

Research Report

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Entomological Efficacy of Lambdacyhalothrin-Treated Mosquito Nets in *Anopheles gambiae* Pyrethroid Resistance Areas. The First Village-Scale Comprehensive Trial Carried Out in Northern Côte d'Ivoire

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In tribute to Dr Dossou-Yovo

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Abstract

The efficacy of lambdacyhalothrin-treated nets (« λITN ») on malaria transmission and disease was assessed, for the first time at villages level, in *Anopheles gambiae* pyrethroid resistance areas of N Côte d'Ivoire. The base line data were obtained during a survey on rice cultivation, malaria transmission and morbidity, considering villages in three agroecosystems of Northern Côte d'Ivoire: (i) uncultivated lowlands “R0”, (ii) lowlands with one annual rice cultivation, in the rainy season, “R1” and (iii) developed lowlands with two annual rice cultivation cycles, “R2”. It appeared that the density of “main vectors”, mainly *An. gambiae* and *An. funestus* in R0; mainly *An. gambiae* in R1 and R2, increased with rice cultivation but the average yearly entomological inoculation rate (around 150 infective bites/human being) were almost similar.

The study on lambdacyhalothrin-treated mosquito nets (λITN) entomological and epidemiological efficacy was then carried out in this zone of *An. gambiae* high level of *kdr* resistance to deal with the burning issue of efficacy of treated nets when vectors are pyrethroid resistant.

From the list of villages surveyed during the study “rice cultivation and malaria” four paired villages (four villages of R2; two villages in R1 and two villages in R0) were selected and matched according to demographic, sociological, and ecological criteria. Among each pair, a village was randomly allocated to receive lambdacyhalothrin insecticide treated mosquito nets while the four other villages remained in their original state for one year, and considered as “control”, they received λITNs the following year.

The entomological impact of the treated nets was evaluated simultaneously with epidemiological studies in the same villages. Epidemiological information was already published showing that lambdacyhalothrin-treated nets induced a 56% reduction of malaria disease in children below 60-month-old. Up to now entomological data were not yet published as the first author passed after a long disease. We decided to publish the data in attrition to Dr Dossou-Yovo.

Entomological surveys were based on night capture on human being inside their houses. The main vectors were always *An. gambiae* in the three agroecosystems plus *An. funestus* in R0.

After implementation of λITN the “entomological inoculation rate” (EIR) due to *An. gambiae* decreased in the four villages receiving λITN, by an average of 90%; while in the villages without λITN the EIR naturally decreased by 47%; meaning a 48% gained efficacy with λITN implementation.

Adding data of the two main vectors showed that installation of λITN reduced the entomological inoculation rate two times more than what naturally occurred without such nets (relative reduction of 52.8%). This entomological level efficacy was remarkably similar the 56% reduction of the malaria incidence rate, a level comparable to the one observed almost everywhere with implementation of classical Long-lasting insecticide-treated nets in insecticide susceptible vectors areas.

Keywords Malaria vector control at village scale level; Kdr pyrethroid resistant *Anopheles gambiae*; Entomological evaluation; lambdacyhalothrin-treated nets

1 Introduction

Since their first entomological evaluation in the experimental huts of Soumouso field station (Burkina Faso) (Darriet et al., 1984) then their first epidemiological evaluation in a savanna village of Burkina Faso showing a reduction of 50% of plasmodic index in protected children (Carnevale et al., 1988) and splenic index in Mali (Ranque et al., 1984), insecticide-treated nets (with pyrethroid) were tested in several countries, with different eco-epidemiological facies (Lengeler et al., 1996; Lengeler, 1998, 2000, 2004) showing a reduction of malaria transmission (by 80%) and malaria morbidity (by 50%).

Large-scale trial of insecticide treated nets in Kenya (Nevill et al., 1996), Ghana (Binka et al., 1996) and The Gambia (Alonso et al., 1993; D'Alessandro et al., 1995) showed they could reduce by 17% the overall infant mortality and could be “a new method of preventing malaria deaths” (Lengeler et al., 1996). Insecticide treated nets became the actual first new operational method of malaria vector control (Lengeler, 2004).

But the need for their regular retreatment, every 6-8 months hampered their sustainability in spite of the development of tablets such as deltamethrin (K-OTAB®) (Sharma et al., 2006) or ICON(®) Maxx 'dip-it-yourself' sachet kit for long-lasting treatment of polyester nets contains a slow-release capsule suspension of lambda-cyhalothrin plus binding agent (Tungu et al., 2015) make it easier the retreatment of nets.

Therefore were developed industrialized long-lasting insecticide-treated nets (“LLINs”) (Robert, 2020) such as initially Olyset Net® treated with permethrin (WHOPES, 2001) and PermaNet® 2.0 treated with deltamethrin (WHO, 2001).

Since then a lot of LLINs were developed, each one being treated with a pyrethroid (permethrin, deltamethrin, alpha-cypermethrin, lambdacyhalothrin) (WHO, 2020).

Large-scale distribution of LLINs since years 2000 induced a striking reduction of malaria morbidity and related malaria deaths and it was estimated that between 2000 and 2015 some 400 million of malaria cases and several hundred thousand deaths were averted in Africa thanks to the large scale distribution of insecticide-treated nets (Bhatt et al., 2015).

More than three billion of LLINs were already distributed and it is scheduled a regular distribution of three hundred million of LLINs with the ultimate goal of malaria elimination.

But the fast spreading of pyrethroid resistance is of great concern (Hemingway and Ranson, 2000; Hemingway et al., 2002; Ranson et al., 2011; Ranson and Lissende, 2016) and it is feared a malaria disaster as insecticide resistance would derail malaria control (Hemingway et al., 2016). It was recently reported that “Reduced efficacy of insecticide-treated nets and indoor residual spraying for malaria control in pyrethroid resistance area, Benin” (N'Guessan et al., 2007) but several co factors could intervene for the analysis pyrethroid resistance influence on the efficacy of LLINs (Kleinschmidt et al., 2018).

To overcome the issues of pyrethroid resistance several approaches were developed (WHO, 2020) such as bitreated nets with two insecticides (Guillet et al., 2001); or treated with a pyrethroid and chlorfenapyr (CPF) (Accrombessi et al., 2023; Zahouli et al., 2023; Sih et al., 2024), or piperonyl butoxide (PBO) (N'Guessan et al., 2010; Protopopoff et al., 2018), or pyriproxyfen (PPF) (9). (N'Gufor et al., 2014, 2015).

Malaria is still endemic in Côte d'Ivoire and the National Malaria Control Programme developed a comprehensive plan including case-management (based on Artemisin Combined Therapy drugs, Rapid Detection Tests etc) and vector control based on large-scale distribution of long-lasting insecticide treated nets.

But *Anopheles gambiae* pyrethroid resistance, observed in 1993 (Elissa et al., 1993) is now present all over the country (Chandre et al., 1999; Edi et al., 2012, 2014; Koffi et al., 2013; Camara et al., 2018).

“Three resistance mechanisms were identified, involving high allelic frequencies of *kdr* L1014F mutation (range = 0.46-1), relatively low frequencies of *ace-1* R (below 0.5) and elevated activity of insecticide detoxifying enzymes, mainly mixed function oxidases (MFO), esterase and glutathione S-transferase (GST) in almost all study sites.” (Camara et al., 2018).

In the Korhogo Department “the frequency of the *kdr* allele in the *An. gambiae* s.s. population (frequency range: 90.70-100%) was very high” (Zogo et al., 2019) and “the *ace-1* frequency in the *An. gambiae* s.s. population ranged from 15.18 to 30.05%” and they were observed in both *An. gambiae* and *An. coluzzii* with some variations and time and space (Camara et al., 2018).

A great lot of studies were devoted to this issue in insecticide resistance in Côte d’Ivoire (Koffi et al., 2012, 2013; Edi et al., 2012, 2014; Camara et al., 2018; Zogo et al., 2019; Kouassi et al., 2020; Meiwald et al., 2022; Kouamé et al., 2023; Lucas et al., 2023; N’Dri et al., 2023).

Most of them were done in experimental huts (Koffi et al., 2012, 2013, 2015; Oumbouke et al., 2019; Clegban et al., 2021) but entomological and epidemiological influence of LLINs at village-scale in area where *An. gambiae* is pyrethroid resistant remained to be done.

A comprehensive evaluation of the influence of rice cultivation on malaria transmission and morbidity in Korhogo area (“West Africa Rice Development in Africa WARDA project”) (Henry et al., 2003; Dossou-Yovo et al., 2024) procured reliable base line data for an implementation, and evaluation, of a vector control operation based of full coverage of communities with lambdacyhalothrin-treated mosquito nets against *An. gambiae* population with high level of *kdr* based insecticide resistance (Henry et al., 2005).

With the support from Zeneca® Company (for entomological studies), TDR-WHO (for parasitological and clinical studies) and IRAC (for genetical background studies of resistance) and with official clearance of national authorities a lambdacyhalothrin-treated mosquito nets programme was developed in eight villages of the previous 24 villages of the programme rice cultivation and malaria in Korhogo area.

This was the first comprehensive trial, at village-scale level, of lambdacyhalothrin-treated mosquito nets against pyrethroid resistant *An. gambiae* in Côte d’Ivoire.

New vector control operation in Korhogo area are scheduled starting with actualized data base collection (Zogo et al., 2019) to be compared to which one collected during this first trial and checked if a “long-trends in *Anopheles gambiae* insecticide resistance occurred in Korhogo area and whole Côte d’Ivoire.” (Edi et al., 2014)

2 Materials and Methods

The areas of the surveys were already presented (Henry et al., 2003; 2005; Dossou-Yovo et al., 2024).

The study was conducted in the Department of Korhogo, (between 9°10’ and 9°40’ N and between 5°20’ and 5°60’ W) northern Côte d’Ivoire.

“The Korhogo area is characterized by a Sudanese climate with a unimodal rainfall regimen from May to November. The annual rainfall varies from 1,200 to 1,400 mm, while the mean annual temperature ranges from 21 to 35 C. The minimum temperatures can drop to 16° C due to the Harmattan wind during December and January. The natural vegetation is mainly a mixture of savannah and open forest characterized by trees and shrubs that are approximately 8-15 m in height.” (Zogo et al., 2019).

2.1 Village’s studied

For the rice cultivation and malaria project 24 villages were classified according to the farming systems in their surrounding valleys within a two kms radius and were randomly selected: eight villages with no rice cultivation (called “R0”); eight villages with one cycle of rice cropping during the rainy season (called “R1”); and eight

villages with water control that permits two cycles of rice cropping per year (called “R2”). The three categories of farming practice are referred as “agroecosystems” (Henry et al., 2003).

Eight villages were randomly selected and paired for receiving lambdacyhalothrin treated nets (λ ITN) the first year, the four other were considered as “control” and distribution of λ ITN was scheduled for these four villages the next year (Table 1a).

Table 1a. Paired villages for the λ ITN first distribution.

Agroecosystem	Villages with λ ITN	Villages without λ ITN
R0	Folofonkaha	Ounatickaha
R1	Fapaha	Bingebougou
R2	Nongochenekaha	Gbahouakaha
R2	Nambekaha	Kaforo

Before λ ITN implementation an in-depth census was made to precise the number of houses, inhabitants and every “sleeping-unit” in each of the eight villages randomly selected (Table 1b).

Table 1b. Populations of the eight villages and sleeping units* (*=anything thing used for sleeping, mattress, cardboard, etc).

Villages	Population	Nb houses	Nb beds	Nb sleeping units*
Folofonkaha	765	441	148	449
Ounatickaha	720	284	95	454
Fapaha	1,513	862	269	820
Bingebougou	1,305	630	369	724
Nongochenekaha	674	337	84	383
Gbahouakaha	605	269	74	242
Nambekaha	568	314	122	321
Kaforo	447	305	91	235

All R1 and R2 villages were situated in the Department of Korhogo where all lowlands were cultivated.

The R0 village was situated in the Department of Katiola, where lowlands were not at all farmed.

2.2 Entomological evaluation

Entomological evaluation was done by night catches on human beings (“NC HB”) inside houses, in the same houses as for the “rice cultivation and malaria programme”. Night catches were done from 10 pm to 5 am, as it appeared, in analyzing previous data of the rice cultivation and malaria programme, that about 90% of transmission occurred during that period and the aim of λ ITNs programme was to check the influence of λ ITNs on malaria parasite transmission.

Mosquitoes were determined at species level; *Anopheles* ♀ were determined according to morphological criteria and classical keys, (Gillies and De Meillon, 1968) then with biological method (Touré et al., 2012).

For the λ ITN evaluation, *An. gambiae* and *An. funestus* only (as they previously appeared to be the main vectors) were dissected (ovaries, salivary glands) in the field at the time of collection.

2.3 λ ITN implementation

Impregnations of mosquito nets were done directly in the villages with local health workers. Mosquito nets were allocated for each sleeping unit and received a double code, with the name of the village and the Number of house and «bed» with both a permanent marker and a water soluble one to check any wash.

The first impregnation was done in Nambekaha village with regional authorities and representative of national authorities (National Malaria Control Programme). 1,958 Mosquito nets (gift from Zeneca®, ordered in SiamDutch® Co) were treated with lambdacyhalothrin (“ λ ”) presented as ICON® CS 2.5, at the targeting dosage of 15 mg a.i./m² and distributed to populations. This operation lasted 10 days.

The other villages received λ ITNs treated with the same protocol.

After the first installation of λ ITNs four entomological surveys were done.

As scheduled, retreatment was done six months later, with same treatment and insecticide (still to get 15 mg a.i./m²).

After retreatment of λ ITNs, four more surveys were done.

The amount of insecticide used for treatment and retreatment and coverage are presented in the Table 2.

The entomological inoculation rate was calculated with Ross' formula $h = ma.s$ where ma =landing rate per human being per night and s = sporozoite index.

Table 2 Insecticide treatments of nets.

Villages	Nets treated	N° liters. Icon®	N° retreated	N° liters. Icon®
Nambékaha (R2)	320	4.2	295	3.1
coverage	99.7%		92.2%	
Nongochénékaha (R2)	374	3.7	298	3.3
coverage	97.7%		80%	
Fapaha (R1)	820	8.2	640	6.3
coverage	100%		78%	
Folonfokaha (R0)	444	4.5	371	4.0
coverage	99%		84%	
Total	1,958	20.6	1,604	16.7

For first treatment 20.6 l were used to treat 1,958 mosquito nets; for the retreatment, 16.7 l were used to treat 1,604 mosquito nets i.e. a total of 37.2 liters to treat 3,562 nets meaning an overall average concentration of 17.4 mg a.i./m² for a target dosage of 15 mg a.i./m².

According to the surface of one net (# 15 m²) 29,370 m² were treated the first time then 24,060 m² the next one.

According to the amount of insecticide used the expected average dosage of impregnation was 17.5 mg a.i./m² the first time then 17.4 mg a.i./m² for the redipping.

Treatment was done by «mass dipping» by lots of ten mosquito nets, the initial solution being composed of 49 l. of water and one liter of Icon® CS 2.5. After dipping mosquito nets dried in shadow places in the villages and were distributed to populations according to the census.

3 Results

3.1 Nets coverage

A check done three months after the second treatment of nets showed that 80% of the population were still protected which is the level requested to obtain a community protection moreover the personal protection (Table 3) and nets were still in good condition.

Table 3 Treatments of nets and coverage

Village	Nambékaha	Nongochénékaha	Fapaha	Folonfokaha
Population	568	674	1,513	765
N° houses	222	239	554	441
N° sleeping units	321	383	810	449
λ ITN given	320	374	820	444
Coverage	99.7%	97.7%	100%	99%
λ ITN retreated	295	298	640	371
Coverage rate retreat	92.2%	80%	78%	84%
λ ITN actually in used	299	329	779	339
ITN used/ITN given	93.4%	88%	95%	76%
People under ITN	471	568	1,271	606
% protected	83%	85%	84%	79%
Shape of ITN	++	++	++	++

3.2 Entomological data

Before implementation of λ ITNs eight entomological surveys were done in the eight villages representing 72 men/nights/villages *i.e.* a total of 576 men/night.

During λ ITNs experimentation eight surveys were done, «matched» in time with the previous one, representing 48 men/night/village *i.e.* 384 men/night.

A total of 31,276 mosquitoes were collected when landing on human beings inside houses (Table 4), 22,931 the first year considered as data base collection, and 8,345 the year of the trial.

-1,901 mosquitoes were collected in the two villages of lowlands without rice cultivation («R0»);

-3,520 mosquitoes were collected in the two villages of lowlands with rainy rice, one crop/year («R1»);

-25,855 mosquitoes were collected in the four villages of lowlands with irrigated rice, two crops/year («R2»).

Table 4 Mosquitoes collected while landing on human beings inside houses (Year 1= base line data, “B” for before; Year 2= vector control, after vector control, “A” for after)

Village	Agrosystem	λ ITN	Year 1	(Before)	Year 2	(After)	Total
			Total	ma	Total	ma	
Folofonkaha	R0	+	579	8.1	127	2.6	706
Ounatiekaha	R0	-	468	6.5	727	15.1	1,195
Fapaha	R1	+	1,818	25.2	491	10.2	2,309
Binguebougou	R1	-	914	12.7	297	6.2	1,211
Nongochenekaha	R2	+	3,449	47.9	294	6.1	3,743
Ggahouakaha	R2	-	1,577	21.9	654	13.6	2,231
Nambekaha	R2	+	8,298	115.3	2,403	50.1	10,710
Kaforo	R2	-	5,825	80.9	3,352	69.8	9,180
Total			22,931	39.8	8,345	21.7	31,276

The mosquitoes sample was composed of 5 genera and 28 species (Annex).

In the four villages where λ ITN were implemented 14,144 mosquitoes were caught before vector control *i.e.* an average human landing rate of 24.6 bites/Human Being/Night and 3,315 the year with λ ITN *i.e.* an average biting rate of 8.6 bites/Human Being/Night *i.e.* a 64.8% reduction.

In the four “control villages 8,784 mosquitoes were caught the first year *i.e.* 15.3 bites/Human Being/Night and 5,030 *i.e.* 13.1 bites/Human Being/Night the second year *i.e.* almost the same landing rate.

3.2.1 Entomological data dealing with *Anopheles gambiae*

Biological analysis showed that the *An. gambiae* s.l. sample was mainly composed of *An. gambiae* (97%) and few *An. coluzzii* (3%) (Touré et al., 2012); therefore further analysis gathered the two species and quoted as *An. gambiae*.

3.2.1.1 Evolution of the landing rate

Before vector control 11,351 specimen of *An. gambiae* were caught when landing on human being (HB) inside houses during the night (N) meaning a human landing rate of 39.4 bites/Human Being/Night; while the second year 2,393 specimen were caught meaning a landing rate of 12.5 bites/Human Being/Night *i.e.* a decrease of 68.3% (Table 5a).

Table 5a Evolution of the landing rate (ma) of *An. gambiae* inside human houses after implementation of lambda-delta-cyhalothrin treated ITNs (Before= 72 “men night”/village i.e. a total of 288 “men night” collection; After= 48 “men night”/villages i.e. a total of 192 “men night”; Diff= difference; Agrosyst= agrosystem)

Vector control		Before		After		
Villages	Agrosyst	Total	ma	Total	ma	Diff
Folofonkaha	R0	224	3.1	72	1.5	-51.6%
Fapaha	R1	1,071	14.9	394	8.2	-45.0%
Nongochenekaha	R2	2,925	40.6	182	3.8	-90.6%
Nambekaha	R2	7,131	99	1,745	36.4	-63.2%
Total		11,351	39.4	2,393	12.5	-68.3%

After implementation of lambda-delta-cyhalothrin treated nets the aggressivity of *An. gambiae* remained similar in two villages and decreased by # 50% in two villages (Table 5b).

Table 5b Evolution of the landing rate (ma) of *An. gambiae* inside human houses without implementation of lambda-delta-cyhalothrin treated mosquito nets (Before= 72 “men night”/village i.e. a total of 288 “men night collection”; after= 48 “men night”/villages i.e. a total of 192 men night collection)

Vector control		Before		After		
Villages	Agrosyst	Total	ma	Total	ma	Diff
Ounatiekaha	R0	279	3.1	187	3.9	=
Binguebougou	R1	758	10.5	259	5.4	-48.6%
Gbahouakaha	R2	691	9.6	465	9.7	=
Kaforo	R2	4,048	56.2	1,302	27.1	-51.8%
Total		5,776	20.1	2,213	11.5	-42.5%

In the four villages with λ ITNs the landing rate was 37.8% lower than in villages which remained without λ ITNs.

3.2.1.2 Evolution of the infectivity

Sporozoites were detected in 189 of the whole sample of 11,634 salivary glands dissected (overall sporozoite index s.i.=1.62%). Before λ ITNs implementation sporozoites were detected in 162 of the 8,928 salivary glands dissected (s.i.=1.81%) while the following year sporozoites were observed in 27 of the 2,706 salivary glands dissected (s.i.= 0.99%).

After λ ITNs implementation in the four villages the sporozoite index of *An. gambiae* significantly decreased from 1.95% (n=5,854) to 0.64% (n=1,397) (Table 6a) ($\chi^2=12.88$; p=0.0003; OR=0.29 [0.14-0.59]).

Table 6a Evolution of sporozoite index in the four villages where λ ITNs were implemented (D= number of salivary glands dissected; SG+= salivary glands with sporozoites; si= sporozoite index).

		<i>An. gb</i> before			<i>An. gb</i> after		
Villages	Agrosyst	D	SG+	si	D	SG+	si
Folofonkaha	R0	185	12	6.49%	67	1	1.49%
Fapaha	R1	672	31	4.61%	271	1	0.37%
Nongochenekaha	R2	1,930	57	2.95%	149	1	0.67%
Nambekaha	R2	3,067	14	0.46%	910	6	0.66%
Total		5,854	114	1.95%	1,397	9	0.64%

In the four villages which remained without λ ITNs the sporozoite index of *An. gambiae* remained similar: 1.56% (n=3,074) and 1.45% (n=1,309) (Table 6b) ($\chi^2= 0.0738$; p= 0.78; OR= 0.93 [0.54-1.58]).

Table 6b Evolution of sporozoite index in the four villages without λ ITNs (“control villages”) (D= number of salivary glands dissected; SG+= salivary glands with sporozoites; si= sporozoite index).

		<i>An. gb</i> before			<i>An. gb</i> after		
Villages	Agrosyst	D	SG+	si	D	SG+	si
Ounatiekaha	R0	254	13	5.12%	143	9	6.29%
Binguebougou	R1	687	18	2.62%	175	5	2.86%
Gbahouakaha	R2	440	7	1.59%	241	1	0.41%
Kaforo	R2	1,693	10	0.59%	750	4	0.53%
Total		3,074	48	1.56%	1,309	19	1.45%

3.2.1.3 Evolution of the entomological inoculation rate

The evolution of the entomological inoculation rate (EIR) according to year, and implementation of λ ITNs, or not, was estimated in combining Table 5a and 5b (landing rate) and 6a and 6b (sporozoite index) for each village (Table 7a, Table 7b).

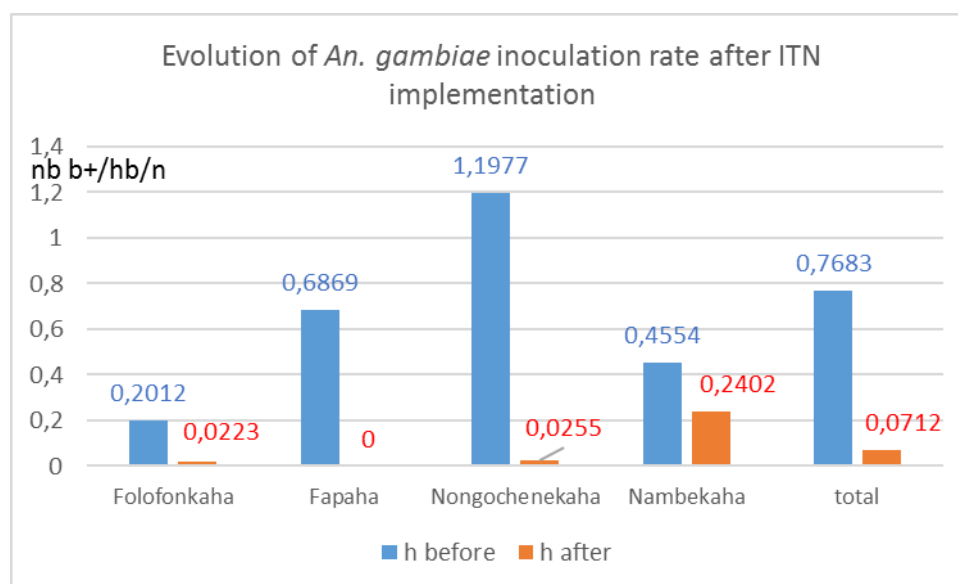
After the implementation of λ ITNs the entomological inoculation rate sharply decreased in each village (Graph 1a); by an average of 90.7%, (Table 7a) except in Nambekaha (R2 agroecosystem) where the inoculation decreased but with a value similar as in villages without λ ITNs.

Table 7a Evolution of the *An. gambiae* inoculation rate (h) with λ ITNs implementation (ITN+) (B=>A: difference between h before ("B") and after ("A") λ ITNs implementation)

Agrosyst.		Before			After			B=>A
Villages	Agrosyst.	ma	si (%)	h	ma	si (%)	h	Diff. (%)
Folofonkaha	R0	3.1	6.49	0.2012	1.5	1.49	0.0223	-88.9
Fapaha	R1	14.9	4.61	0.6869	8.2	0.37	0.0302	-95.6
Nongochenekaha	R2	40.6	2.95	1.1977	3.8	0.67	0.0255	-97.9
Nambekaha	R2	99	0.46	0.4554	36.4	0.66	0.2402	-47.2
Total		39.4	1.95	0.7683	12.5	0.64	0.0805	-89.5

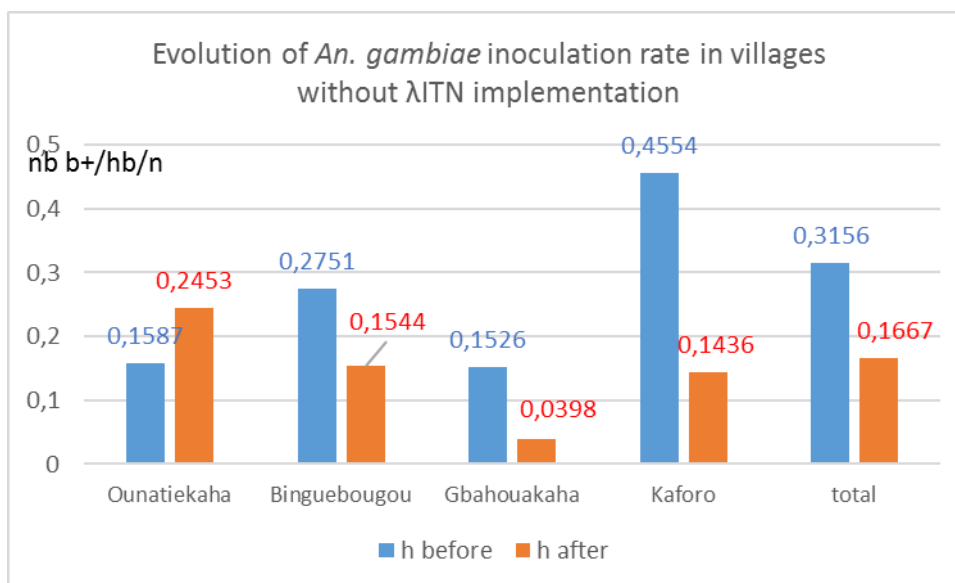
Table 7b Evolution of the *An. gambiae* inoculation rate (h) without λ ITNs implementation (ITN-) (B=>A: difference between h before, (=year 1) ("B") and after ("A") (=year 2))

Agrosyst		Before			After			B=>A
Villages	Agrosyst	ma	si (%)	h	ma	si (%)	h	Diff. (%)
Ounatiekaha	R0	3.1	5.12	0.1587	3.6	6.29	0.2453	-54.6
Binguebougou	R1	10.5	2.62	0.2751	5.4	2.86	0.1544	-43.9
Gbahouakaha	R2	9.6	1.59	0.1526	9.7	0.41	0.0398	-73.9
Kaforo	R2	56.2	0.59	0.3304	27.1	0.53	0.1436	-56.7
Total		20.1	1.56	0.3156	11.5	1.45	0.1667	-46.8



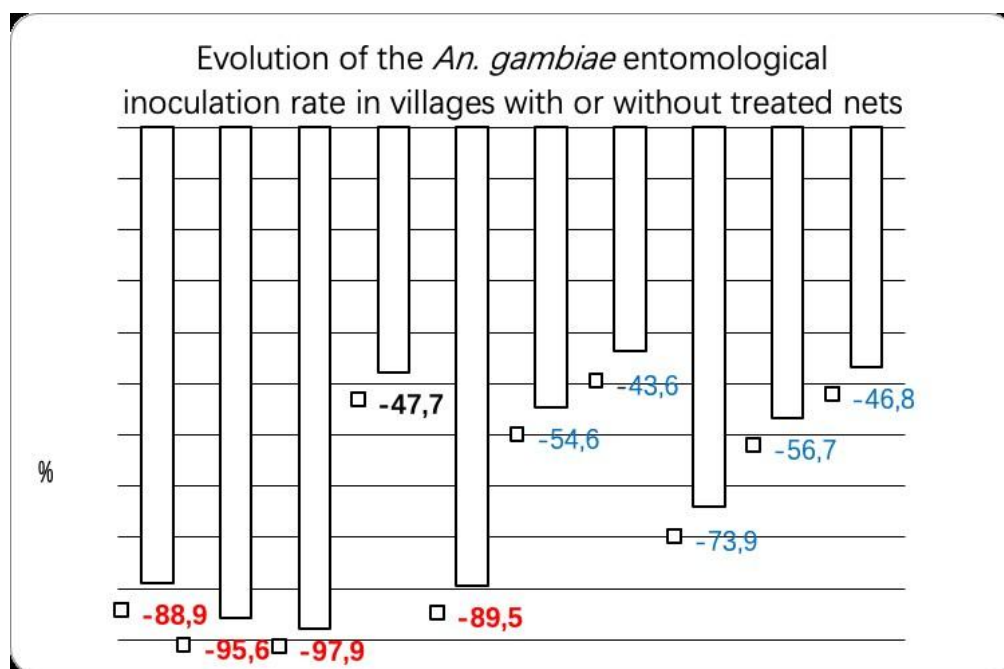
Graph 1a Evolution of *An. gambiae* inoculation rate after λ ITNs implementation (nb b+/hb/n = number of infective bites/human being/night).

In villages which remained without treated nets (Table 7b) the inoculation rate decreased in each village (Graph 1b); with an average of 46.8% and almost the same values except in one village (Gbahouakaha) where the decrease reached 74%.



Graph 1b Evolution of the *An. gambiae* inoculation rate in the four villages without λITNs implementation (nb b+/hb/n= number of infective bites per human being per night).

The entomological inoculation rate of *An. gambiae* decreased in the eight villages but two times mores in villages furnished with lambdacyhalothrin treated nets than in villages without these nets (Graph 2).



Graph 2 Evolution of the entomological inoculation rate by *An. gambiae* in villages furnished (in red) or not (in blue) with lambdacyhalothrin treated nets.

3.2.2 Entomological data dealing with *Anopheles funestus*

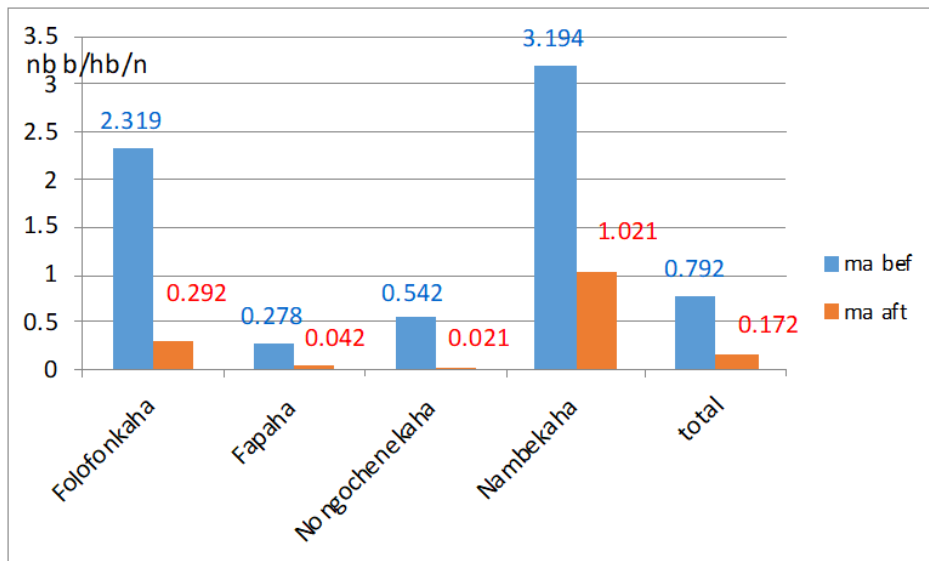
3.2.2.1 Evolution of landing rate

During the two years of the trial 1,082 ♀ *An. funestus* were collected (Table 8a and 8b); 671 ♀ the first year and 411 ♀ the second year.

After implementation of λITNs the landing rate decreased in each village (Graph 3a) with an average of 78.3%.

Table 8a Evolution of the landing rate of *An. funestus* in the villages after λ ITNs implementation ITN-+ (VC = vector control; diff = difference between before and after; C= number of specimens caught).

Villages	VC	Before		After		diff
		C	ma	C	ma	
Folofonkaha	R0 ITN-+	167	2.319	14	0.292	-87.4%
Fapaha	R1 ITN-+	20	0.278	2	0.042	-85.0%
Nongochenekaha	R2 ITN-+	39	0.542	1	0.021	-96.2%
Nambekaha	R2 ITN-+	230	3.194	49	1.021	-68.0%
Total		456	0.792	66	0.172	-78.3%



Graph 3a Evolution of *An. funestus* landing rate inside houses where λ ITNs were installed (nb b/hb/n= number of bites per human being per night).

In villages without λ ITNs installation the landing rate decreased in three villages (Table 8b) but it greatly increased in one, Ounatiekaha (Graph 3b).

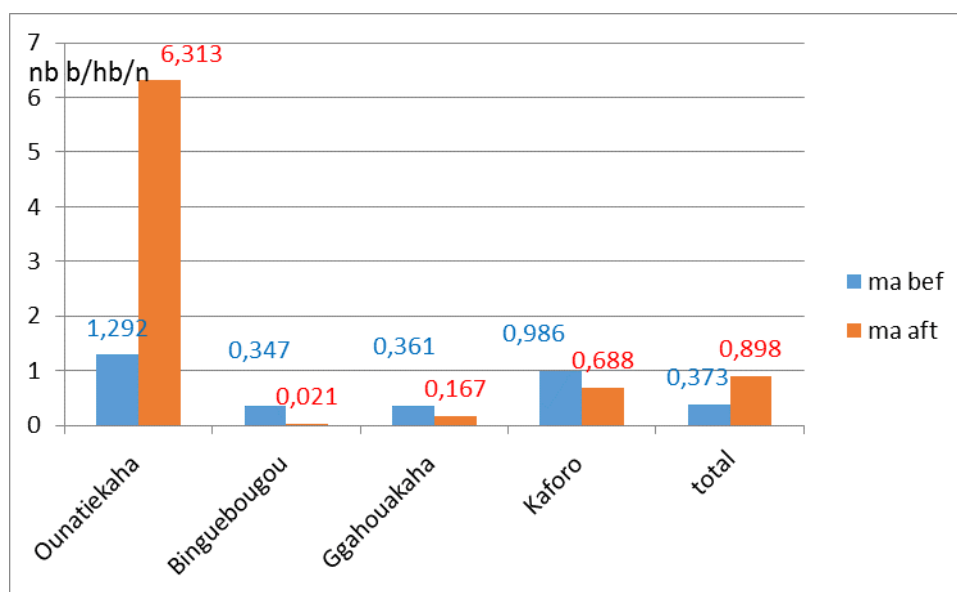
Table 8b Evolution of the landing rate of *An. funestus* in the villages without λ ITNs implementation (ITN--) (VC = vector control; diff = difference between before and after; C= number of specimens caught).

	VC	Before		After		diff
		C	ma	C	ma	
Ounatiekaha	R0 ITN--	93	1.292	303	6.313	x 4.9
Binguebougou	R1 ITN--	25	0.347	1	0.021	-93.9%
Ggahouakaha	R2 ITN --	26	0.361	8	0.167	-53.7%
Kaforo	R2 ITN --	71	0.986	33	0.688	-30.2%
Total		215	0.373	345	0.898	X 2.4

Gathering data from the four villages without λ ITNs the landing rate *increased* by two times.

3.2.2.2 Evolution of the infectivity

In the four villages before λ ITNs implementation sporozoites were detected in 11 of the 395 salivary glands dissected (s.i.= 2.78%) while not a single salivary gland was found with sporozoites among the 23 specimens dissected (Table 9a) meaning that sporozoite index became lower than the detectability threshold of the method used.



Graph 3b Evolution of *An. funestus* landing rate inside houses of villages without λITNs. (nb b/hb/n= number of bites per human being per night).

Table 9a Infectivity of *An. funestus* dissected in villages where λITNs were installed (D= number of specimens dissected; SG+= number of salivary glands with sporozoites); VC= vector control) (λITN+= installation λITNs).

		Before			After		
Villages	VC	D	SG+	s	D	SG+	si
Folofonkaha	R0 λITN++	153	10	6.54%	13	0	0
Fapaha	R1 λITN++	12	0	0%	0	0	0
Nongochenekaha	R2 ITN++	27	0	0%	0	0	0
Nambekaha	R2 ITN++	203	1	0.49%	10	0	0
Total		395	11	2.78%	23	0	0

In villages without λITNs installation the sporozoites remained at the same value (Table 9b), respectively s.i. = 2.42% (n=165) the first year and s.i.= 3.45% (n=232) the second year ($\chi^2= 0.345$; $p= 0.557$; OR= 0.69 [0.21-2.35]).

Table 9b Infectivity of *An. funestus* dissected in villages without λITNs (D= number of specimens dissected; SG+= number of salivary glands with sporozoites; VC= vector control) (λITN--= no λITNs installed).

		Before			After		
Villages	VC	D	SG+	s	D	SG+	si
Ounatiekaha	R0 λITN--	82	4	4.88%	205	7	3.41%
Binguebougou	R1 λITN--	14	0	0%	1	0	0%
	R2 λITN --	17	0	0%	6	0	0%
Ggahouakaha	R2 λITN --	52	0	0%	20	1	5,00%
Kaforo							
Total		165	4	2.42%	232	8	3.45%

3.2.2.3 Evolution of the inoculation rate

Combining the aggressivity (Table 8a, Table 8b) and infectivity (Table 9a, Table 9b) it is possible to estimate the evolution, from year to year, of the daily entomological inoculation rate in villages furnished in λITNs (Table 10a) and villages which remained without λITNs installation (Table 10b).

Table 10a Evolution of the *An. funestus* inoculation rate (h) before vs after λITNs implementation.

Villages ITN-+	h before	h after
Folofonkaha	0.151	0
Fapaha	0	0
Nongochenekaha	0	
Nambekaha	0.0049	
Total	0.0278	0

Table 10b Evolution of the *An. funestus* inoculation rate (h) in villages without λITNs implementation.

Villages ITN--	h before	h after
Ounatickaha	0.0487	0.0341
Binguebougou	0	0
Ggahouakaha	0	
Kaforo	0	0
Total	0.0242	0.027

Two points clearly appeared:

-The first year, when villages were “as they were”, the overall daily entomological inoculation rates were similar; respectively: 0.0278 infected bite/human being/night in villages “targeted for λITNs” and 0.0242 in villages “targeted for control.”

-The second year, after implementation of λITN, the sporozoites index in the four villages furnished became so low that it was not possible to estimate the inoculation rate while in villages which remained without λITNs the inoculation rate remained at the same value as the previous year (respectively 0.0242 and 0.0270 infected bites/human being/night).

3.2.3 Overall evolution of the entomological inoculation rate

Adding the inoculation rate by *An. gambiae* and *An. funestus* procure a proxy of the overall inoculation rate due to these main vectors in villages before and after implementation of λITNs or not (Table 11a, Table 11b).

After λITN implementation the daily inoculation rate decreased by 91% (Table 11a).

Table 11a Evolution of the inoculation rate (h) due to both *An. gambiae* and *An. funestus* after implementation of λITNs (h gb bef= h by *An. gambiae* before λITNs implementation; h gb aft= h by *An. gambiae* after λITNs implementation; h fu bef= h by *An. funestus* before λITNs implementation; h fu aft= h by *An. funestus* after λITNs implementation; h total = sum h by *An. gambiae* and *An. funestus* before and after; diff = difference of inoculation rate before vs after λITNs implementation).

Villages ITN-+	h gb bef	h gb aft	h fu bef	h fu aft	total h bef	total h aft	diff
Folofonkaha	0,2012	0,0223	0,151	0	0,3522	0,0223	-93,70%
Fapaha	0,6869	0	0	0	0,6869	0	
Nongochenekaha	1,1977	0,0255	0	0	1,1977	0,0255	-97,90%
Nambekaha	0,4554	0,2402	0,0049		0,4603	0,2402	-47,80%
Total	0,7683	0,0712	0,0278	0	0,7961	0,0712	-91,10%

Table 11b Evolution of the inoculation rate (h) due to both *An. gambiae* and *An. funestus* in villages which remained without λITNs implementation (λITN- = initial situation; ITN-- = no installation of λITNs; gb= *Anopheles gambiae*; fu= *Anopheles funestus*; diff= difference between year 1 and year 2).

Villages ITN--	h gb λITN-	h gb λITN--	h fu λITN-	h fu λITN--	total h year 1-	total h λITN year 2--	diff
Ounatickaha	0,1587	0,2453	0,0487	0,0341	0,2074	0,2794	+34,70%
Binguebougou	0,2751	0,1544	0	0	0,2751	0,1544	-43,90%
Ggahouakaha	0,1526	0,0398	0		0,1526	0,0398	-73,90%
Kaforo	0,4554	0,1436	0	0	0,4554	0,1436	-68,50%
Total	0,3156	0,1667	0,0242	0,0270	0,3398	0,1937	-43%

In villages where λ ITNs were not installed the inoculation rate decreased by 43% on the average, (Table 11b) at different level according to villages or even increased from year to year (R0).

This means that installation of λ ITNs reduced the inoculation rate two times more than what naturally occurred without such nets (relative reduction of 52.8%).

4 Discussion

A great lot of trials were devoted to lambdacyhalothrin treated mosquito nets (Das et al., 1993; Somboon et al., 1995; Jawara et al., 1998; Marbiah et al., 1998; Buttaporn et al., 1999; Kamolratanakul et al., 2001; Asidi et al., 2005; Vatandoost et al., 2006; Sahu et al., 2008) alone, or in combination, against insecticide-resistant *Anopheles gambiae*.

Insecticide resistance is one of the main issue to get efficient vector control and new approaches are needed for “averting a malaria disaster” (Hemingway et al., 2016).

In Côte d’Ivoire pyrethroid resistance is present all over the country (Chandre et al., 1999; Koffi et al., 2013; Zoh et al., 2018) and several tests were undertaken to evaluate new models of LLINs (Oumbouke et al., 2019); in experimental huts of field station where *An. gambiae* populations are resistant with different mechanisms (Koffi et al., 2012, 2013, 2015).

In *An. gambiae* populations of Northern Côte d’Ivoire (Touré et al., 2012) “the frequencies of the resistant allele at kdr mutation L1014F locus were very high and varied from 0.82 to 0.96. Before the trial, the genotype frequencies of specimens at kdr locus did not show any difference. However, they significantly increased in mosquitoes from treated villages (0.94) compared to those from control villages (0.87) on month 14 ($P= 0.13$). In contrary, a significant differences was observed before and after the trial between mosquitoes from villages with two cycles of rice cultivation per year and those from villages without rice cultivation ($P= 0.008$ and $P= 0.012$). Nine out of twelve populations of field mosquitoes showed Hardy-Weinberg disequilibrium at kdr locus ($P<0.05$). The S and M molecular forms of *An. gambiae* (= *An. gambiae* s.s. and *An. coluzzii* respectively) were sympatric in Korhogo area and the S form (*An. gambiae* s.s.) represents 97%. No hybrid was observed. All individuals of the M form (= *An. coluzzii*) were susceptible at kdr locus.”

Trials done in experimental huts in Côte d’Ivoire (in WHOPES phase II protocol) and Bénin clearly showed that pyrethroid resistance could reduce the efficacy of classical Long-Lasting Insecticide treated Nets (Kolaczinsky et al., 2000; N’Guessan et al., 2007).

But even in such situations permethrin or deltamethrin or lambdacyhalothrin treated mosquito nets could still procure some protection (Henry et al., 1999).

Actually, a first trial, in Kafine village (Côte d’Ivoire), with permethrin treated nets (Olyset® Nets) (Doanio et al., 1999) where local *Anopheles gambiae* population is highly resistant to pyrethroid with kdr allelic frequency > 90%, it was observed an interesting reduction of incidence of malaria morbidity (Henry et al., 1999) even if no entomological impact was noticed (Doanio et al., loc.cit.) showing that these treated nets procured an actual personal protection against the main vector (Henry et al., loc.cit.).

With these information it was decided to test the eventual influence of lambdacyhalothrin-treated mosquito nets at larger scale in villages of Korhogo area where previous entomological and epidemiological studies were done to precise the eventual influence of rice cultivation on malaria transmission and morbidity (Henry et al., 2003; Dossou-Yovo et al., 2024) and where local *Anopheles gambiae* population is pyrethroid resistant (likely due to large-scale use of insecticide on cotton culture) and the average kdr allelic frequencies are # 80%. (Chandre et al., 1999; Touré et al., 2012).

After one year of getting base-line data, during the programme “rice cultivation and malaria” (Henry et al., 2003; Dossou-Yovo et al., 2024) some 2,000 lambdacyhalothrin treated mosquito nets were distributed in four villages (3,500 people) in two steps, first impregnation then redipping six months latter while four paired villages of similar agroecosystems remained without such vector control.

Comprehensive evaluation was undertaken, with simultaneous entomological and epidemiological surveys, to get a double evaluation: before/after and here/there.

Entomological data were obtained by classical night catches of mosquitoes when landing of human beings and epidemiological studies were done exactly as for the rice cultivation and malaria surveys.

Two key points must be kept in mind and are of paramount importance for the vector control approach in malaria control programme.

Even in An. gambiae with high level of kdr based pyrethroid resistance, lambdacyhalothrin-treated mosquito nets conferred an actual reduction of biting rate, infectivity, and inoculation rates which was decreased by 90% while in control villages the inoculation rate decreased by 43% meaning a 53% more reduction obtained by treated nets than natural variations.

The concomitant similar reduction of incidence rate of malaria crisis among children (Henry et al., 2005) showed that lambdacyhalothrin-treated mosquito nets could actually confer efficient both personal protection and collective prevention even if vectors are insecticide resistant.

Actually “clinical malaria observed by active case detection reported that; in the treated villages, the incidence was significantly lower than in the control villages: 0.8 versus 1.8 clinical malaria attacks per child per year ($P < 0.001$). *The protective clinical efficacy of treated nets was 56% (95% CI, 25-75%).*” (60) (Henry et al. loc.cit.) *This figure of 56% of malaria morbidity is to be compared to the 53% reduction of the entomological inoculation rate obtained after introduction of lambdacyhalothrin treated nets.*

These results demonstrate the interest of integrated multidisciplinary field surveys and the correspondence between reduction of transmission and malaria morbidity.

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Authors

J. Dossou-Yovo was involved in the protocol, field surveys, first analysis of data and first report.

J. Chabi was involved in field surveys.

G. Carnevale was involved in writing and English editing.

P. Carnevale, was involved in preparing the protocol, monitoring field surveys, statistical analyses of data and writing the document.

Conflict of Interest

Authors declared they have no conflict of interest.

No AI used.

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Annex

Mosquitoes genera and species composition of samples caught when landing on human beings inside houses during Korhogo trial

Anopheles gambiae
An. coluzzii
An. funestus
An. nili
An. pharoensis
An. brohieri
An. ziemanni
An. obscurus
An. paludis
An. coustani
Aedes aegypti
Ae. furcifer
Ae. gr. palpalis
Ae. tarsalis
Ae. cumminsi
Ae. luteocephalus
Ae. circumluteolus
Ae. vittatus
Ae. opok
Ae. argenteopunctatus
Mansonia africana
Mansonia uniformis
Culex quinquefasciatus
Cx. gr. decens
Cx. annulioris
Cx. nebulosus
Cx. bitaeniorhynchus
Coquillettidia sp.

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