

# Assessment of the risk of mosquito-borne diseases, diversity and *Plasmodium falciparum* transmission in the Centre Hospitalier Universitaire Sourô Sanou (CHUSS) of Bobo-Dioulasso, Burkina Faso

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## Abstract

### Background:

Mosquito-borne diseases remain a major health concern threatening more than 40% of people worldwide. The presence of the pathogens and their vectors in the same area constitute key factors for the maintenance of vector-borne diseases. Seeing this, hospitals appear to be an area having high risk of mosquito-borne diseases transmission. The aim of this study is to assess mosquito-borne disease vector diversity and *Plasmodium falciparum* transmission at the CHUSS.

**Method:** We collected mosquitoes outside the hospital buildings using the Human Landing Catch technique and BG traps during four consecutive days. Specimens belonging to the *Anopheles gambiae* complex were identified using molecular techniques as well as detection of *Plasmodium falciparum* infection and blood meal source.

**Results:** Overall *Culex quinquefasciatus*, *Anopheles gambiae* s.l. and *Aedes aegypti* were the most abundant mosquito found in the study area. *Anopheles arabiensis* was the most abundant malaria vector (70.16%, n = 207), while *Aedes aegypti* was the main arbovirus vector, with a positivity index of 100%. Further, PCR-based blood meal identification showed that the majority of blood meals from *Anopheles gambiae* s.l. were mixed, from both human and goat hosts (42.11%, n=16). Of 282 *Anopheles gambiae* s.l. tested, (21.98%) were found carrying *Plasmodium* sporozoites. *Anopheles arabiensis* played a major contribution in malaria transmission in CHUSS, being responsible of 17.02% infection rate.

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**Conclusion:** In our study, we observed a high density of mosquitoes associated with a risk of disease transmission. The *Plasmodium falciparum* infection rate was very high. This study should alert decision-makers about the risk of mosquito-borne disease transmission in hospitals.

**Key words:** Transmission, vector-borne diseases, hospital, CHUSS, Bobo-Dioulasso and Burkina Faso

## **Évaluation du risque liés aux maladies transmises par les moustiques, de la diversité et de la transmission du *Plasmodium falciparum* au Centre hospitalier universitaire Sourô Sanou (CHUSS) de Bobo-Dioulasso, Burkina Faso**

### **Résumé**

**Introduction :** Les maladies transmises par les moustiques demeurent un important problème de santé publique, menaçant plus de 40 % de la population mondiale. La coexistence des agents pathogènes et de leurs vecteurs dans une même zone constitue un facteur clé dans le maintien des maladies à transmission vectorielle. Dans cette optique, les hôpitaux apparaissent comme des zones à haut risque de transmission des maladies dont les vecteurs sont des moustiques. L'objectif de cette étude est d'évaluer la diversité des vecteurs de maladies transmises par les moustiques et la transmission de *Plasmodium falciparum* au CHUSS.

**Méthodes :** Les moustiques ont été collectés à l'extérieur des bâtiments de l'hôpital en utilisant la technique de capture sur homme et les pièges BG pendant quatre jours consécutifs. Les moustiques appartenant au complexe *Anopheles gambiae* ont été identifiés à l'aide de techniques moléculaires, ainsi que pour la détection de l'infection à *Plasmodium falciparum* et la détermination de l'origine du repas de sang.

**Résultats :** *Culex quinquefasciatus*, *Anopheles gambiae* s.l. et *Aedes aegypti* ont été les moustiques les plus abondants dans la zone d'étude. *Anopheles arabiensis* était le vecteur du paludisme le plus abondant (70,16 %, n = 207), tandis qu'*Aedes aegypti* représentait le principal vecteur des arbovirus, avec un indice de positivité égal à 100%. Par ailleurs, l'identification de l'origine des repas de sang par PCR a révélé que la majorité des repas sanguins de *Anopheles gambiae* s.l. et des repas mixtes provenaient de l'homme et de la chèvre (42,11 %, n = 16). Sur les 282 *Anopheles gambiae* s.l. testés, 21,98 % se sont révélés porteurs de sporozoïtes de *Plasmodium*. *Anopheles arabiensis* a joué un rôle majeur dans la transmission du paludisme au CHUSS, avec un taux d'infection de 17,02 %.

**Conclusion :** En conclusion, nous avons observé une forte densité de moustiques, associée à un risque significatif de transmission de maladies. Le taux d'infection à *Plasmodium falciparum* était particulièrement élevé. Ces résultats devraient inciter les décideurs à prendre en compte le risque de transmission des maladies vectorielles au sein des hôpitaux.

**Mots-clés :** Transmission, maladies vectorielles, hôpital, CHUSS, Bobo-Dioulasso et Burkina Faso.

## Background

Mosquito-borne diseases remain a major health concern affecting more than 40% people worldwide (1). Parasitic mosquito-borne diseases headed by malaria, are responsible for over 400,000 deaths each year. Transmitted by *Anopheles* mosquitoes, malaria is endemic in the whole tropical area especially the sub-Saharan Africa where more than 90% of its deaths occurred (2). In Burkina Faso, malaria is still endemic and responsible for about 8.3 million cases and 16 184 deaths countrywide in 2024 (3). Another *Anopheles*-borne disease is the lymphatic filariasis spread around the world associated with the presence of its vectors(4). In recent years, an outbreak of arboviruses especially dengue, chikungunya and zika was observed worldwide causing severe epidemics in many urban areas (1,5,6). First reported in 1925 (7,8), Dengue epidemic is now reported each year since 2013 in Burkina Faso affecting many people mostly in the urban area of the country (9,10). Recent investigations drew attention about the presence of additional arboviruses especially Zika and chikungunya virus in Burkina Faso, despite the weakness of the surveillance system (11,12). Unfortunately, a new outbreak of Chikungunya is fully confirmed in the Centre-Est part of the country in 2023 and more than 80 cases were reported (13). Despite the efforts of the surveillance system, the prevalence of the diseases remains underestimated due to the symptomatology similar to malaria and limited knowledge of health workers about febrile illnesses other than malaria.

At the community level, the long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS) remain the main tools recommended by the World Health Organization (WHO) for vector control (14). These tools have contributed to a significant decline in malaria incidence and mortality (15). In addition to these tools, coils, sprays and anti-mosquito ointment are widely used by people and seem to be efficient to avoid diurnal mosquito bites. Seeing the bio-ecology of *Aedes* mosquito, the government of Burkina Faso recommends the larval sources management and spatial sprays as first vector control strategy to fight against the outbreak of arboviruses in the country (16). These strategies were shown to be efficient in the reduction of mosquito density in the urban areas where more polluted environments are present and offering suitable conditions for mosquito proliferation.

As it's known, mosquitoes are found everywhere they have suitable conditions of there development including health centers where there is a high concentration of people. In most health centers in Burkina Faso,

no surveillance is made to understand the diversity of mosquito community and how they contribute to the disease transmission. Mostly, no care was taken to avoid the contact between mosquitoes and the patients suffering from vector borne-diseases. The presence of the pathogens and their vectors in the same area constitute key factors for the maintenance of vector-borne diseases. Seeing this, the health centers like hospitals appear to be an area having high risk of mosquito-borne diseases transmission. Understanding the mosquito diversity and their potential role in disease transmission within the health centers could guide the implementation of control strategy to limit the spread of the vector-borne disease in urban areas. In this study, we investigated the mosquito diversity and the potential role in pathogens transmission at the Centre Hospitalier Universitaire Sourô Sanou (CHUSS) of Bobo-Dioulasso.

## II. Methods

### Sampling site

Mosquito sampling took place at the CHUSS of Bobo-Dioulasso (11°17'1019''S, 4°30'1005''E). This hospital is the referral center of the Guiriko, Tannounyan, Bangui and Djoro health regions. The CHUSS has visit hours between 07:00 am-08:45 am and 6:00 pm-8:00 pm and people who frequent this place, whether they are patients, visitors, sick guards, are found inside and outside the buildings, even sleeping there. Located in the center of the Bobo-Dioulasso city, the CHUSS has the same climatic characteristics as the city, namely: a savannah climate with a dry winter with a rainy season from June to early October and a dry season from October to May. The rainfall in Bobo-Dioulasso is much higher in the rainy season than in the dry season. Over the course of the year, the average temperature in Bobo-Dioulasso is 27.7°C and the average rainfall is 1140 mm (17).



**Figure 1:** Study site

## Mosquito collection

Mosquitoes were collected in September and October 2021 using Human Landing Catches (HLC) and the BG-Sentinel trap. Sampling was performed outside the building during (04) four consecutive days. The HLC was carried out at (06) six points from 06pm to 09 am with a change of collector workers team. A total of (12) twelve volunteers were use and the procedure was for a person to collect mosquitoes that landed on him with a hemolysis tube and put into an hourly bag. BG traps were placed in six (06) points at least 30 meters apart and operated 24 hours a day during the collection period. Mosquitoes were collected at 3-hour intervals in each trap.

After the collection, mosquito samples were identified using the morphological keys of for *Anopheles* species(18), Huang *et al.* (2004) for *Aedes* species (19) and Jupp *et al.* (1996) for *Culex* species (20). Mosquitoes were classified according to the physiological state of their abdomen (unfed, gravid, semi-gravid and fed). After dissection of *Anopheles gambiae* s.l., the head-thorax were stored at -20°C for subsequent analysis.

## Molecular analysis

Genomic DNA was extracted from head- thorax following the 2% of CetylTrimethyl Ammonium Bromide (2% CTAB) extraction protocol. The extracted DNA is used to perform PCR. Mosquito species identification within the *Anopheles gambiae* s.l. complex was performed using the PCR SINE 200X6.1 locus method according to Santolamazza *et al.* (21). The primers used were: S200X 6.1F: TCG-CCT-TAG-ACC-TTG-CGT-TA and S200X 6.1R: CGC-TTC-AAG-AAT-TCG-AGA-TAC. Expected fragment sizes were: *An. coluzzii*: 479 bp (presence of the SINE 200X 6.1 gene), *An. gambiae* s.s.: 249 bp (absence of the SINE 200X 6.1 gene), and *An. arabiensis*: 223 bp (absence of the SINE 200X 6.1 gene). Sporozoite detection was conducted by PCR following the protocol of Morlais *et al.* (2004) (22) using primers 1P: GGA-ATG-TTA-TTG-CTA-ACA-C and 2P: AAT-GAA-GAG-CTG-TGT-ATC. This PCR targets the gene encoding the circumsporozoite protein specific to *Plasmodium falciparum*, with an expected fragment size of 501 bp. Blood meal origin in engorged mosquitoes was determined by two multiplex PCR targeting the cytochrome-b gene of common vertebrate hosts, following Kent and Norris (23). Specific primers allowed the identification of DNA from potential hosts, including human, goat, sheep, donkey, poultry

(chicken), dog, cattle, and pig (23). All the PCR were performed using the 5x FIREPol<sup>®</sup> Master mix following the manufacturer's instructions.

### Data analysis

The results were processed and analyzed using the software Microsoft<sup>®</sup> Excel<sup>®</sup> 2016 MSO (Version 2206 Build 16.0.15330.20216) and RStudio 2022.07.1 +554. Vector abundance, relative proportion of the different species of malaria parasite and vectors, human biting rates (HBR) and entomological inoculation rates (EIR) were estimated for *Anopheles gambiae* s.l. mosquitoes. A statistical analysis was conducted to compare mosquito abundance between *Culex*, *Aedes* and *Anopheles*. To assess differences in abundance, a rate ratio was estimated using a Poisson regression model. This method allowed for evaluating inter-species variation while accounting for count-based data. The analysis aimed to determine whether the observed differences in mosquito populations were statistically significant. We also calculate index for *Aedes* mosquitoes: the Positive index (number of positive traps divided by the total number of traps inspected, often multiplied by 100) and the Density index (Total number of adult *Aedes* captured divided by the total number of positive traps).

## II. Results

### Composition of mosquitoes species and *Aedes* indexes

A total of 3813 mosquitoes (*Aedes*, *Anopheles* and *Culex*) were collected during the study period, of which 12.36% (n=479) was collected using BG traps and 87.44% (n=3334) using HLC method (Table I). *Anopheles* were predominantly collected using HLC (n=308; 99.03%), while *Aedes* mosquitoes were more frequently captured with BG-Sentinel traps (n=111; 61.33%). About seven species of mosquito were collected at different proportions depending on the collection method in the study area. *Cx. quinquefasciatus* was the dominant species (86.78%, n=3309) of the total collected mosquitoes, followed by *An. gambiae* s.l. (8.05%, n=307) and *Ae. aegypti* accounting for (4.61%, n=176). The other mosquito collected were *Cx decens* (0.29%, n=11), *Ae. vittatus* (0.08%, n=3), *An. pharoensis* (0.08%, n=3), *Ae. vexans* (0.05%, n=2) and *Cx. tigripes* (0.05%, n=2). Statistical analyses showed a significantly higher densities of *Culex* species than those of the *Anopheles* species (RR=0.32; IC95%: [0.20-0.53]; p < 0.0001) and the genus *Aedes* (RR=0.11; IC95%: [0.06 - 0.21]; p < 0.0001).

The positivity index reached 100%, and the density index was 18.6. These remarkably high values highlight a substantial mosquito

abundance in this facility, reflecting a widespread distribution of vectors within the hospital environment.

**Table I:** Mosquitoes collected by Human Landing Catch (HLC) and BG sentinel trap in Centre Hospitalier Universitaire Sôrou Sanou (CHUSS).

Species	BG_trap		HLC		Total	
	n	%	n	%	n	%
<i>An. gambiae</i> s.l.	2	0.42	305	9.15	307	8.05
<i>An. pharoensis</i>	0	0	3	0.09	3	0.08
<i>Ae. aegypti</i>	109	22.75	63	2	176	4.61
<i>Ae. vittatus</i>	0	0	3	0.09	3	0.08
<i>Ae. vexans</i>	2	0.42	0	0	2	0.05
<i>Cx. quinquefasciatus</i>	354	73.90	2955	88.63	3309	86.78
<i>Cx. decens</i>	11	2.30	0	0	11	0.29
<i>Cx. tigripes</i>	1	0.21	1	0.03	2	0.05
Total	479	12.56	3334	87.44	3813	100

n, samples size; BG trap, BG sentinel trap ; HLC, Human Landing Catches: *An.*, *Anopheles* ; *Ae.*, *Aedes* ; *Cx.*, *Culex*

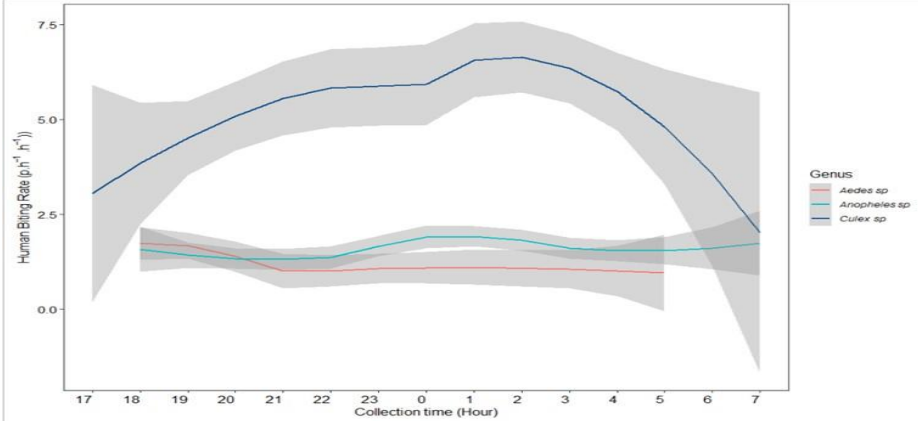
### ***An. gambiae* s.l. species composition**

Among 295 *Anopheles gambiae* s.l. used for species identification (BG and HLC), 284 individuals were successfully analysed for molecular identification of *Anopheles gambiae* s.l. Three species (*An. arabiensis*, *An. gambiae* s.s. and *An. culuzzii*) have been identified. *Anopheles arabiensis* was the most abundant (81.5%, n=207), followed by *Anopheles gambiae* s.s. (40.47%, n=52) and *Anopheles coluzzii* (9.84%, n=25).

### **Time activity of mosquitoes in the Centre Hospitalier Universitaire Sôrou Sanou (CHUSS)**

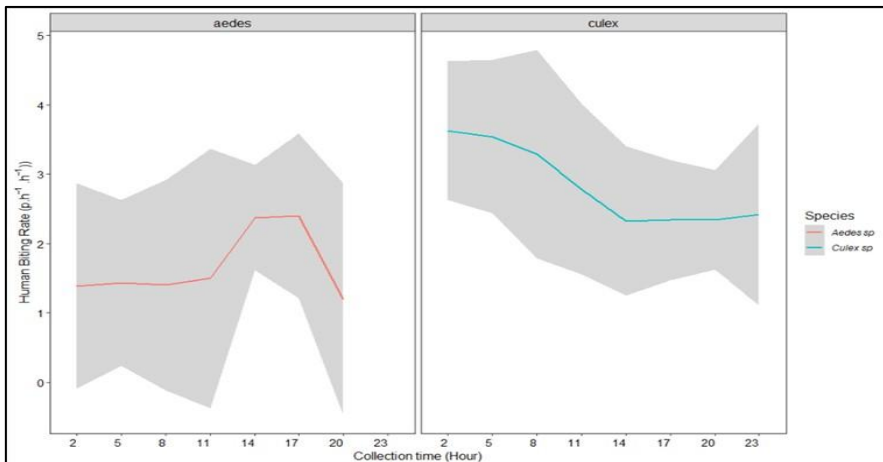
The mosquito activities varied depending on the collection method and collection times. Using the HLC method, mosquitoes were collected throughout the night. The mosquito species had an early biting activity (*Aedes*, *Culex* and *Anopheles*) from 06:00 pm to 08:00 am (Figure 2). The hourly biting cycles of *Culex* species were more marked, with a peak between 08 :00 pm and 03 :00 am. *Anopheles* species were biting

during all the collection time (08:00 pm to 08:00 am) with peaks observed at 10:00 pm, 12:00 am and 03:00 am (Figure 2). On the other hand, *Aedes* species were more active during the day with a peak at 06:00 pm, which lasted until 08:00.



**Figure 2:** Hourly biting rate of mosquito in Centre Hospitalier Universitaire Sôrou Sanou (CHUSS) of Bobo-Dioulasso using Human Landing Catches (HLC). (*1-hour interval*)

In contrast, although BG traps operated all night (Figure 3), *Culex* was the only genus captured during all time intervals. *Aedes* showed no activity between 20:00 and 02:00, with peak activity observed between 14:00 and 17:00.



**Figure 3:** Time activity of *Aedes* and *Culex* mosquito from BG-Sentinel traps (3-hour intervals)

### Blood meals origin

A total of 38 females feed belonging to the *An. gambiae* complex were tested for the origin of the blood meal. Polymerase chain reaction-based blood meal identification showed that most of blood meals from

*Anopheles gambiae* s.l. were mixed blood from human and goat hosts (42.11%; n=11). *An. arabiensis* was the only species that took a blood meals in human (23.68%; n=9). The proportion of meals taken from animals was 34.21% (n=13) (Table 2).

**Table II:** *Anopheles gambiae* s.l. blood meal source

Species	Animals										Human	Mixed	Total		
	Goat		Cow		Dog		Pig		Others						
	n	%	n	%	n	%	n	%	n	%					
<i>An. arabiensis</i>	0	0.00	0	0.00	0	0.00	0	0.00	10	26.36	9	23.68	13	34.21	32
<i>An. gambiae</i> s.s.	0	0.00	0	0.00	0	0.00	0	0.00	2	5.26	0	0.00	3	7.89	5
<i>An. coluzzii</i>	0	0.00	0	0.00	0	0.00	0	0.00	1	2.63	0	0.00	0	0.00	1
Total	0	0.00	0	0.00	0	0.00	0	0.00	13	34.21	9	23.68	16	42.11	38

Abbreviations: n, number of blood-fed *Anopheles* females; Other, other animals not determined; Mixed, animal and human meal

### ***Plasmodium falciparum* infection and entomological inoculation rates**

A total of 282 *Anopheles gambiae* s.l. captured (both BG and HLC), were used for the determination of *Plasmodium falciparum* infection. The PCR revealed that 62 (21.98%) *Anopheles gambiae* s.l. were positive for *Plasmodium falciparum* infection (Table 3). *An arabiensis* has the highest infection rate (17.02%; n=48) followed by *An. gambiae* s.s. (4.25%; n=12). Among the 24 mosquitoes belonging to *An. coluzzii*, only 2 (0.71%) were infected by *Plasmodium falciparum*.

**Table III:** *Anopheles gambiae* s.l. infection rate at Centre Hospitalier Universitaire Sôrou Sanou (CHUSS)

Species	<i>Plasmodium falciparum</i> infection		
	n	Positive	SR
<i>An. arabiensis</i>	207	48	17.02
<i>An. gambiae</i> s.s.	51	12	4.25
<i>An. coluzzii</i>	24	2	0.71
Total	282	62	21.98

Abbreviation: n, sample size; positive, number of *Anopheles gambiae* s.l. infected by *Plasmodium falciparum*; SR, Sporozoites rate

### **III. Discussion**

The current study was carried out to assess vector diversity, the risk of mosquito-borne diseases and the level of *Plasmodium falciparum* transmission in the CHUSS of Bobo-Dioulasso.

## **Risk of mosquito borne-diseases**

Entomological data from HLCs and BG trap showed a high density of mosquitoes at the CHUSS dominated by of *Culex quinquefasciatus*, followed by *Anopheles gambiae* s.l. and *Aedes aegypti*. These mosquito species are the most common in the city of Bobo-Dioulasso, with a high frequency (24,25). This hospital is very visited, as it is the referral center for several regions of the country. The presence of these species of mosquito at this hospital, together with the high volume of patients and visitors increase human-vector contact and poses a risk of mosquito-borne disease transmission in this area (26). Several studies have shown that, where there is a high density of *Aedes aegypti* and a high degree of contact between mosquitoes and humans, there is a high risk of arboviruses such as dengue and chikungunya (27). It is also well known that a high positivity rate suggests a wide distribution of mosquito breeding sites, while a high-density index indicates a greater risk of disease transmission. Altogether, these findings highlight the potential risk of dengue transmission within the hospital. (Moreover, multiple investigations have revealed the presence of the dengue and chikungunya viruses in the region (11,12). In addition, the hospital has been described as the most likely place for the transmission of the dengue virus, due to the expansion of the vectors *Aedes* and the presence of dengue virus carriers in that area (28). *An. arabiensis* was the main malaria vector in CHUSS as well as in the city of Bobo-Dioulasso. These species had become more common in Bobo-Dioulasso, followed by *An. coluzzii* and a little fraction of *An. gambiae* s.s. (25). The predominance of *An. arabiensis* at the CHUSS increases the risk of malaria in this hospital due to its exophilic behaviour. On the other hand, HLC revealed intense mosquito biting activity, with a peak at times when the hospital population is asleep or immobile without any protection. The mosquito activity remains intense when humans are not active and the risk of transmission of the pathogen becomes high (29). Analyses to determine the origin of the blood meal revealed the presence of human and goat blood in the mosquito's abdomen. Human was the main host on which the anopheles took their blood meal at this hospital center. The lack of domestic animals and the low degree of control tool use would increase human-vector contact. These results reflect the risk of mosquito borne diseases in the hospital and the need to adapt locally vector control strategies.

## ***Plasmodium falciparum* transmission**

*Plasmodium falciparum* sporozoites detection by PCR revealed that between the 62 *An. gambiae* s.l. found positive, 48 were *An. arabiensis*,

12 *An. gambiae* and 2 *An. coluzzii*, and the infection rate was 21.98%. This high infection rate is assumed to be due to ease access of vectors to *Plasmodium* carriers. Hospitals are indeed areas where it is more likely to find a person potentially carrying *Plasmodium*. These results suggest that the populations (patients, companions) frequenting this hospital, the majority of whom stay and sleep outside the buildings, are highly exposed to infesting mosquito bites. However, it is important to emphasize that the number and timing of collections may have influenced our results.

### **Conclusion**

This preliminary study highlights the potential risk of vector-borne diseases within CHUSS, where high mosquito diversity and density were observed, particularly *An. gambiae* s.l. and *Aedes aegypti*. The detection of *Plasmodium* infection in vectors demonstrates the potential for malaria transmission during hospital stays. While the presence of *Aedes* further suggests a risk of arboviral diseases. These findings emphasize the importance of considering hospitals and other highly frequented public places in vector surveillance and control strategies, since transmission dynamics in such settings may differ from those observed within households. The limitations of this study include the short survey duration and the absence of *Aedes* screening for dengue and chikungunya viruses.

### **Acknowledgement**

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### **Authors Contribution**

KRD was the principal scientific investigator of the study. DDS and KRD designed the study. DDS, VSE, NKL coordinated the lab and field study. VSE, NKL, DSR, JJT, DOK process the identification and mosquitos' preparation. NKL, VSE, SM, DOK and BR analyse the samples by PCR. DDS and NKL analyzed the data and drafted the manuscript. KRD, HSA critically read the manuscript. All authors read and approved the final manuscript.

### **Ethics declarations**

Ethical approval for this study was granted through protocol number of 43-2021/CEIRES by the Comité d'Ethique Institutionnel pour la Recherche en Sciences de la Santé (CEIRES).

## Competing interest

The authors declare no competing interest.

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