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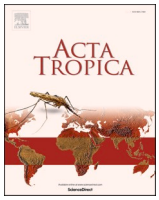

Residual malaria transmission in Western Burkina Faso: Vector Behavior, insecticide resistance, and the efficacy limits of next-generation LLINs

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ABSTRACT

An entomological surveillance was carried out in two districts of western Burkina Faso to assess the impact of mass-distributed next-generation long-lasting insecticidal nets (LLINs) (Piperonyl Butoxide (PBO) LLINs and Interceptor® G2) on *Anopheles gambiae* s.l. populations, focusing on insecticide resistance trends and residual malaria transmission patterns, along with their environmental and operational determinants. Hourly indoor and outdoor mosquito collections were conducted across four households per district form August–October 2023 using Human Landing Catch and Pyrethrum Spray Catch. All collected mosquitoes were morphologically identified. Molecular analysis was performed on *Anopheles gambiae* s.l. to determine species composition, blood meal sources, *Plasmodium falciparum* infection rates, and insecticide resistance mutations. Seven *Anopheles* species were recorded, with the *An. gambiae* s.l. complex being predominant. Species composition varied significantly by month (August–October), with *An. coluzzii* being the dominant species, followed by *An. arabiensis*. Early and late biting behaviors were observed among vector populations. Entomological inoculation rates were 0.875, 0.437, and 0.063 infectious bites/person/month in August, September, and October, respectively. *Kdr-west* and *kdr-east* mutations were detected across all members of the *An. gambiae* s.l. complex, though at varying frequencies. This study highlights the diversity and behavioral adaptability of the *Anopheles gambiae* s.l. complex. Despite widespread use of LLINs and indoor residual spraying (IRS), substantial residual malaria transmission persists. These findings offer critical evidence for optimizing vector control and resistance management strategies in Burkina Faso.

1. Background

The growing spread of insecticide resistance among malaria vector populations poses a serious threat to the efficacy of current vector control strategies (WHO, 2024). In Burkina Faso, this challenge is particularly acute, with *Anopheles gambiae* s.l. exhibiting high resistance levels to nearly all insecticide classes used in public health (Dabiré et al., 2008; Namountougou et al., 2019). Since 1999, several malaria-endemic countries across Africa have launched entomological surveillance combining insecticide susceptibility testing with molecular diagnostics, aiming to adapt and enhance malaria control strategies (Koekemoer et al., 2002; Gimnig et al., 2003).

Despite decades of effort by Burkina Faso's National Malaria Control Programme (NMCP), malaria remains a significant public health concern. Current vector control relies primarily on LLINs and IRS, with support from the President's Malaria Initiative (PMI) since 2007 (WHO, 2020, 2019, 2017). Encouragingly, recent evaluations have reported a progressive decline in malaria morbidity and mortality, prompting the adoption of pre-elimination goals in the central and northern regions (Soma et al., 2020, 2021; Namountougou et al., 2023). However, localized pockets of transmission persist, sustained by widespread resistance across all four major insecticide classes (Russell et al., 2010, 2011; Kibret et al., 2014; Protopopoff et al., 2018). In response, the NMCP adopted a zonal, targeted approach within its 2016–2020

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strategic plan to better tailor interventions to local vector dynamics.

A major gap remains in understanding the drivers of residual malaria transmission, particularly those occurring outdoors and beyond the protective reach of LLINs and IRS. This underscores the need for intensified research into the behavioral and ecological factors underpinning such transmission. Effective supplementary interventions must target outdoor biting populations, including both anthropophilic and zoophilic mosquitoes (Kiware et al., 2012; Gleave et al., 2017).

This study seeks to enhance understanding of the entomological determinants and resistance mechanisms contributing to residual malaria transmission in western Burkina Faso. The findings aim to inform the development of sustainable and locally adapted vector control strategies, support the NMCP's decision-making, and foster a coordinated policy framework to manage residual transmission. Moreover, the research will contribute to capacity building and regional collaboration in malaria vector surveillance and control.

2. Materials and methods

2.1. Sampling sites and rationale

The study was conducted in August–October 2023 in two ecologically distinct zones of Burkina Faso—the Sudanian (Banfora) and Sudan–Sahelian (Houndé) zones—chosen for their heterogeneous and extensive pyrethroid resistance profiles, which may accelerate the emergence of resistant mosquito populations (Fig. 1).

Banfora (10° 36′ N, 4° 45′ W), located in the Cascades Region's Comoé Province and spanning approximately 934 km^2, is home to about 153,574 residents across 15 sectors and 22 villages. It experiences a tropical savanna (Aw) climate with approximately 1125 mm of annual rainfall, peaking in August (~320 mm) and averaging 120 rainy days per year.

Houndé (11° 30′ N, 3° 31′ W), the capital of Tuy Province, hosts around 329,253 residents in five sectors and features a similar savanna climate with an annual mean temperature of ~28.4 °C, 729 mm of rainfall, and roughly 102 rainy days annually.

Both sites display prolonged rainy seasons—from late April/May to early October—with peak precipitation in August, favoring the perennial breeding of malaria vectors. While LLINs coverage and IRS interventions are well-established, persistent malaria transmission remains problematic, likely exacerbated by intensive cotton cultivation and consequent continuous pesticide usage, thereby applying substantial selection pressure on mosquito vectors.

These ecological and demographic characteristics render Banfora and Houndé optimal locations to study the interplay between vector dynamics, insecticide resistance, and residual malaria transmission in areas deploying next-generation LLINs.

2.2. Experimental protocols

This study aimed to improve malaria vector control and reduce insecticide resistance through two core objectives: (i) characterizing vector diversity and transmission during peak rainy seasons in districts deploying next-generation LLINs (PBO-treated and Interceptor® G2), and (ii) evaluating supplementary intervention tools to reinforce surveillance–response strategies and target residual transmission toward

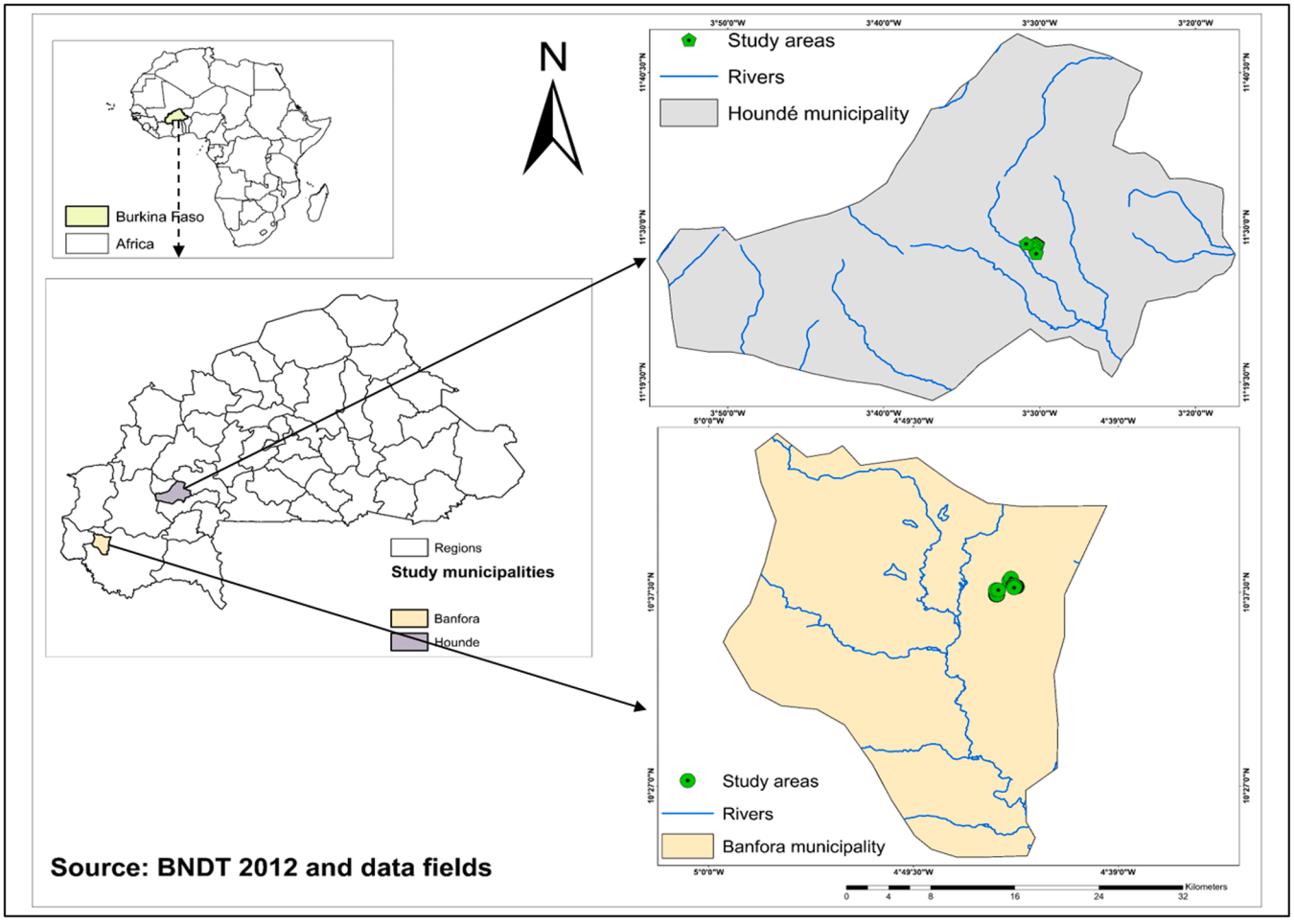

Fig. 1. Map of study area.

malaria elimination.

2.3. Field collections

2.3.1. Mosquito sampling

Sampling occurred during across four households per site. Human Landing Catches (HLCs) were conducted hourly from 17:00 to 09:00 indoors and outdoors under medical supervision, following established guidelines (Gimnig et al., 2013). Two teams of eight volunteers rotated between indoor and outdoor positions on alternating two-hour shifts (17:00–01:00, 01:00–09:00) to minimize collector bias. Collection involved aspiration of mosquitoes landing on exposed lower legs using mouth aspirators and torches (Silver, 2008). Sampling points were spaced at least 250 m apart, with indoor and outdoor stations separated by ≥20 m to avoid interference.

2.3.2. Residual resting collections

Once per month, Pyrethrum Spray Catches (PSC) were conducted in 10 randomly selected houses per site, over two consecutive mornings (totaling 20 houses per site), to collect blood-fed and resting *Anopheles* spp. These specimens were analyzed to determine host blood sources and calculate human blood index, following established methodologies (Kent and Norris, 2005; Das et al., 2017).

2.3.3. Morphological and parity assessment

All collected mosquitoes were morphologically identified to genus and species using standard taxonomic keys (Gillies and De Meillon, 1968; Gillies and Coetzee, 1987). A subset of 50 nulliparous females per month per site from *Anopheles gambiae* s.l. underwent ovarian dissection according to the Detinova method to determine parity rates and estimate mosquito population age structure (Detinova et al., 1962; Detinova and Gillies, 1964). Nulliparous females were selected each month at each site from collections obtained by human landing catches. After morphological identification, they were distinguished from parous females by ovarian dissection following the standard method described by Detinova et al. (1962). When the number of collected nulliparous females exceeded 50 in a given month at a site, a simple random sampling was performed to retain a representative subset of 50 specimens. Conversely, when the total number of nulliparous females collected was ≤ 50, all available individuals were included in the analysis. This method, which relies on identifying coiled versus stretched tracheolar skeins, is a well-established proxy for vector age and longevity. A subsample of *Anopheles* females was taken and stored in individual tubes containing silica gel and preserved for molecular identification of species within the *Anopheles* complex (Scott et al., 1993; Koekemoer et al., 2002).

Anopheles gambiae s.l. specimens were preserved individually in silica gel and processed for species identification using multiplex Polymerase chain reaction (PCR) protocols (Scott et al., 1993; Koekemoer et al., 2002; Santolamazza et al., 2008). Genomic DNA was extracted from head–thorax tissue using 2 % Cetyl trimethyl ammonium bromide (CTAB) buffer. Detection of insecticide resistance alleles (*kdr* L1014F and 1014S) was determined according to Martinez-Torres et al. (1998) and Ranson et al. (2000). PCR assays were employed to detect *Plasmodium* sporozoites (*P. falciparum, P. malariae, P. ovale*), adapting methods from Santolamazza et al. (2008).

2.5. Entomological parameters

We calculated standard entomological metrics—human biting rate (HBR), sporozoite rate, entomological inoculation rate (EIR), and parity rate—based on field data. These indices provide quantitative measures of malaria transmission intensity and were computed in accordance with recognized formulas from epidemiological studies.

2.5.1. Evaluation of the frequency of the various entomological parameters measured

Density

Density is obtained by dividing the number of species considered by the total number of houses surveyed. It was estimated for each site, by the collections from simultaneous night captures on human bait indoor and outdoor dwellings (1 volunteer inside and 1 volunteer outside per district and per month).

m (Anopheles density) = number of females *Anopheles* collected/ number of houses.

2.5.1.1. Average human biting rate. This parameter corresponds to the number of bites of a given species of mosquito received by humans indoor and/or outdoor human dwellings over a given period. Knowledge of this parameter makes it possible to pinpoint places and periods of maximum transmission risk, in order to adapt individual prevention practices and/or plan control operations adapted to the behavior of the vectors in question. HBR, estimated as the number of *Anopheles* bites per unit of time.

This parameter can be quantified using two alternative methods depending on the collection technique used, in particular by the application of pyrethroids (PYs) or by night capture. In the present study, the assessment of HBR, was calculated for the night capture method using the following formula:

$$HBR = \frac{\text{Total number of female Anopheles collected (fasting)}}{\text{Number of catchers X Number of days}}$$

2.5.1.2. Parity rate (PR). It corresponds to the percentage of *Anopheles* pares females (having laid eggs at least once) in relation to the total number of females dissected after capture. It is estimated by reading the ovarian tracheoles. This parameter reflects the proportion of potentially dangerous females that have taken at least one blood meal from humans.

$$\text{PR} = \frac{\text{Number of parous female Anopheles}}{\text{Total number of female Anopheles dissected}} \text{X } 100$$

2.5.1.3. Sporozoite infection rate (SIR). This is the number of female *Anopheles* carrying sporozoites in their salivary glands divided by the total number of mosquitoes dissected or tested by PCR.

$$\text{SIR} = \frac{\text{Number of female Anopheles carrying at least one sporozoite}}{\text{Total number of female Anopheles tested}} \text{X100}$$

2.5.1.4. Entomological inoculation rates. It represents the number of infecting bites received by humans per unit of time (night, month or year). It is equal to the HBR multiplied by the infection rate. The EIR, expressed as the number of infested bites/human/night (b h^{-1} n^{-1}), is calculated by multiplying the infestation rate by the HBR. The annual EIR, expressed as the number of bites infested/man/year, is calculated by multiplying the average annual infestation rate by the average annual HBR over 365 days.

$$\text{EIR } \left(\text{b } h^{-1} n^{-1}\right) = \text{ HBR x SIR}$$

2.5.1.5. Endophagy or exophagy rate (ER). This is the proportion of female malaria mosquitoes that bite inside or outside homes. It is expressed as a percentage (%).

$$\text{ER} = \frac{\text{Number of female Anopheles caught indoors}}{\text{Total number of female Anopheles captured}}$$

2.6. Laboratory bioefficacy trials

Next-generation LLINs were retrieved from field sites and replaced with new nets, after which WHO cone bioassays were performed to

evaluate their lethal efficacy against resistant *Anopheles* populations. LLINs exhibiting ≤80 % mortality in the cone test were subsequently subjected to more comprehensive tunnel assays, integrating both behavioral and biological components of mosquito interaction with the net.

2.6.1. WHO cone bioassay

Following WHO protocols, net samples were exposed to batches of 2–5-day-old, non-blood-fed female *Anopheles* mosquitoes for 3 min within standardized cone chambers. Knockdown was assessed at 60 min (KD_{60}), and mortality after a 24-hour holding period with sugar solution. Nets failing to achieve ≥80 % mortality were advanced to the tunnel test for further evaluation.

2.6.2. WHO tunnel assay

Conducted under controlled laboratory conditions (27 ± 2 °C; 75–80 % Relative Humidity), tunnel tests involved glass chambers measuring 25 × 25 × 60 cm. A 20 × 20 cm fragment of LLINs was affixed one-third along the length, pierced with nine 1 cm-diameter holes (eight peripherals and one central). A restrained guinea pig served as bait at one end. A cohort of 5–8-day-old, unfed female *Anopheles* (n = 100) was introduced into the tunnel at 18:00, and collections occurred at 09:00 the next morning. Mortality and blood-feeding rates were recorded by section, and overall mortality was computed across the entire chamber.

Blood-feeding inhibition was calculated by comparing the proportion of engorged mosquitoes in treated versus control tunnels. Untreated nets were used as negative controls. The assay adhered to WHO criteria for exit of tunnels assays, including mortality ≥80 % and feeding inhibition ≥90 %. Abbott's correction was applied when control mortality exceeded 5 %, and assays were repeated if control mortality surpassed 20 %.

This tiered bioassay approach ensured robust evaluation of the nets' operational efficacy against resistant vector populations, combining standard lethality assessments with realistic behavioural challenges.

2.7. Statistical analysis

All analyses were conducted using RStudio (R v.4.4.3) with the lme4 and emmeans packages. Entomological metrics—namely ma, EIR, SIR, ER and PR—were evaluated through Generalized Linear Mixed Models (GLMMs) via the glmer function in lme4.

- Count data (ma, EIR): Modeled using a negative binomial distribution to account for overdispersion, with month, capture position (indoor/outdoor), and species as fixed effects, and household as a random intercept.
- Proportion data (SIR, ER, PR): Analyzed with binomial GLMMs with the same fixed and random structure.

Model selection employed backward stepwise elimination, retaining only those covariates with $p < 0.05$. Effect estimates were reported as incidence rate ratios (IRR) for negative binomial models and odds ratios (OR) for binomial models, each accompanied by 95 % confidence intervals.

To compare multiple levels of categorical predictors (e.g., different months), post hoc contrasts were performed using Tukey-adjusted pairwise comparisons with the emmeans package.

Comparisons of species composition across survey months were conducted using Pearson's chi-squared tests with simulated p-values for robustness. Biting activity metrics—such as peak aggressiveness and median hour of capture (MHC, the time at which 50 % of bites occur)—were assessed using Kruskal–Wallis tests, followed by post hoc Dunn tests corrected for multiple comparisons via the VIA package.

This analytical framework rigorously quantifies the spatiotemporal dynamics of malaria vectors and evaluates the impact of environmental and operational factors on residual transmission.

2.8. Ethics approval and consent to participate

All participants were recruited on a voluntary basis and gave their informed consent after the purpose, potential risks and benefits of the study were explained to them. All HLC activities were accompanied by rigorous medical monitoring, chemoprophylaxis, and access to diagnosis and treatment. Prior to the start of activities, the project was submitted to the Institutional Ethics Board of the IRSS/Centre Muraz in Burkina Faso for approval (Ref. No 008–2022/CEIRES, dated January 20, 2022). For all activities involving volunteers, the data were processed anonymously after informed consent had been obtained.

3. Results

3.1. Composition of the vector species fauna

A total of 4393 female mosquitoes, belonging to five genera, were collected in the districts of Banfora and Houndé during the months of August, September, and October 2023. The most prevalent genus was *Culex* spp. ($n = 2861$; 65.12 %), followed by *Anopheles* spp. ($n = 1279$; 29.38 %), *Phlebotomus* spp. ($n = 105$; 2.39 %), *Mansonia* spp. ($n = 76$; 1.73 %), and *Aedes* spp. ($n = 72$; 1.63 %) (Table 1).

Among the *Anopheles* specimens collected, 86.24 % ($n = 1103/1279$) belonged to the *An. gambiae* sensu lato complex, while 0.31 % ($n = 4/1279$) were part of the *An. funestus* group. Additional species included members of the *An. nili* complex (2.27 %, $n = 29$), *An. rufipes* (7.04 %, $n = 90$), and *An. squamosus* (0.70 %, $n = 9$). *An. pharoensis* and *An. coustani* were represented equally, each accounting for 1.72 % ($n = 22/1279$) of the *Anopheles* population.

The distribution of *Anopheles* species varied significantly between the two districts (Pearson's Chi-squared test: $\chi^2 = 4.8508$; df = 1; $p = 0.0276$), with a marked predominance of the *An. gambiae* s.l. complex throughout the collection period—96.40 % ($n = 321/333$) in August, 89.42 % ($n = 642/718$) in September, and 61.40 % ($n = 140/228$) in October—regardless of location.

A significant temporal variation was observed in the species composition of *Anopheles* populations across the sampling months (Pearson's Chi-squared test: $\chi^2 = 75.3312$; df = 2; $p < 2.2 \times 10^{-16}$), with the highest abundance recorded in September (Banfora: $n = 276$; Houndé: $n = 366$). This pattern was further supported by a fixed-effects model (Estimate = 0.88567; $Z = 6.537$; $p < 6.26 \times 10^{-11}$), confirming significant monthly fluctuations in vector composition.

3.2. Trophic behavior of vectors

3.2.1. Mean human biting rate (bites per human per night; b. h^{-1}. n^{-1})

The mean HBR of the *Anopheles* species collected were 22.93 and 26.56 bites per human per night (b. h^{-1}. n^{-1}) in Banfora and Houndé, respectively. When analyzed by month, the average HBR across both districts was 11.37, 28.25, and 9.87 b h^{-1}. n^{-1} for August, September, and October, respectively. A statistically significant difference in the average HBR between the two districts was observed during the survey period (Tukey's test: Ratio $R = 0.769$ [0.609; 0.971], Z-ratio = –2.202; $p = 0.0276$).

In Banfora, the monthly mean biting rates were 8.31, 14.00, and 0.62 b h^{-1}. n^{-1} for August, September, and October, respectively. In contrast, Houndé recorded 3.06, 14.25, and 9.25 b h^{-1}. n^{-1} for the same months. Statistical comparisons revealed no significant difference between districts in August (Tukey's test: $R = 0.189$ [0.857; 1.764], Z-ratio = 1.343; $p = 0.3714$), whereas significant differences were found in September ($R = 0.412$ [0.300; 0.566], Z-ratio = –6.537; $p < 0.0001$) and October ($R = 0.0335$ [0.241; 0.466], Z-ratio = –7.759; $p < 0.0001$) (Fig. 2). Regardless of the district, the highest vector aggressiveness was recorded in September, with mean biting rates of 14.00 b h^{-1}. n^{-1} in Banfora and 14.25 b h^{-1}. n^{-1} in Houndé.

Table 1
Diversity of culicid fauna in two districts (Banfora and Houndé) during August-September and October.

Collection method	Species collected	Banfora				Hounde				
		August n (%)	September n (%)	October n (%)	(n) %	August n (%)	September n (%)	October n (%)	(n) %	Overall total n(%)
HLC	*Ae. aegypti*	12 (2.11)	24 (4.03)	11 (2.99)	47 (3.07)	2 (0.45)	3 (0.37)	4 (0.69)	9 (0.49)	56 (1.66)
	Ae. vexans	0	0	0	0	0	6 (0.74)	0	6 (0.33)	6 (0.18)
	Ae. vittatus	0	0	0	0	0	2 (0.25)	0	2 (0.11)	2 (0.06)
	An. coustani	0	0	0	0	1 (0.22)	6 (0.74)	15 (2.58)	22 (1.19)	22 (0.65)
	An.s funestus	0	0	0	0	0	0	3 (0.52)	3 (0.16)	3 (0.09)
	An. gambiae s.l	127 (22.36)	195 (32.72)	9 (2.45)	331 (21.61)	45 (10.07)	182 (22.3)	65 (11.19)	292 (15.84)	623 (18.45)
	An. nili	3 (0.53)	25 (4.19)	0	28 (1.83)	0	1 (0.12)	0	1 (0.05)	29 (0.86)
	An. pharoensis	3 (0.53)	4 (0.67)	1 (0.27)	8 (0.52)	3 (0.67)	7 (0.86)	2 (0.34)	12 (0.65)	20 (0.59)
	An. rufipes	0	0	0	0	0	30 (3.68)	56 (9.64)	86 (4.66)	86 (2.55)
	An. squamosus	0	0	0	0	0	2 (0.25)	7 (1.2)	9 (0.49)	9 (0.27)
	Cx. cinereus	1 (0.18)	34 (5.7)	46 (12.5)	81 (5.29)	0	38 (4.66)	6 (1.03)	44 (2.39)	125 (3.70)
	Cx. decens	0	0	0	0	0	1 (0.12)	2 (0.34)	3 (0.16)	3 (0.09)
	Cx. nebulosus	0	0	0	0	0	36 (4.41)	63 (10.84)	99 (5.37)	99 (2.93)
	Cx. quinquefasciatus	389 (68.49)	275 (46.14)	252 (68.48)	916 (59.79)	391 (87.47)	479 (58.7)	286 (49.23)	1156 (62.69)	2072 (61.37)
	Cx. univittatus	26 (4.58)	5 (0.84)	1 (0.27)	32 (2.09)	0	8 (0.98)	0	8 (0.43)	40 (1.18)
	Man. africana	2 (0.35)	9 (1.51)	13 (3.53)	24 (1.57)	0	0	0	0	24 (0.71)
	Man. uniformis	2 (0.35)	15 (2.52)	34 (9.24)	51 (3.33)	0	0	1 (0.17)	1 (0.05)	52 (1.54)
	Phléb. phlébotomus	0	9 (1.51)	0	9 (0.59)	0	0	0	0	9 (0.27)
	Phléb. sergentomya	3 (0.53)	1 (0.17)	1 (0.27)	5 (0.33)	5 (1.12)	15 (1.84)	71 (12.22)	91 (4.93)	96 (2.84)
	Total n (%)	568 (37.07)	596 (38.90)	368 (24.02)	1532 (100)	447 (24.24)	816 (44.25)	581 (31.50)	1844 (100)	3376 (76.84)
PSC	*Ae. aegypti*	3 (1.72)	1 (0.93)	0	4 (1.3)	3 (1.06)	0	1 (0.93)	4 (0.56)	8 (0.79)
	An. funestus	0	0	0	0	0	0	1 (0.93)	1 (0.14)	1 (0.10)
	An. gambiae s.l	90 (51.72)	81 (75.00)	5 (19.23)	176 (57.14)	59 (20.85)	184 (57.86)	61 (56.48)	304 (42.88)	480 (47.20)
	An. pharoensis	0	0	0	0	2 (0.71)	0	0	2 (0.28)	2 (0.20)
	An. rufipes	0	0	0	0	0	1 (0.31)	3 (2.78)	4 (0.56)	4 (0.39)
	Cx. cinereus	1 (0.57)	1 (0.93)	0	2 (0.65)	0	1 (0.31)	1 (0.93)	2 (0.28)	4 (0.39)
	Cx. quinquefasciatus	69 (39.66)	25 (23.15)	21 (80.77)	115 (37.34)	219 (77.39)	132 (41.51)	41 (37.96)	392 (55.29)	507 (49.85)
	Cx univittatus	11 (6.32)	0	0	11 (3.57)	0	0	0	0	11 (1.08)
	Total n (%)	174 (56.49)	108 (35.06)	26 (8.44)	308 (100)	283 (39.91)	318 (44.85)	108 (15.23)	709 (100)	1017 (23.15)
Overall total n (%)		742 (16.89)	704 (16.02)	394 (8.96)	1840 (41.88)	730 (16.61)	1134(25.81)	689 (15.68)	2553 (58.11)	4393 (100)

n: number of individual species; %: percentage of species; HLC: Human Landing Catch; PSC: Pyrethrum Spray Catch.

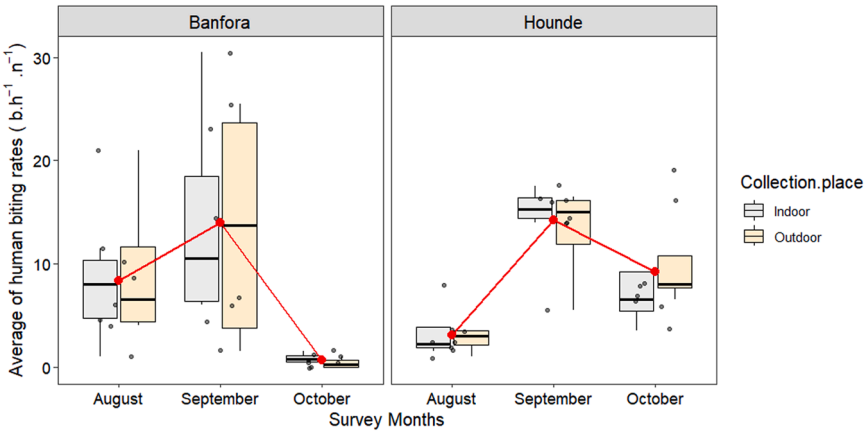

Fig. 2. Average human biting rates of *Anopheles* mosquitoes in Banfora and Houndé during the survey months. The boxes indicate the 1st-3rd quartiles and the horizontal black line represents the median of the aggressive densities. The upper whiskers extend to the highest value not exceeding 1.5*IQR from the hinge of aggressive densities per survey. The small black dots indicate the aggressive densities (ma) recorded at each collection point and the red dots the average 'ma' per survey.

3.2.2. Biting patterns and hourly activity profiles of vector populations

Overall, the number of *Anopheles* mosquitoes collected both indoors and outdoors varied between the two districts across the sampling months (Table 2). The estimated rates of endophagy (i.e., proportion of indoor biting) with corresponding 95 % confidence intervals were 46.70 % [39.33–54.22] in August, 52.88 % [48.16–57.54] in September, and 45.57 % [37.70–53.66] in October. When disaggregated by district, the endophagy rates in Banfora were 42.85 % [34.41–51.73], 51.34 %

Table 2
Comparison of indoor (endophagy) and outdoor (exophagy) mosquito collections in Banfora and Houndé.

Districts	Species	August		T (n)	September		T (n)	October		T (n)	Overall Total
		Ind	Out		Ind	Out		Ind	Outd		
Banfora	*An. gambiae* s.l.	55	72	127	97	98	195	6	3	9	331
	An. nili	1	2	3	15	10	25	0	0	0	28
	An. pharoensis	1	2	3	3	1	4	1	0	1	8
	Total	57	76	133	115	109	224	7	3	10	367
	% Ind [95 % CI]	42,85 [34,41 ; 51,73]			51,34 [44,61 ; 58,03]			70,00 [35,37 ; 91,91]			-
Houndé	*An. coustani*	0	1	1	3	3	6	4	11	15	22
	An. funestus	0	0	0	0			2	1	3	3
	An. gambiae s.l.	26	19	45	97	85	182	33	32	65	292
	An. nili	0	0	0	0	1	1	0	0	0	1
	An. pharoensis	2	1	3	3	4	7	1	1	2	12
	An. rufipes	0	0	0	19	11	30	24	32	56	86
	An. squamosus	0	0	0	2		2	1	6	7	9
	Total	28	21	49	124	104	228	65	83	148	425
	% Ind [95 % CI]	57,14 [42,29 ; 70,88]			54,39 [47,68 ; 60,94]			43,92 [35,85 ; 52,30]			-
Overall Total		85	97	182	239	213	452	72	86	158	792
% Ind [95 % CI]		46,70 [39,33 ; 54.22]			52,88 [48,16 ; 57.54]			45,57 [37,70 ; 53,66]			-

Ind : Indoor; Out: Outdoor; 95 % CI : 95 % Confidence Interval; T : Total.

[44.61–58.03], and 70.00 % [35.37–91.91] for August, September, and October, respectively (Chi-squared = 0.34877, df = 1, $p = 0.5548$). In Houndé, the corresponding rates were 57.14 % [42.29–70.88], 54.39 % [47.68–60.94], and 43.92 % [35.85–52.30] (Chi-squared = 0.30118, df = 1, $p = 0.5831$). No statistically significant differences in endophagy rates were observed between the two districts over the course of the study period (Tukey's test: Ratio $R = 0.9598$ [0.768–1.198], Z-ratio = –3.622, $p = 0.7172$).

The hourly biting profiles revealed notable differences in *Anopheles* spp. activity between months and across districts (Fig. 3). Marked fluctuations in biting activity were observed throughout the night, indicating defined periods of high and low vector aggressiveness. In general, the highest biting activity occurred during the early evening hours, between 6:00 pm. and 10:00 pm., with the exception of October in Houndé, where peak biting began earlier, between 5:00 pm. and 9:00 pm. After 10:00 pm., biting activity declined progressively, reaching minimum levels between 3:00 a.m. and 6:00 a.m. Peaks in mosquito aggressiveness were observed around 10:00 pm., 1:00 a.m., and 3:00 a.m., which likely reflect the host-seeking behavior and circadian rhythms of the vector species involved.

Hourly activity profiles also varied depending on whether biting occurred indoors or outdoors. Outdoor biting tended to exhibit more pronounced peaks, particularly between 10:00 pm. and 3:00 a.m., whereas indoor biting remained relatively stable, albeit at lower intensities. These differences suggest distinct resting and host-seeking behaviors among the vector populations. Although the general temporal trends were similar across months and districts, statistically significant differences in biting patterns were detected (Kruskal-Wallis test: $\chi^2 = 55.317$, df = 5, $p = 1.123 \times 10^{-10}$).

However, no statistically significant differences were observed when comparing the two districts, Banfora and Houndé (Dunn's post hoc test following Kruskal-Wallis: $Z = 1.0878$; $p = 0.2767$). In Banfora, mosquito biting activity was significantly higher in the evening during August compared to September and October (Dunn's test: $Z = 3.5686$; $p = 5.38 \times 10^{-3}$).

Conversely, significantly lower evening biting rates were recorded in September compared to October ($Z = –4.6315$; $p = 5.45 \times 10^{-5}$), suggesting heightened vector activity during the early part of the

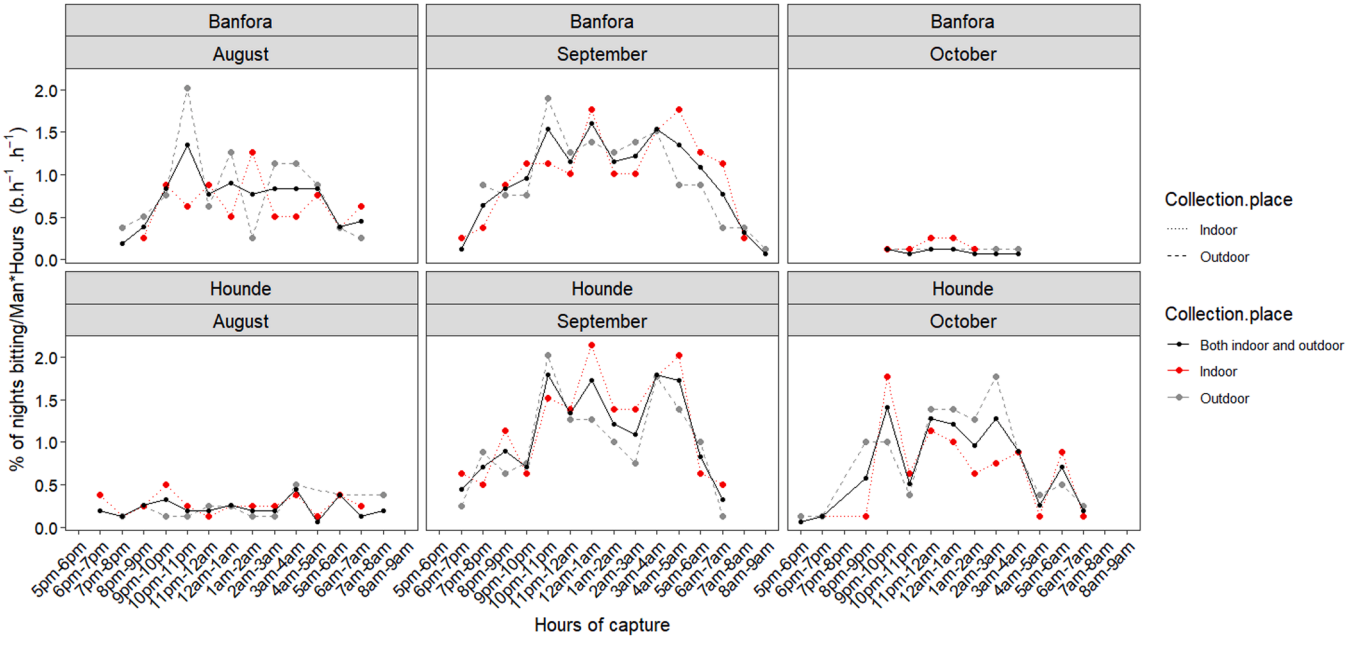

Fig. 3. Mean Hourly biting activity profiles of *Anopheles* spp. mosquitoes in Banfora and Houndé, August–October.

transmission season.

In Houndé, hourly biting dynamics appeared more consistent across the months, with less pronounced temporal fluctuations (Dunn's test: $Z = 3.5686$; $p = 5.38 \times 10^{-3}$). A comparison between months revealed only modest differences ($Z = -2.3028$; $p = 0.3194$), indicating greater temporal stability in biting behavior.

These observed differences may be attributable to environmental or ecological factors, such as microclimatic conditions, land use patterns, or variations in vector species composition between the two areas.

3.2.3. Parous rate of malaria vector populations

A total of 250 female mosquitoes belonging to the *Anopheles gambiae* s.l. complex were dissected to assess their physiological age and estimate parity rates (Fig. 4). The overall parous rates were 82.59 % (95 % CI: [76.47–87.42]) in Banfora and 87.76 % (95 % CI: [74.54–94.42]) in Houndé over the entire study period (August to October). Statistical comparison between districts revealed no significant difference in parous rates (Chi-squared = 0.4368, df = 1, $p = 0.5087$; Fisher's Exact Test: $p = 0.1177$).

In October, parous rates reached 100 % (95 % CI: [59.77–100]) in both districts, with similarly high rates observed in Houndé during September (95 % CI: [85.87–100]), indicating a predominance of older, potentially more infectious females during this period.

3.3. Molecular identification of species within the anopheles gambiae s.l. complex

Molecular identification of members of the *An. gambiae* s.l. complex was performed on specimens collected through both HLC and PSC methods, yielding a total of 462 individuals: 239 from HLC and 223 from PSC (Table 3).

Among the HLC samples, 45.19 % (108/239) originated from Banfora and 54.81 % (131/239) from Houndé. No statistically significant difference in collection proportions between the two districts was observed (Pearson's Chi-squared test: $\chi^2 = 0.8107$, df = 1, $p = 0.3679$). In Banfora, *An. arabiensis* was the predominant species, accounting for 50.93 % (55/108), followed by *An. coluzzii* (33.33 %; 36/108), and *An. gambiae* s.s. (15.74 %; 17/108). Conversely, in Houndé, *An. coluzzii* was the most abundant species (54.20 %; 71/131), while *An. arabiensis* and *An. gambiae* s.s. were less prevalent, comprising 24.43 % (32/131) and 21.37 % (28/131), respectively. Species composition did not differ significantly between districts during the survey period (August to October) (Pearson's Chi-squared test: $\chi^2 = 2.0185$, df = 2, $p = 0.3645$).

For specimens collected via PSC, 46.19 % (103/223) were identified in Banfora and 53.81 % (120/223) in Houndé (Pearson's Chi-squared test: $\chi^2 = 1.1331$, df = 1, $p = 0.2871$). *An. coluzzii* was the predominant species in both districts, representing 53.40 % (55/103) of identified specimens in Banfora and 70.83 % (85/120) in Houndé. This was followed by *An. gambiae* s.s. (37.86 % in Banfora; 16.76 % in Houndé) and *An. arabiensis* (8.74 % in Banfora; 12.50 % in Houndé). Notably, the distribution of species varied significantly between the months of September and October (Tukey's multiple comparisons of means:

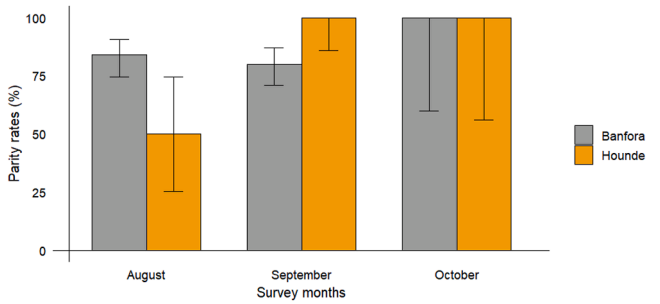

Fig. 4. Parity rate of anopheles collected during the months of survey.

Estimate = 0.9096 [0.058–1.760], $Z = -3.362$, $p = 0.0329$), indicating temporal variation in species prevalence.

3.3.1. Trophic preferences and atypical feeding behavior of malaria vectors

To assess host-feeding preferences, a total of 545 blood-fed *Anopheles* females were analyzed, including 241 from Banfora and 304 from Houndé (Table 4). Blood meal sources tested included humans, goats, cattle, and pigs.

In Banfora, the proportion of mosquitoes feeding on humans was 15.00 % (3/20) for *An. arabiensis*, 18.55 % (23/124) for *An. coluzzii*, and 22.68 % (22/97) for *An. gambiae* s.s. In Houndé, these values were higher across all species: 34.21 % (13/38) for *An. arabiensis*, 30.45 % (67/220) for *An. coluzzii*, and 32.61 % (15/46) for *An. gambiae* s.s.

These findings suggest heterogeneous anthropophagic tendencies among species and between districts, which may reflect differences in local host availability, vector ecology, or behavioural plasticity. The relatively high proportion of non-human blood meals indicates a degree of zoophily, which may influence local transmission dynamics and the effectiveness of human-targeted vector control strategies. The overall human blood index was statistically significant between Banfora and Houndé (OR = 0.54; 95 % CI [0.35; 0.82]; p-value = 0.003219).

3.3.2. Evaluation of SIR and EIR indoors and outdoors

The detection of *Plasmodium falciparum* sporozoites in the head-thorax of female *Anopheles* mosquitoes revealed variation in SIR and EIR across months and species (Table 5).

The mean sporozoite index (SI) for all species combined across both districts was estimated at 17.28 % (14/81; 95 % CI: [10.58–26.95]) in August, 7.07 % (7/99; 95 % CI: [3.46–13.88]) in September, and 1.69 % (1/59; 95 % CI: [0.04–9.09]) in October, indicating a declining trend in infection rates over the transmission season.

Correspondingly, EIRs were estimated at 0.875 [95 % CI: 0.70–1.04], 0.437 [0.35–0.52], and 0.063 [0.05–0.07] infectious bites per person per night (b. h^{-1}. n^{-1}) for August, September, and October, respectively. Transmission intensity was significantly elevated during the months of August (Rate Ratio [RR] = 0.13 [0.10–0.18], $p < 0.0001$) and September (RR = 0.69 [0.52–0.71], $p < 0.0001$), indicating a seasonal peak in vector infectivity at the beginning of the rainy season.

3.3.3. Allele frequencies of kdr-West and kdr-East mutations

The genotypic composition and allele frequencies of the *kdr*-west and *kdr*-east mutations were determined monthly (August, September, and October) in *An. arabiensis, An. coluzzii*, and *An. gambiae* s.s. (Table 6).

In *An. arabiensis*, the frequency of the *kdr*-west allele was 0.486 in August, 0.550 in September, and decreased to 0.231 in October. Significant deviations from Hardy–Weinberg equilibrium (HWE) were observed in August and September (exact HWE test: $p < 0.05$), consistent with ongoing selection pressure. However, the October population showed no significant deviation (exact HWE test: $p > 0.05$), suggesting a return to equilibrium and a reduction in resistance allele frequency. The exact G-test indicated statistically significant temporal variation in allele frequencies in August ($p = 0.0023$) and September ($p = 0.0035$), but not in October ($p = 0.5257$).

In *An. coluzzii*, the *kdr*-west allele frequency was 0.355 in August ($p = 0.0031$), increased significantly to 0.605 in September ($p = 0.0023$), and then declined slightly to 0.519 in October ($p = 0.6576$). As observed in *An. arabiensis*, significant deviations from HWE were noted in August and September (exact HWE test: $p < 0.05$), suggesting strong selection for resistance alleles during this period. The relatively high frequencies of the resistant allele point to ongoing insecticide pressure likely contributing to the spread of resistance.

3.3.4. Frequency and stability of kdr-West and kdr-East mutations in an. gambiae s.s

In *Anopheles gambiae* s.s., the frequency of the *kdr*-west mutation remained stable across the study period, with no significant temporal

Table 3
Species composition of the *An. gambiae* s.l. complex during surveys in the Banfora and Hounde districts.

Collection Method	Molecular form for *An. gambiae* s.l identified	Banfora				Hounde				Overall total n (%)
		August n (%)	September n (%)	October n (%)	Total n (%)	August n (%)	September n (%)	October n (%)	Total n (%)	
HLC	*An. arabiensis*	31 (62)	19 (38.78)	5 (55.56)	55 (50.93)	5 (16.13)	16 (32)	11 (22)	32 (24.43)	87 (36.40)
	An. coluzzii	12 (24)	20 (40.82)	4 (44.44)	36 (33.33)	21 (67.74)	23 (46)	27 (54)	71 (54.20)	107 (44.77)
	An. gambiae s.s	7 (14)	10 (20.41)	0	17 (15.74)	5 (16.13)	11 (22)	12 (24)	28 (21.37)	45 (18.83)
Total n (%)		50 (46.30)	49 (45.37)	9 (8.33)	108 (45.19)	31 (23.66)	50 (38.17)	50 (38.17)	131 (54.81)	239 (100)
PSC	*An. arabiensis*	6 (12.50)	3 (6.25)	0	9 (8.74)	4 (11.11)	3 (6.12)	8 (22.86)	15 (12.50)	24 (10.76)
	An. coluzzii	26 (54.17)	26 (54.17)	3 (42.86)	55 (53.40)	20 (55.56)	43 (87.76)	22 (62.86)	85 (70.83)	140 (62.78)
	An. gambiae s.s	16 (33.33)	19 (39.58)	4 (57.14)	39 (37.86)	12 (33.33)	3 (6.12)	5 (14.29)	20 (16.76)	59 (26.46)
Total n (%)		48 (46.60)	48 (46.60)	7 (6.80)	103 (46.19)	36 (30)	49 (40.83)	35 (29.17)	120 (53.81)	223 (100)

n: number of individual species; %: percentage of species.

Table 4
Blood meal origins of *An. gambiae* s.l from the Banfora and Hounde districts.

Districts	Species	Animals					Human		Mixed		Unknown		Total
		Goat	Cattle	Pigs	Other	%	n	%	n	%	N	%	n
Banfora	*An. arabiensis*	5	0	0	4	45.00	3	15.00	1	5.00	7	35.00	20
	An. coluzzii	41	0	0	14	44.35	23	18.55	14	11.29	32	25.81	124
	An. gambiae s.s.	31	0	1	8	41.23	22	22.68	18	18.56	17	17.53	97
Total		77	0	1	26	43.23	48	19.92	33	13.69	56	23.24	241
Hounde	*An. arabiensis*	9	0	0	6	39.47	13	34.21	8	21.05	2	5.26	38
	An. coluzzii	58	1	0	27	39.09	67	30.45	50	22.73	17	7.73	220
	An. gambiae s.s.	8	0	0	12	43.47	15	32.61	6	13.04	5	10.87	46
Total		75	1	0	45	39.80	95	31.25	64	21.05	24	7.89	304

n: number of blood-fed Anopheles females, Other: other animals not determined, Mixed fed on both animal and human.

Table 5
Entomological parameters of transmission: sporozoite infection rate (*P. falciparum* infection) and entomological inoculation rate.

Districts	Species	August			September			October		
		n	SIR (n/%)	EIR	n	SIR (n/%)	EIR	n	SIR (n/%)	EIR
Banfora	*An. arabiensis*	31	6 (19.35)	0.375	19	2 (10.52)	0.125	5	0	0
	An. coluzzii	12	5 (41.67)	0.313	20	10	0.125	4	0	0
	An. gambiae s.s	7	0	0.000	10	0	0	-	-	-
Hounde	*An. arabiensis*	5	0	0.000	16	2 (12.5)	0.125	11	1 (9.09)	0.063
	An. coluzzii	21	3 (14.28)	0.188	23	1 (4.34)	0.0625	27	0	0
	An. gambiae s,s	5	0	0.000	11	0	0	12	0	0
Total % 95 % CI		81	14 (17.28) [10.58; 26.95]	0.875	99	7 (7.07) [3.46; 13.88]	0.437	59	1 (1.69) [0.04; 9.09]	0.063

n: Number of individuals tested; SIR: Sporozoite Infection Rate; EIR: Entomological Inoculation Rate; [95 % CI]: 95 % Confidence Interval, "-": not determined.

variation observed between August ($p = 0.9442$) and September ($p = 0.7386$, exact G-tests). The resistant allele remained predominant throughout, indicating a stable adaptation to insecticide selection pressure. In October, the distribution of genotypes was also stable ($p = 0.0507$), and in all months, no significant deviation from Hardy–Weinberg equilibrium (HWE) was detected ($p > 0.05$), suggesting a genetically equilibrated population.

3.3.5. Distribution and dynamics of kdr-East mutation

In *An. arabiensis*, the *kdr*-east mutation exhibited significant deviations from HWE in both August and September ($p < 0.05$), attributable to a deficit in heterozygotes. The exact G-test confirmed highly significant variation in allele distributions across these months ($p = 0.0001$ for August; $p = 0.0002$ for September), suggesting directional selection or assortative mating affecting this locus.

In *An. coluzzii*, the *kdr*-east allele was initially detected at a frequency of 0.118 in August ($p = 0.1619$), followed by a significant increase to 0.125 in September ($p < 0.0001$), and a further significant increase in October ($p = 0.0020$). Deviations from HWE were observed in both September and October ($p < 0.05$), also driven by a deficit of heterozygotes, indicating non-random mating or ongoing selective pressure for the resistant allele.

For *An. gambiae* s.s., the *kdr*-east allele frequency was consistent in August and October (0.333), with no significant change detected (exact G-tests: August $p = 0.5447$; October $p = 0.0507$). The stable frequency and conformity with HWE in these months suggest a balanced or

Table 6
Allelic and genotypic frequencies at the *kdr* L1014F (*kdr-west*) and *kdr* L1014S (*kdr-east*) locus in *An. gambiae* s.l. populations.

Species	Months		Genotypes of *kdr-West*						Genotypes of *kdr-East*				
		N	SS	RS	RR	f(1014F)	p(HW)	N	SS	RS	RR	f(1014S)	p(HW)
An. arabiensis	August	35	5	26	4	0.486	0.9977	22	10	2	10	0.500	0.9999
	September	30	1	25	4	0.550	0.9965	13	7	0	6	0.462	0.9998
	October	13	8	4	1	0.231	0.4743	9	8	1	0	0.056	0.2371
An. coluzzii	August	31	11	18	2	0.355	0.9969	17	14	2	1	0.118	0.8381
	September	38	1	28	9	0.605	0.9977	24	21	0	3	0.125	1.0000
	October	26	7	11	8	0.519	0.3424	10	7	0	3	0.300	0.9980
An. gambiae s.s	August	12	2	6	4	0.583	0.0558	6	3	2	1	0.333	0.4553
	September	21	4	12	5	0.524	0.2614	9	4	0	5	0.556	0.9996
	October	11	3	3	5	0.591	0.9493	3	2	0	1	0.333	0.9493

N: number of mosquitoes; SS: sensitive homozygote; RS: heterozygote; RR: resistant homozygote; f(1014F): frequency of the kdr-west mutation; f(1014S): frequency of the kdr-east mutation; p(HW): Hardy-Weinberg test for rejection or acceptance of the Hardy-Weinberg equilibrium hypothesis.

stabilized resistance trait. However, in September, the allele frequency rose to 0.556 ($p = 0.0004$), accompanied by a significant deviation from HWE ($p < 0.05$), again due to a heterozygote deficit.

3.4. Coverage of pyrethroid-PBO nets, resistance intensity, and impact on vector control efficacy

Insecticide bioassays were conducted to assess the effectiveness of two next-generation insecticide-treated nets (ITNs): Interceptor® G2 and PermaNet® 3.0, within the Banfora and Houndé health districts (Fig. 5A–D). Mortality rates were evaluated using both the susceptible laboratory strain An. gambiae Kisumu and field-collected *An. gambiae* s. l. populations from each site.

These assays revealed clear differences in susceptibility between laboratory and field populations, indicating the presence and intensity of pyrethroid resistance in the latter. While both ITNs induced high mortality in the Kisumu strain, reduced efficacy was observed in the wild populations, reflecting ongoing insecticide resistance in natural settings despite the inclusion of the synergist PBO in these net formulations.

These results underscore the need for integrated resistance management strategies and ongoing monitoring of the operational effectiveness of control tools in areas with established pyrethroid resistance.

3.5. Evaluation of the efficacy of LLINs based on WHO criteria

According to World Health Organization (WHO) guidelines, a LLINs is considered effective if it induces a mortality rate of ≥80 % or a knockdown rate of ≥95 % in tested mosquito populations. Additionally, bioassay results are deemed invalid if the mortality rate in the untreated control group reaches or exceeds 20 % (WHO, 2016;WHOPES, 2016).

Bioassays conducted using the susceptible *Anopheles gambiae* Kisumu

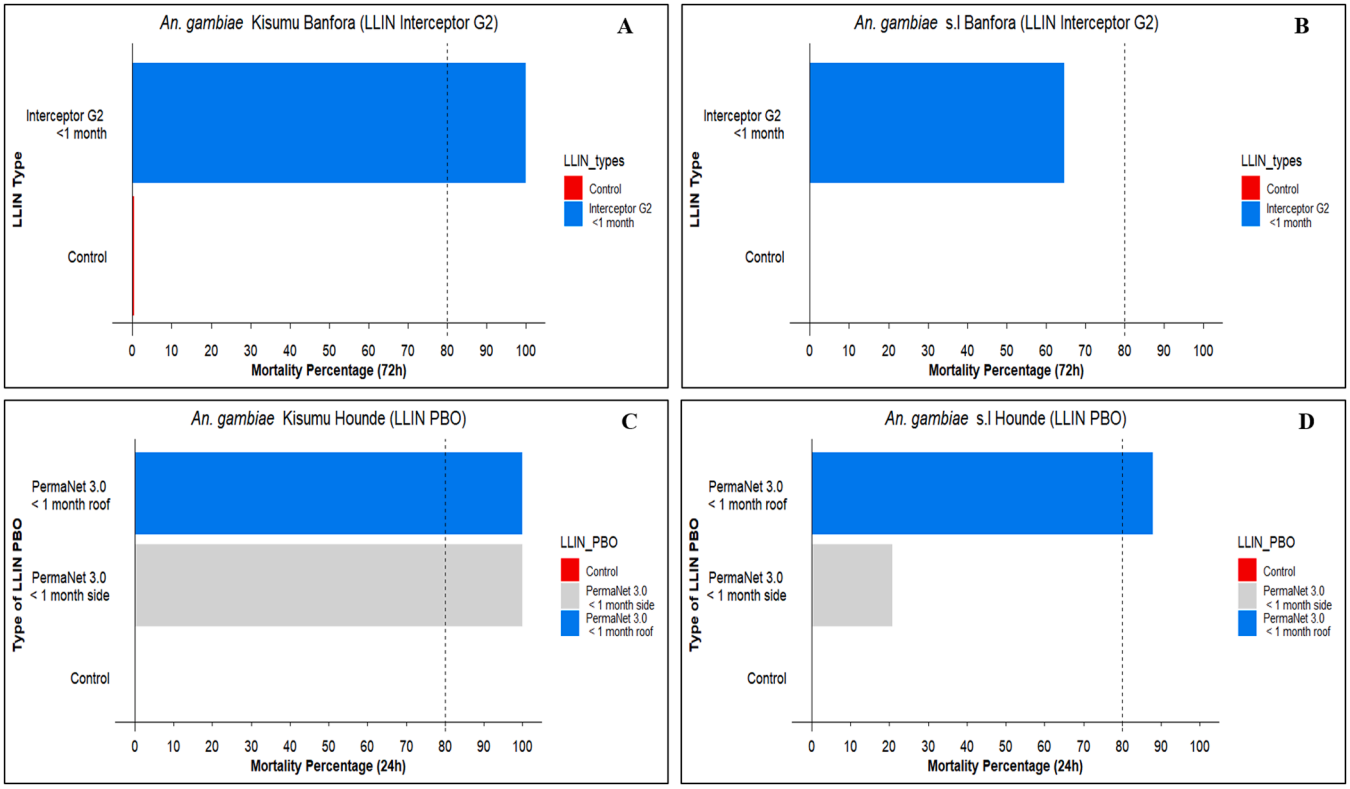

Fig. 5. Bioefficacy trials of Interceptor G2 and PermaNet 3.0 LLINs on natural *Anopheles gambiae* s.l population. Panels A and B show the percentage mortality after 72 h for *An. gambiae* s.l. de Banfora mosquitoes exposed to Interceptor G2 (<1 month old), compared to a control group of *An. gambiae* Kisumu. Panels C and D illustrate the percentage mortality of *An. gambiae* s.l. de Hounde mosquitoes exposed to PermaNet 3.0 (<1 month old) on the roof and sides over 24 h, compared to a control group. The vertical dotted lines represent the 80 % mortality threshold.

strain demonstrated complete efficacy for both tested LLINs. Interceptor® G2 (Fig. 5A) and PermaNet® 3.0 (Fig. 5C) induced 100 % mortality, confirming the intrinsic insecticidal potential of both net types under controlled conditions.

Conversely, mortality rates observed in wild *An. gambiae* s.l. populations were notably lower, reflecting varying levels of resistance. Interceptor® G2 nets (less than one month in use) induced a mortality rate of 64.67 % in Banfora (Fig. 5B), falling below the WHO efficacy threshold.

For PermaNet® 3.0, roof samples (<1 month old) tested against wild *An. gambiae* s.l. from Houndé achieved a mortality rate of 87.84 %, thus meeting the WHO criteria for effectiveness (Fig. 5C). However, side panel samples from the same nets induced significantly lower mortality (approximately 20.74 %), far below the efficacy threshold (Fig. 5D). This stark contrast between net sections suggests heterogeneity in insecticide distribution or rapid depletion of insecticidal activity on side panels.

These findings highlight not only the presence of pyrethroid resistance in field populations but also the importance of considering both net age and sampling location (roof vs. sides) when evaluating the real-world performance of LLINs. Continuous monitoring and quality assessment of deployed nets are essential to ensure sustained protection against malaria vectors in endemic areas.

4. Discussion

Malaria remains a formidable public health challenge despite extensive efforts by national control programmes. LLINs and IRS, supported by the President's Malaria Initiative since 2007, have significantly reduced malaria transmission in Africa. Yet, these interventions have reached their protective limit: residual transmission persists even when LLINs/IRS coverage exceeds 80 % (Bhatt et al., 2015; WHO, 2015a; Namountougou et al., 2023). This persistent transmission is compounded by the relative paucity of entomological data needed to decipher its underlying mechanisms.

Our entomological surveillance revealed pronounced monthly and spatial variation in vector species composition in the Banfora and Houndé districts. These fluctuations are influenced by ecological and climatic factors conducive to breeding, particularly temporary, sunlit pools created by irrigated agriculture and market gardening. These anthropogenic habitats appear to sustain the high diversity and abundance of the *Anopheles gambiae* s.l. complex, confirming prior observations that most effective malaria vectors thrive in tropical African environments (Namountougou et al., 2023).

We documented seven *Anopheles* species, with *Anopheles gambiae* s.l. comprising over 86 % of captures. Secondary vectors included *An. rufipes, An. nili*, and *An. funestus*, the latter being less prevalent and confined to wooded, humid savannah—habitats less prevalent in our study zones. While *An. funestus* historically contributed significantly to malaria transmission, its role here appears limited outside specific ecological niches (Dabiré et al., 2008, 2007).

Molecular assays confirmed the presence of *An. coluzzii, An. arabiensis*, and *An. gambiae* s.s. across both study districts, with *An. coluzzii* predominating (44.8 % via HLC and 62.8 % via PSC), followed by *An. arabiensis* and *An. gambiae* s.s. These findings align with earlier studies linking *An. coluzzii* to stable breeding habitats (Della Torre et al., 2005, 2002, 2001) and *An. arabiensis* to drier, agricultural landscapes (Somda et al., 2018). However, we observed a stronger presence of *An. arabiensis* than previously reported in Banfora, particularly in villages where irrigated agriculture and vegetable practices have intensified, while less cultivated areas showed lower proportions of this species (Badolo et al., 2012; Namountougou et al., 2019) . These observations suggest that its emergence is likely associated with local habitat modifications rather than a generalized phenomenon across the entire district. Similar findings in Bobo-Dioulasso, from an ongoing study on the influence of dry and rainy seasons on human exposure to malaria vector bites, indicate that *An. arabiensis* can dominate in urban and agricultural environments, benefiting from flexible trophic and feeding behaviors. Such adaptations allow this species to sustain malaria transmission throughout the year, particularly during the rainy season, highlighting the key role of agricultural intensification and the creation of favorable microhabitats in its spatial dynamics and emergence.

Vector density and biting activity peaked in September and October, with HBR averaging 22.9 bites per person per night in Banfora and 26.6 in Houndé—consistent with patterns observed in Sudanian regions and underscoring the strong influence of rainfall on vector proliferation (Epopa et al., 2019; Soma et al., 2020). Notably, biting activity began early in the evening, peaked between 18:00–22:00 and again around 01:00, reflecting crepuscular and nocturnal behavior. This has significant implications for residual transmission, as these periods fall outside conventional LLINs/IRS protection (Shutt et al., 2010; Diabate and Tripet, 2015).

These observations on seasonal biting trends can be further detailed by examining monthly data. The average biting rate in Banfora in October was 0.62 bites per person per night ($b.h^{-1}.n^{-1}$), compared to 9.25 $bh^{-1}.n^{-1}$ in Houndé. The peak biting activity was observed in September, with 14 $bh^{-1}.n^{-1}$ in Banfora and 14.25 $bh^{-1}.n^{-1}$ in Houndé. The relatively low rate recorded in October in Banfora may reflect the impact of the dengue outbreak response plan, which was launched during this period. Indeed, intensified indoor and outdoor spraying, the use of bio-larvicides, geolocation of case foci, and community sanitation campaigns may have contributed to reducing mosquito density and, consequently, the frequency of bites. Although other ecological or seasonal factors may also influence this dynamic, the temporal association between the implementation of the response plan and the marked reduction in biting rates suggests a beneficial effect of these interventions on reducing human exposure to vectors.

In addition to seasonal variations, changes in vector behavior also influence malaria transmission dynamics. Outdoor biting rates are increasing, evidencing a shift in vector behavior to evade indoor interventions (Howell and Knols, 2009). This exophagic trend diminishes the protective efficacy of LLINs and IRS, highlighting the need for interventions addressing outdoor exposures.

To mitigate this residual transmission, complementary measures can be considered. The use of spatial repellents within households and in peri-domestic areas can help deter mosquitoes active early in the evening or outdoors, thereby reducing human–vector contact. Targeted larval source management, particularly of temporary pools created by irrigation and market gardening, can further decrease adult mosquito densities. In addition, outdoor spraying applied to shelters, peri-domestic walls, or around water points may enhance protection against exophilic vectors. Integrating these approaches into a combined strategy is essential to effectively reduce residual transmission and strengthen the overall impact of current malaria control interventions.

High parity rates (>80 %) indicate that older, potentially infectious females dominate the vector populations. SIRs also peaked in August—41.7 % in *An. coluzzii* and 19.4 % in *An. arabiensis* in Banfora—corresponding to high EIRs of 0.31 and 0.38 infectious bites per person per night, respectively. These findings corroborate documented seasonal transmission patterns in the Sudanian zone (Soma et al., 2020). In Houndé, *An. arabiensis* became the main infective vector later in the rainy season, while *An. gambiae* s.s. remained scarce and carried no sporozoites, reinforcing its minimal role in local transmission (Baldet et al., 2003). Moreover, our data indicate the zoophagic tendencies of *Anopheles* mosquitoes, particularly towards goats. This behavior may impact human malaria transmission by diverting mosquitoes away from their primary human hosts. However, we acknowledge that the trophic analysis was based on a relatively limited number of blood-fed specimens, which may restrict the representativeness of the results. In addition, the local availability of animal hosts (goats, cattle, pigs) or the differential accessibility of human versus animal baits during collections likely influenced the feeding profiles observed. Therefore, the relatively

high proportion of non-human blood meals may reflect not only genuine behavioral plasticity of the vectors but also ecological constraints specific to the local context.

The high prevalence of *kdr-west* resistance alleles across *An. gambiae* s.l., with genotype frequencies in Hardy–Weinberg equilibrium, indicates sustained selective pressure from pyrethroid exposure (Pinto et al., 2006; Ranson et al., 2000). Seasonal fluctuations in *kdr-west* frequencies in *An. coluzzii* and *An. arabiensis* suggest dynamics influenced by agricultural practices and insecticide deployment (Edi et al., 2012; Nkya et al., 2013; Gnankiné et al., 2013) . The observed *kdr-east* increase in *An. gambiae* s.s. in September (frequency = 0.556) may represent new selection or gene flow, though heterozygote deficits signal potential inbreeding or selection bias (Martinez-Torres et al., 1998; Weetman et al., 2012).

One of the main limitations of this study lies in the fact that the resistance analysis focused solely on the *kdr*-west (L1014F) and *kdr*-east (L1014S) mutations. Other potentially important resistance mechanisms, such as super-kdr mutations or non–target-site mechanisms (e.g., metabolic resistance mediated by cytochrome P450 monooxygenases, glutathione S-transferases, and esterases), were not investigated. This limitation constrains the scope of interpretation, as phenotypic resistance is often multifactorial and may result from complex interactions between target-site and metabolic mechanisms. Future biochemical and transcriptomic analyses will be required to better characterize the contribution of detoxification pathways and to provide a more comprehensive understanding of resistance dynamics in local *Anopheles* populations (Ingham et al., 2021; Kleinschmidt et al., 2018; Tchouakui et al., 2020).

Another important limitation concerns the sample size. Although the selection of individuals was designed to ensure sufficient statistical power, larger samples would allow for more robust and generalizable results (Kleinschmidt et al., 2018). In addition, the study period was limited to three months during the rainy season. This temporal constraint may not fully capture the annual variations in species composition, biting behavior, or resistance trends. Longitudinal studies conducted over multiple seasons have shown that Anopheles population dynamics and resistance allele frequencies can fluctuate with seasonality, agricultural practices, and the intensity of insecticide use (Gnankiné et al., 2013; Nkya et al., 2014a, 2014b; Edi et al., 2014). This limitation should be taken into account when interpreting the results and suggests that year-round monitoring would be necessary to obtain a more comprehensive and representative picture of vector dynamics and resistance. Finally, the absence of longitudinal monitoring prevents capturing the temporal dynamics of insecticide resistance and its potential impact on the operational effectiveness of bed nets and other interventions. Future studies incorporating continuous surveillance would provide a better understanding of the persistence and evolution of resistance across different epidemiological settings (Nkya et al., 2014a, 2014b; Edi et al., 2014).

Despite phase III compliance in laboratory assays, our field-based bioefficacy trials revealed suboptimal performance of Interceptor® G2 and PermaNet® 3.0 nets against resistant vector populations. Mortality rates did not meet WHO thresholds—64.7 % for G2 in Banfora, and 20.7 % on the sides of PermaNet 3.0 (despite high roof-side efficacy)—underscoring the critical role of mosquito–net contact behavior and insecticide distribution (Oxborough et al., 2019; Sovegnon et al., 2024). These findings parallel regional reports of *kdr* prevalence and metabolic resistance, necessitating continuous surveillance and integrated management strategies (Namountougou, 2013; Namountougou et al., 2013; Ranson and Lissenden, 2016).

The reduced efficacy observed against field mosquitoes may be influenced by several operational factors that extent beyond the controlled conditions of laboratory assays. In this study, the tested nets were new (<1 month old), which excludes the possibility of decreased effectiveness due to prolonged physical wear or repeated washing.

The observed reduction in efficacy for Interceptor® G2 and PBO-LLINs could therefore be attributed to other factors, notably insecticide distribution. Net impregnation can be heterogeneous, with higher concentrations often on the roof than on the side panels. This variation can affect mosquito exposure and reduce overall effectiveness. For example, the roof of PBO-LLINs is often treated with a mixture of pyrethroid and PBO, whereas the side panels contain only pyrethroid, leading to variable efficacy depending on the contact area (Tungu et al., 2010; Machange et al., 2024). Additionally, microdistribution and insecticide degradation may differ between the upper and lateral sections, further reducing exposure to the most toxic areas. In Interceptor® G2 nets, this heterogeneity can also affect the balance between alpha-cypermethrin and chlorfenapyr, particularly if the surface dose of chlorfenapyr is lower or declines more rapidly on the side panels.

Behavioral adaptations of mosquitoes may further limit contact with the most treated areas. Some populations exhibit feeding or resting patterns favoring the side panels, which reduces the effectiveness of PBO-LLINs, especially if these areas contain less synergist (Sanou et al., 2021). Behaviors such as early biting, exophily, or excito-repellency can also reduce the time mosquitoes spend in contact with the net, decreasing exposure to PBO or chlorfenapyr and overall lethality.

Finally, physiological resistance plays a key role. Increased tolerance or metabolic resistance to the insecticides used (pyrethroids, PBO, chlorfenapyr) could explain the reduced efficacy observed, even with new nets. Several studies report growing resistance among Anopheles populations. In Tanzania, pyrethroid-resistant mosquitoes partially or fully restored susceptibility after pre-exposure to PBO, confirming the involvement of P450-mediated metabolic resistance (Kabula et al., 2024). In Cameroon, an escalation of pyrethroid resistance associated with overexpression of detoxification genes has also been documented (Tene-Fossog et al., 2022). These findings suggest that even new nets may have reduced effectiveness against mosquito populations capable of metabolizing or tolerating the insecticides used.The high prevalence of the *kdr* allele in our study areas is likely a key factor underlying the suboptimal operational performance of insecticide-treated nets, despite their compliance under phase III conditions. By altering the target site of pyrethroids (voltage-gated sodium channels), this mutation reduces knockdown efficacy and allows mosquitoes to survive and continue biting after net contact. Consequently, even new nets such as PBO-LLINs show limited performance, since the synergist PBO primarily counteracts metabolic resistance but does not directly mitigate *kdr*-mediated target-site resistance. These findings highlight that genetic resistance is not only a molecular observation but also translates into tangible operational failures in the field. Therefore, systematic integration of genetic monitoring (*kdr* alleles and detoxification genes) into entomological surveillance programs is essential to anticipate efficacy loss and guide the deployment of novel vector control tools based on alternative insecticidal modes of action. The widespread of pyrethroid resistance in two targeted areas calls into an urgent need to integrate plants derived products as an alternative control against Anopheles populations (Gnankiné and Bassolé, 2017; Balboné et al., 2022a, 2022b).

Overall, our study highlights the adaptive behavior of malaria vectors, ecological influences on species distribution, and the operational limits of current vector control tools, reinforcing the need for context-specific and dynamic intervention strategies that address both indoor and outdoor transmission.

5. Conclusion

Entomological surveillance conducted in the Banfora and Houndé districts has provided critical insights into malaria transmission dynamics through multifaceted parameter assessment. Our findings reveal a distinct diversity within the *Anopheles gambiae* s.l. complex, with *An. coluzzii* and *An. arabiensis* predominating. Both spatial and temporal variations in species distribution and behavior correlate closely with local ecological conditions and seasonal shifts. Despite high coverage and utilization of LLINs and robust IRS implementation, entomological

indicators of malaria transmission remain elevated, underscoring persistent residual transmission.

Notably, the detection of early and late biting phenotypes among principal vector species likely contributes significantly to transmission that occurs outside protected hours. The presence of both *kdr*-west (L1014F) and *kdr*-east (L1014S) mutations across the *An. gambiae* s.l. complex, with differing frequencies among species, highlights the genetic complexity underpinning resistance. These findings, combined with the vectors' behavioral plasticity, point to the urgent need for malaria control policies that are context-specific and informed by local entomological evidence.

Seasonal fluctuations in infection rates further emphasize the importance of targeting interventions in sync with transmission peaks. This study contributes essential, up-to-date entomological data to guide malaria control strategies in the targeted districts. It demonstrates that an integrated, localized control approach—combining real-time entomological surveillance, context-driven vector control interventions, and reinforced community education and prevention—remains pivotal for achieving sustainable reductions in residual malaria transmission and advancing toward elimination goals.

Author statements

We, the undersigned, confirm that this manuscript is an original work and has not been published before. Furthermore, it is not currently under consideration for publication elsewhere.

We would like to draw the publisher's attention to the following publications, authored by one or more of us, that relate to specific elements of the submitted manuscript. Relevant copies of these publications are provided as attachments. We would also like to draw the editor's attention to potential conflicts of interest and the significant financial contributions made to support this research. We confirm that there are no conflicts of interest relating to this publication, and that no significant financial support has been provided for this work that could affect its outcomes.

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Ethics approval and consent to participate

This study received ethical clearance from the Ethical Research Committee of the Institut de Recherche en Sciences de la Santé (IRSS), under reference number 008–2022/CEIRES, dated January 20, 2022.

Consent for publication

Not applicable.

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Availability of data and materials

The datasets used during the current study available from the corresponding author on reasonable request.

CRediT authorship contribution statement

Kouamé Wilfred Ulrich Kouadio: Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Data curation. **Miriam Félicité Amara:** Formal analysis, Data curation. **Dieudonné Diloma Soma:** Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Roch Kounbobr Dabiré:** Validation, Resources, Project administration, Funding acquisition, Conceptualization. **Abdoulaye Diabaté:** Validation, Resources, Project administration, Funding acquisition, Conceptualization. **Olivier Gnankiné:** Writing – review & editing, Validation, Supervision. **Moussa Namountougou:** Writing – original draft, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

All the authors have read and accepted this version of the manuscript. The authors also declare there are no conflicts of interest.

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Data availability

Data will be made available on request.

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