     | 412              | 20.6                  |
| Mean             | 96.6             | 4.87                  |

Epidemiological survey

Information relating to medical consultations for vector-borne diseases were obtained at the Kovié Health Center. Information was exclusively about malaria (Table 3). The variations in positive thick blood smear (TBS) by year and age group are shown in Figure 3. Results showed that malaria cases were higher in ≥5 years; 579 cases in 2018, 594 cases in 2019, and 165 cases in 2020. However, in <5 years and PW, the TBS revealed that the highest number of malaria infections was recorded in 2019. Analyses showed a significant difference (F= 9.88623; ddf = 2; p = 0.02831) between the values collected in <5 years, ≥5 years, and PW per year. Malaria prevalence by age group is shown in Table 3.

**Table 3: Malaria prevalence between 2018 and 2020**

| Years   | Age groups | Positive TBS | Negative TBS | Total | Prevalence (%) |
|---------|------------|--------------|--------------|-------|----------------|
| 2018    | <5 years   | 100          | 51           | 151   | 66             |
|         | ≥5 years   | 579          | 235          | 814   | 71             |
|         | PW         | 81           | 53           | 134   | 60             |
| 2019    | <5 years   | 88           | 55           | 143   | 62             |
|         | ≥5 years   | 594          | 248          | 842   | 71             |
|         | PW         | 54           | 114          | 168   | 32             |
| 2020    | <5 years   | 26           | 39           | 65    | 40             |
|         | ≥5 years   | 165          | 108          | 273   | 60             |
|         | PW         | 19           | 30           | 49    | 39             |

PW: pregnant women, TBS: thick blood smear

Discussion

In this study, mosquito fauna in Kovié is predominantly comprised of Anopheles, followed by Culex. Similar trends were reported by Djegbe et al. [18] in Benin and Adja et al. [19] in Côte d'Ivoire. Aedes and Mansonia were less represented. The presence of mosquitoes is aggravated by irrigated rice cultivation related breeding sites. Indeed, Kovié is a peri-urban locality located in a lowland. Rice cultivation in lowland has created a very favorable ecosystem for the proliferation of An. gambiae s.l. Several studies have shown the impact of irrigated rice cultivation on the proliferation of Anopheles mosquitoes, especially An. gambiae [20,21]. The diversity of mosquito fauna at Kovié is very low as indicated by Simpson's diversity index value (Ď) = 0.4. This low index could be explained by the short period of collection. Despite such a low index, all the genera of medical importance (Anopheles, Culex, and Aedes) are represented; this represents a threat to the health of the local population. The presence of Culex in the breeding sites could be explained by the presence of ions and organic compounds in the breeding sites because of the use of organic fertilizers in the rice fields. It has been shown that Culex prefers breeding sites rich in organic compounds and adapts to polluted environments with high ionization [22-24]. In addition, An. coluzzii could adapt to organic-rich and highly mineralized breeding sites [25]; this
could explain the proliferation of this species in Kovié. The absence of *Mansonia* mosquitoes during the larval surveys, although collected during the SC is probably related to the Dipping method[^10] used in collecting the larvae. In fact, this collection method is not appropriate for the collection of *Mansonia* larvae which tend to cling to plants. Hence the need to combine adult collection methods to increase the probability of collection. The high number of female mosquitoes recorded is an important factor in the epidemiology and transmission of mosquito-borne diseases given their role as vectors. The fact that An. coluzzii, Cx. poicilipes, Cx. pipiens, and Ma. uniformis were collected in houses explains the anthropophilic behavior of these species. *Anopheles coluzzii* is highly anthropophilic as reported by Dossou-Yovo[^26]. Most of the houses in Kovié have rooms with ventilation openings, which contribute to increasing the number of mosquitoes entering rooms, thus increases the likelihood of infectious bites. In Kovié, malaria is the main cause of consultation, especially among children <5 years, considered as vulnerable population together with PW. The National Malaria Control Program (NMCP) reported that malaria is the leading cause of mortality in pediatric services[^27]. In addition, 2020 data indicated a slightly low prevalence rate in PW and <5 years; this could be explained by the special attention the local community gives to malaria prevention by avoiding mosquito bites through an effective use of mosquito nets, the main vector control tool adopted by the NMCP in the country. High prevalence of malaria (especially in 2018 and 2019) at Kovié could be attributed to the high number of An. coluzzii – the only representative sibling of *Anopheles gambiae* s.l. – recorded in the area and to the reduction of LLINs effectiveness. Indeed, Diuk-Wasser et al.[^28] reported that there is a quadratic relationship between mosquito abundance and malaria transmission. *Anopheles coluzzii* was reported in 2013 and 2016 by Amoudjì et al.[^29] in our study area. However, two other members of the complex including *An. gambiae* and *An. arabiensis*, the main malaria vectors, have been recently reported in Togo[^29-31].

**Conclusion**

Mosquitoes collected at Kovié were predominantly represented by *Anopheles coluzzii*. Data from the health center showed that malaria is the main cause of consultation, especially among children <5 years and PW. Though, malaria prevalence was slightly low in 2020, it was high in 2018 and 2019.

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**References**

1. Tchuinkou D, Tchuente M, Sereno D, Simard F, Bousses P, Kegne P et al. « Projet PAPILLON : Identification rapide des vecteurs impliqués dans les maladies à transmission vectorielle » www.ird.fr 2015; Accessed 2021.
2. Githeko AK, Lindsay SW, Confalonieri UE, Patz JA. "Changement climatique et maladies à transmission vectorielle: une analyse régionale." Bull World Health Org 2001;78(9):1136-1147.
3. World Health Organization (WHO). World Malaria Report. World Health Organization.; Geneva, Switzerland 2020.
4. Paty M-C, Noël H, Septfons A, De Valk J, Franck F, Cochet A et al. Situation des arboviroses en France et dans le monde. Bull veille sanitaire, N°2 Spécial arboviroses 2014, 2-6.
5. World Health Organization (WHO). Les maladies à transmission vectorielle dans le monde. www.who.int/campaigns/world-health-day/2014/factsheets/fr. Published 2014. Accessed May 25, 2021.
6. Faye O, Fontenille D, Hervé JP, Diack PA, Diablo S, Mouchet J. Le paludisme en zone sâhélienne du Sénégal. 1. Données entomologiques sur la transmission. *Ann Soc Belge Med Trop* 1993;73:21-20.
7. FAO. La démarche de qualité liée à l’origine du riz de de Kovié, Togo 2012, 26.
8. Glé KE. Qualification des produits agricoles locaux et Indications Géographiques en Afrique de l’Ouest : Cas du riz de Kovié au Togo. Thèse de doctorat en économie de l’agriculture et des ressources (Agro Campus Ouest-France) et de doctorat en agroéconomie ESA, Univ Lomé 2010, 366.
9. Aboa K, Kpemoua K, Dantsy-Barrier H. Situation de Référence du riz, ITRA-CRAF, 2006, 37.
10. Service MW. Mosquito ecology, field sampling methods vector biology and control. 2nd ed. Liverpool School of Tropical Medicine 1993.
11. Desfontaine MA, Tchikangwa I, Le Goff G, Robert V, Carnevale P. Influence of the alimentation des larves d’*Anopheles gambiae* (Diptera, Culicidae) sur le développement primaginal en insectarium. Bull Liais Doc Oceac 1991;98:12-14.
12. Gillies MT, De Meillon B. *The Anophelinae of Africa south of the Sahara*. Publication of the South African Institute for Medical Research 1968:54:343.
13. Gillies MT, Coetsee M. A supplement to the *Anophelinae* of Africa south of the Sahara. Publication of the South African Institute for Medical Research. 1987;55:143.
14. Edwards FW. Clé des *Culicinæ* adultes de la région éthiopienne. Clé simplifiée de détermination des larves de *Culicidarum* en région éthiopienne. ORSTOM. Labo. Entomol. Med. S. S. Bondy 1941.
15. Scott JA, Brogdon WG, Collins FH. Identification of single specimens of the *Anopheles gambiae* complex by polymerase chain reaction. Am J Trop Med Hyg 1993;49:520-529.
16. Collins FH, Mendez MA, Rasmussen MO, Mehaffey PC, Besansky NJ, Finnerty V. A ribosomal RNA gene probe differentiates member species of the *Anopheles gambiae* complex. Am J Trop Med Hyg 1987;37:37-41.
17. Favia G, Lanfrancotti A, Spanos L, Sidén C, Noël H, Septfons A, De Valk J, Franck F, Cochet A et al. Field collection method is rapid. The Insect Biology Center (CRAF, 2006[^26]).
18. Djebe I, Missihoun A, Djuoka R, Akogbeo M. Surveillance Entomologique: Dynamique de la population et de la résistance aux insecticides chez *Anopheles gambiae* s.l. en milieu de riziculture irriguée au Sud Bénin. J Applied Biosci 2017;111:10934-10943.
19. Adja M, Assare R, Assi S, Ngoran K, Yapi A. Etude du comportement au repos et des préférences trophiques de
Anopheles gambiae dans la ville d'Adzope, Côte d'Ivoire. Eur Sci J 2015;11(3):1857-7431.
20. Sissoko MS, Dicko A, Briet OJ, Sissoko M, Sagara I, Keita HD et al. Malaria incidence in relation to rice cultivation in the irrigated Sahel of Mali. Acta Trop 2004;89:161-170.
21. Koudou BG, Adja AM, Matthys B, Cissé G, Koné M, Tanner M et al. Pratiques agricoles et transmission du paludisme dans deux zones éco-épidémiologiques au centre de la Côte d'Ivoire. Bull Soc Path Exot 2007;100:1-3.
22. Louah M. Écologie des Culicidae (Diptères) et état du paludisme dans la péninsule de Tanger. Thèse de Doctorat d’État ès sciences, Université de Tétouan, Maroc 1995.
23. Himmi O. Les Culicidés (Insectes, Diptères) du Maroc : systématique, écologie et études épidémiologiques pilotes. Thèse de Doctorat d’État, Faculté des sciences, Université Mohammed V, Maroc 2007.
24. El Ouali Lalami A, Hindi T, Azzouzi A, Elghadrāoui L, Maniar S, Faraj C et al. Inventaire et répartition saisonnière des Culicidae dans le centre du Maroc. Entomol faun 2010;62(4):131-138.
25. Mbida Mbida AM, Etang J, Ntonga Akono P, Moukoko CE, Awono-Ambene P, Tagné D, Talipouou A, Ekoko W, Binyang JR, Tchoffo R, Lehman G, Mimpfoundi, R. Nouvel aperçu sur l’écologie larvaire d’*Anopheles coluzzii* dans l’estuaire du Wouri, Littoral-Cameroun. Bull Soc Pathol Exot 2017;110:92-101.
26. Dossou-Yovo J. Étude éthologique des moustiques vecteurs du paludisme en rapport avec les aspects parasitologiques de la transmission du paludisme dans la région de Bouaké. Thèse de doctorat en Entomologie Médicale. Université de Cocody, Abidjan, Côte d’Ivoire. 2000; 150.
27. PNLP. Programme National de Lutte contre le Paludisme. Ministère de la Santé et de l'Hygiène Publique du Togo. Rapport annuel 2005.
28. Diuk-Wasser MA, Toure MB, Dolo G, Bagayoko M, Sogoba N, Traore SF, Manouki N, Taylor CE. Vector abundance and malaria transmission in rice-growing villages in Mali. Am J Trop Med Hyg 2005;72:725-731.
29. Amoudji AD, Ahdjji Dabla KM, Hien AS, Apétogbo YG, Yaméogo B, Soma DD et al. Insecticide resistance profiles of *Anopheles gambiae* s.l. in Togo and genetic mechanisms involved, during 3-year survey: is there any need for resistance management? Malar J 2019;18:117.
30. Ahdjji-Dabla KM, Amoudji AD, Nyamador SW, Apétogbo GY, Chabi J, Glitho IA et al. High Levels of Knockdown Resistance in *Anopheles coluzzii* and *Anopheles gambiae* (Diptera: Culicidae), Major Malaria Vectors in Togo, West Africa: A 2011 Monitoring Report. J Med Entomol 2019;56(4):1159-1164.
31. Ahdjji-Dabla KM, Romero-Alvarez D, Djègbè I, Amoudji AD, Apétogbo YG, Djouaka R et al. Potential roles of environmental and socio-economic factors in the distribution of insecticide resistance in *Anopheles gambiae sensu lato* (Culicidae: Diptera) across Togo, West Africa. J Med Entomol 2020;57(4):1168-1175.