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Three-year trends in malaria transmission parameters under deployment of Interceptor[®]G1, Interceptor[®]G2 and PermaNet[®]3.0 insecticide-treated bed nets in South-Western Burkina Faso

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Abstract

Background In South-Western Burkina Faso, where malaria remains highly endemic, the widespread resistance of *Anopheles* mosquitoes to pyrethroid is reducing the effectiveness of insecticide-treated nets (ITNs). This study assessed 3-year trends in key entomological indicators following the deployment of standard pyrethroid-only (Interceptor[®]G1), dual-AI pyrethroid-chlorfenapyr (Interceptor[®]G2) and pyrethroid-PBO (PermaNet[®]3.0).

Methods A longitudinal entomological study was conducted from August 2019 to February 2022 in three malaria-endemic health districts, each receiving a different ITN type. Weekly mosquito sampling was conducted in three villages per district using Human Landing Catches at two households and CDC light traps at six households. Mosquitoes were morphologically and molecularly identified, and *Plasmodium falciparum* infection was detected by PCR. Key indicators included mosquito density, biting behaviour, sporozoite infection rate (SIR), and entomological inoculation rate (EIR).

Results A total of 73,101 female *Anopheles* mosquitoes were collected. The dominant vector species was *An. gambiae* s.l. (> 59%), with *An. coluzzii* prevalent in Banfora (65.5%) and *An. gambiae* s.s. in Gaoua (91%) and Orodara (83.3%). In Banfora, where Pyr-CFR Interceptor G2 nets were deployed, *Anopheles* density declined by 88.7% over the 3 years. In Gaoua (Pyr-only ITNs), mosquito density declined initially but rebounded in year 3. In Orodara (Pyr-PBO ITNs), densities remained relatively stable. Trends in SIR and EIR mirrored those in vector density, with decline in year 2 followed by resurgences in Banfora and Gaoua during year 3.

Conclusion Dual-AI nets, particularly Pyr-CFR Interceptor[®]G2, were associated with sustained reductions in mosquito density and transmission indicators over 2 years. However, resurgence of entomological indices by the third year highlights the need for improved ITN durability and complementary vector control strategies. Continuous entomological

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surveillance and integrated, locally adapted interventions remain essential for sustaining malaria control in areas with high insecticide resistance.

Keywords Dual-active ingredient nets, Piperonyl butoxide (PBO), Chlorfenapyr, Malaria transmission, Vector control

Background

Malaria, caused by *Plasmodium* parasites transmitted by *Anopheles* mosquitoes, remains one of the deadliest vector-borne diseases in sub-Saharan Africa. According to the World Health Organization (WHO) malaria report 2023, the disease causes over 600,000 deaths each year, with the majority occurring among children under 5 years of age [1]. In Burkina Faso, malaria accounts for about 40% of outpatient consultations, with the south-western area experiencing persistent hyper-endemic transmission. The major vectors in the country are *Anopheles (An.) gambiae sensu lato* (s.l.) and *An. funestus* s.l. [2].

Insecticide-treated bed nets (ITNs) have played a central role in reducing malaria transmission, accounting for more than 50% of the decline in malaria cases observed in Africa between 2000 and 2015 [3]. In Burkina Faso, national mass distribution campaigns conducted in 2010 and 2013 increased household ownership from 19% to over 90%, contributing to a 51% decrease in uncomplicated malaria incidence and an 18% reduction in child mortality [4, 5]. Furthermore, a stepped-wedge trial of Olyset Duo ITNs demonstrated a 12% reduction in clinical malaria incidence over 18 months [6].

Despite this gain, progress in malaria control has stalled since 2015, largely due to the emergence and spread of resistance to pyrethroid insecticides. Pyrethroid resistance is now widespread in 85% of malaria-endemic countries, including Burkina Faso, where *Anopheles* populations commonly exhibit both knockdown resistance (*kdr*) mutations and metabolic resistance mechanisms, such as overexpression of cytochrome P450 enzymes [7, 8].

To address this growing challenge, next-generation ITNs incorporating dual-active ingredients (dual-AI) have been developed. Two key types have shown promise: (i) Pyrethroid-PBO (Pyr-PBO) nets (e.g., PermaNet®3.0), which combine a pyrethroid with the insecticide synergist piperonyl butoxide (PBO) that inhibits metabolic detoxification enzymes; and (ii) pyrethroid-chlorfenapyr (Pyr-CFR) nets (e.g., Interceptor®G2), which combine a pyrethroid with chlorfenapyr, a pro-insecticide that disrupts mitochondrial oxidative phosphorylation [9]. Pyr-PBO ITNs have the potential to reduce human biting rates (HBR) by killing mosquitoes prior to blood feeding, while Pyr-CFR ITNs cause delayed mortality, potentially reducing

mosquito survival across successive gonotrophic cycles [9–11]. These nets may also impact *Plasmodium* (*P.*) transmission by shortening mosquito lifespan and reducing sporozoite development [12, 13]. In addition, chlorfenapyr may directly affect *P. falciparum* development within the mosquito, further lowering infection prevalence [14].

However, the entomological evidence base for these dual-AI ITNs remains limited. Most existing studies have relied on experimental hut trials, focusing primarily on mosquito mortality, and fail to capture broader community-level impacts [11]. Moreover, few studies have directly compared PBO and chlorfenapyr based ITNs in real-world, high-transmission settings [1]. Although cluster-randomised trials in countries such as Benin and Tanzania have shown significant reductions in malaria prevalence and case incidence following deployment of dual-AI nets [15, 16], more data are needed on their entomological performance across diverse ecological zones with intense pyrethroid resistance, such as South-Western Burkina Faso.

In response to this need, the Government of Burkina Faso, in collaboration with international partners, launched an initiative in 2019 to transition from pyrethroid-only to dual-AI ITNs in routine mass distribution campaigns. That year, 242,254 Interceptor® G2 nets were distributed in Banfora district, 108,331 PermaNet® 3.0 nets in Orodara district and 119,989 pyrethroid-only (Pyr-only) Interceptor® G1 nets in Gaoua district, which served as the comparator. This targeted deployment creates a unique opportunity to assess the community-level entomological effects of Pyr-CFR, Pyr-PBO and Pyr-only ITNs in three distinct epidemiological zones.

Key entomological metrics such as vector density, HBR, sporozoite infection rate (SIR), and entomological inoculation rate (EIR) are direct indicators of malaria transmission intensity and provide insights into ITN efficacy under operational conditions [17, 18]. This study, conducted as part of the New Nets Project, aimed to assess the entomological effect of next-generation ITNs by analyzing 32 months of longitudinal vector surveillance data from the three study districts. Trends in vector abundance, HBR, *Anopheles gambiae* complex species composition, SIR, and EIR were assessed across study years. Seasonal variation and changes in mosquito biting time and location (indoor vs outdoor) were also examined as potential markers of behavioural adaptation.

By linking ITN type to community-level entomological outcomes, this study provides operational evidence to support informed decision-making by the National Malaria Control Program (NMCP), particularly for ITN selection in high resistance areas. The findings will also contribute to refining malaria transmission models and insecticide resistance management strategies in line with WHO Global Technical Strategy 2023–2030.

Methods

Study design

A quasi-experimental study was undertaken to assess the effect of next-generation dual-AI ITNs on key entomological parameters of malaria transmission in South-Western Burkina Faso, including year-to-year comparisons within each ITN type. The study was implemented in collaboration with Burkina Faso NMCP, which, with the support of donors and key stakeholders, selected both the type of ITNs to be distributed and the target locations for their deployment [13]. The distribution of ITNs took place during the peak malaria transmission season between June and October 2019 and followed a phased rollout strategy. Pyr-PBO ITNs were distributed first in June 2019, followed by Pyr-only ITNs in August 2019, and finally, dual-AI Pyr-CFR ITNs in October 2019 [13]. The selection of distribution areas was guided by malaria incidence data and patterns of insecticide resistance. The targeted distribution prioritized regions and districts with high levels of resistance to pyrethroids, ensuring that dual-AI ITNs were allocated to the targeted area [13]. As a result, this study primarily focused on the Western districts of Burkina Faso, where insecticide resistance poses a significant challenge to malaria control efforts and all three types of ITN were distributed.

Study areas

The study was conducted in three health districts located in South-Western Burkina Faso: Banfora, Gaoua, and Orodara, all characterized by high levels of pyrethroid resistance [19–21] and malaria transmission [6]. Pyr-PBO PermaNet®3.0 and dual-AI Pyr-CFR Interceptor®G2 ITNs were deployed in Orodara and Banfora respectively while Pyr-only Interceptor®G1 ITNs were deployed in Gaoua. In each health district, three villages were selected for the study: Tengrela, Tiéfora and Panga in Banfora; Diéri, Kourinion and Tin in Orodara; and Sibera, Holly, and Doudou in Gaoua, as presented in the map (Fig. 1). The study area is characterized by a humid savannah climate with a distinct rainy season from May to October and a dry season from November to April. Annual rainfall ranges between 900 and 1200 mm, supporting seasonal agriculture. Average annual temperatures are around 27 °C, with minimal variation

throughout the year. Relative humidity fluctuates significantly, averaging approximately 25% in the dry season and rising to 85% during the rainy season. These climatic conditions, typical of Southwestern Burkina Faso, sustain the year-round presence of malaria vectors.

Outcomes

The following primary and secondary entomological outcomes were defined a priori to assess the impact of different ITN types on malaria vector populations, malaria transmission dynamics, and vector behavioural patterns across the study districts. Primary outcomes included:

- Vector density, defined as the number of *Anopheles* mosquitoes collected per house per night using Centers for Disease Control and Prevention Light Traps (CDC-LT).
- Human biting rate (HBR), defined as the number of mosquito bites per person per night, measured through human landing catches (HLC).
- *Plasmodium* sporozoite infection rate (SIR), defined as the proportion of *Anopheles* mosquitoes found positive for *P. falciparum* sporozoites, as determined by PCR analysis.
- Entomological inoculation rate (EIR), the key indicator of malaria transmission intensity, calculated as the product of HBR and SIR [22], and expressed as the number of infective bites per person per year.

Secondary outcomes included:

- Biting behaviour and behavioural trends, assessed through analysis of indoor versus outdoor biting (endophagy rate), hourly biting patterns, and potential temporal shifts in biting behaviour or biting times in response to ITN deployment.
- *Anopheles* species diversity, defined as the number and relative abundance of *Anopheles* species collected in each health district.
- Species composition of the *Anopheles gambiae* complex, defined as the relative abundance of each *An. gambiae* complex species within the collected samples, determined through molecular identification of individual specimens.

Mosquito collection

Adult mosquitoes were collected weekly for more than three consecutive years (August 2019 to February 2022) in the nine villages. Two collection methods were used: Human Landing Catches (HLC) and Centers for Disease Control and Prevention Light Traps (CDC-LT). In each village, two houses were selected for HLC, and six houses

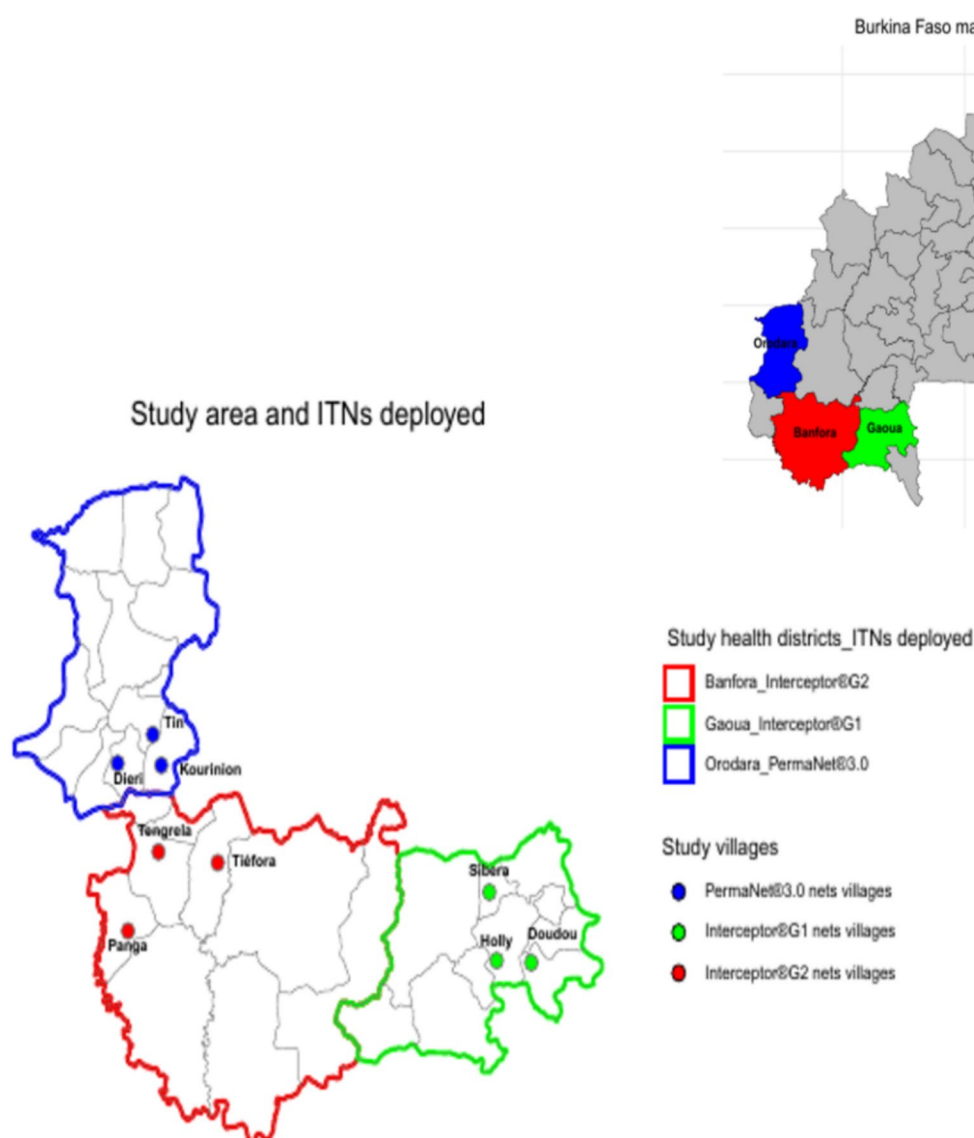


Fig. 1 Study area with insecticide treated bed nets deployed

for CDC-LT at each collection timepoint to ensure spatial representativeness, with houses evenly distributed across the village. Sampling was conducted weekly at night on a weekly schedule; HLC took place simultaneously indoors and outdoors from 19:00 to 6:00, while CDC-LT were deployed indoors from 20:00 to 6:00. This sampling strategy resulted in 72 nights of CDC-LT and 48 nights of HLC per health district each month. For the HLC, host-seeking mosquitoes were collected by trained personnel using mouth-operated aspirators and flashlights as they landed on the exposed legs of collectors. Collections were carried out both indoors (within five meters of the house entrance) and outdoors in the peri-domestic environment. During each collection hour, mosquitoes

were captured for 45 min, followed by a 15-min rest period. To minimize potential bias, collectors rotated hourly between indoor and outdoor collection sites. For CDC-LT, traps consisted of a motorized fan powered by a 6 V battery with an incandescent light bulb connected to a collection chamber. The trap was placed beside the sleeper's bed, positioned at the same height as the sleeper's feet, and suspended to avoid contact with the floor. The sleeper, resting under a intact bed net, served as the bait; mosquitoes attracted by the light drawn into the collection chamber. All adult volunteers who participated as HLC collectors or CDC-LT sleepers provided written informed consent prior to enrolment. In the event of suspected or confirmed malaria, they had prompt access to

parasitological diagnosis and were treated free of charge in accordance with the Burkina Faso National Malaria Control Program case management guidelines.

The processing of mosquitoes

Morphological identification

Mosquitoes collected in the field were kept in cups, with cotton pads soaked in a 10% sugar solution placed on top of the HLC collection cups to provide nourishment. The mosquitoes were then placed in a humidified cool box and transported to the entomology laboratory in each health district for morphological identification. Identification was conducted using established dichotomous morphological keys [23, 24]. Mosquitoes were identified to the species or species complex level and further classified based on their physiological status into four categories: unfed, fed and gravid. Unfed mosquitoes were characterized by empty abdomens, indicating they had not recently taken a blood meal. Fed mosquitoes exhibited distended abdomens visibly engorged with blood, signifying a recent feeding event. In contrast, gravid mosquitoes contained fully developed eggs, indicating they were at the final stage of the gonotrophic cycle and ready to oviposit.

A subsample of five unfed *An. gambiae* s.l. and *An. funestus* s.l. per night from HLC collections, both indoors and outdoors, was randomly selected at each time slot (19:00 to 22:00; 22:00 to 02:00; and 02:00 to 06:00) for dissection to determine their physiological age (parous or nulliparous), following the method described by Detinova [25]. Only mosquitoes belonging to the *Anopheles* genus were retained and stored in labeled 1.5 mL Eppendorf tubes containing silica gel at room temperature for subsequent molecular analysis.

Molecular identification of *Anopheles gambiae* complex species

To ensure a representative sample for molecular analysis, approximately 35% of the collected *An. gambiae* s.l. females were selected each year, considering factors such as health district, village, collection month, trapping method (HLC vs. CDC-LT), and collection position (indoor vs. outdoor). Molecular identification of *Anopheles gambiae* complex species was performed using a standard Polymerase Chain Reaction (PCR) protocol described by Fanello et al. [26]. DNA was extracted from the head and thorax of each *An. gambiae* s.l. specimen using the DNAzol reagent method [27]. The extracted DNA was then amplified, and the resulting fragments were separated by electrophoresis on a 2% agarose gel prepared with TBE buffer. After staining with Red-Safe, DNA bands were visualized under an ultraviolet

transilluminator to confirm species identification within the *Anopheles gambiae* complex.

Plasmodium falciparum detection

The DNA extracts used for *Anopheles gambiae* complex species identification were also analysed for *P. falciparum* detection using a standard PCR as described by Snounou et al. [28].

Data analysis

Data were recorded in Microsoft Excel and analysed using R statistical software version 4.4.1 [29]. The composition of *Anopheles* and *Anopheles gambiae* complex species was analysed by calculating the proportion of each species collected per year and comparing these proportions across years and study districts. Vector density and human biting rate (HBR) were analysed by district, year, and season. Temporal and spatial variation in vector density and HBR were assessed using a linear model with collection year as the explanatory variable. The model estimated differences in mean mosquito density and HBR across years, using the first year as the reference category. Differences in mosquito abundance between indoor and outdoor collections were assessed for each morphologically identified species using a generalized linear model with a quasi-Poisson distribution. The model included capture position, species, year, and their two-way interactions. Predicted indoor/outdoor biting ratios and corresponding confidence intervals were derived from these models. *Plasmodium*-sporozoite infection rate (SIR) and entomological inoculation rate (EIR) were estimated for each study health district and year, in accordance with the definitions described in the Entomological Outcomes section. To account for seasonal variation in malaria transmission, the EIR was calculated separately for the rainy season (May to October) and the dry season (November to April). For each season, the mean daily EIR was estimated and then multiplied by the total number of days in that season to obtain the seasonal EIR. Annual EIR was calculated by summing EIR values from both the rainy and dry seasons. Effect size reporting. For primary count outcomes (density, HBR, EIR), models included the log of exposure as an offset and report incidence rate ratios (IRR) with 95% cluster-robust confidence intervals (CI). For binary/proportion outcomes (SIR; indoor-biting/endophagy), binomial GLMs with a logit link report adjusted odds ratios (aOR) with 95% cluster-robust CI; we also provide marginal risk differences (RD) with 95% CI derived from model-based marginal means. Inference uses two-sided $\alpha = 0.05$. Cluster adjustment: village for all analyses; households nested within villages where repeated household observations were present.

Year-to-year comparisons for these variables were performed using proportion tests or generalized linear models with a binomial family and a logit link function. All statistical analyses were conducted with 95% confidence intervals (95% CI) and a significance level of 5% ($\alpha=0.05$).

Results

Anopheles species abundance and density

Between July 28, 2019 and February 27, 2022, a total of 73,101 female *Anopheles* mosquitoes were collected. Of these, 23.4% (17,083) were captured using CDC_LT, while 76.6% (56,013) were collected through HLC. The mosquito densities were distributed as follows: 34.9% (25,511/73,101) in year 1, 38.2% (27,934/73,101) in year 2, and 26.9% (19,656/73,101) in year 3. The collections were unevenly distributed among the districts: Banfora accounted for 46.3% (33,846/73,101), Gaoua for 35.3% (25,816/73,101), and Orodara for 18.4% (13,439/73,101).

During the 3-year study period, the highest mean *Anopheles* mosquitoes' densities were recorded between August and October across all three health districts (Fig. 2).

In Banfora, where dual-AI Pyr-CFR Interceptor®G2 ITNs were deployed, the mean nightly *An. gambiae* s.l. density per house declined markedly over time, from 6.90 mosquitoes per house per night in year 1 (95% CI 4.67–9.13) to 2.62 in year 2 (95% CI 1.49–3.75) and 1.92 in year 3 (95% CI 1.06–2.78). Model-based contrasts from negative binomial models confirmed large reductions in density relative to year 1 (year 2 vs year 1: IRR=0.39, 95% CI 0.27–0.55; $P<0.0001$; year 3 vs year 1: IRR=0.28, 95% CI 0.17–0.45; $P<0.0001$), corresponding to approximately 60–70% lower densities.

In Gaoua, which received standard Pyr-only Interceptor®G1 ITNs, mean nightly density dropped sharply from 5.28 mosquitoes/house/night in year 1 (95% CI 3.67–6.89) to 1.43 in year 2 (95% CI 0.95–1.90), before partially rebounding to 2.75 in year 3 (95% CI 1.68–3.83). The model-based effect sizes indicated a substantial reduction in year 2 vs year 1 (IRR=0.27, 95% CI 0.23–0.32, $P<0.0001$) and a smaller but still statistically significant decrease in year 3 vs year 1 (IRR=0.52, 95% CI 0.29–0.94, $P=0.03$).

In contrast, Orodara where Pyr-PBO Permanet®3.0 ITNs were distributed, showed more modest change in mean nightly density, which increased from 1.48

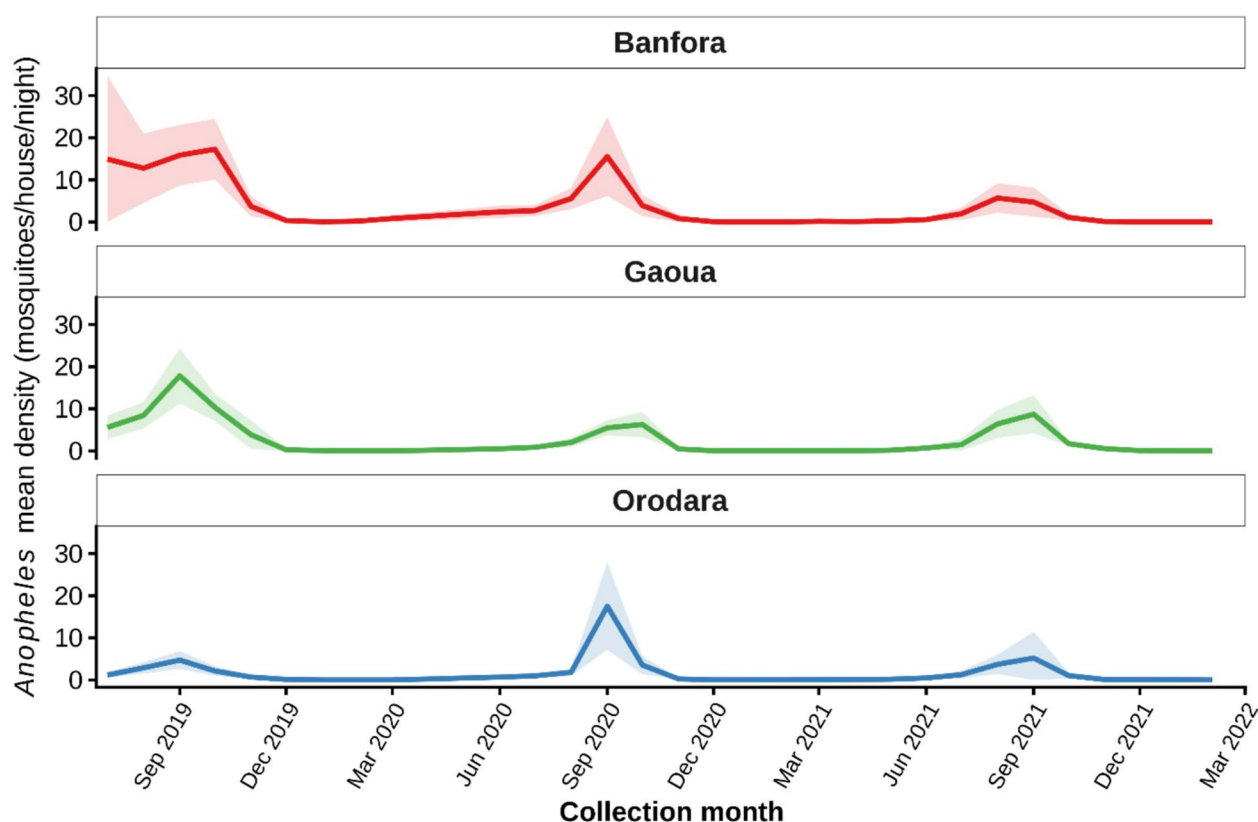


Fig. 2 *Anopheles* mosquitoes mean density per night in each health district across the study period

mosquitoes/house/night in year 1 (95% CI 0.99–1.97) to 2.20 in year 2 (95% CI 0.98–3.41) and then decreased slightly to 1.62 in year 3 (95% CI 0.59–2.66). Consistent with these means, the model indicated a significant increase in year 2 vs year 1 (IRR=1.49, 95% CI 1.24–1.79; $P<0.0001$), whereas year 3 did not differ significantly from year 1 (IRR=1.10, 95% CI 0.65–1.86, $P=0.73$). Effect sizes for mosquito density (IRR with cluster-robust 95% confidence intervals) are summarized in Table 1.

In terms of reduction percentage, Banfora experienced a 70.4% (95% CI 22.7% to 91.6%) decline in mosquito density from year 1 to year 2, and an 88.7% (95% CI 70.1% to 97.3%) reduction by year 3. In Gaoua, reductions were 79.6% (95% CI 42.4% to 95.7%) in year 2, and 78.5% (95% CI 39.9% to 95.5%) in year 3, compared to year 1. In Orodara, however, the reduction was more modest and statistically inconclusive, with a 48.1% decrease (95% CI –61.9% to 89.8%) from year 1 to year 3, indicating substantial variability.

District-level *Anopheles* mean density fell after the 2019 ITN campaign, then rebounded in year 3, most prominently in Banfora. Each panel is one district. The line is the monthly mean density (mosquitoes/house/night); the shaded ribbon is the 95% CI (mean $\pm$ 1.96 $\times$ SE across collection nights in that month). Months are aggregated over all villages in the district.

Anopheles species diversity

Across the three study districts, a total of eleven distinct *Anopheles* species or morphologically indistinguishable species complexes were identified. Among them, *An. gambiae* s.l. was consistently the most abundant species throughout the study (Fig. 3). In Gaoua, seven additional *Anopheles* species were detected, including, *An. funestus* s.l., *An. rufipes*, *An. nili*, *An. ziemanni*, *An. pharoensis*, *An. coustani*, and *An. moucheti*. In Banfora, six other species were recorded: *An. funestus* s.l., *An. nili*, *An. pharoensis*, *An. coustani*, *An. obscurus*, and *An. flavicosta*. In Orodara, four additional species were observed: *An. nili*, *An. pharoensis*, *An. wellcomei*, and *An. flavicosta*.

Figure 3 illustrates the distribution and relative proportions of *Anopheles* species across health districts and collection years.

Molecular identification of *Anopheles gambiae* complex species

Over the 3-year study period, a total of 60,643 female *An. gambiae* s.l. mosquitoes were collected, of which approximately 35% (21,366) were analysed for molecular identification. The success rate of molecular identification was 83.4% (8898/10,672) in Banfora, 89.1% (5389/6049) in Gaoua, and 83.7% (3889/4645) in Orodara. *An. gambiae* sensu stricto (s.s.) was the dominant specie in both Gaoua (91.0%, $n=4906$) and Orodara (83.3%, $n=3239$), whereas in Banfora, *An. coluzzii* was the most prevalent specie (65.5%, $n=5833$). Table 2 presents the species composition of *Anopheles gambiae* complex in each health district over the 3 years of the study.

Anopheles mosquito's biting behaviour

Throughout the 3-year study period, the highest monthly mean HBR of *Anopheles* mosquitoes were consistently recorded during the peak transmission season, between August and October (Fig. 4).

In Banfora (dual-AI Pyr-CFR Interceptor® G2), HBR declined in year 2 relative to year 1 and then rose in year 3. Model-based contrasts indicated a statistically significant reduction of about 13% in year 2 vs year 1 (IRR=0.87; 95% CI 0.81–0.93; $P<0.0001$), while HBR did not differ significantly from year 1 (IRR=1.21; 95% CI 0.54–2.69; $P=0.64$). In Gaoua (Pyr-only Interceptor® G1), HBR dropped sharply in year 2 and only partially rebounded in year 3, remaining lower than year 1 (year 2 vs year 1: IRR=0.59; 95% CI 0.47–0.73; $P<0.0001$; year 3 vs year 1: IRR=0.69; 95% CI 0.59–0.81; $P<0.0001$). In Orodara (Pyr-PBO PermaNet® 3.0), HBR increased markedly in year 2 compared with year 1 (IRR=1.80; 95% CI 1.55–2.10; $P<0.0001$) and returned to levels similar to year 1 in year 3 (IRR=1.01; 95% CI 0.74–1.36; $P=0.96$). Effect sizes for all district-specific contrasts (IRR with

Table 1 Effect sizes for mosquito density by district (vs year 1)

District_ITN type	Year comparison	IRR (95% CI)	P value	N village	Nights	Model/offset/cluster
Banfora_Pyr-CFR	Year 2 vs year 1	0.39 (0.27–0.55)	7.04×10^{-8}	9	336	NB/log(nights)/village
Banfora_Pyr-CFR	Year 3 vs year 1	0.28 (0.17–0.45)	1.69×10^{-7}	9	336	NB/log(nights)/village
Gaoua_Pyr-only	Year 2 vs year 1	0.27 (0.23–0.32)	5.99×10^{-53}	9	340	NB/log(nights)/village
Gaoua_Pyr-only	Year 3 vs year 1	0.52 (0.29–0.94)	0.03	9	340	NB/log(nights)/village
Orodara_Pyr-PBO	Year 2 vs year 1	1.49 (1.24–1.79)	2.76×10^{-5}	9	341	NB/log(nights)/village
Orodara_Pyr-PBO	Year 3 vs year 1	1.10 (0.65–1.86)	0.73	9	341	NB/log(nights)/village

IRR incidence rate ratio from Poisson/negative binomial models with log link and log (nights) offset. 95% confidence intervals are cluster-robust at the village level ($\alpha=0.05$). Counts were aggregated by district $\times$ village $\times$ year; exposure is the number of collection nights

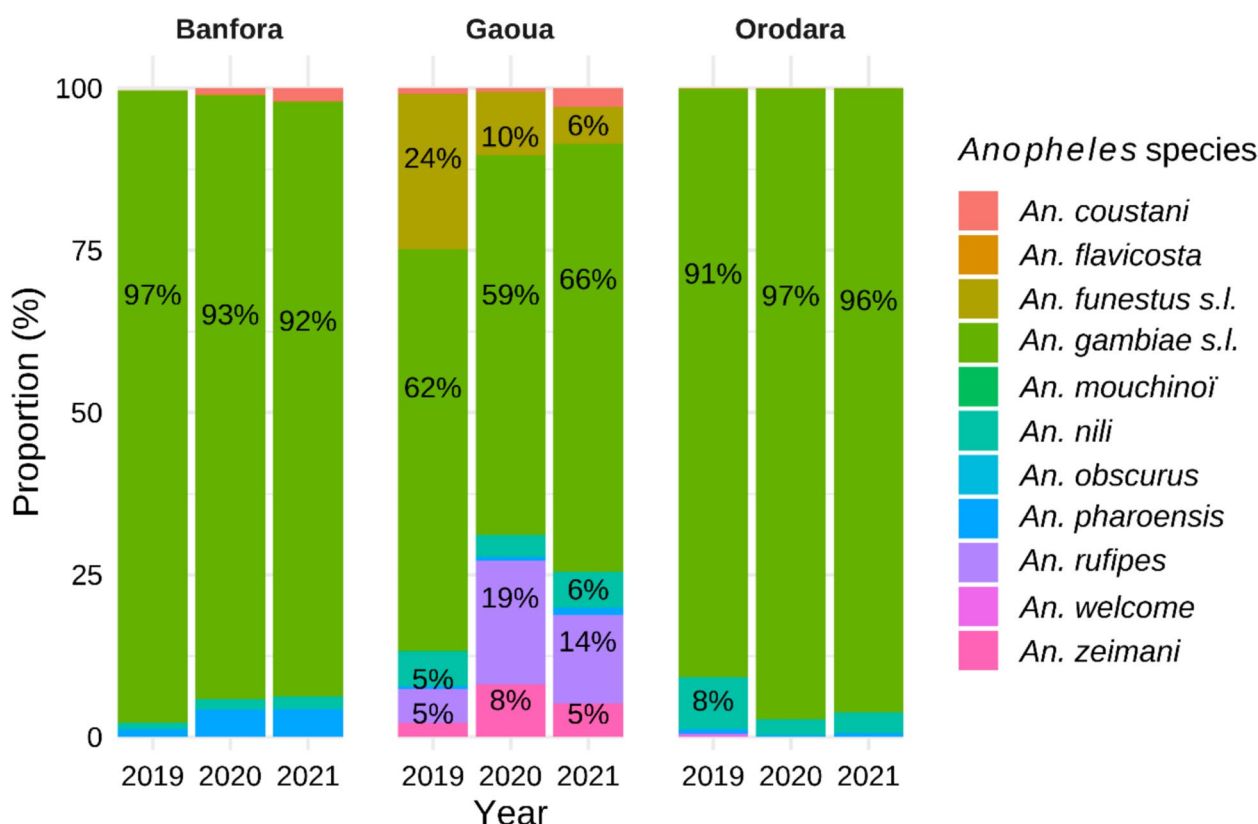


Fig. 3 Distribution and relative proportions of *Anopheles* species across health districts and collection years

Table 2 *Anopheles gambiae* complex species composition in each health district over the three years of the study

<i>Anopheles gambiae</i> complex species	Banfora_Pyr-CFR			Gaoua_Pyr-only			Orodara_Pyr-PBO		
	Year 1	Year 2	Year 3	Year 1	Year 2	Year 3	Year 1	Year 2	Year 3
<i>An. gambiae</i> s.s. (n, %)	1389 (34.0)	1168 (51.9)	396 (15.4)	2718 (91.9)	955 (91.7)	1233 (88.6)	1280 (81.4)	1347 (90.5)	612 (73.9)
<i>An. coluzzii</i> (n, %)	2686 (65.8)	1055 (46.9)	2091 (81.5)	195 (6.6)	82 (7.9)	129 (9.3)	292 (18.6)	134 (9.0)	205 (24.8)
<i>An. arabiensis</i> (n, %)	9 (0.2)	26 (1.2)	78 (3.0)	44 (1.5)	4 (0.4)	29 (2.1)	0 (0.0)	8 (0.5)	11 (1.3)
Total successfully analysed	4084	2249	2565	2957	1041	1391	1572	1489	828

Pyr-only District with standard ITN Interceptor®G1 (Pyrethroid-only ITN) deployment; Pyr-CFR District with Interceptor®G2 ITN (Pyrethroid + chlorfenapyr ITN) deployment; Pyr-PBO District with PermaNet®3.0 ITN (Pyrethroid + synergist PBO ITN) deployment. n is the number of mosquitoes collected; % is the proportion in percentage of the mosquito collected

cluster-robust 95% CI from negative binomial models with log (person-nights offsets) are summarized in Table 3.

District-level *Anopheles* mean human biting rate fell after the 2019 ITN campaign, then rebounded in year 3, with district-specific magnitude and peaks aligning with the rainy season. Each panel is one district. The line shows the monthly mean HBR (bites/person/night); the shaded ribbon is the 95% CI (mean $\pm 1.96 \times$ SE across collection nights in that month). Values aggregate all villages within each district.

The endophagy rate of *An. gambiae* s.l. varied significantly between health districts ($\chi^2=90.15$, $df=2$, $P<0.0001$) and across the three collection years ($\chi^2=30.25$, $df=2$, $P<0.0001$). In Banfora, no significant variation in the endophagy rates were observed over the three years ($\chi^2=2.36$, $df=2$, $P=0.3068$). The recorded indoor biting rates were 49.2% (95% CI 45.9–52.5) in year 1, 47.6% (95% CI 45.2–49.9) in year 2, and 49.2% (95% CI 46.4–52.1) in year 3. In Gaoua, endophagy rate increased significantly ($P<0.0001$), rising from 47.5% (95% CI 44.0–50.9) in year 1 to 53.8% (95% CI 51.0–56.5) in year

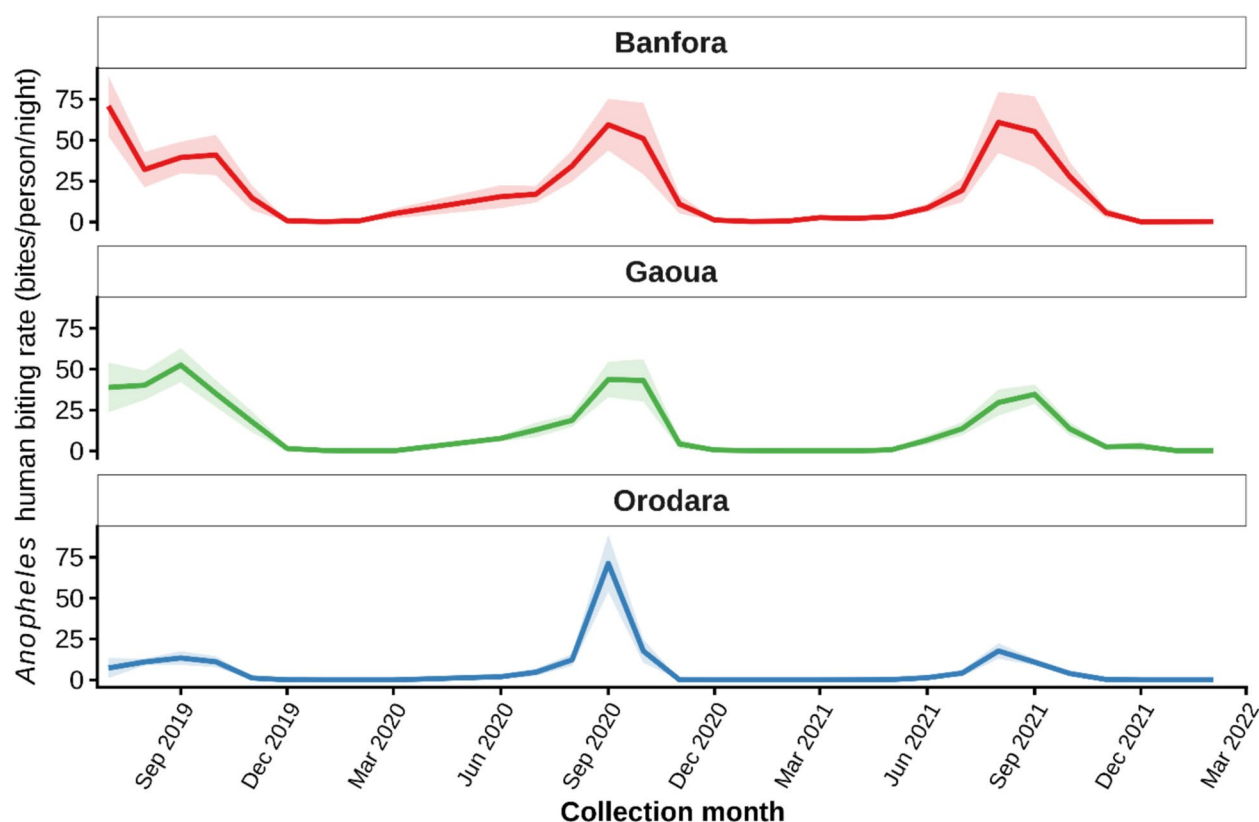


Fig. 4 *Anopheles* mosquitoes mean human biting rate per night in each health district across the study period

Table 3 Effect sizes for human biting rate by district (vs Year 1)

District_ITN type	Year comparison	IRR (95% CI)	P-value	N village	Person-nights	Nights	Model	Model/offset/cluster
Banfora_Pyr-CFR	Year 2 vs year 1	0.87 (0.81–0.93)	1.11×10^{-4}	9	677	677	NB/log	NB/log(person_nights)/village
Banfora_Pyr-CFR	Year 3 vs year 1	1.21 (0.54–2.69)	0.64	9	677	677	NB/log	NB/log(person_nights)/village
Gaoua_Pyr-only	Year 2 vs year 1	0.58 (0.47–0.73)	1.80×10^{-6}	9	682	682	NB/log	NB/log(person_nights)/village
Gaoua_Pyr-only	Year 3 vs year 1	0.69 (0.59–0.81)	5.29×10^{-6}	9	682	682	NB/log	NB/log(person_nights)/village
Orodara_Pyr-PBO	Year 2 vs year 1	1.80 (1.55–2.10)	2.88×10^{-14}	9	682	682	NB/log	NB/log(person_nights)/village
Orodara_Pyr-PBO	Year 3 vs Year 1	1.01 (0.74–1.36)	0.96	9	682	682	NB/log	NB/log(person_nights)/village

IRR incidence rate ratio from negative binomial models with log link and log(person-nights) offset. 95% confidence intervals are cluster-robust at the village level ($\alpha=0.05$). Counts were aggregated by district $\times$ village $\times$ year; exposure is the number of person-nights

2, and 57.3% (95% CI 54.0–60.7) in year 3. In Orodara, the endophagy rate also increased from 35.1% (95% CI 32.0–38.2) in year 1 to 42.8% (95% CI 38.8–46.8) in year 2, reaching 45.1% (95% CI 41.5–48.7) in year 3. These trends likely reflect combined effects of changing species mix and temporal shifts in population level behaviour rather than fixed species-specific indoor preferences.

No *Anopheles* species showed a statistically significant indoor-outdoor difference in abundance (all $P>0.60$). Predicted indoor/outdoor ratios clustered

near 1.0, indicating broadly similar biting across positions. Apparent deviations in rarer species should be interpreted cautiously due to small sample sizes and wide confidence interval. A compact visual summary is provided in an Additional file 1 Figure S1.

Across species, biting was predominantly nocturnal, with most activity between 22:00 and 05:00. Peaks occurred around midnight to 01:00 for *An. gambiae* s.s. and *An. coluzzii*; earlier peaks (21:00–23:00) were observed for a few species. Detailed hourly curves by species are provided in Additional file 2 Figure S2; here

we retain the key message that the critical exposure window aligns with typical sleeping hours.

Sporozoite infection rate (SIR) and entomological inoculation rate (EIR)

A total of 21,429 *An. gambiae* s.l. head–thoraxes were tested for *P. falciparum* infection over the 3-year study period. The detailed year-to-year SIR results for each health district are summarized in Table 4, and the corresponding year effects (odds ratios and 95% confidence intervals from district-specific binomial GLMs) are presented in Additional file 3 Table S1.

In Banfora, the SIR decreased significantly from 2.4% in year 1 to 0.9% in year 2 (OR for year 2 vs year 1 = 0.36, 95% CI 0.23–0.53), and then increased to 1.9% in year 3, with SIR in year 3 remaining similar to year 1 (OR = 0.79, 95% CI 0.57–1.10). In Gaoua, SIR was relatively stable between year 1 (3.6%) and year 2 (OR = 0.85, 95% CI 0.58–1.21), but rose sharply to 15.4% in year 3 (OR = 4.89, 95% CI 3.90–6.16, relative to year 1). In Orodara, SIR remained close to 1% in years 1 and 2 (1.0% in both years; OR for year 2 vs year 1 = 1.09, 95% CI 0.58–2.11), before increasing to 3.7% in year 3 (OR = 4.02, 95% CI 2.24–7.52, relative to year 1).

In Banfora, the EIR varied significantly over time ($\chi^2 = 17.779$, $df = 2$, $P < 0.001$), with 49 infective bites per person per year (95% CI 29–69) recorded in year 1. This value declined to 31 (95% CI 6–55; $P = 0.002$) in year 2 and subsequently increased to 65 (95% CI 37–94; $P < 0.001$) in year 3. Consistent with these patterns, the linear model fitted to nightly EIR values indicated a modest decrease in mean EIR between year 2 and year 1 (difference = -0.33 infective bites/person/night, 95% CI -0.73 – 0.08) and a slightly higher mean EIR in year 3 compared with year 1 (difference = $+0.25$, 95% CI -0.23 – 0.72 ; Additional file 3 Table S2).

In Gaoua, the EIR decreased from 123 infective bites/person/year (95% CI 32–214) in year 1 to 41 (95% CI 9–74; $P = 0.013$) in year 2 and increased to 251 in year 3 (95% CI 150–351; $P < 0.001$). The corresponding model-based contrasts showed a small, imprecise reduction in mean nightly EIR between year 2 and year

1 (difference = -0.51 infective bites/person/night, 95% CI -1.63 – 0.61), followed by a pronounced increase in year 3 relative to year 1 (difference = $+2.00$, 95% CI 0.87 – 3.13).

In Orodara, no statistically significant variation in EIR was observed across years ($\chi^2 = 2.124$, $df = 2$, $P = 0.346$). The annual EIR increased from 6 infective bites/person/year (95% CI 2–10) in year 1 to 24 (95% CI 3–44) in year 2, and to 28 (95% CI 9–47) in year 3. Model-based differences in mean nightly EIR were smaller and more uncertain in this district, with estimates of $+0.22$ infective bites/person/night (95% CI -0.11 – 0.55) for year 2 versus year 1 and $+0.33$ (95% CI -0.02 – 0.67) for year 3 versus year 1 (Additional file 3 Table S2). The detailed year-to-year EIR results for each health district are summarized in Table 5.

Discussion

This study provides new evidence on the entomological effect of the 2019 ITN distribution campaign in three distinct ecological settings in South-Western Burkina Faso. Significant spatial and temporal variations were observed in vector abundance, species composition, biting behaviour, SIR and EIR, reflecting the influence of both ecological conditions and vector control interventions.

This operational, district-level quasi-experimental evaluation involved a non-random allocation of ITN types and was not designed or powered for formal head-to-head product comparisons. Our inferences

Table 5 Yearly entomological inoculation rate (EIR) by health district

District_ITN type	Year 1 EIR [95% CI]	Year 2 EIR [95% CI]	Year 3 EIR [95% CI]
Banfora_Pyr-CFR	49 [29–69]	31 [6–55]	65 [37–94]
Gaoua_Pyr_Only	123 [32–214]	41 [9–74]	251 [150–351]
Orodara_Pyr_PBO	6 [2–10]	24 [3–44]	28 [9–47]

Pyr-only District with standard ITN Interceptor®G1 (Pyrethroid-only ITN) deployment; *Pyr-CFR* District with Interceptor®G2 ITN (Pyrethroid + chlorfenapyr ITN) deployment; *Pyr-PBO* District with PermaNet®3.0 ITN (Pyrethroid + synergist PBO ITN) deployment. EIR is the number of infectives bites per person per year and 95% CI is 95% confidence intervals

Table 4 Yearly sporozoite infection rates (SIR) by health district

District_ITN type	Year 1 SIR % (n/N) [95% CI]	Year 2 SIR % (n/N) [95% CI]	Year 3 SIR % (n/N) [95% CI]
Banfora_Pyr-CFR	2.4% (111/4653) [2.0–2.9]	0.9% (29/3366) [0.6–1.2]	1.9% (54/2839) [1.4–2.5]
Gaoua_Pyr_Only	3.6% (122/3407) [3.0–4.3]	3.1% (40/1309) [2.2–4.1]	15.4% (233/1516) [13.6–17.3]
Orodara_Pyr_PBO	1.0% (16/1678) [0.5–1.5]	1.0% (23/2213) [0.7–1.6]	3.7% (34/912) [2.6–5.2]

Pyr-only District with standard Interceptor®G1 ITN (Pyrethroid-only ITN) deployment; *Pyr-CFR* District with Interceptor®G2 ITN (Pyrethroid + chlorfenapyr ITN) deployment; *Pyr-PBO* District with PermaNet®3.0 ITN (Pyrethroid + synergist PBO ITN) deployment. n is the number of infected mosquitoes; N is total number of mosquitoes tested; SIR % is the percentage of infected mosquitoes, and 95% CI denotes the 95% confidence interval for the proportion. Odds ratios and 95% confidence intervals for temporal changes in SIR (year 2 vs year 1 and year 3 vs year 1 within each district), derived from district specific binomial GLMs, are provided in Additional file 3 Table S1

therefore emphasize within-district temporal trends rather than causal differences between ITN types. Apparent cross-district differences should be considered hypothesis-generating and warrant evaluation in appropriately powered randomized or matched comparative designs.

Potential confounding and ecological heterogeneity. Interpretation of between-district contrasts may be influenced by baseline mosquito density, ecological variability (e.g., rainfall, permanence of breeding sites, housing structure, presence of livestock), and programmatic factors such as ITN coverage/previous-night usage, attrition/retention, and net physical integrity/bio-efficacy. Where such indicators were available, we summarize them; where they were unavailable, we explicitly acknowledge these gaps as limitations that could bias cross-district comparisons.

The *An. gambiae* complex was the predominant malaria vector group across all three study districts, consistent with its well established role in malaria transmission throughout West Africa [30–32]. However, sibling species composition varied by district, likely influenced by local ecological factors and selective pressures. In Banfora (dual-AI IG2 ITNs), *An. coluzzii* was dominant, whereas *An. gambiae* s.s. prevailed in Gaoua (Pyr-only ITNs) and Orodara (PBO ITNs). These patterns align with the distinct ecological niches of the two species, *An. coluzzii* typically breeds in more permanent or semi-permanent water bodies, while *An. gambiae* s.s. is associated with transient, rain-dependent habitats [33–35].

A notable shift in Banfora between year 2 and 3 showed a decline in *An. gambiae* s.s. and a rise in *An. coluzzii*, which may reflect combined effects of environmental variation and selection pressure from dual-AI ITNs, as *An. gambiae* s.s. tends to be more endophilic and anthropophilic [35, 36]. Such shifts have also been documented in other West African settings [37, 38]. In contrast, species composition remained stable in Gaoua and Orodara, suggesting that *An. gambiae* s.s. is both ecologically well adapted and resistant to pyrethroids, an assertion supported by documented high resistance levels in the region [20, 21, 39].

Biting behaviour also varied across study sites and years. Most bites occurred between 22:00 and 05:00, consistent with peak sleeping hours and supporting the continued relevance of ITNs [40]. Despite variations in indoor biting rates (endophagy), no *Anopheles* species showed statistically significant indoor versus outdoor biting preferences, suggesting that observed trends likely reflect ecological dynamics rather than inherent behaviour traits [36, 41, 42]. The apparent exophagy in some fewer common species should be interpreted cautiously due to small sample size. Sustained behavioural

surveillance is essential for detecting such trends, which could influence intervention success [43].

Regarding ITN effect, the largest within district reduction in vector density was observed in Banfora following deployment of dual-AI Interceptor®G2 nets, which combine pyrethroids and chlorfenapyr, a compound effective against resistant mosquitoes due to its unique mode of action [44–46]. Mean *Anopheles* density declined by 88.7% over the 3-years. In Gaoua, where standard Pyr-only Interceptor®G1 nets were used, density initially dropped then rebounded by year, likely due to high levels of pyrethroids [7, 47, 48]. In Orodara, where Pyr-PBO PermaNet®3.0 nets were used, effects on mosquito density were limited, likely due to the coexistence of metabolic and target-site resistance mechanisms [49, 50]. Moreover, year 1 mosquito density in Orodara were consistently low, complicating interpretation. Because no formal between-product tests were undertaken and allocation was at district level, we refrain from inferring product superiority across districts.

Trends in SIR and EIR were complex and context dependent. In Gaoua, both decreased in year 2 but increased again in year 3, possibly due to behavioural adaptations, resistance evolution, or declining net condition and usage. In Banfora, dual-AI nets achieved strong reductions in transmission in year 2, followed by a rebound in year 3, potentially linked to net degradation or behavioural plasticity in vectors [21, 51, 52]. A recent durability study found that Pyr-only Interceptor®G1 and dual-AI Pyr-CFR Interceptor®G2 nets did not reach the 36 months performance benchmark, in contrast to the Pyr-PBO PermaNet®3.0 [53], emphasizing the need for operational durability monitoring [53–55]. In Orodara, the EIR increased over time, despite stable mosquito densities, suggesting that vector abundance alone does not reliably predict transmission risk. Such dissociations underscore the need to monitor both infection and vector density indicators for accurate malaria risk assessment [17, 56, 57].

Several non-exclusive mechanisms could plausibly account for the rebounds observed in year 3. First, ITN durability declines over time, through physical attrition (loss, tears) and insecticidal decay, reducing both personal protection and community effects [58, 59]. Findings from a parallel ITN durability cohort conducted in the same three districts after the 2019 mass campaign for 36 months support this interpretation [53]; (Additional file 4 Table S3). By the end of follow-up, only 29–61% of households retained at least one cohort net. Total attrition reached 55% in Orodara and 70% in Banfora, of which 20–41% was due to wear and tear. Furthermore, just 37.1–53.8% of nets remained in serviceable condition at 36 months. These deterioration patterns align

with a progressive reduction in both effective coverage and net integrity by Year 3, likely undermining entomological impact. Second, behavioural adaptations can increase exposure outside typical net-use windows (earlier-evening or outdoor biting), making any loss of net efficacy more consequential [56, 60]. Third, resistance dynamics, spanning target-site mutations and upregulated detoxification pathways, continue to evolve under selection pressure, eroding pyrethroid-only performance; while dual-AI (e.g., chlorfenapyr–pyrethroid) and PBO nets can partially mitigate this, benefits vary by context and may attenuate as active ingredients decline [12, 15, 53]. Finally, inter-annual ecological variability (rainfall, temperature, larval habitat availability) and operational factors (use, maintenance, competing interventions) likely contributed to the district-specific heterogeneity. Together, these processes provide a coherent explanation for the year 3 resurgence and its differing magnitude in Banfora (Interceptor® G2), Gaoua (Interceptor® G1), and Orodara (PermaNet® 3.0).

The findings highlight the potential value of next-generation ITNs in areas with intense pyrethroid resistance. In our study, both dual-AI Pyr-CFR (Interceptor®G2) and Pyr-PBO (PermaNet®3.0) nets were associated with reductions within their respective districts over time. However, the study was not designed for direct product-to-product comparisons; consequently, we do not claim superiority of one ITN type over another. The resurgence of entomological indicators in year 3, particularly in high transmission settings, highlights the limitations of relying exclusively on ITNs, even those with improved formulations.

To maximize impact, malaria control programs should adopt an integrated vector management strategy tailored to local entomological and epidemiological contexts. This includes combining ITNs with interventions such as indoor residual spraying, larval source management, and environmental modification [3]. Monitoring ITN physical condition and bio-efficacy is essential to ensure consistent protection between distribution rounds. Moreover, routine entomological surveillance including assessments of vector density, species composition, infection rates, biting patterns, and insecticide resistance is critical for guiding timely, evidence-based response to emerging threats.

This study is among the few longitudinal evaluations of ITN effectiveness in operational settings across multiple ITN types and ecological contexts in West Africa. Its strengths include the use of multiple entomological indicators (density, species composition, SIR, EIR), multi-year data collection, and a robust sampling design covering peak and off-peak transmission periods. However, several limitations should be acknowledged. First, the

absence of random allocation of ITN types limits the ability to directly attribute differences in outcomes to specific ITNs, and no formal statistical comparisons between ITN types were conducted. Second, operational challenges such as net attrition, variations in household use, or unmeasured confounders (e.g., local interventions or environmental changes) may have influenced outcomes. Third, while entomological data were comprehensive, parasitological, and clinical outcomes were not assessed, which limits conclusions about epidemiological impact. Lastly, small sample size for some species may reduce the reliability of behavioural interpretations, particularly regarding biting location. Consequently, between-district contrasts should be interpreted with caution given these potential confounders and data gaps and are best viewed as descriptive and hypothesis-generating. Despite these limitations, the study provides valuable insights into the real-world performance of next-generation ITNs and highlights the need for adaptive, integrated approaches to sustain malaria control in the face of rising insecticide resistance.

Conclusion

This study provides operational that dual-active, particularly Pyr-CFR Interceptor®G2 nets, can substantially reduce malaria vector densities and entomological indicators of transmission in high resistance settings such as South-Western Burkina Faso, with effects sustained for up to 2 years post-deployment. However, variability in effect across districts and the resurgence of transmission indices in the third year, underscore the complexity of vector dynamics and limitations of relying solely on ITNs. While standard pyrethroid-only nets also showed early effectiveness, their limited long-term impact raise concerns about their standalone use. These findings highlight the value of next-generation ITNs as part of an integrated vector management strategy and reinforce the importance of tailoring interventions to local resistance patterns and ecological conditions. Continued research is needed to assess how factors such as ITN durability, usage, and community coverage affect long-term effectiveness and sustainability of malaria control.

Abbreviations

CDC-LT	United State Center for Diseases Control and Prevention Light Trap
ITN	Insecticide treated bed net
Dual-AI	Dual active ingredients
EIR	Entomological inoculation rate
HBR	Human biting rate
HLC	Human landing catches
<i>kdr</i>	Knockdown resistance
NMCP	National Malaria Control Program
PBO	Piperonyl butoxide
SIR	Sporozoite Infection Rate
PCR	Polymerase chain reaction
95% CI	95% Confidence interval
Pyr-CFR	Pyrethroid + chlorfenapyr

Pyr-only Pyrethroid-only
Pyr-PBO Pyrethroid + PBO

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s12936-025-05732-9>.

Additional file 1: Figures S1. Predicted abundance of *Anopheles* species by collection position. Model-based predictions of *Anopheles* species abundance by indoor versus outdoor collection position, and temporal profiles of hourly biting activity over the course of the study.

Additional file 2: Figures S2. Hourly biting activity of *Anopheles* species recorded during the study period. For each species, the number of bites collected per hour is shown. Dashed vertical lines indicate the peak biting hour for each species. The shaded area corresponds to the critical biting window between 22:00 and 05:00. This period was defined based on typical human sleeping patterns in the study area and represents the hours when most people are indoors and asleep. It is also the time when the use of bed nets is expected to provide maximum personal protection against mosquito bites. The *Anopheles gambiae* complex species combined accounted for the highest total number of bites recorded during the study period.

Additional file 3: Tables S1. Year-to-year changes in *Anopheles gambiae* s.l. sporozoite infection rate by health district, expressed as odds ratios from district-specific binomial generalized linear models. Year 1 is the reference category within each district. **Table S2.** Year-to-year changes in *Anopheles gambiae* s.l. entomological inoculation rate by health district, expressed as differences in mean EIR from linear models with year as a categorical predictor. Year 1 is the reference category within each district. District-specific model outputs summarizing temporal changes in SIR and EIR across the three-year follow-up period.

Additional file 4: Table S3. Cohort ITN retention and physical condition in the three study districts. Summary of cohort ITN survival, attrition and physical condition over 36 months in Banfora, Gaoua and Orodara following the 2019 mass ITN campaign [53].

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Author contributions

WMG, AS, AG, CG, JW, SNF, MR, KT, HR designed the Burkina Faso study; AG, WMG, AS, SD, SZ, GT, KA, KD, JS, PD, JM, CF and MT led data collection activities and contributed critical datasets for the analysis; JDC, AS, WMG, OG, MT analyzed and interpreted the data; MT and WMG wrote the first draft of the manuscript. All authors contributed to the final interpretation of the findings, reviewed and approved the final manuscript.

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in the design of the study, the collection, analysis, or interpretation of data, or the writing of the manuscript.

Data availability

The datasets used and/or analysed during the current study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate

This study protocol was reviewed and approved by the Institutional Bioethical Committee of the Centre National de Recherche et de Formation sur le Paludisme (CNFRP) and registered under reference N°2019/003/MS/SG/INSP/CIB. Ethical clearance was also obtained from the Ethical Committee for Research in health of Burkina Faso health Ministry under reference 2019-3-029. Before field activities, study objectives and procedures were explained to community leaders in each study village, and community permission was obtained. Written informed consent was then obtained from all household heads where mosquito collections were conducted, and from all field personnel (collectors and supervisors) involved in mosquito sampling through CDC_LT and HLC methods. All Collectors and supervisors who participated in the study were provided with free malaria treatment in accordance with the Burkina Faso National Malaria Control Program guidelines. Participant information was handled confidentially in accordance with approved protocols.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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