Research Article

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Influence of Partial and Full Coverage on Long Lasting Deltamethrin Treated Nets ("δLLIN") on *Plasmodium falciparum* Parasitaemia in 2 Villages around Balombo Town (Benguela Province, Angola)

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ABSTRACT

During two consecutive years 40 random cross sectional parasitological surveys were done in 4 villages around Balombo town (Benguela Province, Angola): 2 villages receiving classical Long lasting deltamethrin treated nets Perma©Net 2.0 and 2 villages remaining as control. The LLIN distribution were done in 2 steps: first year at least one net/house; second year one net/sleeping unit to get full coverage.

The current analysis focus on *Plasmodium falciparum* infections (prevalence, parasitaemia) in children ≤5 years considered as "at risk group". Plasmodial infections were observed in 1059 of the 2806 thick blood films made in the field (=37.7%) on symptomless patients then microscopically examined in the medical department of the Angolan Sonamet Company in the framework of its "Malaria Control Programme". *P. falciparum* prevalence actually significantly decreased from year 1 ("partial coverage") to year 2 ("full coverage") in LLIN villages (from #41% to #34%) but a significant decrease was also noticed in control villages (from #42% to #24%) where the drop was higher than in LLIN villages.

Several in-depth analyses were done comparing the evolution of *P. falciparum* parasite load in LLIN *versus* control villages. Comparison of parasitological surveys done at the same period of the years to avoid the influence of seasonal variations showed that *P. falciparum* parasitaemia significantly decreased after the complete distribution of LLIN but a significant decrease was also noticed in "control" villages. On the other hand entomological evaluation based on regular use classical CDC Light traps inside human houses showed a reduction of #50% of the "number of main vectors (*An. gambiae+An. funestus*) by trap after full "coverage" in LLIN while a "natural" reduction of 40% was noticed in control villages.

The similitude of parasitological data in "LLIN" and in "control" villages raised the issue of the actual use of LLIN, already observed elsewhere, to have is not to use and distribution of LLIN must be done along with sensibilization campaigns and regular monitoring to gain an actual reduction of malaria parasite prevalence and load.

INTRODUCTION

The WHO Global Technical Strategy for Malaria 2016-2030 identified 3 main pillars: Pillar 1. Ensure universal access to malaria prevention, diagnosis, and treatment; Pillar 2. Accelerate efforts towards elimination and attainment of malaria-free status; Pillar 3. Transform malaria surveillance into a core intervention [1]. Vector control, one of the key components of pillar 1 "is an essential component of malaria control and elimination" and "National

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malaria control programmes need to ensure that all people living in areas where the risk of malaria is high are protected through the provision, use and timely replacement of long-lasting insecticidal nets or, where appropriate, the application of indoor residual spraying".

According to Bhatt et al. [2], "it can estimate that 663 million cases of malaria have been averted in sub-Saharan Africa since 2001 as a direct result of the scale-up of 3 key interventions: ITNs, ACTs and IRS. It is estimated that 69% of the 663 million fewer malaria cases attributable to interventions were due to the use of mosquito nets, 21% due to ACTs and 10% due to indoor spraying". Therefore some 524 million cases were averted thanks to vector control and 457 million cases with large scale implementation of insecticide treated mosquito nets.

Some groups, such as children below 5 years old and pregnant women, are "particularly susceptible to malaria illness, infection and death" and are considered as "at risk groups which deserve a high priority of protection". On the other hand, universal ITN coverage is recommended, which is expected to provide community protection, and it was estimated that in 2017," about half of the population at risk of malaria in Africa was protected by impregnated mosquito nets"; however, "protection by persistent spray did not exceed 3% in 2017" [3].

To assess the ITN coverage one indicator was "at least one home-impregnated net" [4]. In this double concept «one net/house» and «universal coverage» we developed a program of distribution of long-lasting insecticide treated nets ("LLIN") in two villages (Caala and Cahata) in a 2 steps process: first at least one ITN per house and then, one ITN per sleeping unit as part of a general vector control programme in the Balombo region (Benguela Province, Angola) implemented to compare 4 vector control methods [5,6]. One of the problems for the epidemiological evaluation of vector control is the choice of indicators considering that the aim of the control is to reduce the "burden" of malaria that can be understood in terms of «malaria-parasite» and/or "malaria-disease".

Several methods can be used for epidemiological surveys [7] mainly "passive detection case" in Health Center or "active detection case" directly in communities [8]. One of the problems in Health Centre is often the well-known lack of reliability of data, from both clinical and parasitological examinations [9-11] with some overdiagnosis of "malaria" reported as high as 85% [12] and its consequences on drug pressure and the risk of increasing drug resistance.

As early as 1949 it was considered that "when diagnoses are based on symptomatology alone it is likely of any errors will be commonly inclusive rather than exclusive and as a consequence malaria will appear to be of exaggerated importance in the community" [13]. As there is no health center in the 8 villages of the trial, the population must go to the Hospital of the nearby city, Balombo, with a Pediatrics department and a laboratory for some biological analysis and there is obviously a classical "bias of recruitment" so that it is not possible to get reliable database from it. Active detection case has been shown to be reliable in bed net assessment studies [14,15] but requires resources that are not always available.

"For an extended period, the detection of the malaria parasite in blood smears was the only available laboratory aid to diagnosis" [16] but in areas of permanent malaria endemicity the classical cross-sectional surveys show plasmodial prevalence of 50% and more in symptomless carriers, especially in the areas studied [6,17,18]. And "the opinion is entertained that if in any ambulatory group in whom 50 per cent infection is found on a single examination, the group is actually nearly 100 per cent infected [13].

An additional criterion was then proposed taking into account the level of parasitaemia [19,20] with a "pyrogenic threshold" or "parasite threshold" or "pyrogenic level" or "pyrexia level" or "clinical threshold") [13,21,22] which is variable according to the ages, epidemiological conditions and other factors. It has been the subject of several studies [23-25] and even controversies considering that "the determination of this level in falciparum infections is practically impossible" [16].

The objective of our study was not to determine this pyrogenic threshold in the targeted populations but to analyze the evolution of the parasite prevalence rate and densities of *P. falciparum* in the "at risk" group, i.e. below 5 years old children depending on the treated net coverage and comparing with 2 other close villages of the region, Barragem and Chisséquélé without organized vector control operations and considered as "control".

MATERIALS AND METHODS

The ecological situation of Balombo, and the surrounding villages involved in the long-term malaria vector control program, were already presented [5,6]. The present analysis deals with 4 (of the 8 initially studied for the comprehensive evaluation of 4 methods of vector control), two which received impregnated nets and two closed villages remaining as "control".

First census

A house census was done in each village by the local health worker. Every house received a number and was localized by GPS for mapping with classical software. Distributions of deltamethrin treated LLIN Perma©Net© 2.0 ("δLLIN") were done in Caala village (14°47'E; 12°25'S; 238 houses; 792 inhabitants; 469 sleeping units) and Cahata village (14°48'E; 12°20'S; 138 houses; 484 inhabitants; 442 sleeping units). A sleeping unit is "anything" used by inhabitants to sleep: mattress, piece of cardboard, loincloth, grass skirt, etc. Two villages of the same area served as "control" for the first 2 years: Barragem (14°47'E, 12°21'S; 134 houses, 620 inhabitants) and Chisséquélé (14°47'E; 12°22'S; 181 houses; 418 inhabitants) few kilometers from Caala and Cahata, far enough to avoid any flight of mosquitoes from village to village.

LLIN distribution process

The distribution of LLINs in Caala and Cahata were done following a two steps process according to the objectives in coverage:

a. February 2007: the objective was "at least 1 LLIN/house": distribution of 360 LLIN in Caala and 310 in Cahata; = "partial coverage".

b. February 2008: the objective was a full coverage with 1 LLIN/sleeping unit: distribution of 277 LLIN in Caala and 195 in Cahata.

c. December 2008: complementary distribution of 49 LLIN in Caala and 25 in Cahata.

Therefore, 686 LLIN were given in Caala for 469 sleeping units and 530 LLIN for 442 sleeping units in Cahata allowing a complete coverage in LLIN in both villages. An imperative for Roll Back

Malaria is to monitor progress in coverage on nets. Two indicators that are of potential value are (i) the proportion of households that have one or more nets and (ii) the proportion of children under 5 years of age who use (i.e., sleep under) a net and they were fulfilled with this process.

Parasitological surveys

Random cross sectional surveys (CSS) were made every 2 months, following the same protocol as the one implemented in Côte d'Ivoire for the evaluation of the efficacy of lambdacyhalothrin treated nets ("λITN") in areas where local *An. gambiae* population is resistant to insecticide with a high kdr level mechanisms [14]. Field made blood thick films were Giemsa colored *in situ* then microscopically examined in Lobito in the Medical Department of the Angolan Sonamet© Society which sponsored a "Malaria Control Program" (MCP) including this village scale vector control trial in Balombo.

Parasite density was estimated in counting the number of parasite *versus* 200 white blood cells with a further evaluation of parasitaemia/ml of blood considering 8000 white blood cells/ml. A percentage of 10% of these blood films was double blind checked in Yaoundé (OCEAC Parasitology Department).

From the initial listing of patients of the field surveys, we then extracted data of "under five children" (≤5) considered as the "at risk" group to analyze the evolution of prevalence and parasite load for *P. falciparum* (only) according to the 2 steps implementation of LLIN coverage in Caala and Cahata and comparing to the situation observed in the 2 closed control villages.

Statistical analysis

Percentages were compared with the classical Chi2 test (CDC EpiInfo software) and parasitaemia were analyzed with the non-parametric Mann-Whitney test with GraphPad software. As well underlined by Bruce-Chwatt [26] a useful malariometric index is the parasite density index considering 10 classes of parasitaemia with a geometric increase (less than 100par/µl; 101 to 200; 201 to 400; 401 to 800 etc), each parasitaemia belongs to one class. The parasite density index is then calculated by multiplying the number of individuals of each class of parasitaemia by the numero of class of parasitaemia, adding the 10 number obtained then dividing this figure by the total number of positive slides (Annex 1).

RESULTS

Evolution of *P. falciparum* infections prevalence

Overall Plasmodial prevalence

40 random regular cross-sectional surveys (CSS) were done in the four villages in 2007-2008 and among the 2806 thick blood films done from children ≤5 years, 1059 were found positive with *Plasmodium falciparum* i.e., an overall Plasmodic Prevalence of 37.7% with variations in time and space (Table 1).

Table 1: *P. falciparum* prevalence among children ≤5 years old in villages with LLIN (Caala and Cahata) and control villages (Chisséquélé and Barragem) (CSS= cross sectional surveys, parameter; Pf+ = blood films with *Plasmodium falciparum*; n= number of thick films examined; VC= vector control; 11 or 9 CSS= number of cross sectional surveys done; "Chisséq". = Chisséquélé; Y2007 and 2008 = years 2007 and 2008).

Villages	Caala 11 CSS			Cahata 11 CSS			Chisséq. 9 CSS			Barragem 9 CSS		
Parameter	Pf+	n	%	Pf+	n	%	Pf+	n	%	Pf+	n	%
Y 2007	184	412	44.7	204	406	50.2	79	216	36.6	103	219	47.0
Y 2008	144	447	33.2	137	376	36.4	99	400	24.7	109	330	33.5
villages	328	859	38.2	341	782	43.6	178	616	28.9	212	549	38.6
VC+ or -	669P+/1641 = 40.8%						390P+/1165 = 33.5%					

The results of the 40CSS are reported in Figure 1 where it appeared that the highest level of *P. falciparum* infections (>50%) were usually noticed during the year "one" then appeared some kind of "stabilization" at a lower level.

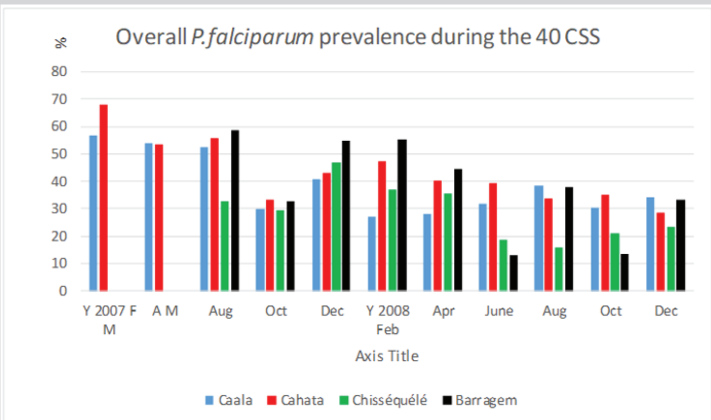

Figure 1: Results of *P. falciparum* prevalence noticed during each one of the 40CSS done in the 4 villages during the 2 consecutive years. (FM = February, March; AM = April, May).

In LLIN villages (Figure 2a) a sharp drop occurred soon after the first distribution of LLIN the first year (from #60-70% to #30%) then a stabilization at around 35% with an increase during the rainy season which is well marked in Cahata.

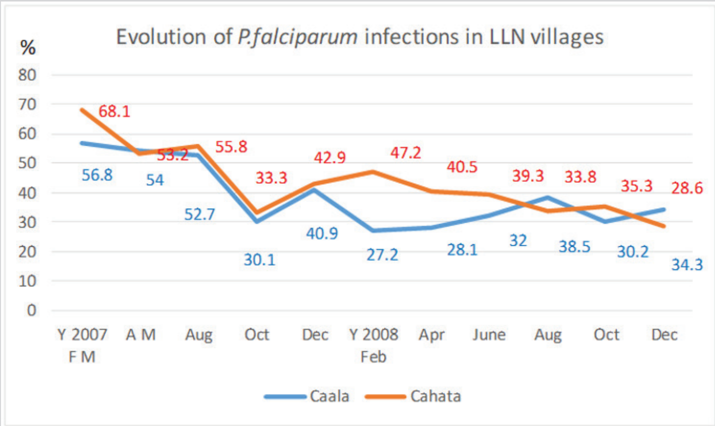

Figure 2a: Evolution of *P. falciparum* infections in LLIN villages.

In control villages (Figure 2b) the increase of rainy season is well marked in Barragem and Plasmodial infections remained stabilized in Chisséquélé.

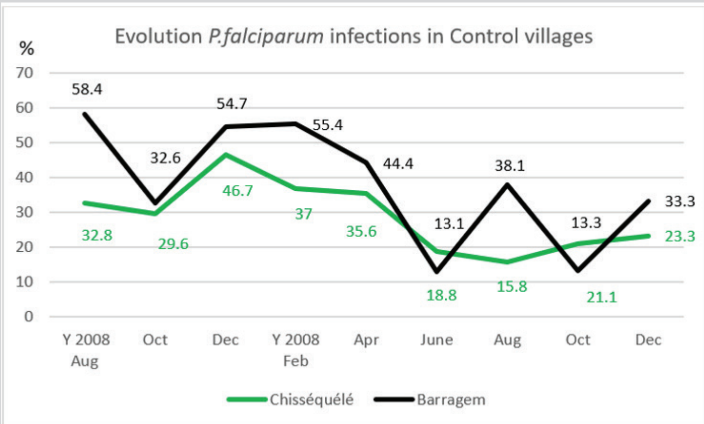

Figure 2b: Evolution of *P. falciparum* infections in control villages.

In every village the overall Plasmodial index dropped significantly from 2007 to 2008 (Figure 3):

I. in Caala: $\chi 2=14.07$; P-values=0.00018; OR=0.59 [0.45-0.78]

II. in Cahata: $\chi 2=15.14$; P-values=0.00099; OR=0.57 [0.43-0.76]

III. in Chisséquélé: $\chi 2=9.54$; P-values=0.0021; OR=0.57 [0.39-0.82]

IV. in Barragem: $\chi 2=10.89$; P-values=0.00096; OR=0.55 [0.39-0.79]

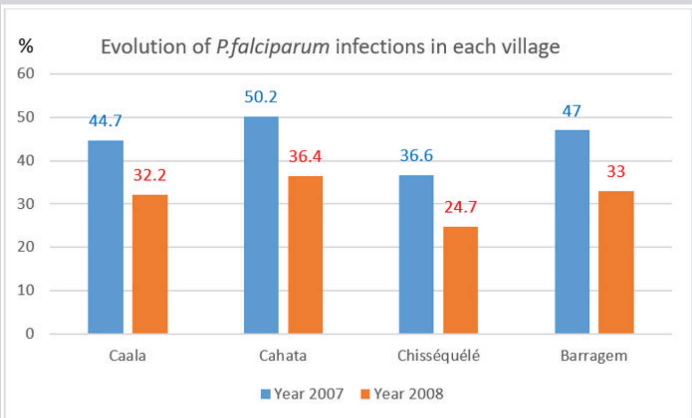

Figure 3: Evolution of *P. falciparum* infections diagnosed in children ≤5 years each year in each village.

From year to year the overall *P. falciparum* infections significantly and almost similarly, decreased, by 28% in LLIN villages (χ2=30.0; OR=0.57 [0.47-0.71]) and by 32% in control villages (χ2=21.8; OR=0.55[0.43-0.71]) (Figure 4a).

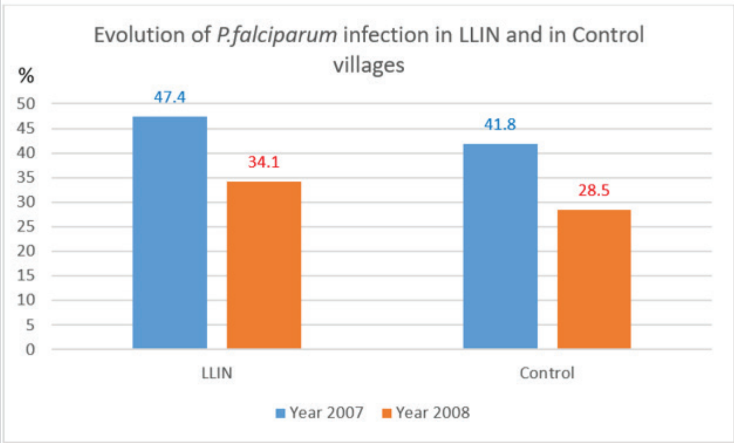

Figure 4a: Evolution of *P. falciparum* infections in villages with LLIN and control villages.

In 2007 the overall *P. falciparum* infections were similar in LLIN and control villages (χ2=3.58; P-values=0.058; OR=0.79 [0.63-1.08]) while in 2008 *P. falciparum* infections were significantly lower in control than in LLIN villages (χ2=5.72; P-values=0.0167; OR=0.77 [0.62-0.95]) (Figure 4b).

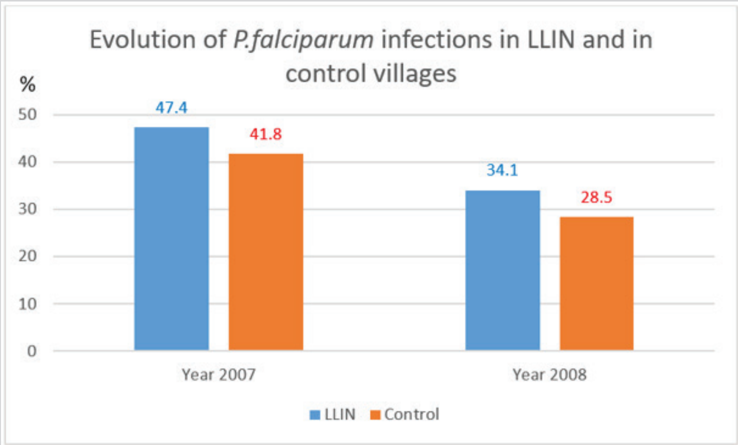

Figure 4b: *P. falciparum* infections in LLIN and in control villages in years 2007 and 2008.

With the parameter "prevalence" it thus did not appear any special impact of LLIN when considering the overall data.

Comparison of the evolution during the 36 CSS done at the same period in 2007 and 2008 in the 4 villages

For operational reasons 2CSS (March and May) were done in the 2 "LLIN" villages (Caala and Cahata) but not in the 2 control villages (Chisséquélé and Barragem) in year 2007.

Therefore, to be relevant, the comparison "year to year" could deal with data of the same period of the year.

Data gained during the 3 common CSS of year 2007 (August, October, and December) in the 4 villages and the 6 simultaneous CSS regularly done every 2 months in year 2008 (February, April, June, August, October and December) are gathered in Table 2.

Table 2: *P. falciparum* prevalence observed during CSS done at the same period of the year in the 4 villages (LLIN = Long Lasting Insecticide treated Nets).

Years	Villages	LLIN		Control	
		Caala	Cahata	Chisséq.	Barragem
Y 2007	n	301	221	216	219
	Pf+	123	92	79	103
	%	40.9%	41.6%	36.6%	47.0%
Y 2008	n	447	376	400	330
	Pf+	144	137	99	109
	%	33.2%	36.4%	24.7%	33.0%

In LLIN villages the *P. falciparum* infections significantly dropped from 2007 to 2008; respectively from 41.2% (n=522) to 34.1% (n=823) (χ2=6.81; P-values=0.0090; OR=0.74 [0.59-0.93]) while it also dropped significantly in control villages: from 41.8% (n=435) to 28.5% (n=730) (χ2=21.80; OR=0.55 [0.43-0.72]) (Figure 5a).

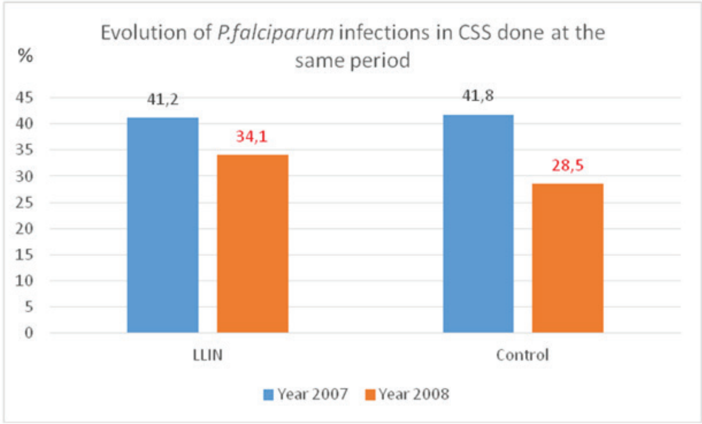

Figure 5a: Evolution of *P. falciparum* infections noticed during CSS done at the same period of the year in LLIN and in control villages.

Thus, the natural decrease appeared more important in control villages (-32%) than in LLIN villages (-17%).

In 2007 the levels of *P. falciparum* prevalence were comparable in LLIN and in control villages (χ2=0.0415; P-values=0.84; OR=0.97 [0.75-1.26]) while in 2008 they were statistically lower in control than in LLIN villages (χ2=5.72; P-values=0.0167; OR=0.77 [0.62-0.96]) (Figure 5b).

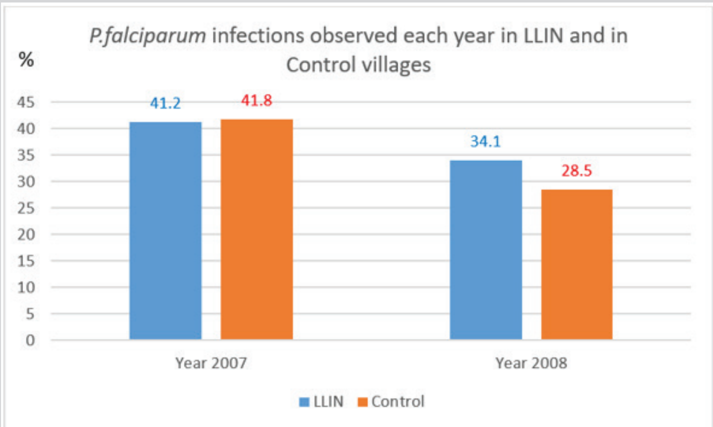

Figure 5b: *P. falciparum* infections noticed in LLIN and control villages during the 9 CSS done at the same periods of the year.

With these 36 CSS done simultaneously in LLIN and in control villages *P. falciparum* prevalence did not appeared as a relevant indictor to measure the eventual impact of LLIN distribution, partial *versus* total coverage even if a significant drop was noticed.

Comparison on the CSS done simultaneously in August-October and December in 2007 and in 2008 in the 4 villages.

Comparing these 24 CSS done simultaneously in LLIN and control villages seemed pertinent to avoid seasonal variations for a better analysis of the evolution of *P. falciparum* infections in children ≤5 years old according to the coverage with LLIN in Caala and Cahata and not any organized implementation of vector control in Chisséquélé and Barragem. Results of these simultaneous CSS are gathered in the Table 3.

Table 3: Evolution of *P. falciparum* infections diagnosed in children ≤5 years old simultaneously in the 4 villages (n = number of blood thick films examined, Pf+= number of *P. falciparum* positive blood films).

Years	Parameters	Caala	Cahata	Chisséquélé	Barragem
Y 2007	n	301	221	216	219
	Pf+	123	92	79	103
	%	40.9%	41.6%	36.5%	47.0%
Y 2008	n	227	220	188	168
	Pf+	80	71	37	50
	%	35.2%	32.3%	19.7%	29.8%

P. falciparum infections were diagnosed in 635 of the 1760 thick blood films i.e. a Plasmodic Prevalence of 36.1% with variations from year to year according to villages and vector control, or not, implementation.

P. falciparum infections remained similar in Caala (χ2= 1.728; P-values= 0.188; OR= 0.79 [0.55-1.12]); but significantly decreased in Cahata (χ2= 4.14; P-values= 0.042; OR= 0.67 [0.46-0.98]), in Chisséquélé (χ2= 14.01; P values< 0.05; OR=0.42 [0.27-0.67]) and in Barragem (χ2= 11.86; P-values < 0.05; OR= 0.45 [0.33-0.61]) (Figure 6).

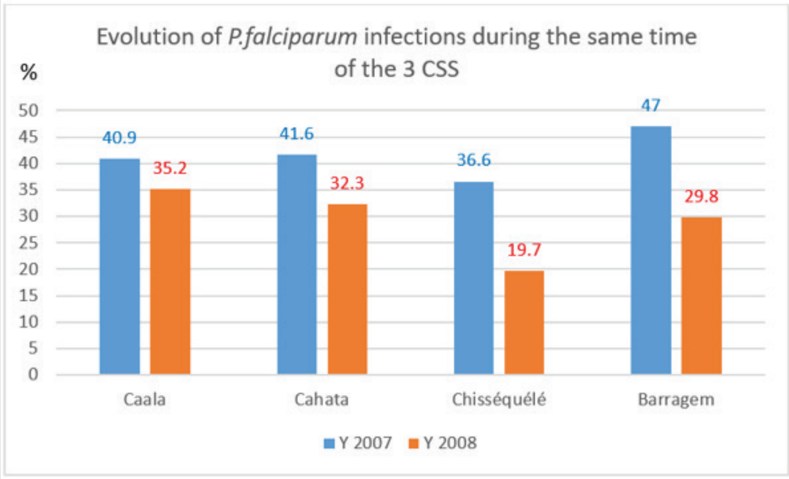

Figure 6: *P. falciparum* infections noticed during the 3 CSS done at the same period (August, October, and December) in the 4 villages in 2007 and 2008.

Considering the situations "LLIN" *versus* "control" from year 2007 to 2008 the *P. falciparum* infections significantly decreased, by 18% LLIN villages, from 41.2% (n=522) to 33.8% (n=447) (χ2=5.62; P-values=0.0177; OR=0.73 [0.56-0.95]) and by 41% in control villages: from 41.8% (n=435) to 24.4% (n=356) (χ2=26.41; P-values<0.05; OR=0.45 [0.33-0.61]) (Figure 7).

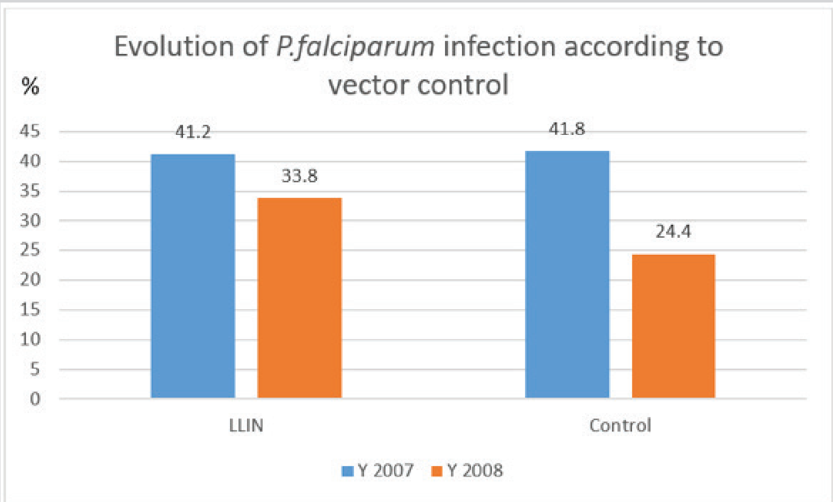

Figure 7: Evolution of *P. falciparum* infections noticed during the CSS simultaneously done in the 4 villages.

On the other hand, *Plasmodium* prevalence were similar in LLIN and in control villages in 2007 (χ2=0.0415; P-values=0.838; OR=0.97 [0.75-1.26]) but were significantly lower in control than in LLIN villages in 2008 (χ2=8.29; P-values=0.00397; OR=0.63 [0.48-0.86]).

Considered altogether the overall *Plasmodium falciparum* infections in children ≤5 years old significantly decreased from year 2007 (Pf=41.5%; n=957) to 29.6% (n=803) in 2008 (χ2=26.56; OR=0.59 [0.49-0.72]).

Therefore, whatever the results of CSS were compared (overall; same 9 periods, same 3 periods) it did not appeared any particular impact of LLIN from partial to full coverage with the "plasmodial prevalence" as indicator.

Evolution of *P. falciparum* parasitaemia

Evolution in LLIN villages

Analyzing the 669 *P. falciparum* positive thick blood films noticed during the 11 Cross Sectional Surveys (CSS) done simultaneously in 2007 (5 CSS/village) and in 2008 (6 CSS/village) in both Caala and Cahata shows that the *P. falciparum* parasite load in under 5 children significantly decreased with the full coverage in "P2.0" LLIN in each village (Figure 8): in Caala (P-value=0.0131) and in Cahata (P-value<0.0001) and from year 2007 to year 2008 (P-value<0.0001).

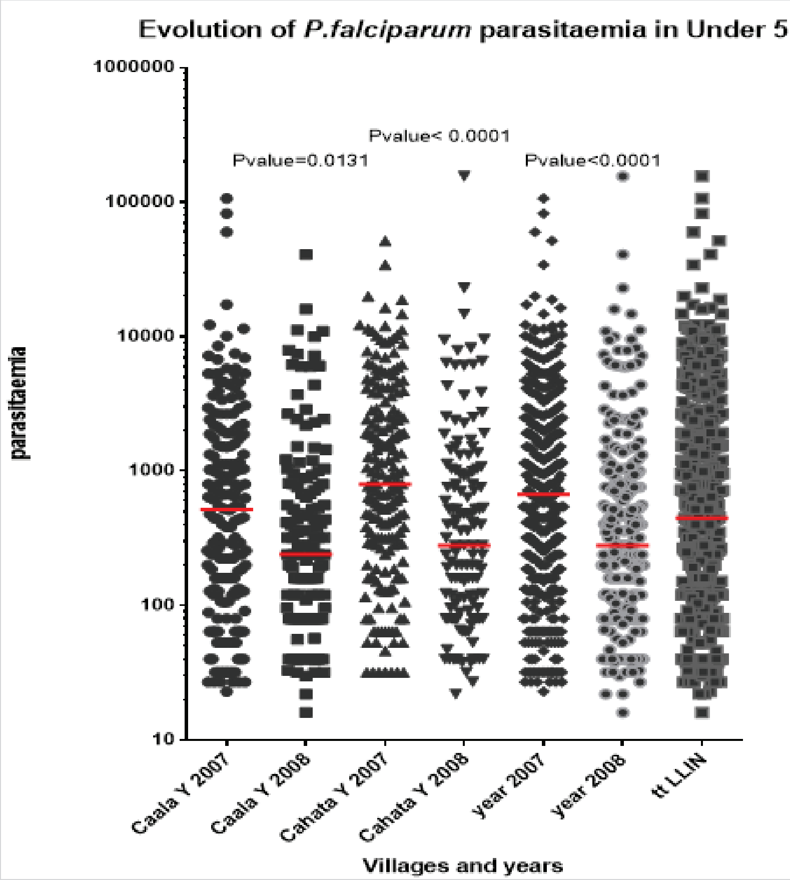

Figure 8: Evolution of *P. falciparum* parasite load in ≤5 years old children in villages with LLIN (the horizontal bar mean the median value).

All main parasitological indicators noticed during these CSS are gathered in the Table 4.

Table 4: *P. falciparum* parasitaemia in ≤ 5 children in the 2 villages with LLIN (*= 5 CSS; ** = 6 CSS; nb Pf+= number of *P. falciparum* positive thick films analyzed; arith. mean= arithmetic mean; SD= standard deviation; geom. mean= geometric mean; nb ≤ 100= number of parasitaemia ≤ 100 par./µl; nb 101-1000 = number of parasitaemia between 101 and 1000 par./µl etc).

Villages	Caala		Cahata		LLIN		LLIN total
Years	Y 2007*	Y 2008**	Y 2007*	Y 2008**	Y 2007	Y 2008	
Nb Pf+	184	144	204	137	388	281	669
Arith mean	2833	1388	2745	2432	2786	1897	2413
SD	10905	4111	5388	13544	8454	9893	9090
Geom mean	494	305	845	359	655	280	491
median	518	240	800	280	671	218	448
Nb ≤100 (%)	39 (21.2%)	40 (27.8%)	25 (12.2%)	38 (27.7%)	67 (17.3%)	78 (27.8%)	142 (21.2%)
Nb 101-1000 (%)	78 (42.4%)	76 (52.8%)	86 (42.1%)	64 (46.7%)	164 (42.3%)	140 (49.8%)	304 (45.4%)
Nb 1001-10000 (%)	60 (32.6%)	24 (16.7%)	80 (39.2%)	32 (23.3%)	139 (35.8%)	56 (19.9%)	195 (29.1%)
Nb 10001 1000000 (%)	6 (3.3%)	4 (2.8%)	13 (6.4%)	2 (1.4%)	20 (5.2%)	6 (2.1%)	26 (3.9%)
Nb ≥100001(%)	1 (0.5%)	0	0	1 (0.7%)	1 (0.3%)	1 (0.4%)	2 (0.3%)

Considering the 3 main classes of parasite densities (≤1000 par./µl=low density; 1001-10000par./µl=medium density; ≥100001 par./µl=high density) it appeared, for both villages, a significant decrease (by almost 45%) of medium densities (from #36% to #20%) and an increase of #32% of low densities with full coverage in LLIN (Figure 9).

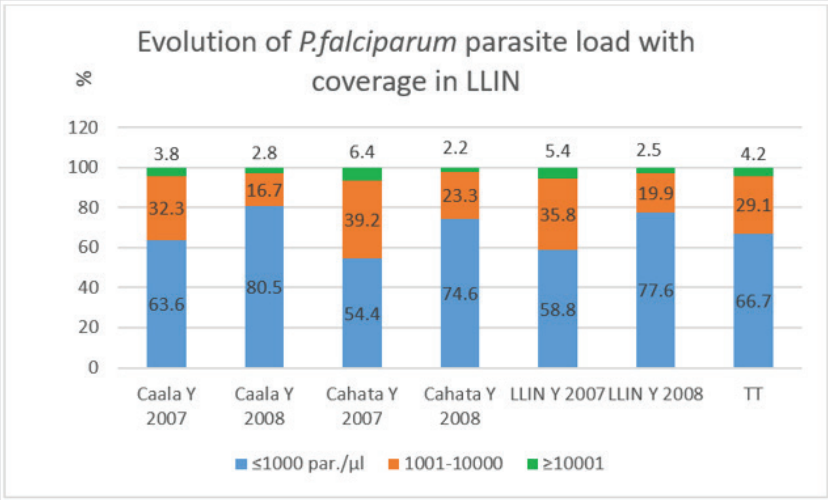

Figure 9: Evolution of the 3 main classes of *P. falciparum* densities from year 2007 to year 2008 in each village (LLIN Y 2007 = partial coverage in LLIN in 2007; LLIN Y 2008= full coverage in LLIN in 2008).

Evolution in the 2 control villages

Analyzing the *P. falciparum* parasite load of the positive thick films noticed in the 2 control villages during the 2 years 2007 and 2008 (Table 5a) showed that in Chisséquélé the parasitaemia remained at the same level (respective median 384 and 320 in Years 2007 and 2008) while it significantly decreased in Barragem (respective median 868 and 267) showing interesting "natural" variations (Figure 10a).

In Year 2007 the parasitaemia was significantly higher in Barragem than in Chisséquélé (P-value=0.0020) while they were similar in 2008 (P-value=0.3649).

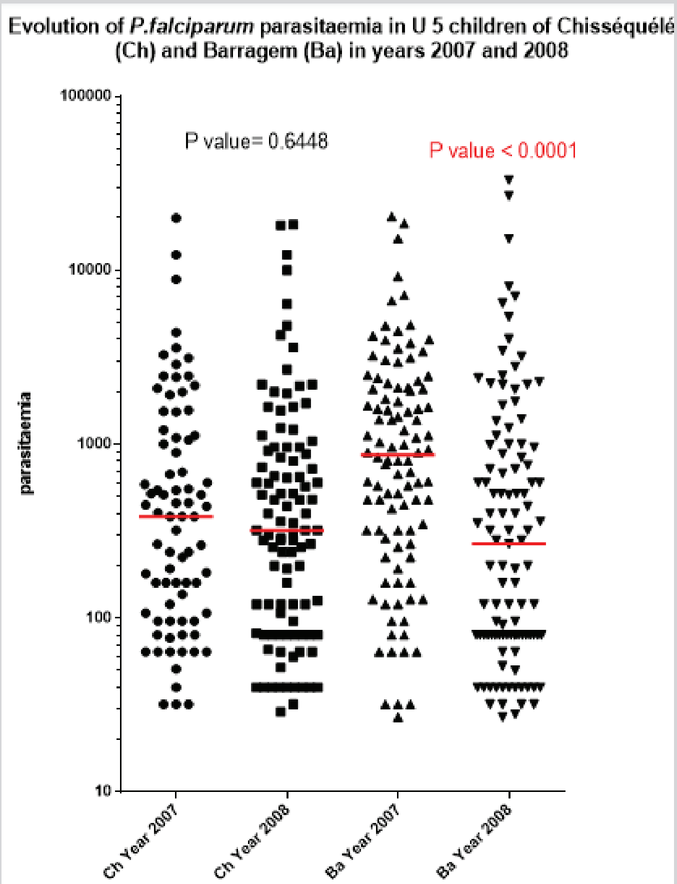

Figure 10a: Evolution of *P. falciparum* parasite load in ≤5 years old children in control villages.

Comparing the data of the CSS done at the same period of the year, August, October, December, in years 2007 and 2008 (Table 5b) it appeared that the *P. falciparum* parasitaemia of ≤5 years old children significantly decreased in both villages (Figure 10b) and from year to year (P-value<0.0001) (Figure 10b).

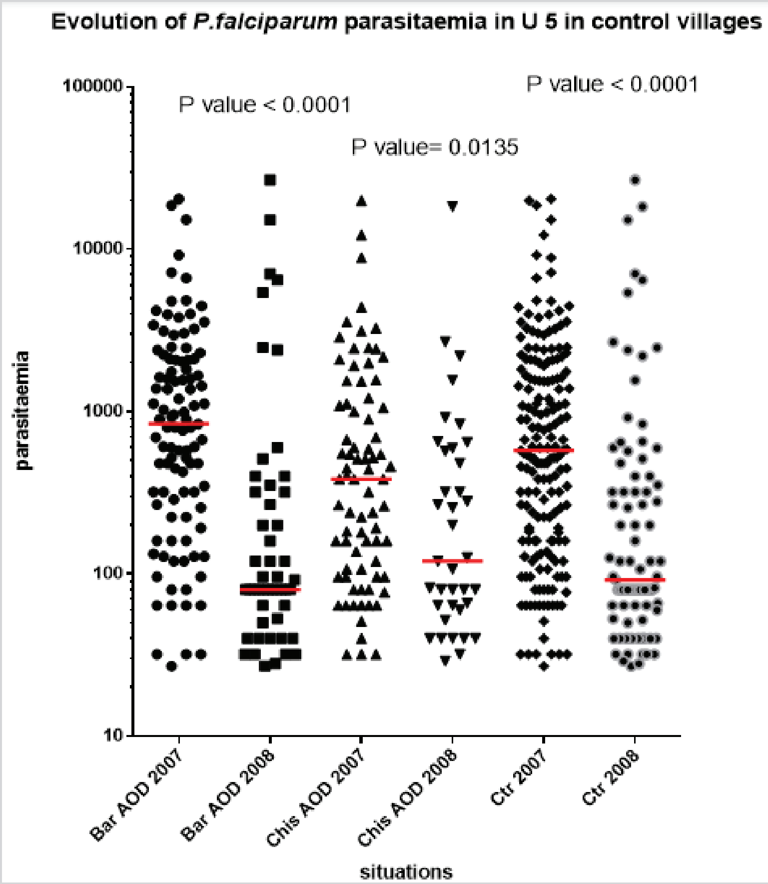

Figure 10b: Evolution of *P. falciparum* parasitaemia in children ≤5 years old during surveys done at the same period (August, October and December) in years 2007 and 2008 in the 2 control villages.

Table 5a: *P. falciparum* parasitaemia in ≤ 5 children in the 2 control villages (* AOD= CSS done in August, October and December; ** CSS done in February, April, June, August, October, December; n= number of positive thick films examined; nb ≤ 100= number of parasitaemia ≤ 100 par./µl; nb 101-1000 = number of parasitaemia between 101 and 1000 par./µl etc).

Villages	Chisséquélé			Barragem			Σ Y 2007	Σ Y 2008
Years	Y 2007 3 CSS AOD*	Y 2008 3 CSS AOD*	Y 2008 6 CSS**	Y 2007 3 CSS AOD*	Y 2008 3 CSS AOD*	Y 2008 6 CSS	6 CSS AOD*	12 CSS
Nb Pf+	79	37	99	103	50	109	182	208
Arith mean	1250	877	1273	1897	1434	1487	1616	1385
SD	2823	3008	3045	3284	4475	4408	3101	3813
Geo mean	387	188	356	735	168	293	556	321
med	384	120	320	840	80	267	564	310
≤100	19 (24.1%)	17 (45.9%)	26 (26.3%)	12 (11.6%)	29 (58%)	43 (39.4%)	31 (17%)	69 (33.2%)
101-1000	38 (48.1%)	16 (43.2%)	51 (51.5%)	45 (43.7%)	14 (28%)	42 (38.5%)	83 (45.6%)	93 (44.7%)
1001-10000	20 (25.3%)	3 (8.1%)	19 (19.2%)	43 (41.7%)	5 (10%)	21 (19.3%)	63 (34.6%)	40 (19.2%)
≥10001	2 (2.53%)	1 (2.7%)	3 (3%)	3 (2.9%)	2 (4%)	3 (2.7%)	5 (2.7%)	6 (2.9%)

Table 5b: Results of surveys done at the same period of the year (August, October, December) in the 2 control villages during the years 2007 and 2008.

Barragem+ Chisséq.	Year 2007	Year 2008	Total
Nb Pf+	182	87	269
Arith mean	1616	1197	1480
SD	3101	3908	3381
Geom mean	556	177	384
median	564	92	384
≤100	31 (17%)	46 (52.9%)	77 (28.6%)
101-1000	83 (45.6%)	30 (34.5%)	11 (42%)
1001-10000	63 (34.6%)	8 (9.2%)	71 (26.4%)
≥10001	5 (2.7%)	3 (3.5%)	8 (3%)

Considering the 3 main classes of parasite load (≤1000; 1001-10000; ≥10001) it appeared, in both villages, a sharp reduction of the "medium density" with an average from about 35% to 9% while low densities increased (from 63% to 87% of parasitaemia) and high densities remained at the same values (#3%) (Figure 10c).

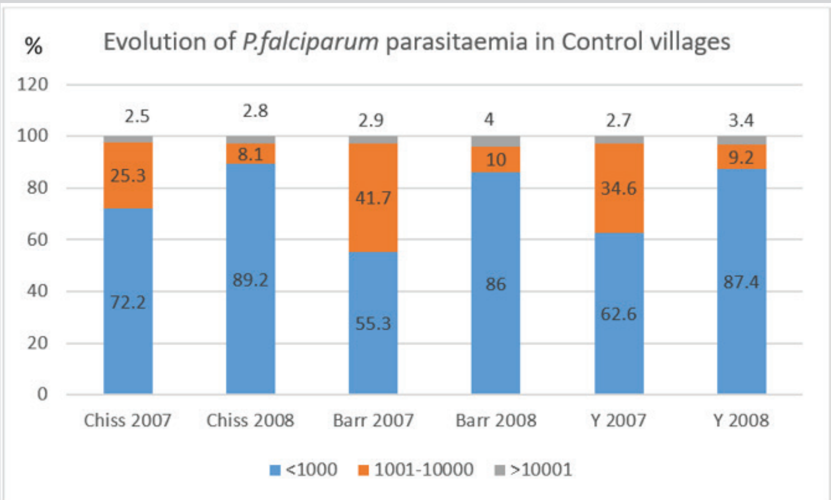

Figure 10c: Evolution of the 3 main classes of *P. falciparum* densities from year 2007 to year 2008 during the CSS done in August-October and December in each control village.

Simultaneous evolution of *P. falciparum* parasitaemia in LLIN and control villages.

a) During the 3 simultaneous CSS of August, October and December of 2007 and 2008.

The analysis of the parasite load of the *P. falciparum* positive thick blood films observed during the 24 simultaneous CSS in the 4 villages in 2007 and 2008 (Table 6a) showed that, from year to year:

i. the parasitaemia did not decreased in Caala (P-value=0.2598) but significantly decreased in the 3 other villages (Figure 11a) in Cahata (P-value<0.0001), in Chisséquélé (P-value=0.0128) and in Barragem (P-value<0.0001)

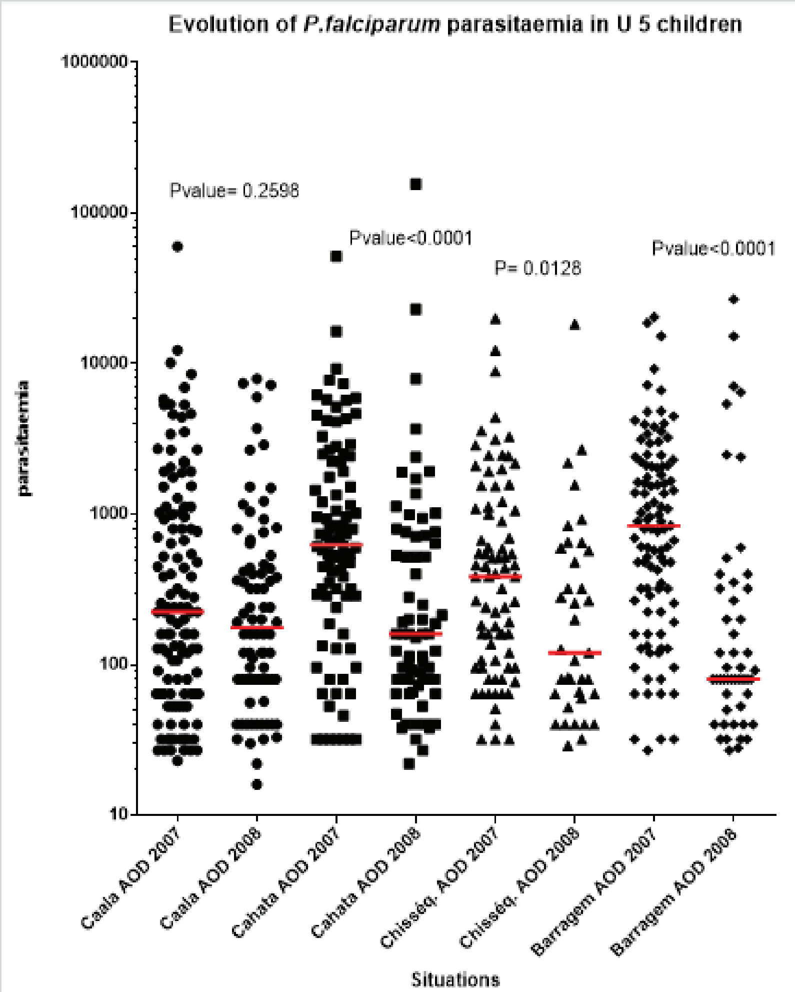

Figure 11a: Evolution of *P. falciparum* parasitaemia during simultaneously done CSS in August, October and December in each village every years.

Table 6a: Results of the CSS simultaneously done in August, October and December in each village every years.

Villages	Caala		Cahata		Chisséq		Barragem	
Years	2007	2008	2007	2008	2007	2008	2007	2008
Nb Pf+	123	80	92	71	79	37	103	50
Arith mean	1571	741	2193	3040	1250	877	1897	1434
SD	5689	1617	5784	18638	2823	3008	3284	4475
Geom mean	300	215	633	227	387	188	735	168
median	224	176	627	160	384	120	840	80

ii. Adding parasitaemia of each year for the 2 LLIN villages and the 2 control villages (Table 6b) showed that the parasitaemia significantly decreased from year to year in LLIN villages (P-value=0.0004) (respective median =440 and 160) and in the control villages (P value<0.0001) (respective median =576 and 92). For the couple of years, 2007+2008, parasite load were similar between LLIN and control villages (P=0.1397) (Figure 11b) (respective median =268 and 368).

iii. In 2007 the parasitaemia were similar in LLIN and in Control villages (P-value=0.0598; respective median =440 and 576); in 2008 also the parasitaemia were similar in LLIN and in Control villages (P-value=0.0969; respective median =160 and 97).

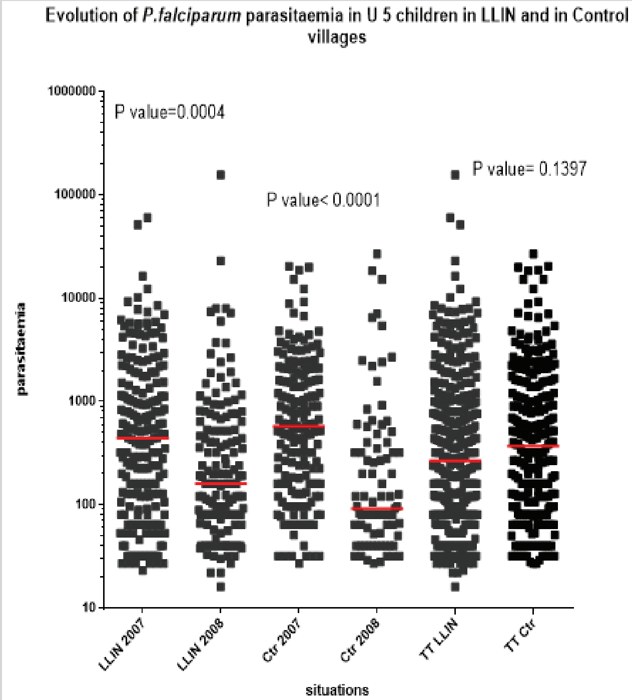

Figure 11b: Evolutions of *P. falciparum* parasitaemia noticed in ≤5 children during the CSS simultaneously done in August, October and December in the LLIN and in the control villages in 2007 and 2008.

Table 6b: Evolution of *P. falciparum* parasitaemia in ≤5 years children of villages with LLIN and control villages from year 2007 to year 2008.

VC	LLIN		Control		LLIN	Control
Years	**2007**	**2008**	**2007**	**2008**	**2007+2008**	**2007+2008**
n	215	151	182	87	366	269
Arith mean	1838	1822	1616	1197	1831	1480
SD	5725	12838	3101	3908	9234	3381
Geom mean	413	221	556	386	319	384
Median	440	151	564	92	366	384

Therefore, for the same period of the years, the *P. falciparum* parasite load in U 5 children seem having been comparable in villages with or without LLIN.

The evaluation of the parasite density index by Bruce-Chwatt [26]'s method shows that, from year 2007 to year 2008, it decreased from 3.68 to 2.75 in LLIN villages and from 4.02 to 2.49 in control villages (Annex 1).

Comparing the situation for the 2 years, 2007+2008 shows that parasite density index was slightly lower in LLIN than in control villages, respectively: 3.3 *versus* 3.5 (Annex 2).

Further detailed analysis of the *P. falciparum* parasite load of the positive blood slides obtained during the 24 CSS simultaneously done in August, October and December in the 4 villages are reported in Annex 3 where it appeared a decrease in the 25% percentile, of median value and of 75% percentile from year to year while the means and minimum values remained of the same order (with great SD to be noticed).

Comparison for 6 CSS of the full year 2008

In 2008 the Plasmodial prevalence were similar in LLIN and in control villages (respectively 34.1% n=823 and 28.5%, n=730) (X^2=5.72; P-value=0.0168).

Analyzing the distribution of *P.falciparum* parasite load of the 489 positive blood films observed in children ≤5 years old during the 24 CSS regularly done in 2008 (Figure 12) showed similar level between Caala and Cahata (P-value=0.4437; median Caala =240; n=144; median Cahata =280, n=137); between Chisséquélé and Barragem (P-value=0.3114; median Chisséquélé=320, n=99; median Barragem=267, n=109) and between total LLIN *versus* total control (P-value=0.9100; median LLIN =280, n=281; median control=310, n=208).

Plasmodial prevalence and parasitaemia were similar in LLIN and control villages in 2008.

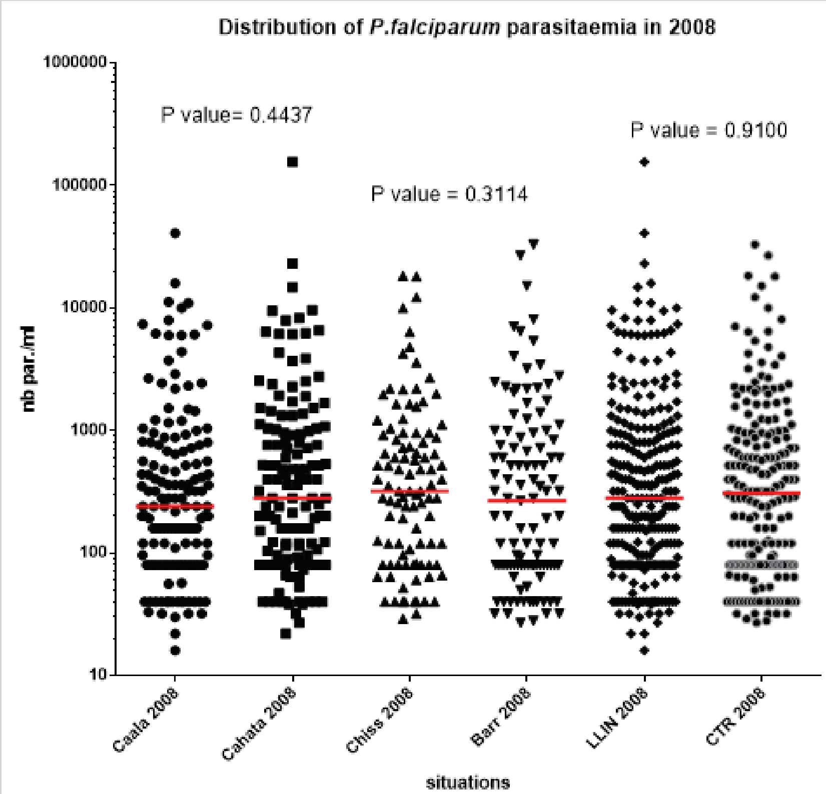

Figure 12: Distribution of *P. falciparum* parasite load noticed during the 6 CSS simultaneously done in 2008 in the 4 villages.

The distribution according to the 3 main classes (low density=≤1000 par./µl; medium density=1001-10000 and high density =≥10001 par./µ) shows remarkable similar values between LLIN and control villages (Table 7).

Table 7: Distribution on 3 main classes of parasitaemia of *P. falciparum* infections noticed in Year 2008 in the ≤5 years old children of the villages with LLIN or without.

Classes	LLIN		Control	
	n	%	n	%
≤1000	218	77.60%	162	77.80%
1001-10000	56	19.90%	40	19.20%
≥10001	7	2.50%	6	2.90%
Total	281		208	

The calculation of the Bruce-Chwatt [26]'s parasite density index (PDI) procures the respective and quite similar values: for LLIN villages PDI=3.277 and for control villages PDI=3.279.

Evolution of Entomological situation

With a regular use of classical CDC Miniature Light traps inside the houses it was possible to get an evolution of the "density/traps" of the main vectors *An. gambiae+An. funestus* in the four villages in the 2 years, 2007 and 2008 (Table 8).

Table 8: Evolution of the densities/traps of the main vectors, *An. gambiae+An. funestus* in each village each year.

	Y 2007	Y 2008	Diff
Caala	0.56	0.42	-25%
Cahata	1.29	0.43	-66.70%
Tt LLIN	0.89	0.42	-52.80%
Chiss.	0.54	0.4	-25.90%
Barragem	0.58	0.25	-56.90%
TT Ctr	0.56	0.32	-42.90%

It thus appeared (Figure 13) a sharp reduction (by #53%) of the number of main vectors in LLIN village and a natural reduction of #43% in control villages showing that LLIN were actually efficient against the main vector.

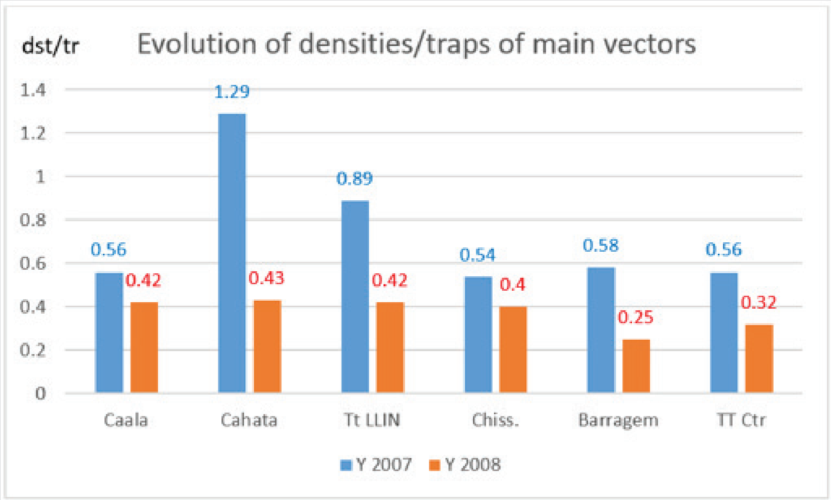

Figure 13: Evolution of densities/trap of the main vectors *An. gambiae+An. funestus* in each village for each year.

DISCUSSION AND CONCLUSION

In 2007 a long-term village scale malaria vector control program was implemented in 8 villages around Balombo town (Benguela Province, Angola) to compare the efficacy of 4 methods: the deltamethrin treated long lasting insecticide treated nets (LLIN) Perma©Net 2.0; inside residual spraying with lambdacyhalothrin; deltamethrin treated insecticide treated plastic sheeting (ITPS) used either alone or associated with LLIN. Each method was implemented in 2 villages.

For ethical reasons distribution of LLIN started immediately in 2 villages, Caala (792 inhabitants) and Cahata (484 inhabitants) in 2 steps; first, in February 2007: 670 LLIN to procure "at least one net /house" (360 LLIN for 238 houses in Caala and 310 LLIN for 138 houses in Cahata) then in February 2008: 227 more LLIN in Caala and 195 more in Cahata to cover every "sleeping units" (469 in Caala and 442 in Cahata), with some supplementary LLIN distributed in December 2008 (49 in Caala and 25 in Cahata). It thus appeared that an actual complete coverage was obtained.

The 6 other villages remained as control for these 2 years then in December 2008 vector operations were implemented according to the plan of action.

The comprehensive evaluation included entomological and parasitological parameters with regular Cross Sectional Parasitological surveys (CSS) on randomly selected population from the number of the houses following the methodology developped in Côte d'Ivoire for the evaluation of the efficacy of lambdacyhalothrin treated nets against pyrethroid resistant *An. gambiae* population [13].

A first parasitological surveys was done by cluster sampling method in this area showing an overall plasmodic index of 70% in children <15 years old [18] and another parasitological survey before vector control implementation showed similar plasmodic index in the different main classical age groups (<5 years old; 2-9y) to get the "endemic index" and <15y [17] which was thus selected to increase the sample size for some statistical analysis [6] and the class 1-15 years old was already used to evaluate the vector control operations in Equatorial Guinea [27,28].

The present first parasitological analysis of *Plasmodium falciparum* prevalence and parasite load in children ≤5 years old was done to compare the situation in 2007 and 2008 in the 2 villages with LLIN *versus* 2 "closed" villages: Barragem (620 inhabitants, 134 houses) and Chisséquélé (418 inhabitants, 181 houses) still not involved in vector control operations and considered as control. It appeared that after a full coverage in LLIN the plasmodial prevalence sharply decreased in these villages, but an almost similar decrease was also noticed in control villages.

Several indicators can be used for malaria survey [13,16] with blood examination to confirm the actual presence of the *Plasmodium* (prevalence, incidence, parasite load) and its species, the "proportion of persons in a given community who harbor the parasites" is called the parasite rate and "it must be precised for a relatively narrow range of age groups" [13].

Malaria health statistic showed that 86% of death for malaria involved children below 5 years old [29] who are considered as "at risk" group with pregnant women and must receive efficient protection such as ITN/LLIN. This age group "U-5" is often used for studies of malaria and ITN [30-32].

In Nigeria a study of 270 outpatients (from November 2012 to December 2013) showed that 40.1% had positive microscopically examined blood slides [31] with a "significant association between those with fever and parasitaemia" while "there was no significance difference between ownership of ITN". A key observation which is similar to our analysis of the parasitological situation of U-5 in the 2 villages with LLIN not significantly different from the one noticed in the 2 control villages during this Balombo program.

This could be due, in some part, to a "not enough regular use of nets by children which raise the well-known issue of the discrepancy between "to have *versus* to use" the nets [30,32-36].

Analyzing the data collected by surveys in 14 countries Korenromp et al. [37] highlighted that "the proportion of children under 5 years of age who slept under a net during the night preceding a survey was considerably lower than the proportion of households that possess a net. This was especially true of ITNs". Not all mosquito nets owned by African households are being used for young children"; "in a typical household possessing nets, half the children will not be sleeping under these nets"; the average number of nets in a household would typically not suffice to cover all residents";"use was lower than possession because: (i) nets were scarce (mean 1.8 per possessing household); (ii) nets were not always used for children and (iii) use was lower during hot, dry months than during cool rainy months, and many surveys had been conducted in the dry season". "The discrepancy between possession and use by children was remarkably consistent across countries and sub regions". "For example, in a survey in rural Burkina Faso with use levels of 96% for adults and 23% for children under 5 years in households with nets, the discrepancy between use and net possession by children, which had not been measured here, must have been at least 4.2-fold [38].

Recent surveys in Sahel and Delta regions of Nigeria on use of nets 6 months after large distribution of treated nets among the country revealed a great difference in the use of nets by children ≤5 years: those of Niger Delta "were four times more likely to sleep under treated nets than those of the Sahel savanna region" [39]. Still in Nigeria a study of 270 outpatients (from November 2012 to December 2013) showed that 40.1% had a positive microscopically examined blood slide [31] with "a significant association between those with fever and parasitaemia." "Although 191 (71.5%) of the households possessed at least one mosquito net, only 25.4% of the under-5 children slept under any net the night before the survey. No statistically significant reduction in malaria parasitaemia was observed with the use of mosquito nets among the under-5 children" "Mosquito net utilization among the under-fives was low despite high net ownership rate by households"; they considered that "the high discrepancy between ownership and utilization of ITN calls for great concern" and the observations in Caala and Cahata were well in line with these comments.

For Iwuafor et al. [31] "assessment of the impact of ITN coverage and/or utilization on health outcomes is usually difficult" using logistic regression analysis they "observed a 32% reduction in malaria parasitaemia among under-5 users which was not statistically significant". This is in the line which what was noticed during the first steps of the Balombo program.

A cross-sectional survey was conducted from March to August 2016 in twelve health facilities selected from three area councils in Abuja, Nigeria [40] to evaluate the "Long-lasting insecticidal net use and asymptomatic malaria parasitaemia among household members of laboratory-confirmed malaria patients attending selected health facilities" and it appeared that "proportions of households that owned and used at least one LLIN were 44.8% and 33.6%, respectively. Parasitaemia was detected in at least one family member of 102 (95.3%) index malaria patients. Prevalence of asymptomatic malaria parasitaemia among study participants was 421/602 (69.9%). No association was found between individual LLIN use and malaria parasitaemia among study participants".

Free distribution of nets is obviously a necessary but in fact not sufficient condition to ensure their regular use and, therefore, to get the expected impact; regular monitoring and awareness campaigns must be done along with the scaling-up of LLIN. The fact that plasmodial indices decreased even in close villages showed that campaigns to increase public awareness and actual participation is a key element in the Malaria Control Program.

AUTHOR'S CONTRIBUTION

PC designed the study protocol; participated to field surveys, analyzed data and drafted the manuscript; VF and JCT carried out the field experiments and laboratory studies; SM checked data and finalized the document. All authors read and approved the final manuscript.

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Annex 1: Evaluation of the *P. falciparum* parasite density index each year in the 2 villages with LLIN compared to the 2 control village, calculated with the Bruce-Chwatt (1985) method (*freq. = numero of the class x number of parasitaemia belonging to this class). [data dealing with the 3 Cross Sectional Surveys done at the same time, August, October, December in both 2007 and 2008 years].

VC	LLIN				Control			
Classes	Y 2007 Nb Pf+	Freq*	Y 2008 Nb Pf+	Freq*	Y 2007 Nb Pf+	Freq*.	Y 2008 Nb Pf+	Freq*
1 < 100	54	54	57	57	31	31	46	46
2 101-200	25	50	31	62	22	44	10	20
3 201-400	26	78	16	48	19	57	11	33
4 401-800	36	144	20	80	34	136	7	28
5 801-1600	24	120	12	60	28	140	3	15
6 1601-3200	20	120	6	36	26	156	4	24
7 3201-6400	20	140	3	21	13	91	1	7
8 6401-12800	7	56	4	32	5	40	2	16
9 12801-25600	1	91	1	9	4	36	2	18
10 ≥25601	2	20	1	10	0	0	1	10
Total	215	791	151	415	182	731	87	217
Parasite Density Index	791/215= 3.649		415/151=2.748		731/182= 4.016		217/87=2.494	

Annex 2: Evaluation of the *P. falciparum* parasite density index (PDI) in the LLIN and control villages, calculated with the Bruce-Chwatt's (1985) method (*freq. = numero of the class x number of parasitaemia belonging to this class) [data dealing with the 3 Cross Sectional Surveys done at the same time, August, October, December in both 2007 and 2008 years].

Vector Control	LLIN		Control	
Years 2007+2008	Number Pf+	Freq.	Number Pf+	Freq.
Class 1	111	111	77	77
Class 2	56	112	32	64
Class 3	42	126	30	90
Class 4	56	224	41	164
Class 5	36	180	31	155
Class 6	26	156	30	180
Class 7	23	161	14	98
Class 8	10	80	6	48
Class 9	3	27	7	63
Class 10	3	30	1	10
Total	366	1207	269	949
PDI	1207/366=	3.298	949/269=	3.528

Annex 3: Detailed statistics of *P. falciparum* parasitaemia unregistered during the simultaneous CSS of August October and December 2007 and 2008 in LLIN and control villages (mean= arithmetic mean; SD= standard deviation).

	LLIN 2007	LLIN 2008	Ctr 2007	Ctr 2008	Tt LLIN	Tt Ctr
Nb Pf+	215	151	182	87	366	269
minimum	23	16	27	27	16	27
25% percentile	96	80	160	60	80	81
median	440	160	564	92	268	384
75% percentile	1440	533	1776	400	967.8	1413
maximum	60000	156000	20408	26800	156000	26800
mean	1838	1822	1616	1197	1831	1480
SD	5725	12838	3101	3908	9324	3381
sum	395076	275128	294097	104154	670204	398251

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