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Research Article

INCREASING RISK EXPOSURE TO MALARIA AS RESULT OF WATER TABLE RISING IN NIAMEY CITY, NIGER

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ABSTRACT

Rising groundwater occurring in Niamey city, leads to the formation of waterholes facilitating the proliferation of mosquitoes which are responsible of numerous vector-borne diseases including malaria, transmitted by *Anopheles* species. This study aims to determine how this phenomenon influence the risk of exposure to malaria. Doing so, we carried out over a nine-month period, an entomological monitoring in 3 different areas of Niamey city: 2 of them were permanently flooded, while the third one, never flooded serves as a control. Mosquitoes were collected using CDC light traps and insecticide sprays throughout 72 sampling locations across all the areas. Specimens were identified using a morphological identification key. Species abundances and entomological parameters were analyzed using R software (v.4.4.0) and compared using a chi-square test. A total of 1,071 specimens of *Anopheles gambiae* complex were collected, including 43.79% females. Abundances were found higher: i) in flooded sites: 56.21% and 25.68% respectively, while it was only about 7% at the non-flooded ($p < 0.001$); ii) during the rainy season (85.8%) but, tends to decrease during the dry seasons (cold: 13.91% and hot: 0.28%; $p < 0.001$). Females were more abundant in flooded sites, up to 54.16% and Human-Blood Index, varied from 34.1% to 20% between sites and from 41.7% to 29.9% between seasons ($p < 0.001$). Besides being low (0.83%), the overall infectivity rate, was limited to the flooded sites and only during the rainy season. These insights could serve malaria prevention strategies by focusing on mosquito control in high-risk areas and periods.

Keywords: Flooding, Mosquitoes, Transmissibility, Malaria, Niamey.

INTRODUCTION

Acting as vectors for numerous pathogens, including viruses, bacteria and parasites, mosquitoes play a major epidemiological role (Pombi and Montarsi, 2022). Such pathogens are transmitted to humans and animals through mosquito bites, which make them responsible for widespread and deadly diseases such as Dengue, Zika, Chikungunya, West Nile and Malaria (Benelli and Mehlhorn, 2016; Onen *et al.*, 2023). The latter is of particular concern, as it is a deadly vector-borne disease caused by a protozoan parasite, *Plasmodium spp*., and transmitted to humans by *Anopheline* mosquitoes (Sinka *et al.*, 2012; WHO, 2024). It causes an estimated 249 million cases globally, and results in more than 608,000 deaths every year. Most of the deaths occur in children under the age of 5 years (WHO, 2024). For instance, in 2020, Africa region accounted for 95% of the world's malaria cases, making it the most affected continent (Kouamé *et al.*, 2023). In Niger, malaria is endemic and represents the primary cause of mortality (Soumaila *et al.*, 2022). It is one of the countries with the highest per capita mortality rates globally (Ibrahima *et al.*, 2024; Aminou *et al.*, 2025). In 2021, over 4.5 million cases of malaria were documented, resulting in 4,170 deaths (ICF, 2023). Nationwide control efforts have resulted in a 7.9% reduction in the number of cases and a 25.9% reduction in the number of deaths between 2015 and 2019 (MSP, 2021). However, the incidence rate per 1,000 inhabitants increased from 0.04% in 2019 to 0.09% in 2020, probably due to the significant rainfall amounts recorded in 2020, which would have increased anopheline mosquito breeding sites (Ale *et al.*, 2023; Ibrahima *et al.*, 2024). Among the seventy *Anopheles*

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species known for being vectors and competent for human malaria (Hay *et al.*, 2010), only *An. gambiae s.l.* and *An. funestus* (Labbo *et al.*, 2012) are present in Niger and, the most abundant taxon in Niamey City, is *An. gambiae* (Labbo *et al.*, 2016). In addition, numerous studies (Soumaila *et al.*, 2022; Labbo *et al.*, 2016; Iro *et al.*, 2020) have shown that the high densities of *Anopheles spp.* would be associated with humid conditions that prevailed in the city. The latter is crossed by two important rivers: the Niger River and the Gunti Yena Valley, which have permanent water (Hassane *et al.*, 2016). Such humid environments represent suitable breeding habitats for mosquitoes, thus increasing the risk of malaria transmission (Radl *et al.*, 2024). In the context of the flooding resulting from rising groundwater, which has already affected several districts in Niamey City (Alassane *et al.*, 2021), this study intends to determine how these particular environments influence *Anopheles* species abundance and diversity as well as their role in malaria transmission. So, it aims to assess the risk of exposure to malaria for people living in areas affected by the phenomenon.

MATERIALS AND METHODS

Study areas and mosquitoes sampling

Entomological monitoring was carried out through three different sites, all located in District I of Niamey City (Figure 1). Two of them, SONUCI and DARESSALAM, are along Gunti Yena Valley watershed from upstream to downstream. They were also permanently flooded due to the groundwater rising and rain water falls. As for the third one (KARSAMBA), it is also located in the same valley watershed but has different hydroclimatic conditions from those observed at the two other sites. Indeed, the site is never flooded so we considered it as a control site.

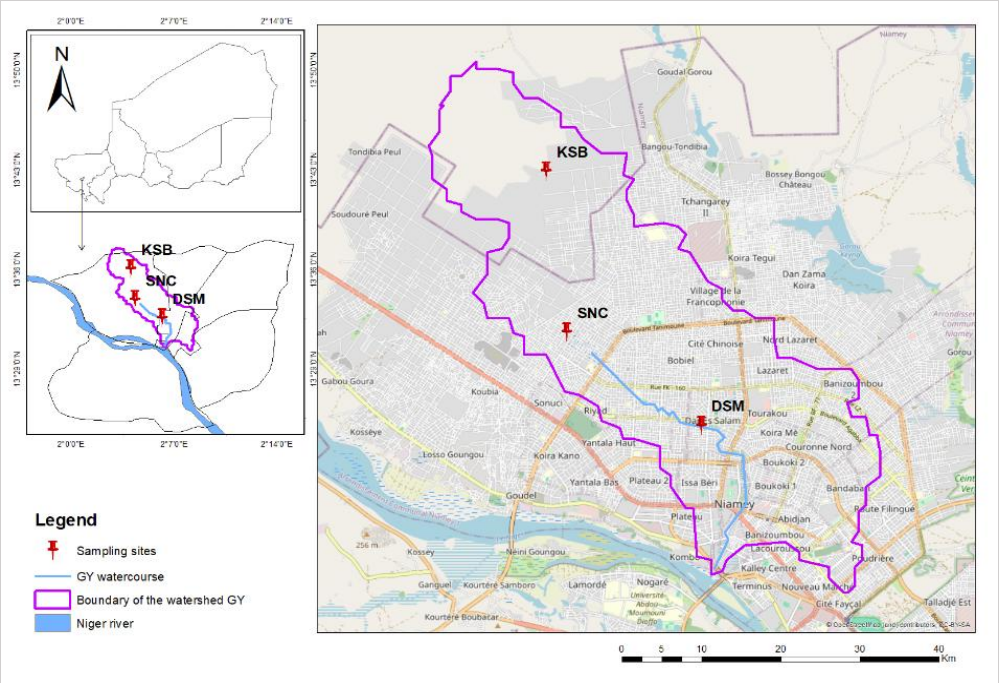

Figure 1. Map of Niamey showing the study's sites (DSM = DARESSALAM; KSB = KARSAMBA; SNC =SONUCI).

Data collecting campaigns were conducted over a nine-month period, from October 2020 to June 2021, including the three seasons of the year: the dry season, divided into cold and hot, and the rainy season. Mosquitoes were collected using two distinct methods according to previously described methods: Pyrethrum Spray and Light traps. For sampling, 24 compounds were randomly selected for each site. These include 20 for Pyrethrum Spray and 4 for Light traps. Each compound was prospected three times due to one collect per season and each collect includes 3 trap-nights. To collect endophilic mosquitoes, we used Pyrethrum Spray method, which consists of indoors spraying insecticide, typically pyrethroids, early in the morning. Prior to spraying, all flat surfaces were covered with white sheets, making it easier to collect all residual mosquitoes that would fall under the insecticide effect, 15 minutes after spraying. Light traps were used mainly to collect nocturnally active mosquitoes, indoors and outdoors. So, 2 light traps (one indoor and the other one, outdoor) were systematically set in each compound. Traps were set in the evening but activated at nightfall and checked the following morning. All captured mosquitoes were collected and preserved in Petri dishes for subsequent identification and further analyses.

Anopheles mosquito species identification

All the specimens collected were systematically identified based on their morphological characteristics. There were

separated into genera, species and sexes. To do so, we used required identification keys (Coetzee *et al.*, 2000). Specimens were observed through a binocular microscope and grouped by genus, species and sex according to their own morphological traits. We also used Xper2 software (Ung *et al.*, 2010), which is a plate form dedicated to taxonomic descriptions and computer-aided-identification. It employs databases containing information on both distinctive characteristics of the species and description details of each species.

Abundances, proportion of females and blood-feeding status

To determine the relative and specific abundances, the total number of specimens per site was counted. Abundances were calculated for both sexes combined and separately and expressed as a percentage according to the following formula AR (%) = (ni/N) × 100 (Turquin, 1974). In addition, two other indices were calculated directly from known percentages: i) the female rate, obtained by dividing the number of females by the total number of individuals, and ii) the proportion of blood-fed females for which female *Anopheles* mosquitoes were first sorted according to their feeding status and then classified into three categories: unfed, blood-fed and gravid (WHO, 2013). For each category, proportions were calculated, based on the following formula: $pi = \frac{ni}{N} \text{x } 100$ (p_i = proportion of category I; n_i = individuals' number of category I; N= total number of females).

Entomological parameters of malaria transmission

Two main entomological parameters: Human Blood Index (HBI) and Infectivity, were determined based on molecular analyses. To this end, two types of PCR were performed based on fed female DNA fragments' amplification using different PCR conditions, depending on the parameter being targeted, either to assay the blood meal sources, or to determine the infectivity rate. Prior to the PCR, DNA was extracted from all female *Anopheles* samples, from the entire crushed contents of the abdomen, on the one hand, and the head-thorax on the other hand, respectively for HBI and infectivity. All DNA extractions were performed using NaOH Tris-saline solution according to the protocol (Rudbeck and Dissing, 1998).

Human Blood Index (HBI)

To amplify samples' DNA fragments, for the trophic preference (Oshaghi *et al.*, 2006), we used a panel of 6 species-specific forward primers corresponding to the selected reference models that were i) Pig: 5′-cctcgcagccgtacatcctc-3' (573 F); ii) Human: 5′-ggcttacttcttcattctctcct-3' (741 F); iii) Goat: 5′-cctaatcttagtacttgtacccttcctc-3' (894 F); iv) Cow: 5′-catcggcacaaatttagtcg-3' (121 F); v) Dog: 5′-ggaattgtactattattcgcaaccat-3' (368 F) and a universal reverse primer common to all species UnRev: 5′-ggttgtcctccaattcatgtta-3' (1025). Multiplex PCR was performed in a final volume of 25 µL including 3 µL of template DNA and amplification reactions started with an initial 95 °C denaturation for five minutes, followed by 40 cycles of 95 °C for 60 s (denaturation), 56 °C for 60 s (hybridization), and 72 °C for 60 s (elongation) and a final extension of 7 min at 72 °C. The protocol used was derived from (Kent and Norris, 2005). Amplified PCR products were separated by electrophoresis into 2% agarose gel stained with Ethidium Bromide. DNA fragments were visualized under UV light through a gel imaging system and corresponding bands were identified compared with those of the reference models whose molecular weights are well-known.

Human Blood index (HBI) was calculated from the proportion of mosquitoes feeding on humans out of all identified blood meals by the following formula (Pappa *et al.*, 2011):

$$HBI = \frac{number\ of\ mosquitoes\ with\ a\ human\ blood\ meal}{Total\ number\ of\ mosquitoes\ gorged} \times 100$$

Sporozoite infection rate

To detect *Plasmodium* DNA in female mosquitoes and test infectivity rate, DNA extracted from head-thorax, was subjected to PCR amplification of COX1 primers pair: ShortCOX1F 5′-agaacgaacgcttttaacgcctg-3' and ShortCOX1R 5′-acttaatggtggatataaagtccatccwgt-3.' Amplification reactions were run at a final volume of 20 µL including 3 µL of template DNA and started with preheating at 94 °C for three minutes, followed by 40 cycles of 94 °C for 30 s (denaturation), 65 °C for 60 s (hybridization), and 72 °C for 60 s (elongation) and a final extension of 10 min at 72 °C. Amplified DNA fragments were separated by electrophoresis on 2% agarose gel stained with Ethidium Bromide and visualized under UV light through a gel imaging system. Corresponding bands were identified with reference to the known size of the *Plasmodium falciparum* sporozoite band. Sporozoite infection rate, as the proportion of mosquito's positive for *Plasmodium falciparum* parasites, was calculated using the following formula:

$$Infectivity\ rate = \frac{number\ of\ Anopheles\ positive\ for\ Plasmodium\ falciparum}{Total\ number\ of\ tested\ Anopheles} \times 100$$

Data analysis

To assess the risk of exposure to malaria transmission, we compared all the parameters (abundances and other entomological parameters) between and within sites and seasons. A chi-square test for comparisons of proportions was performed with R (version 4.0.0) using "ggplot2" packages for graphical visualization.

RESULTS AND DISCUSSION

A total of 1,071 adult *Anopheles* mosquitoes were collected and identified, belonging all to *An. gambiae s.l.* species (Table 1). Total abundance varied considerably from one site to another (χ^2 = 605.5, p <0.001). It was higher in the flooded sites of SONUCI and DARESSALAM, with

67.13% and 25.68% of abundance, respectively while it was very weak as expected in the non-flooded site, KARSAMBA with only 7.18%. Moreover, *Anopheles* abundance varied according to the season (χ^2 = 1356.3, p <0.001). It was about 85.81% in the rainy season, which is six times more than during the cold season (13.91%) and more than ten times more than during the hot season (0.28%) (Table 1).

Table 1. Abundance of *Anopheles gambiae s.l.* by site and season.

Variable	Category	n	Ab (%)	χ^2 [p]
Site	DARESSALAM	275	25.68	*605.51 [<0.001]*
	KARSAMBA	77	7.19	
	SONUCI	719	67.13	
Season	Hot	3	0.28	*1356.9 [<0.001]*
	Cold	149	13.91	
	Rainy	919	85.81	
Total		1071	100	

"n" represents the number of individuals; "Ab" represents abundance expressed as a percentage (%); "χ^2" and "[p]" indicate the test values between sites and between seasons.

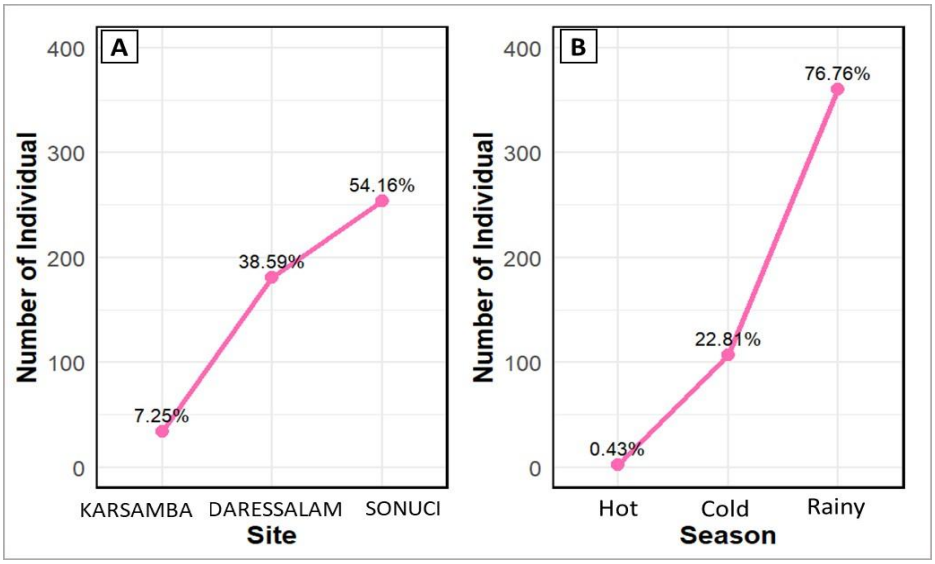

Figure 2. Proportions of female *Anopheles* mosquitoes between sites (A) and seasons (B).

Males were found more abundant (56.2%) compared to females (43.8%) in the total sample of *Anopheles gambiae* collected (χ^2 = 16.52; p <0.001). When comparing the proportion of females between sites (Figure 2A), female abundance was higher in the flooded sites SONUCI (54.16%) and DARESSALAM (38.59%) than in the non-flooded sites, KARSAMBA, with only 7.25% (χ^2 = 106.4; p <0.001). Between seasons (Figure 2B), the proportion of females varied from very low in the cold season (0.43%) to moderate in the hot season (22.81%) and very high in the rainy season (76.76%, χ^2 = 433.26; p <0.001). Depending on the blood-feeding status, female *Anopheles* mosquitoes were classified into 3 categories: gravid, blood-fed or unfed. Note that the proportion of blood-fed females was globally low. However, according to the sites (Table 2), it was found higher in the two flooded sites: DARESSALAM (22.65%) and SONUCI (17.72%), and weak in the non-flooded, KARSAMBA (14.71%) and these relative proportions were significantly different (χ^2 = 32.00; p <0.001). Significant variations (χ^2 = 109.34; p <0.001) were also observed regarding the season. So, in the hot season, all females (n=2) are blood-fed. In rainy and cold seasons, proportions of blood-fed females were respectively 21.39% (n=360) and 11.21% (n=170) (Table 2).

Table 2. Blood-feeding status and rates of female *Anopheles* mosquitoes between sites and seasons.

Variables.	Category	Unfed	Blood-fed	Gravid	Blood-fed (%)	χ^2 [p]	Total
Site	DARESSALAM	114	41	26	22.65	*32.00 [p<0.001]*	181
	KARSAMBA	27	5	2	14.71		34

	SONUCI	173	45	36	17.72		254
	Cold	81	12	14	11.21		107
Season	**Hot**	0	2	0	100.00	*109.34 [p<0.001]*	2
	Rainy	233	77	50	21.39		360
Total		314	91	64	19.40	*240.85 [p<0.001]*	469

"χ^2" and "[p]" indicate the test values between sites and between season

Table 3. Blood meal source and Infectivity rate.

Site	**Origin of blood meals**					**Infectivity rate**		
	Tested	**Unknown**	**Goat**	**Human**	**HBI (%)**	**Tested**	**Positive**	**Rate (%)**
DARESSALAM	41	18	9	14	34.14	181	1	0.55
KARSAMBA	5	4	0	1	20.00	34	0	0
SONUCI	45	29	3	13	28.88	254	2	1.1
χ^2 [p]	*11.214 [0.003]*					*0.33 [=0.56]*		
Season	**Origin of blood meals**					**Infectivity rate**		
	Tested	**Unk**	**Goat**	**Human**	**HBI (%)**	**Tested**	**Positive**	**Rate (%)**
Cold	12	6	1	5	41.66	2	0	0
Hot	2	2	0	0	0	107	0	0
Rainy	77	43	11	23	29.87	360	3	0.83
χ^2 [p]	*27.769 [<0.001]*					*NA*		

Values are compared between sites and seasons. The "χ^2" and "[p]" values represent the results of statistical comparisons. "NA" indicates that the statistical test is not valid; "HBI" = Human Blood Index.

Feeding patterns revealed that 56.04% of blood meals tested were of unknown origin (Table 3). Identified blood meal sources included humans and goats. Human blood meal origin, representing 30.76% across all sites (χ^2=25.34, p<0.001), was more frequent at DARESSALAM (34.1%) and SONUCI (28.9%) than KARSAMBA, 20% (χ^2=11.241, p<0.001). Between seasons, it was more common in Cold (41.7%) and Rainy (29.9%). In terms of infectivity across all sites, the rate remained remarkably low, ranging from 0% (0/34) at Kasamba to 1.10% (2/254) at SONUCI but, there was no significant difference between the three sites (χ^2 = 0.33; p = 0.56). According to seasons, infectivity was only detected during the rainy season (0.83%, 3/360), while no reported cases in neither the cold nor hot seasons (0/2 and 0/107, respectively). So, it was not possible to perform statistical comparisons here, since there was no data available for two seasons (Table 3).

The aim of this study is to determine how humid environments resulting from groundwater rising influence *Anopheles* species abundance and diversity as well as their role in malaria transmission, thus to assess the risk of exposure to malaria for people living in areas affected by the phenomenon. Based on a total of 1,071 individuals of *Anopheles* species collected overall the sites and the study period, we found that *Anopheles'* abundance fluctuated markedly between both sites and seasons. First, regarding to the sites, it was observed that the flooded ones (SONUCI and DARESSALAM) exhibited higher abundance significantly different (p <0.001) than that recorded at the non-flooded site, KARSAMBA. Furthermore, abundance varied significantly (p <0.01) from one season to another throughout the year. The maximum of abundance (85.81%) during the rainy season. It went down less than 1% in hot season. The seasonal variations of *Anopheles* abundance, are well-known and have been mentioned in many studies particularly, in tropical regions where, on the one hand, abundance is significantly higher during the rainy season (Moreno *et al.*, 2007; Kigadye *et al.*, 2010; Rakotoarison *et al.*, 2022), and on the other hand, the number of *Anopheles* mosquitoes decreases drastically during the hot season (Smith *et al.*, 1995; Hima *et al.*, 2025). Our results are consistent with these studies and confirm the key role of climate factors mainly temperature and humidity associated to the year's seasons, in *Anopheles* abundance. As well, same trends were observed for females, whose proportions were significantly higher in flooded sites than those recorded in non-flooded ones. These proportions were also found higher during the rainy season, decreasing in cold season and almost null in hot season. These results show that, in addition to the abundance of *Anopheles* mosquitoes, the female proportion also increases in humid conditions. That is not surprising to the extent that, numerous studies have already demonstrated that female *Anopheles* mosquito's density increases during the rainy season and in wet areas, while it decreases during hot dry periods (Toure *et al.*, 2018; Yaro *et al.*, 2012).

However, this high number of females [which are responsible for transmitting *Plasmodium* to humans (Carnevale and Robert, 2017)] on flooded sites maybe induce an increase in vector competence and exposure to malaria among residents of these areas. Indeed, their high proportion may increase contact with humans, especially with having endophilic behavior within these areas (our unpublished data not presented in this paper).

Females were largely dominated by unfed categories. Feeding rates were <20% for all females, but even low rates remain an indicator of the link between vector and host. The highest rates were observed in flooded sites and during the rainy season (Table 2). However, during the hot season, only two females were captured, both of which were blood-fed (no associated statistics due to the very small sample size). This pattern joins the female's abundance at high-flooded sites and during the rainy season as showed in many studies carried in some African countries where it has been observed that the number of blood-fed mosquitoes increases significantly during the rainy season and in humid areas. Indeed, biting activity increased (more than 5 times) during the rainy season (Dambach *et al.*, 2018; Katusi *et al.*, 2022).

Female *Anopheles* mosquitoes were found to feed on both humans and animals (e.g., goats), with a human blood index of 30%. This behavior is consistent with previous studies showing that *An. gambiae* species' trophic preference, consists of animal hosts including human and the other animals particularly, the domestic ones (Yeshanew *et al.*, 2025; Adja *et al.*, 2015). In addition, Human-Blood Index comparisons between sites and/or seasons generally confirm previous trends, with higher values in flooded areas and during the cold season. In contrast, it has been known that the HBI in *Anopheles* mosquitoes does not vary between different agroecosystems; its trend remains the same regardless of habitat type (Belay *et al.*, 2024). The results obtained here, could be explained by the high female abundance observed in our study's sites and seasons where mosquitoes' diversity increased due to groundwater conditions (Hima *et al.*, 2025). Furthermore, it should be mentioned that *An. gambiae* is the major vector of malaria in Niamey city malaria (Labbo *et al.*, 2016; Ibrahima *et al.*, 2024) so, the higher human-blood index recorded in flooded sites, constitutes an important risk factor for people exposure to malaria (Djedanem *et al.*, 2025; Hima *et al.*, 2025).

Even particularly low (0.83%), the sporozoite index (infectivity rate) obtained in this study, is congruent with the malaria's transmission dynamics as documented in Niamey, which indicated that the highest infectivity rate is about 1.3% (Labbo *et al.*, 2016). This trend of low infectivity rate, is a well-known characteristic of *Anopheles*, as even in areas of higher transmission in West Africa, rates rarely exceed 6% (2.1% in Accra, Ghana, Sabtiu *et al.*, 2025; 6.6% in Burkina Faso, Epopa *et al.*, 2019). Such low rates could be explained by two major biological factors. First, the immune system of *Anopheles* mosquitoes, notably via the protein called "Thioester-containing protein 1: TEP1," eliminates a significant number of ingested parasites during blood meals, potentially limiting their detection (Gildenhard *et al.*, 2019). Second, the parasite requires 10 to 14 days for incubation to reach the salivary glands (Raymond, 2018). However, in our study, we used only head-thorax section for PCR. Probably, the rates might have changed if we had also analyzed abdomens sections. The detection of positive cases exclusively during the rainy season and in flooded sites confirms the hypothesis that the risk of exposure to malaria increases in flooded areas, as the latter provide the necessary humidity to prolong *Anopheles*' survival.

CONCLUSION

Our study's findings showed that the total abundance as well as the proportions of female *An. gambiae s.l.* were very higher in flooded than non-flooded areas, in one hand, and on the other, during the rainy season than the dry seasons, particularly the hot ones. Likewise, as another significant achievement, the study showed that, despite the weak rate observed, infectivity was strongly associated with flooded sites and rainy season. In addition, it became clear that, as a trophic preference, human-blood is the most common. So, as expected, *Anopheles* mosquitoes' abundance as well as the entomological parameters of malaria's transmission were higher in flooded sites and during the rainy season. Altogether, our results suggest that, the risk of exposure to malaria is permanent and very high for people living in areas affected by groundwater rising phenomenon in Niamey City. This should be taken into account in vector-control and surveillance strategies for more efficiency.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest

ETHICS APPROVAL

Not applicable

FUNDING

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AI TOOL DECLARATION

The authors declares that no AI and related tools are used to write the scientific content of this manuscript.

DATA AVAILABILITY

Data will be available on request

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