The Durable Lining: A New Tool for Malaria Vector Control Compared to Other Classical Methods (Long Lasting Insecticide Treated Nets "LLIN" During a Village Scale Long Term Trial in Angola. I. Entomological Evaluation.

Pierre Carnevale[1],* **Jean-Claude Toto[2], Vincent Foumane[2], Filomeno Fortes[3], Guillaume Carnevale[4], Frédérick Gay[5]**

[1] Director of Research, Le Majoral Building 34420 Portiragnes Plage, France (pjcarnevale2001@yahoo.fr)

[2] OCEAC PO 288 Yaoundé Cameroon (jctotofr@yahoo.fr) (Vincent.foumane@yahoo.fr)

[3] National Malaria Control Programme, Luanda, Angola (filomenofortes@gmail.com)

[4] HSE, Batuan, Indonesia (gpcarnevale2001@yahoo.fr)

[5] Sorbonne University, INSERM, Pierre Louis Institute of Epidemiology and Public Health, Pitié Salpétrière Hospital, APHP, F75013, Paris, France (frederick.gay@sorbonne–universite.fr).

*Corresponding author

Pierre Carnevale, Director of Research, Le Majoral Building 34420 Portiragnes Plage, France; **Email:** pjcarnevale2001@yahoo.fr.

Abstract

Background: Deltamethrin ("δ") treated durable lining ("DL") or "insecticide treated plastic sheeting" ("δITPS") were used for a long term village–scale malaria vector control (VC) program in 8 villages around Balombo town (Benguela Province, Angola) either alone (in 2 villages) or associated with Long Lasting deltamethrin Insecticide treated Net ("δLLIN") Permanet© 2.0 (in 2 villages) or following Indoor Residual Spraying with lambdacyhalothrin "λ" ("λIRS") (in 2 villages) while LLIN P2.0 alone were considered as "golden standard" and implemented in 2 villages. 2 models of ITPS were used, "Wall lining" and "Zero Fly" with different deltamethrin concentration. A comprehensive evaluation, entomological and parasitological, was initially planned for 5 years. Entomological evaluation was based upon the regular use of the classical CDC Miniature Light Trap inside human houses.

Results: During these 5 years (2 years before and 3 years after full vector control implementation) 202 CDC "night catching sessions" were done, representing 1880 "trap–night", 1152 *Anopheles* were captured, belonging to 10 species, the main vectors ("MV") were *Anopheles funestus* and *An. gambiae*. After implementation of VC the densities of "main–vector/traps" dropped by # 70%, similarly with the 4 VC methods (#63% with LLIN alone; #69% with the association LLIN +ITPS model "Zero Fly"; # 82% with ITPS model "Wall Lining" alone and #75 % after 2 rounds of λIRS followed by ITPS installation).

28 of the 1133 anopheles analyzed procured an Elisa positive test i.e. a general positivity index of 2.47%; with 8.00% (n= 50) for *An. gambiae*; 3.61% (n= 498) for *An. funestus* and 2.06% for *An. marshalli* (n= 243).

Infectivity of main vectors *An. gambiae + An. funestus* dropped, but not significantly, from 4.53%, (n= 375) to 2.59% (n=193) after vector control implementation.

Overall Inoculation rates decreased by #80% after Vector Control: #–60% with LLIN alone; # 80% with durable lining, similarly alone (–81%), or associated with LLIN (–79%), or following λ IRS (–80%).

Conclusions: The fact that Durable Lining, even alone, were as efficient as Indoor Residual Spraying to reduce biting rate and inoculation rate of main vectors while having a longer lasting activity, and that the association Long Lasting Treated Nets + Durable Lining was not much better than a single method are important information for the National Malaria Control Program and other Malaria Control programs.

Keywords: *Anopheles*, vector control, malaria, Angola, entomological evaluation, deltamethrin treated durable lining, Long Lasting insecticide (deltamethrin) treated Nets (LLIN), lambdacyhalothrin Indoor Residual Spraying.

Introduction

According to the recent WHO Malaria report 2021 (Regional data and trends) "in 2020, six countries in sub–Saharan Africa, accounted for 55% of all malaria cases globally: Nigeria (26.8%), The Democratic Republic of the Congo (12.0%), Uganda (5.4%), Mozambique (4.2%), Angola (3.4%) and Burkina Faso (3.4%)". Increase in mortality rate of between 5% and 25% were seen in eight countries: Angola, The Democratic Republic of the Congo, Guinea–Bissau, Liberia, Namibia, South Sudan and Uganda" [1]

In Angola, the whole population of 31 million inhabitants is at risk of malaria with some 15,928,260 presumed and confirmed (estimated 7,052,636) malaria cases with 11 814 reported (13 425 estimated) deaths

Due to insecticide [2] and drug resistance [3–5] the situation is worsening; 2,548 deaths from malaria were registered in the first quarter of 2020, plus 467 deaths compared to the same period in 2019. From January to March 2020, 2,065,673 cases and 2,548 deaths were registered, 190,398 more cases than in 2019.

Angola is still among the countries with the highest number of malaria cases and deaths (3% of the global cases and deaths) [6]. Between 2015 and 2018, there was an increase in malaria cases, from 200 to 288 per 1000 of the at risk population. According to the National Malaria Control Programme (NMCP) 8% of all reported yearly malaria cases in 2017 were severe malaria.

The strategy of NMCP combines preventive action and case management, mainly national scale distribution of Long lasting insecticide treated nets (LLIN), through different channels, and intermittent preventive treatment (IPT) to pregnant women [7] Rapid Detection tests (RDT) are distributed to improve timely reliable testing diagnosis [8] with treatments based on Artemisin Combined Therapy ("ACT") since 2006 [3,9,10].

The recent Sixteenth meeting Vector Control Advisory Group (VCAG) (2022) of WHO [11] examined 4 kinds of "new" approaches for vector control:

- gene drives and effector genes that aim to reduce or eliminate the potential of *Anopheles gambiae* and *An. coluzzii* to transmit *Plasmodium falciparum* parasites; for the time being "there are currently no active epidemiological trials under way for gene drive constructs that fall into this intervention class"

- Bait stations (attractive targeted sugar baits; ATSBs): which are designed "to attract and kill sugar–seeking mosquitoes. As both male and female mosquitoes feed on plant–derived sugars to maintain energy for survival, ATSBs exploit the almost daily need for sugar by attracting the mosquitoes to a source that also contains a lethal

toxicant". One intervention has been implemented "has been shown to reduce mosquito vector populations and the survivorship of individual mosquitoes, as well as to decrease the proportion of mosquitoes with malaria parasites". The green light has been given to start epidemiological evaluation but several issues were raised (budget, protocol etc)

- Eave tubes (previously lethal house lures): "No WHO recommendation is in place. One trial in Côte d'Ivoire on the combined use of eave tubes + screening was completed, but was unable to assess evidence of the individual impact of eave tubes".

- house modifications such as screens but this cannot be reasonably be considered as a real new method while referring to the well–known trial in Ostia malarious area (Italy) in July–October 1900.

As it can be noticed no one of these methods has yet procured epidemiological data and is included in National Malaria Control Program which are mainly concentrated on IRS and LLIN for adult control while Dr P. Alonso (Director of the WHO Global Malaria Action Program) underlined in his recent interview "we need of new technological tools"

In Angola, due to the failure of the recent Indoor Residual Spraying (IRS) operation [12] an accent for vector control was deliberately given to large scale distribution of Long Lasting Insecticide Treated Nets (LLINs) while researches for new approaches, such as durable lining, deserves special attention [13–15] Actually Insecticide Treated Plastic Sheeting (ITPS) often called "durable lining", represent a new tool, pinned on the wall inside human houses they could easily and usefully replace IRS with their well–known operational issues. ITPS were recently used in refugees camps [16–17] and experimental huts, alone [18] or in combination with LLIN [19,20]. Organophosphate treated ITPS were used with LLIN against pyrethroid resistant *An. gambiae* in Côte d'Ivoire [21] while carbamate–treated ITPS were used in combination with deltamethrin treated LLIN for controlling pyrethroid resistant *An. gambiae* [19,22] .They were recently tested in Huambo area (Angola) close to Balombo, and Nigeria [14], in several other countries in Africa and Asia [13] and some operational issues were raised [23]. Their acceptability was studied in Papua–New Guinea [24] as well as in Equatorial Guinea, Ghana, Mali, South Africa, and Vietnam [13]. The model called "Zero Fly©" (with higher concentration in deltamethrin) was tested in India [25–27].

According to these positive entomological data observed elsewhere and in Angola with Insecticide Treated Plastic Sheetings, a long term village scale malaria vector control programme was

implemented with the National Malaria Control Program in Balombo area (Benguela Province) for a long term village scale comprehensive evaluation of the entomological, parasitological, immunological efficacy and acceptability of ITPS (2 models) either alone or associated with LLIN (« Perma®Net »2.0) or following 2 rounds of lambdacyhalothrin Indoor Residual Spraying and partial data were already published [28–35]. It appeared important to present the "starting point" of this program with the entomological observations while parasitological one will be published soon.

Materials and methods

The National Malaria Control Programme (NMCP) identified eight villages around the Balombo Municipality, in the Benguela Province, central Angola (12°21'S; 14°46'E), 150 km east of Lobito Town and 600 km south–east from Luanda the capital (Figure 1).

It is a humid mountainous area at an altitude of # 1,200 meters with tropical savannah forests (greatly damaged for agricultural purposes) and fast running rivers; there is a long rainy season (October to May) and a short dry season (June to September).

These villages are far enough from each other so that it could not be expected that some mosquitoes could actively fly from one to another one and induce some bias for entomological evaluation.

Census

Before the trial, a comprehensive census was done in each village and each house received a number which was reported on the door, noticed for GPS mapping and used for randomization.
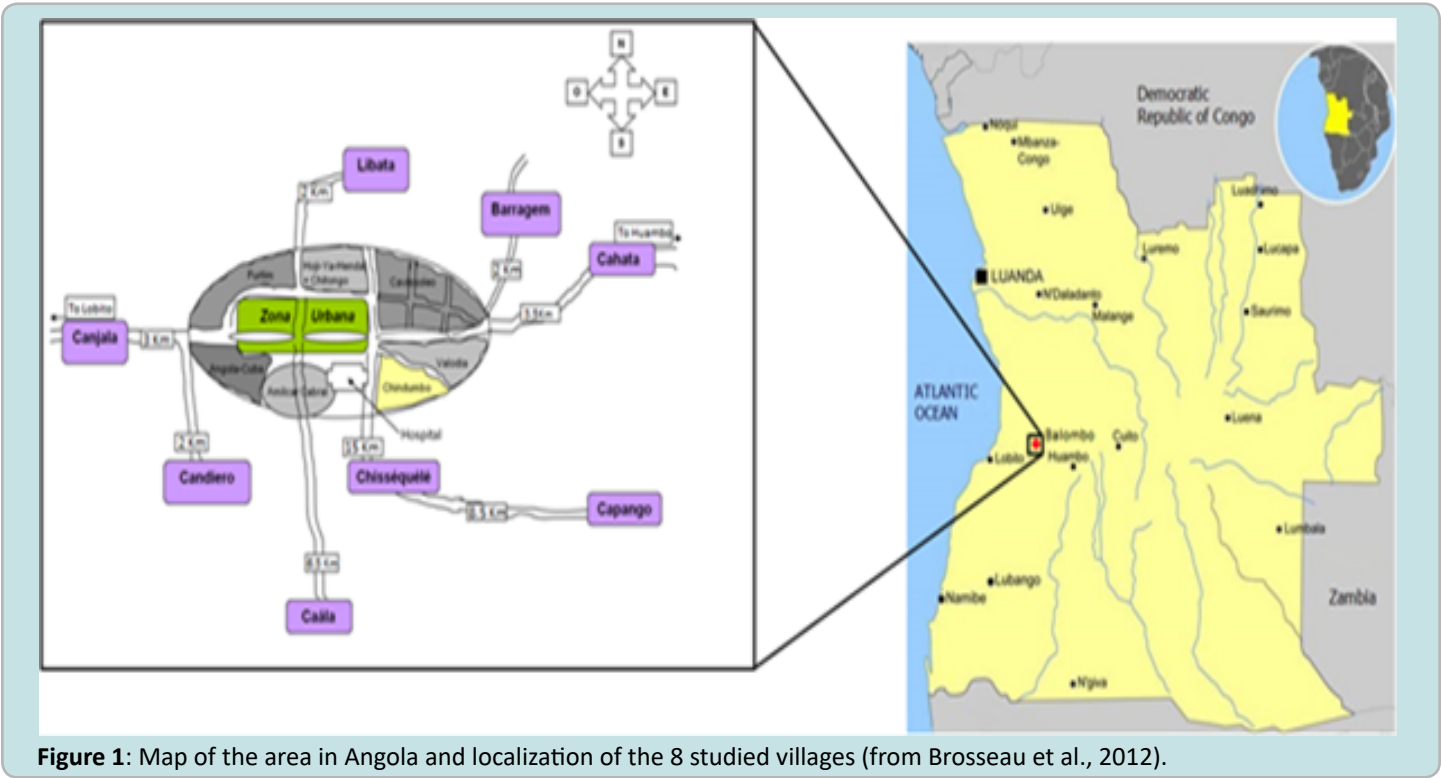

Figure 1: Map of the area in Angola and localization of the 8 studied villages (from Brosseau et al., 2012).

The demographic and geographic situation of each village is indicated Table 1.

The programme included 5322 people in 1600 houses at its beginning.

Villages	Nb Inhabitants	Nb Houses	Longitude E	Latitude S
Caala	792	238	14°47'	12°25'
Cahata	484	138	14°48'	12°20'
Capango	137	60	14°50'	12°24'
Canjala	873	401	14°44'	12°21'
Chisséquélé	418	181	14°47'	12°22'
Barragem	620	134	14°47'	12°21'
Candiero	654	190	14°45'	12°22'
Libata	1344	258	14°46'	12°20'
Total	5322	1600		

Table 1: Census of the 8 targeted villages at the beginning of the trial (February 2007).

Tools used for vector control

4 tools were used for vector control (pictures 1 and 2):

- Long Lasting Insecticide treated Net (LLIN) PermaNet© 2.0: treated at 55 mg a.i. deltamethrin (δ) /m^2; (with a surface of 13 m^2 the total amount of δ insecticide is 0.715 gr a.i. deltamethrin/LLIN P2.0).

- Insecticide Treated Plastic Sheeting (ITPS), model "Wall Lining": treated at 170 mg a.i. δ/m^2.

- ITPS, model Zero Fly©: treated at 360 mg a.i. δ/m^2; (with a size of 19.11 m^2 the total amount of insecticide is 6.88 gr a.i. δ/ ZeroFly).

- Sachets lambdacyhalothrin (λ) Icon© WP: 6.25 gr a.i. λ/ sachet for IRS (targeting 25 mg a.i./m^2 of sprayed surface), 2 rounds followed by installation of ITPS.

Deltamethrin ($C_{22}H_{19}Br_2NO_3$) is a pyrethroid ester insecticide largely used (with other pyrethroid such as permethrin, alphacypermethrin etc) in the manufacture of long–lasting insecticidal mosquito nets.

Lambdacyhalothrin ($C_{23}H_{19}ClF_3NO_3$) in 2.5% capsule suspension formulation it has been approved by experts of the WHO in 2000.

Vector control operations (VCOp)

The details of vector control operations are gathered in Table 2.

LLIN were given at the beginning of the programme in 2 villages (Caala and Cahata) while the 6 other villages remained without vector control during 2 years (2007–2008) ("control villages") (period called "before VC") and the 3 following years (2009–2010–2011) were called "period after VC".

The details of vector control operations done in each village are gathered in Table 2.

Villages	February 2007	February 2008	December 2008	June 2009	January 2010
Caala (469 sleeping units)	δLLIN* 360 LLIN	δLLIN** 227 LLIN	δLLIN** 49 LLIN	xx	xx
Cahata (442 sleeping units)	δLLIN* 310 LLIN	δLLIN** 195LLIN	δLLIN** 25LLIN	xx	xx
Capango	C	C	93 δLLIN +93 ZF	xx	xx
Canjala	C	C	422 δLLIN + 621 ZF	xx	xx
Barragem	C	C	5554 m² WL	xx	xx
Chisséquélé	C	C	5441 m² WL	xx	xx
Candiero	C	C	λIRS 1 191 houses 9500 m²	λIRS 2 176 houses 8750 m²	WL 4914 m²
Libata	C	C	λIRS 1 258 houses 12825 m²	λIRS 2 263 houses 13070 m²	916 ZF 17505 m²

Table 2: Vector control operations (δLLIN = PermaNet 2.0 Long Lasting Insecticide deltamethrin Treated Nets; ZF= Insecticide deltamethrin Treated Plasting Sheeting model Zero Fly; WL= Insecticide deltamethrin Treated Plastic Sheeting model Wall Lining; λIRS 1= Insecticide Residual Spraying with lambdacyhalothrin Round 1; λIRS 2= Insecticide Residual Spraying with lambdacyhalothrin Round 2).[*target 1 LLIN/house; ** target: 1LLIN/sleeping unit] [xx = no vector control done; C= control villages].

The average amount of insecticide /house was greatly different according to the vector control method implemented, from # 1 g a.i. deltamethrin/house with LLIN (almost the same with λ IRS) to 13.1 gr a.i./house with Zero Fly ITPS alone (Table 3).

village	method of VC	amount of a.i./house
Caala	LLIN only "1 net/house"	1,08 gr a.i. δ/house
	LLIN only full coverage	2,06 gr a.i. δ/house
Cahata	LLIN only "1 net/house"	1,61 gr a.i. δ/house
	LLIN only full coverage	2,75 gr a.i. δ/house
Barragem	WL only	7,05 gr a.i. δ/house
Chisséquélé	WL only	5,11 gr a.i. δ/house
Capango	LLIN + ZF	11,77 gr a.i. δ/house
Canjala	LLIN+ ZF	9,92 gr a.i. δ/house
Libata	IRS R1	1,48 gr a.i. λ/house
	IRS R2	1,07 gr a.i. λ/house
	ZF only	13,79 gr a.i. δ/house
Candiero	IRS R1	1,28 gr a.i. λ/house
	IRS R2	1,31 gr a.i. λ/house
	WL only	4,16 gr a.i. δ/house

Table 3: Average amount on insecticide per house according to the various vector control method implemented (δ= deltamethrin; λ= lambdacyhalothrin).

Mosquito collections and biological analysis

Entomological surveys were regularly done (from February 2007 to December 2011) and based on the classical CDC Light Traps (CDC–LT) thanks to their reproducibility, if the same traps are regularly used in the same houses and they are independent of the collector' skill or attractiveness [36–39]. CDC were also used for the evaluation of the efficacy of pyrethroid treated nets in Tanzania [40] and, in conjunction with human landing catches, for the evaluation of LLIN universal coverage in Uganda [41]. In Northern Tanzania "mosquitoes were sampled using CDC miniature light trap" for the evaluation of deltamethrin treated wall lining against *Anopheles gambiae* s.l. [42]

For each session, 10 traps were installed inside initially randomly selected houses, which were kept the same for the whole entomological survey. Traps were switched on at 6 pm and switched off at 6 am the following day.

Mosquitoes were sorted out at the genus level and *Anopheles* species determined with classical keys [43], then isolated in plastic tubes and kept in dry atmosphere until their identification at the species level (*An. gambiae* complex) and classical circumsporozoite ELISA analysis [44,45]. Molecular identification of members of the *An. gambiae* complex was done following the RFLP–PCR assay developed by Fanello *et al.* (2002) [46] which allows, with one amplification and a digestion with *Hha*I restriction enzyme, the differentiation between *An. gambiae* and *An. coluzzii*, as well as the other members of the complex.

Following Sriwichai *et al.* (2015) [39] method, absolute vectors numbers were converted into "capture rates" (i.e.,

number of vector per trap, per year and per village). For each year and each village, the total number of *An. gambiae* and *An. funestus* ("Main Vectors" or "MV") collected was divided by the total number of CDC–LT implemented to get a proxy of the "number of *Anopheles*/trap" considered as a reliable indicator of the "density"/house with a figure of their evolution according to the implementation of each vector control methods.

Combining these data with infectivity rates gave some proxy of the "indoor risk of inoculation" and its evolution according to the vector control methods implemented.

The entomological inoculation rate (h) was calculated with the classical Ross 'formula [47] h= ma.s (with ma = number of bite received in one day by one human being; and s = infectivity rate) considering here the "capture rate" of Main Vectors (MV) for "ma".

Statistical analyses of *Anopheles* samples collected by traps were done by the non–parametric Mann–Whitney test; graphics were prepared with the Graphpad software.

Results

Anopheles species caught

From February 2007 to December 2011, 202 catching sessions with CDC light traps were implemented in the 8 villages representing a total of 1,880 trap–nights which caught 1,152 *Anopheles* specimens, belonging to 10 taxa including 9 species and one species complex (Gambiae) (Table 4). The main *Anopheles* were *An. funestus* (44.7%) abundant in every village, followed by *An. marshallii* (21.0%), *An. maculipalpis* (17.8%), *An. ziemanni* (6.5%), and *An. gambiae* s.l. (4.8%). The other 5 species including *An. pharoensis* (1.9%), *An. nili* (1%), *An. coustani, An. tenebrosus* (0.9%), and *An. hancocki* (0.6%) were scarce.

Only *An. gambiae* and *An. funestus* were considered for further analysis according to the well–known few, if any, vectorial capacities of other species [43].

The molecular analysis of a sub sample of 45 *An. gambiae* s.l. specimens out of the 55 caught showed the preponderance of *An. gambiae* (82.2%) (formerly called "*An.gambiae* S form"), with only 3 specimens of *An. coluzzii* and 5 *An. arabiensis.*

villages	Caala	Cahata	Capango	Canjala	Chiss.	Barr.	Candi.	Libata	Total
An. gambiae	15	11	8	1	8	1	8	3	55
An. funestus	71	74	119	60	32	46	56	55	513
An. marshalli	37	30	79	22	18	11	26	20	243
An. ziemanni	19	4	28	3	6	3	4	8	75
An. maculipalpis	43	24	72	6	27	7	19	8	206
An. hancocki	0	0	5	0	0	1	1	0	7
An. nili	7	0	1	2	1	0	0	0	11
An. pharoensis	3	2	14	0	2	0	1	0	22
An. coustani	0	1	4	0	3	0	2	0	10
An. tenebrosus	2	1	6	0	0	0	1	0	10
total	197	147	336	94	97	69	118	94	1152

Table 4: Anopheline species caught by CDC Light Traps inside human houses of the 8 surveyed villages from 2007 to 2011 (Chiss. = Chisséquélé; Barr. = Barragem; Candi.= Candiero).

In their pyrethrum spray sampling Cuamba *et al.* (2006) [2] found mainly *An. gambiae* M form (= *An. coluzzii*) as resting inside houses but "the highest proportion of S–form (20%) was in samples from Huambo, in the humid highlands". The fact that *An. gambiae* was predominant in humid area or season was also already noticed in Lobito town (Angola) [48].

### Evolution of densities of main malaria vectors according to village

The yearly evolution of the capture rate of the main vectors, *An. gambiae* s.l. and *An. funestus,* in each village during the 5 years of the study, is shown in Table 5.

Table 5: Evolution of density/traps of Main Vectors *An. funestus* + *An. gambiae* according to year and village.

villages\years	Y 2007	Y 2008	Y 2009	Y 2010	Y 2011
Caala	0.56	0.42	0.35	0.12	0.47
Cahata	1.29	0.43	0.18	0.12	0.17
Capango	1	1.1	0.15	0.45	0.23
Canjala	0.5	0.38	0.07	0.1	0.53
Chisséquélé	0.54	0.40	0.05	0	0
Barragem	0.58	0.25	0.25	0.02	0.07
Libata	0.33	0.53	0.15	0.08	0.13
Candiero	0.67	0.47	0.07	0.12	0.30

It clearly appeared (graph– 2a & 2b) that

- densities were very low (less than 1 MV/trap/night);
- a striking drop occurred in 2009, in the 6 initially "control villages", the year following vector control operation;
- an overall increase in 2011, the 3rd year after VC implementation which was not apparent in villages with Durable Lining.
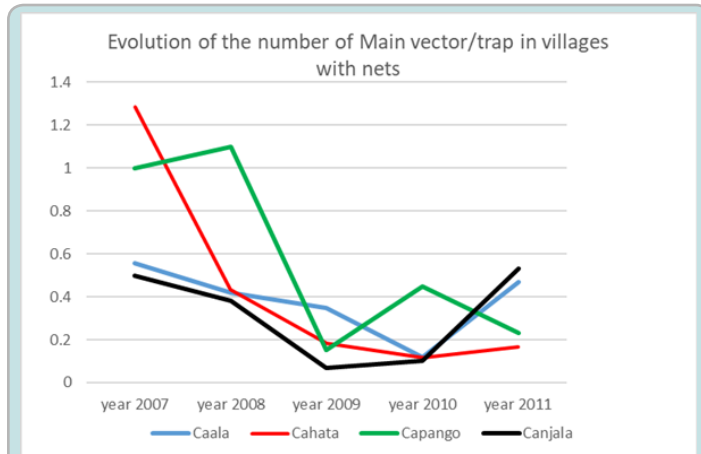

Graph 2a: Yearly Evolution of the "number of Main Vectors/trap" in villages with δLLIN alone (Caala and Cahata) or association δLLIN + ZF (Capango and Canjala).
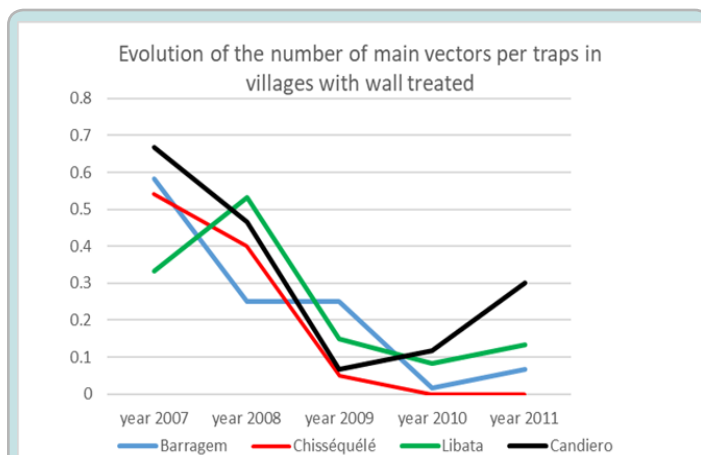
Graph 2b: Yearly Evolution of the "number of Main Vectors/trap" in villages with Wall Lining (Barragem and Chisséquélé) or Indoor Residual Spraying then durable lining (Libata, Candiero).

In the 6 control villages the natural evolution of the densities of MV/trap the first 2 years, i.e. without any implementation of vector control were different **(graph–2c)**, remaining almost the same in Capango, slightly decreasing in Canjala, Chisséquélé; greatly decreasing in Barragem and Candiero; but increasing in Libata.

The first year after Vector Control the drop of the densities of Main Vector/trap was spectacular in every villages except Barragem (graph 2d) where the drop in the densities of MV/Trap was observed in 2010 the following year (graph. 2b).
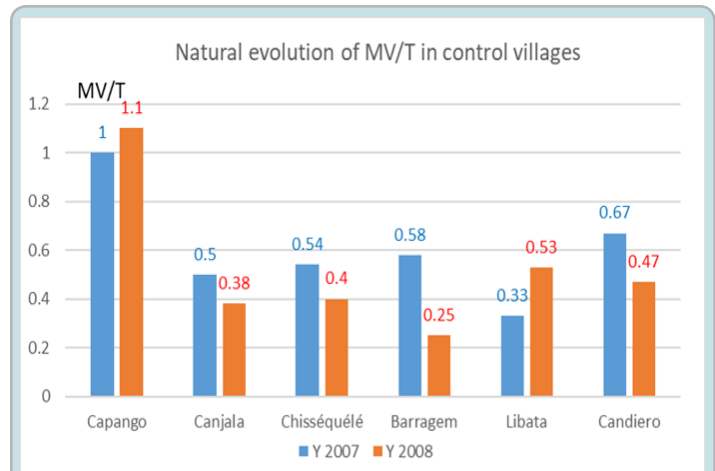
Graph 2c: Natural evolution of the densities of main vector per trap (MV/T) in the 6 control villages before Vector Control implementation.

The first year after Vector Control the drop of the densities of Main Vector/trap was spectacular in every villages except Barragem (graph 2d) where the drop in the densities of MV/Trap was observed in 2010 the following year (graph. 2b).
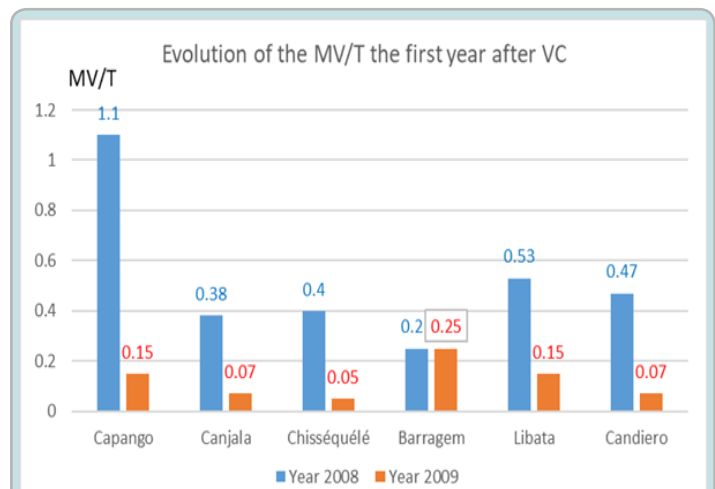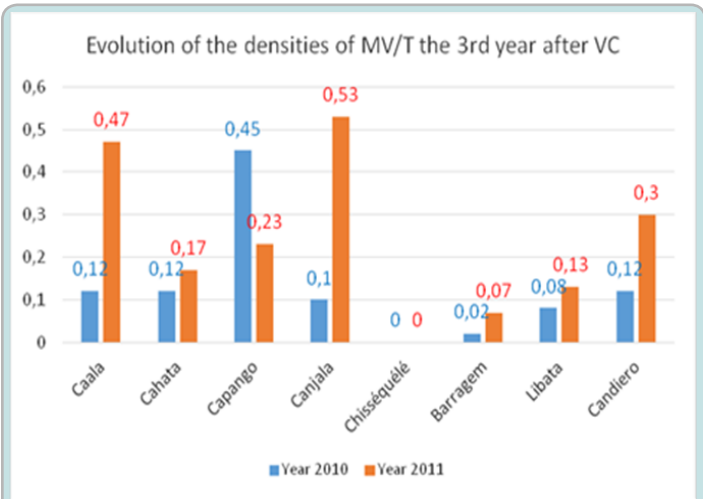

Graph 2d: Evolution of the densities of the Main Vector/trap the first year after Vector Control implementation.

The densities of Main Vector /trap the 3rd year after implementation of Vector Control presented a general increase excepted in Capango (LLIN+ZF) where densities still decreased and Chisséquélé (WL) where densities remained nil (graph 2e).

In the 2 villages where LLIN were given with full coverage in 2008 (Caala and Cahata) 3 years after full coverage the densities of Main Vector increased (in Caala) or slightly increase (in Cahata) (graph. 2a) showing the worrying reduction of their protective efficacy for inhabitants.

In the 2 villages with ITPS only (Barragem and Chisséquélé) the densities remained very low the 3rd year (graph 2e).

Graph 2e: Evolution of the densities of the Main Vectors/trap (MV/T) the 3rd year after complete Vector Control implementation.

When checking the evolution of the average monthly number of main vectors per trap according to the method of vector control it must be highlighted 3 main points: a) the drop in February 2009 soon after VC implementation; b) the long period with very low densities of main vectors inside human houses and c) the increase the third year, but which was not observed in villages with WL (graph 2f).
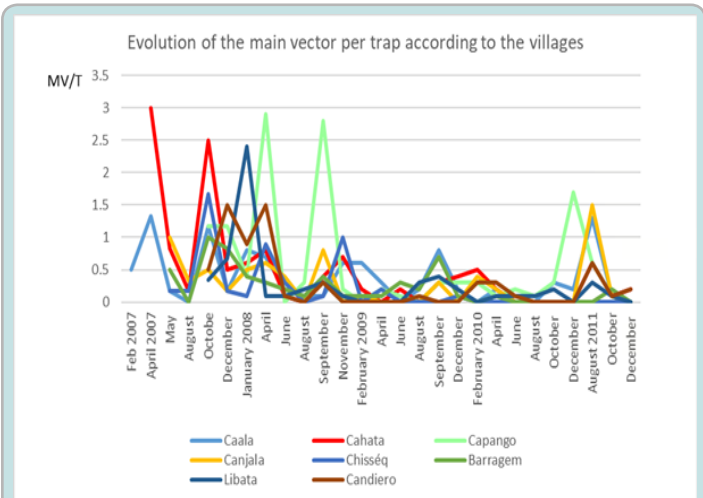
Graph 2f: Long term (5 years) of monthly evolution of densities of main vectors/house according to the method of vector control. (nb MV/t= number of main vector per trap).

When checking the evolution of densities of main vectors/trap before and after implementation of vector control in each village it appeared that the decrease was significant in all villages but Caala (LLIN) and Candiero (IRS) (graph 2g).
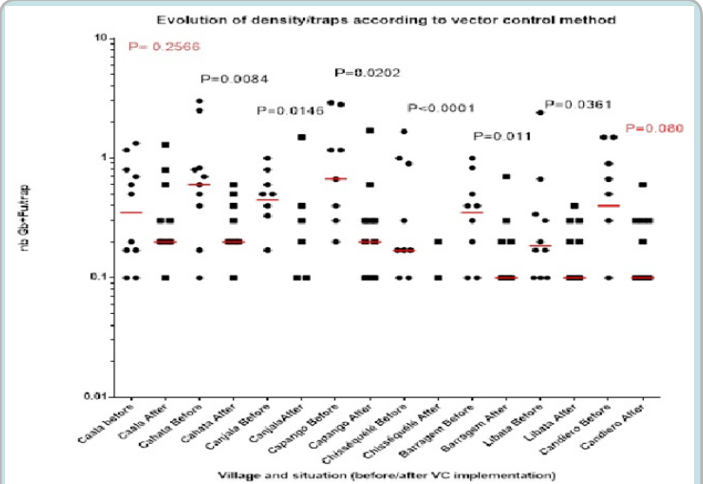
Graph 2f: Long term (5 years) of monthly evolution of densities of main vectors/house according to the method of vector control. (nb MV/t= number of main vector per trap).

Evolution of densities of main malaria vectors according to method of vector control

The four methods of vector control showed the same general trends for the evolution of the average yearly densities of main vectors caught by CDC Light Traps inside human houses (graph. 3a) with the striking drop in 2009 soon after vector control operations and the increase in 2011 i.e. 3 years after complete coverage while in village with WL the densities remained low.
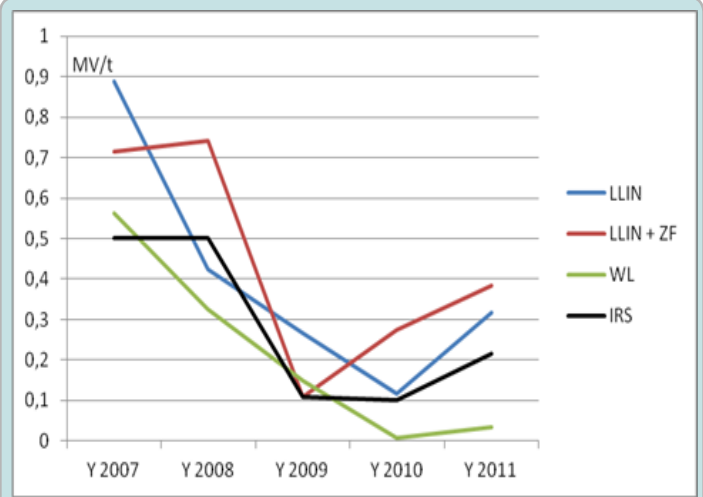

Graph 3a: Evolution of yearly densities of main vectors/trap according to the method of vector control implemented.

Actually every method induced a striking reduction of the main densities/traps (# 71%) (Table 6).

Methods of VC	Years 2007–2008	Years 2009–2010–2011	Diff.
LLIN	0.582	0.217	− 62.8%
LLIN +ZF	0.734	0.23	− 68.7%
WL	0.393	0.070	− 82.2%
IRS then ITPS	0.500	0.127	− 74.7%

Table 6: Evolution of the average of *An. gambiae + An. funestus* caught by CDC Light Traps in paired villages according to the method of vector control implemented, before and after VC implementation.

Statistical analysis of the distribution of densities of main vectors per trap before versus after vector control (graph 3b) showed that the reduction was significant for each method:
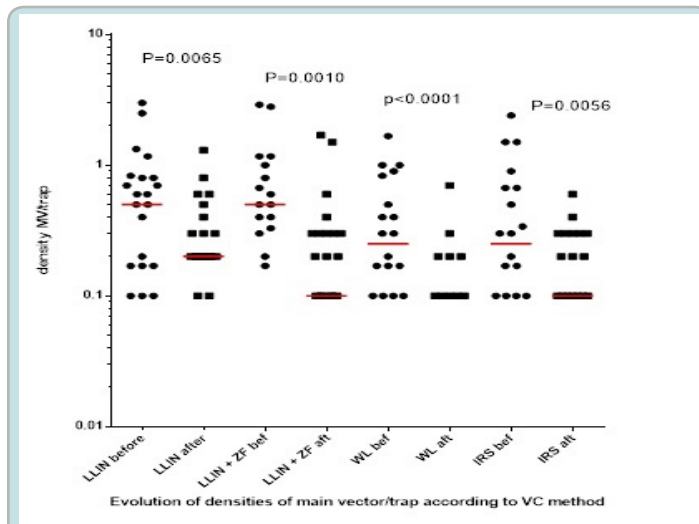
Graph 3b: Evolution of the distribution of the number of main vectors /trap (and median, in red) before/after mplementation of every vector control method.

Each method reduced statistically the number of main vector (MV) by traps and it is worth noticing that durable lining alone reduced also statistically this number of MV/trap

Infectivity of main vectors and its evolution according to VC implemented

28 of the 1133 anopheles analyzed procured an Elisa positive test i.e. a general positivity index of 2.47%; with 8.00% (n= 50) for *An. gambiae*; 3.61% (n= 498) for *An. funestus* and 2.06% for *An. marshalli* (n= 243) (Table 7a).

species	E+	n	s (%)
An. gambiae	4	50	8
An. funestus	18	498	3,61
An. marshalli	5	243	2,06
An. ziemanni	0	75	0,00
An. maculipalpis.	0	206	0,00
An. coustani	0	7	0,00
An. nili	0	11	0,00
An. pharoensis.	1	22	4,55
An. hanckoki	0	10	0,00
An. tenebrosus	0	10	0,00
An. rufipes.	0	1	0,00
total	28	1133	2,47

Table 7a: Infectivity of the different *Anopheles* species for the 5 years of the study (n= number of specimen tested; E+= number of specimen positive with ELISA test).

Elisa tests were performed on 548 specimens of *An.gambiae* and *An.funestus* and 22 appeared positive i.e. an overall infectivity rate of main vectors of 4.01% (table 7b) which is well in the range of the usual sporozoite index reported elsewhere [43].

Data of the number Elisa tests performed and positive one, for each year and each village, are reported Table 7b.

| Villages/ years	Caala	Cahata	Cap.	Canj.	Chiss.	Barr.	Cand.	Lib.	total
Y 2007	0/19	1/36	0/18	1/12	1/13	1/14	1/16	1/8	6/136 4.41%
Y 2008	1/25	1/26	2/66	2/23	1/24	0/15	1/28	3/32	11/239 4.60%
Y 2009	1/21	1/11	0/9	0/4	0/3	1/15	0/4	0/9	3/76 3.94%
Y 2010	0/7	0/7	0/10	0/6	0	0/1	1/4	0/5	1/40 2.50%
Y 2011	0/14	0/5	0/7	0/16	0	0/2	1/9	0/4	1/57 1.75%
total	2/86 2.32%	3/85 3.52%	2/110 1.82%	3/61 4.92%	2/40 5%	2/47 4.25%	4/61 6.55%	4/58 6.89%	22/548 4.01%

Table 7b: Infectivity of *An.gambiae + An.funestus* in each village each year.

From this comprehensible table it is possible to sort out 3 interesting points:

- the evolution of the main vectors infectivity each year (graph 4a)

After a plateau the first 2 years there is remarkable linear evolution of the infectivity (y=0.999x + 5.695; R²=0.9784) between 2008 and 2011.

In 2009, the first year after vector control implementation, the infectivity decreased by 14% compared to 2008, then 36% from 2009 to 2010 then 30% from 2010 to 2011 meaning that in three years after vector control (from 2008 to 2011) the infectivity of main vectors decreased by 62%.
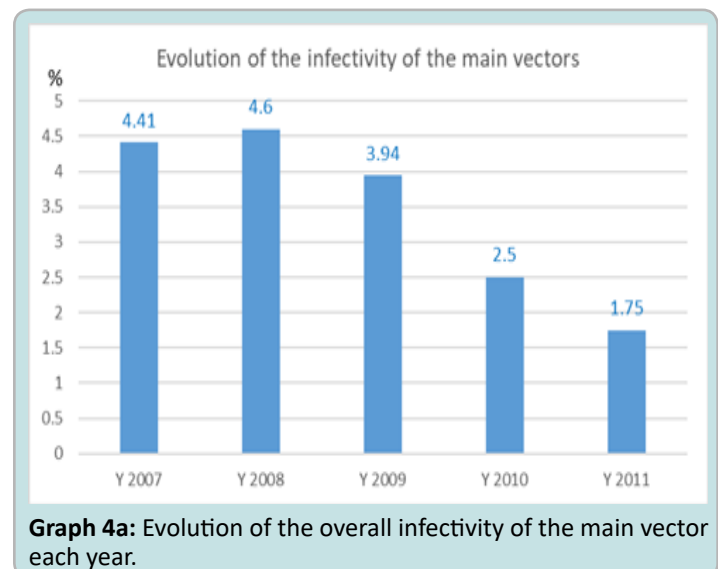

Graph 4a: Evolution of the overall infectivity of the main vector each year.

- a diversity of situation in term of level of infectivity of the main vectors in the surveyed villages.

It appeared villages with low infectivity # 2% (Capango, Caala), "middle" infectivity, on the range of the average (Cahata, Canjala, Barragem) and villages with high infectivity (Candiero, Libata) of main vectors (graph 4b)
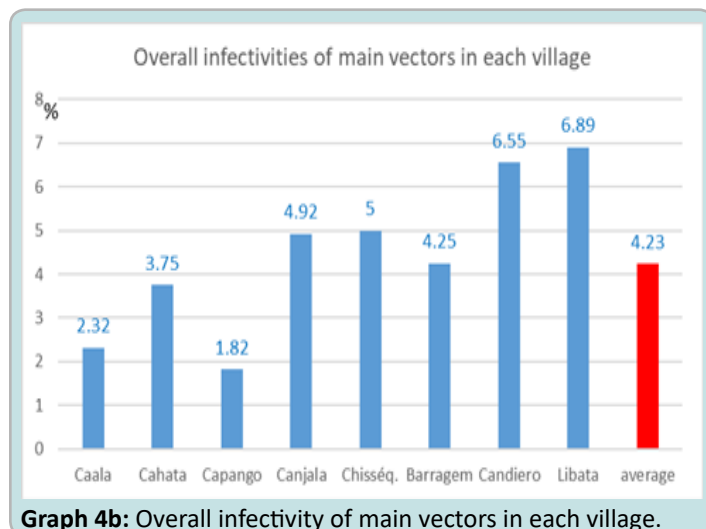
Graph 4b: Overall infectivity of main vectors in each village.

a) due to the low infectivity of vectors in villages we pooled the data of the paired villages according to the method of vector control to compare the infectivity of main vectors before (years 2007–2008)/after (years 2009–2010–2011) vector control operation according to the methods used and it showed a great impact of the association LLIN + ZF (graph 4c) and a general reduction of 36%, from 4.53% (n= 375) to 2.89% (n=173) which is noticeable even if not significant (X^2= 0.83; P= 0.36; OR= 0.63 [0.23–1.73]) (graph. 4c).
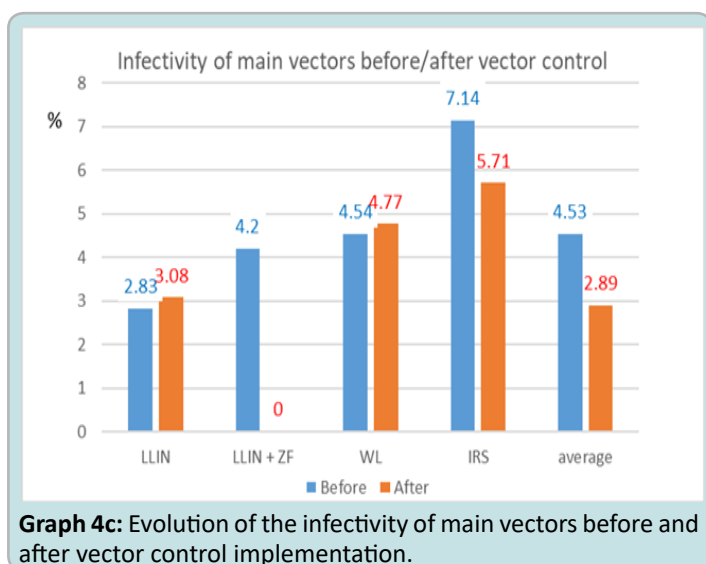
Graph 4c: Evolution of the infectivity of main vectors before and after vector control implementation.

Considering only the 6 villages initially "control" the infectivity dropped form 5.20% (n= 269) before vector control implementation to 2.78% (n=108) after, which is not significant (X^2 corrected= 0.56; P=0.42; OR=0.52 [0.15–1.85]).

It did not seem that the 2 Rounds of λIRS followed by the full

coverage in ITPS had induced a particular reduction of infectivity of vectors in the 2 villages involved.

On the other hand the overall infectivity of *An.gambiae* (4E+/50 specimen analyzed i.e. 8%) was not statistically different from the *An.funestus* one (18D+ /493 i.e. 3.65% (X^2 corrected= 2.21; P= 0.267; OR=0.44 [0.14–1.34]).

Evolution of the "Inside House Inoculation Risk" according to vector control

From the table 6.gathering information on the evolution of the densities of main vector ("ma") with vector control implementation and Table 7b dealing with the evolution of their infectivity ("s") it is possible de make an evaluation of the entomological inoculation rate before and after vector control with the Ross formula h= ma.s (Table 8).

Methods of VC/parameter		LLIN	LLIN+ZS	WL	IRS
Before (years 2007 2008)	ma	0.5824	0.734	0.393	0.5
	s	0.0283	0.0420	0.04545	0.0714
	h_d	0.01648	0.0308	0.0179	0.0357
	h_m	0.494	0.925	0.536	1.07
	h_y	5.933	11.1	6.43	12.85
After (years 2009 2010 2011)	ma	0.217	0.23	0.07	0.127
	s	0.0308	0.0278*	0.0476	0.0571
	h_d	0.0067	0.0064	0.0033	0.00725
	h_m	0.200	0.1918	0.0999	0.2175
	h_y	2.403	2.302	1.2	2.61

Table 8: Evolution of the Entomological Inoculation Rate(h) per day "h_d" per month "h_m" or per year "h_y" according to the method of vector control implemented (* we used the average infectivity rate of main vector in the 6 treated control villages).

It thus appeared a great reduction of the entomological inoculation rate with each one of the vector control method implemented (graph. 5a) which can be estimated at 59.5% with LLIN, and # 79.3% with the association MMIN+ZF, 81.3% with Wall Lining alone and 79.7% with IRS followed by WL with an average of 77% reduction of the entomological inoculation rate after vector control implementation (Graph 5b).
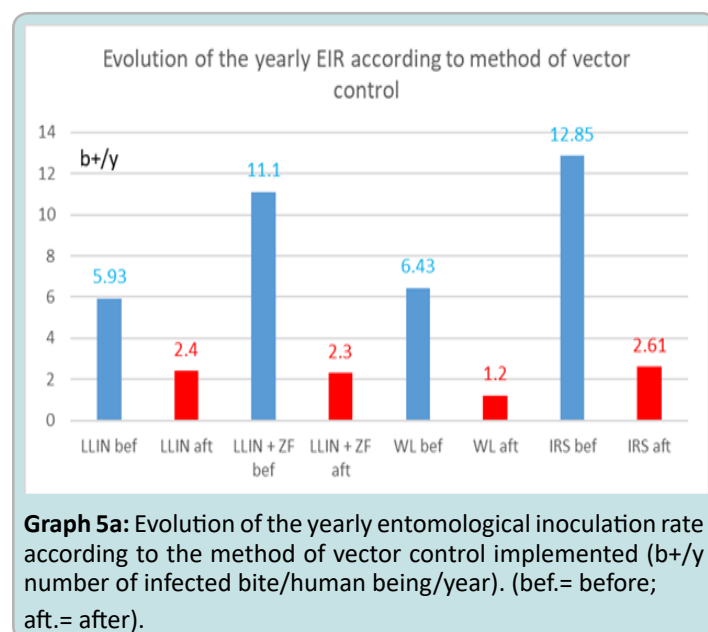
Graph 5a: Evolution of the yearly entomological inoculation rate according to the method of vector control implemented (b+/y number of infected bite/human being/year). (bef.= before; aft.= after).

Graph 5b: Evolution of the Entomological Inoculation Rate according to the method of vector control implemented.
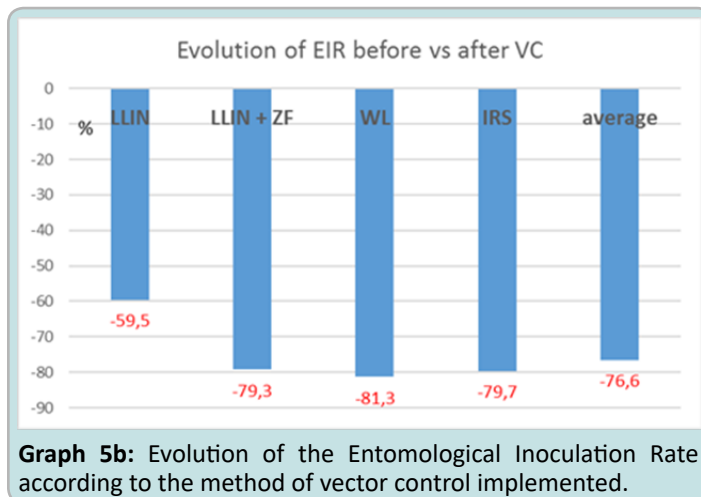
Discussion

This several years trial carried in complete natural field conditions showed that the 4 methods tested induced a clear decrease of the number of main vectors (*An.gambiae* + *An.funestus*) per house in using CDC Light Trap (−63% with δLLIN alone; −69% with LLIN + Durable Lining Zero Fly; −82% with durable lining "Wall Lining" alone and −75% with 2 rounds of λ lambdacyhalothrin followed by WL installation). The four method had same efficacy at that level. It appeared a clear drop the year after complete installation of vector control tools but an increase the 3rd year which is matter of concern. Field surveys showed that in 3 years several nets were torn and thrown outside, discarded in the field, the same for plastic sheetings misused for different purposes such as thread to hang up washed clothes etc. Nevertheless this drop in malaria vector densities in human houses must be kept in mind. The infectivity of vector was in the usual range of other report dealing with these anopheles species [43] and, even not statistically significant, decreased by vector control implementation.

What appeared worth underlining is the drop of entomological inoculation rate by the four tools (−60% with LLIN; and similarly # 80% with the three other methods giving an average of a 78% reduction of inoculation in these villages, a value similar to the one reported elsewhere with Insecticide Treated Nets [49,50]. The similar values on the reduction of inoculation rate obtained by ITPS alone such as IRS is one of the positive input of this program and an interesting asset for the National Malaria Control Program

As it was well underlined [15] «Insecticide–treated durable wall lining (ITWL) may represent a new paradigm for malaria control as a potential complementary or alternate longer–lasting intervention to IRS" and it could also be method to control other vector–borne diseases such as leishmaniasis [51,52] and in urban as well as rural areas [13–15,23]. Their "entomological efficacy and household acceptability" were studied in Africa and South–East Asia [13,53] in Papua New Guinea [24,54].

Several studies were devoted to Insecticide Treated Plastic Sheetings [18], in experimental huts [55,56] or at village level [57].

ITPS were tested treated with pyrethroid permethrin [20] or deltamethrin [42]), or Organophosphate [21] or Carbamate [19,22] in association with pyrethroid treated long lasting nets to deal with the burning issues of pyrethroid resistant vectors.

In Tanzania Emidi *et al* [58] tested the entomological efficacy against *An. gambiae* and *An. funestus* of a novel non–pyrethroid insecticide treated durable wall liner treated with a mixture of two non–pyrethroid insecticides namely abamectin and fenpyroxinate [56,57], showing their deterrent effect.

Durable lining "Zero Fly" was tested with success in India [25–27] where it was considered as "a supplementary vector control intervention and ZVDL (for Zero vector durable lining) has the potential to become a viable alternative to IRS in malaria endemic areas".

According to these studies, the Balombo village scale protocol was implemented to get reliable data for the National Malaria Control Program with its national distribution of Long Lasting Insecticide Treated nets, while the possibility of using the Insecticide Treated Plastic Sheeting (ITPS) could be envisaged due to the "difficulties in organizing first indoor spray programme against malaria in Angola" [12].

Compared to classical IRS the Durable Lining have several advantages such as the actual community involvement as they cut DL at the right dimension and pinned themselves the plastic sheeting on the walls inside their own houses (while for Indoor Residual Spraying there are external team who enter the houses for spraying and this is quite often not well accepted). Being on the wall ITPS did not interfere with movement inside the small room, a point often noticed when nets are hanged over sleeping units such as mattress and other unrolled in entrance or living rooms. This participates to their acceptability as it was well noticed recently in Huambo area [14] close to Balombo, and elsewhere [15,24]. On the other hand they did not induce the "reject behaviour" for claustrophobia as it was observed with bed nets. In Balombo houses several pictures and furniture were noticed hanged on the ITPS showing the appreciation of their "aesthetic value" by populations.

Long lasting efficacy of ITPS has also a great operational advantage, being still efficient several years after their installation compared to the short efficacy of IRS, usually of 3 to 6 months and as recently reported "durable lining can be considered an advanced form of long–lasting IRS where insecticide is gradually released form an aesthetically attractive wall lining to provide vector control for several years"[14].

It has to be underlined that, in the Balombo trial, ITPS alone were as efficient as IRS in reducing the inoculation rate by some 80% and are as efficient alone as associated with LLIN. According to the model of ITPS used the average amount of insecticide inside the house could be different but higher than LLIN alone or IRS only. The amount of insecticide per house increased with Durable Lining (almost 2 times more than classical Permanet 2.0) and the association Permanet2.0 + durable lining model Zero Fly increased by almost 5 times the amount of insecticide compared to full coverage in PermaNet2.0 alone. But it appeared that such increase in amount of insecticide was not followed by a spectacular impact in the density of main vectors caught by CDC Light Trap inside human houses.

According to these positive entomological results obtained by durable lining and their acceptability noticed in several places added to observation done in areas where vectors are

resistant to pyrethroid it could be proposed an association of pyrethroid treated nets (with PBO or pyriproxyfen) and "other than pyrethroid (carbamates, organophosphate)" treated durable lining to deal with the worrying spreading of insecticide resistant vectors [59,60] and participated thus to the targeted malaria elimination.

The data of Balombo trial confirmed the assertion of Chandre *et al* (2010) that "ITPS has a potential role for community control of malaria" [20].

Author contributions

PC designed the protocol; participated to field surveys and specimen determination, analyzed data and drafted the manuscript; JCT did initial training, laboratory analysis and participated to field surveys; VF participated to field surveys; FF participated to the protocol and the follow up; GC participated in writing and check; FG participated and finalized data analyses.

Declaration of Competing Interest

The authors declare that they have no competing interests.

Ethical Approval

The study was done as part of and for the National Malaria Control Program.

Insecticide Treated Mosquito Net (P 2.0) + Zero Fly

«Insecticide Treated Plastic Sheeting» ITPS

Zero Fly
ITPS « Wall Lining »

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