Evolution of plasmodial infection according to age and vector control method implemented: deltamethrin treated durable lining (δDL) in Candiero or lambdacyhalothrin Indoor Residual Spraying (λIRS) in Chisséquélé, 2 villages around Balombo town (Benguela Province, Angola).

Abstract

Background. Indoor Residual Spraying (IRS) and Insecticide Treated mosquito nets (ITNs) are the main tools for malaria vector control and several hundred millions of malaria cases were averted these last decades thanks to their use at large scale.

Due to several operational issues IRS were stopped in Angola and the National Malaria Control Program (NMCP) is based upon large scale distribution of Long Lasting Insecticide Treated Nets (LLIN) through different channels.

On the other hand the recently developped Insecticide Treated Plastic Sheeting (ITPS) could represent an interesting alternative to IRS in term of longer lasting activity, community acceptability and participation, entomological and epidemiological efficacy observed in various experimental and natural conditions.

Methods. In the framework of a long term village scale malaria vector control program in Balombo area (Benguela Province, Angola) 4 methods of vector control were used: deltamethrin treated LLIN alone ("δLLIN"), or associated with δITPS; δITPS alone; lambdacyhalothrin IRS ("λIRS") followed by δITPS with a comprehensive evaluation. Parasitological evaluation was based upon regular random Cross Sectional Surveys ("CSS") to monitor the evolution of plasmodial infections in children ≤15 years such as surveys previously done in this region.

The aim of the present study was to compare 2 parasitological indicators of plasmodial infection: parasite index ("PI") and parasite load("PL") in 2 villages: Candiero, which received 2 rounds of λIRS then δITPS; and Chisséquélé , which received δITPS only and just once; considering 4 age groups recommended in Essential Malariology: 0-2 years old; 3-5 years old; 6-9 years old and 10-15 years old (from which it is easy to analyze as wished the evolution if other age groups sometimes used).

Results.

During the 5 five consecutive years of the study 4792 thick blood films were done and their microscopical examination revealed the presence of plasmodial infections in 925 i.e. an overall Plasmodial Index of 19.3%

Plasmodium were noticed in 475 of the 2513 TBF (PI=18.9%) made in Candiero and 450 of the 2279 TBF done in Chisséquélé (PI=19.7%).

Before vector control (VC) Parasite Index of babies (≤ 2 years old) were significantly lower than PI of the three other older age groups. The impact of VC on plasmodial index was different according to age groups. After VC the overall parasite index significantly dropped by #70% in Candiero (IRS village) and # 60% in Chisséquélé (ITPS village), the decrease was significant in each age group but it was less in babies than in the 3 older groups. This could lead to a "negative" conclusion of the efficacy of VC if targeting only plasmodial prevalence in babies.

On the other hand parasitaemia significantly dropped the first year after VC in babies in the 2 villages and not in all older age group leading to a positive conclusion of the efficacy of VC when targeting

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babies; actually the drop of parasitaemia after VC was noticed in some age groups (but not all) in Candiero but not in Chisséquélé.

Conclusion. These analyze underlined the key issues of the choice of indicator and the targeted age groups because conclusion of the efficacy of one or the other method of vector control could be opposite. Another key point is that, considering the classical age groups (0-5 or 2-9 or $\leq$15 years old) Insecticide treated plastic sheeting appeared as efficient as Indoor Residual Spraying with the double advantage of lasting longer and involving actual community participation.

Key words. Vector control, Inside Residual Spraying, Insecticide Treated Plastic Sheeting, impact on parasite index and parasite load according to age groups.

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I. Introduction

After the discovery of its insecticidal properties, DDT [1] used in indoor residual spraying (IRS), was considered as the magic bullet against malaria vectors and the main tool for an expected global « Malaria Eradication Program" ("MEP")[2].

But, due to several issues, such as operational constraints, acceptability [3, 4], costs/effectiveness, insecticide resistance [5] the Malaria Eradication Program 1955-1969 was stopped for malaria "case management" but the spreading of drug, and insecticide, resistance are of great concern [6] [7] and the Strategy has to be changed with the Amsterdam Conference 1992.

Actually a new interest was devoted to vector control with the availability, and demonstrated efficacy, of Insecticide Treated Nets (ITNs) [8] which became industrialized Long Lasting Insecticide Treated Nets (LLIN).

Thanks to their scaling-up several millions of malaria cases were averted this last decade [9] while IRS maintained its usefulness with some efficacy in certain circumstances.

For exemple DDT IRS were of great efficacy to prevent malaria [10], to stop malaria outbreak in Malagasy [11, 12] or to contain malaria in Southern Africa [13] [14, 15], in Malawi [16]; in Equatorial Guinea [17, 18] were successively used deltamethrin then carbamates, bendiocard and Fludora™ fusion, a formulated combination of clothianidin (a neonicotinoid) and deltamethrin (a pyrethroid) [19-22].

Due to DDT resistance other OC were used such as Dieldrin [5] but resistance was soon observed [23] or pyrethroids such as deltamethrin of alphacypermethrin [18] or lambdacyhalothrin [16] or OP such as pirimiphos methyl [24-27] or chlorfenapyr [28], or neonicotinoids such as clothianidin [29].

One of the issue of IRS is the short term efficacy of insecticide sprayed (3-6 months) and the need of longer lasting efficacy [30].

Due to "difficulties in organizing first indoor spray program against malaria in Angola" [31] IRS were stopped in the country and the National Malaria Control Program planned large scale distribution of long lasting insecticide treated nets (LLIN) but it appeared that, in some field conditions, their efficacy was reduced by their misuse or they are not maintained, quickly have large holes and are removed [32, 33].

To overcome these issues, while targeting a complete protection of houses and inhabitants, a tool called Insecticide Treated Plastic Sheeting (ITPS) was recently developped [34], treated with pyrethroid such as deltamethrin [35] or permethrin [36, 37] or non pyrethroid [38] such as pirimiphos methyl [39] or abamectin or fenpyroxinate [40].

Their acceptability was already studied in Angola [41] and several other countries of Africa and S.E. Asia [42] in Papouasia-New Guinea [43] [44] in South Africa [45] and its efficacy to reduce malaria burden was well demonstrated in refugees camps in Sierra-Leone[46].

It thus appeared worth comparing the efficacy of ITPS and IRS in 2 villages close to Balombo town in the framework of a village scale long term malaria vector control program with comprehensive evaluation [47].

It was already demonstrated that malaria vectors bite more frequently adults than babies [48] and recommended that the parasite rate must be determined for some age groups, infants- babies (0-11

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months), toddlers (12-23 months), small children (2-4 years), juveniles (5-9 years), adolescents (10-14 years) and adults (≥15 years). Actually in some endemic conditions the overall babies and toddlers mortality could be high with the loss of materno transmitted antibodies and the not yet fully developped immune system [49] while teenagers and adults could get a special immunity called « premunition » [50].

Indeed some previous demographic studies also showed how risky is the period <2 years [51] and it was considered that < 5 years old children are « at risk » and must be protected in priority (WHO).

Therefore it was decided to analyze the evolution of plasmodial infection (parasite prevalence and parasite load) with the implementation of house spraying or installation of ITPS during the village scale vector control in Balombo area with a special attention to the situation in different age groups [52, 53] to determine, what, in these local conditions, should be the prior steps of vector control of the National Malaria control Program (NMCP) for the best and fastest impact on the burden of malaria.

II. Materials and Methods

A long term village scale malaria vector control (VC) program was implemented in 8 villages around Balombo (Benguela Province, Angola) to compare 4 methods of vector control inside human houses: Long lasting deltamethrin treated nets (δ LLIN) alone in 2 villages; association δ LLIN + δ treated ITPS (model « Zero Fly ») in 2 villages; δ treated ITPS (called « wall lining ») alone in 2 villages ; and 2 rounds of lambdacyhalothrin IRS (λIRS) followed by installation of δ treated ITPS in 2 villages. Details of these VC operations were described elsewhere [47].

The current parasitological analysis dealt with 2 villages, Candiero and Chisséquélé.

In Chisséquélé (181 houses; 418 inhabitants at the beginning of the trial): in December 2008: 5541 m^2 of δITPS wall lining (concentration 170 mg a.i. deltamethrin /m^2) were pinned on the wall of every sleeping room or place temporary used for sleeping (on mattress etc.) to protect every sleeper.

In Candiero (190 houses; 654 inhabitants at the beginning of the trial): 2 rounds of λIRS at the target dosage of 25mg ai/m^2 were done: in December 2008 then in June 2009, then δITPS wall lining were installed in every houses by inhabitants themselves showing their involvement and acceptability compared to the well-known reluctance to receive people from elsewhere to enter their houses for indoor spraying.

Parasitological cross sectional surveys (« CSS ») were regularly done in a randomly samples of population identified from the number, initially wrote on the door of every houses (checked with GPS), and the random number were obtained with Excel software. Thick blood films (TBF) were done in the field and microscopically examined in the Medical Department of the private Angolese Sonamet© Company which supported the studies in the framework of their «Malaria Control Program» (MCP). 10% of the films were double checked in the parasitology department of the Pan African OCEAC Organization in Yaoundé (Cameroon).

For the analyses according to age groups we followed the protocol developped [14] to monitor the impact of IRS in Southern Africa and considered 4 age groups :

- 0-2 years old ;
- 3-5 years old ;
- 6-9 years old ;
- 10-15 years old.

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With such a 4 age groups it is easy to further calculate if needed the plasmodial situation of the «at the risk group» (0-5 years old; or the group used for « malaria endemicity evaluation » (2-9 years old); or ≤ 15 years old as used for vector control evaluation in Equatorial Guinea.

We considered 2 parasitological indicators: Plasmodial index "PI" (gathering all *Plasmodium* species, *P. falciparum* being largely prevalent over very few *P. malariae* infections [54]) and parasite load ("PL") counting the number of erythrocytes with parasite versus 200 white cells (WC) and reporting for microliter of blood considering 8000 WC/ml). This protocol was carried out for each one of the 4 age groups, for each village, for each year and therefore the 2 years before and the 3 years after vector control to evaluate the impact of these operations.

Percentages were compared with the classical Chi^2 test (CDC EpiInfo software) and parasitaemia were analyzed with the non-parametric Mann-Whitney test with Graph Pad software.

III. Results

III-1. Plasmodial infections: Plasmodial index and its evolution

III-1-1. Overall Plasmodial index and its evolution

Between August 2007 and December 2011 46 CSS were done, 23 CSS by villages, 9 before vector control operations (VCOp) and 14 after. During the 5 five consecutive years of the study 4792 thick blood films were done and their microscopical examination revealed the presence of plasmodial infections in 925 i.e. an overall Plasmodial Index of 19.3% (table 1). All infections, except 1 mixed *P. falciparum*+ *P. malariae* and 2 *P. malariae* alone, were due to *P. falciparum* therefore analyses dealt with all *Plasmodium* infections gathered.

The evolution of plasmodial infections noticed during each CSS showed a general trend similar in the 2 villages (graph. 1) in a quite logarithm evolution.

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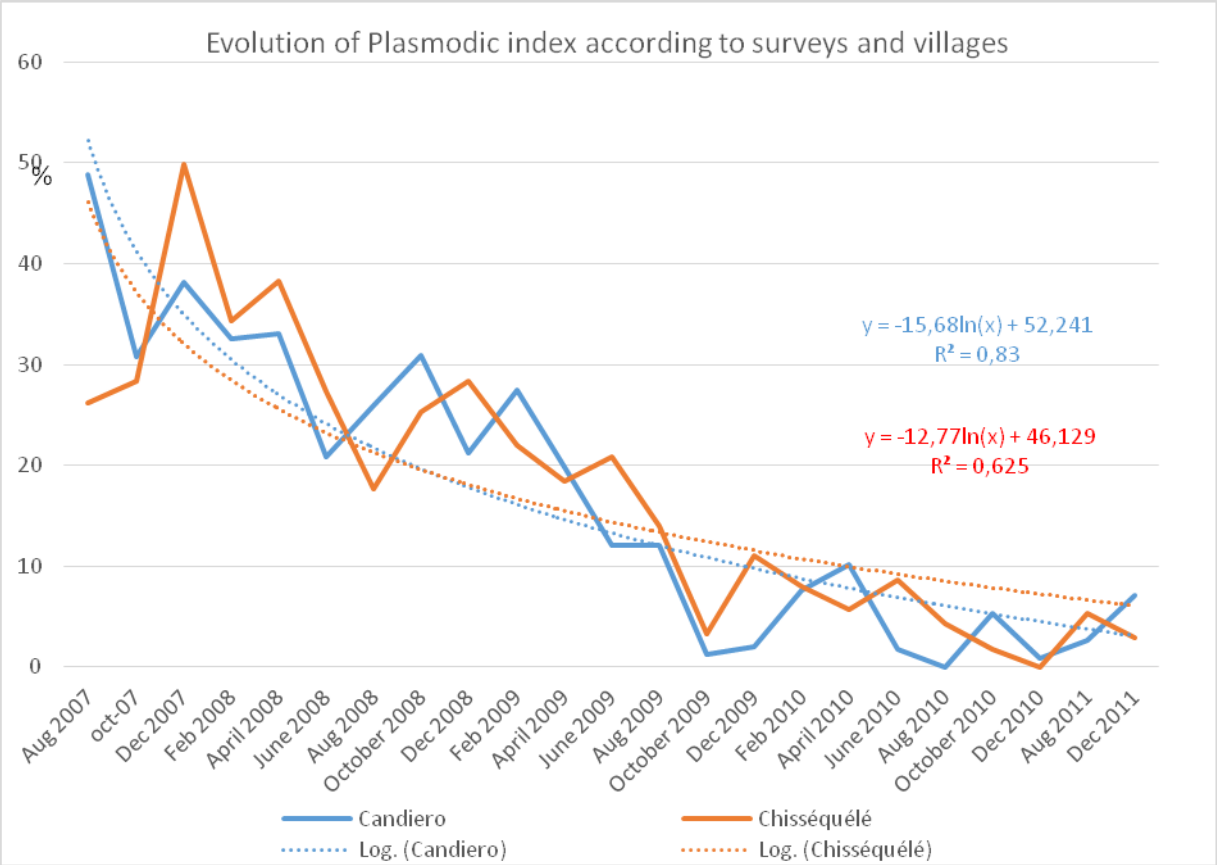

Graph. 1. Evolution of plasmodial prevalence noticed in each one of the 46 CSS carried on during 5 years in the 2 villages.

<u>III-1-2. Yearly evolution of overall plasmodial prevalence</u>.

Data of blood films made, and noticed positive, each year in each village, are gathered in table 1 where it appeared similar level of *Plasmodium* index: 18;9% (n= 2513) for Candiero and 19.7% (n= 2279) for Chisséquélé ($\chi 2$= 0.55; P value= 0.46; OR= 0.95 [0.82-1.09]) giving an overall PI of 19.3% (n= 4792) for the 2 villages gathered.

village	Year 2007		Year 2008		Year 2009		Year 2010		Year 2011		tt		
	n	BF+	n	BF	n	BF+	n	BF+	n	BF+	n	BF+	%
Cand	350	139	748	208	655	94	629	29	131	5	2513	475	*18.9 %*
Chiss	357	121	646	185	601	110	562	29	113	5	2279	450	*19.7 %*
tt	707	260	1394	393	1256	204	1191	58	244	10	4792	925	*19.3 %*
%		*36.8%*		*28.2%*		*16.2%*		*4.9%*		*4.1%*			

Table 1. Thick blood films (n) and *Plasmodium* positive (BF+) noticed every year in every village, Candiero (Cand.) and Chisséquélé (Chiss.).

It is worth underlining the evolution of the PI each year in each village (graph.2a) showing similar trends (graph. 2b) with a clear drop after VC implementation (from #30% to #5%) then a plateau during 2 years at a <5% level.

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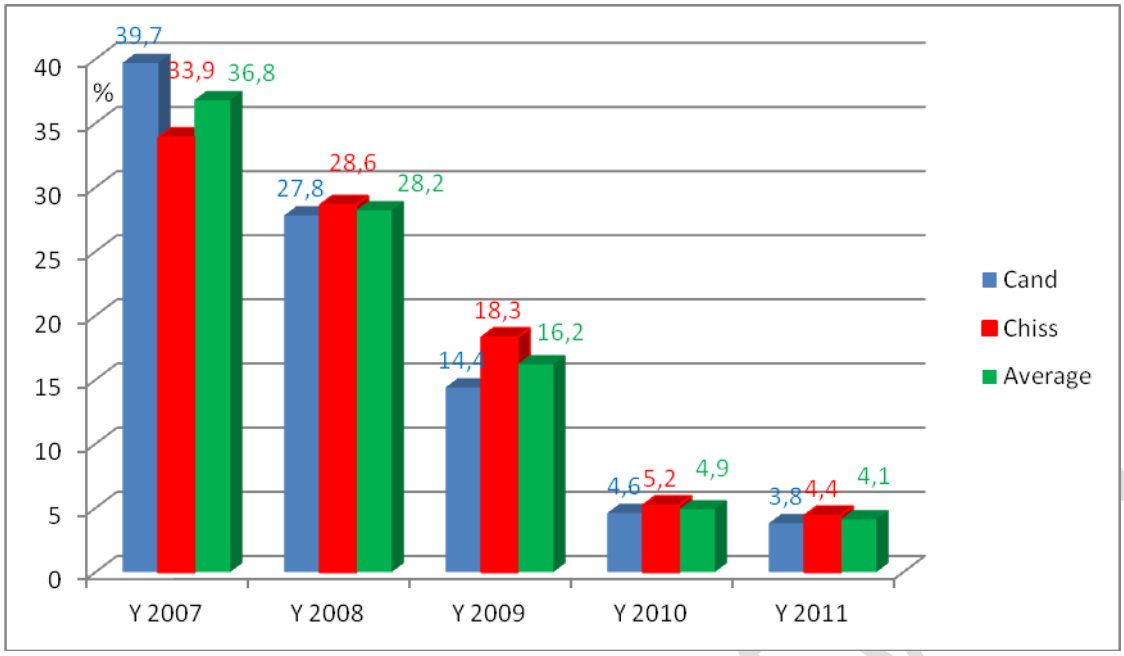
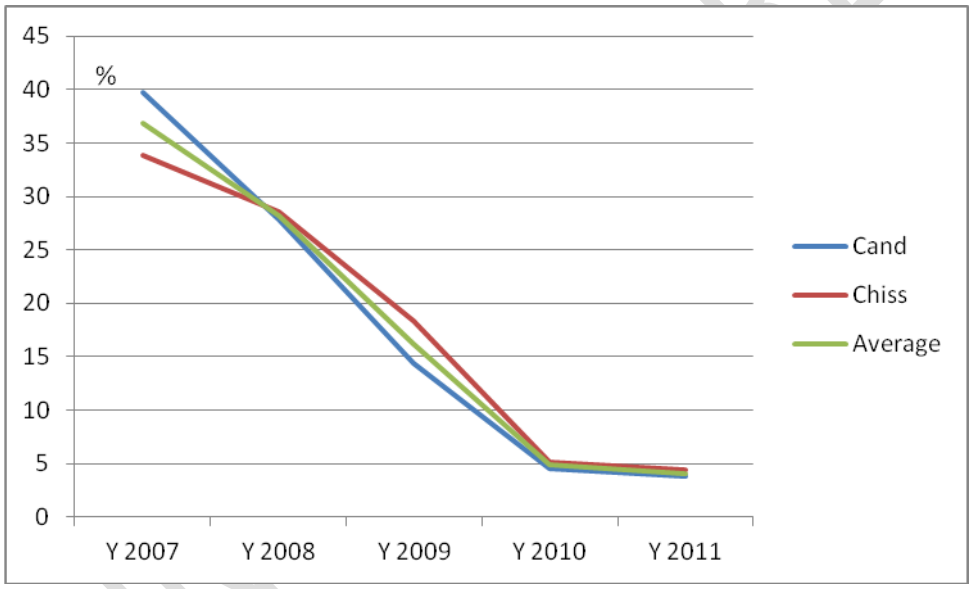


Graph. 2a. Evolution of yearly plasmodial prevalence in village treated with ITPS (Chisséquélé) and with indoor residual spraying (Candiero).



Graph. 2b. Trends in evolution of PI each year in each village.

Actually each year plasmodial index were similar between the 2 villages (table 2).

Years	PI Candiero	PI Chisséq	χ^2	P value	OR
Y 2007	39.7%	33.9%	2.575	0.108	1.28 [0.95-1.74]
Y 2008	27.8%	28.6%	0.118	0.731	0.96 [0.76-1.21]
Y 2009	14.4%	18.3%	3.598	0.0578	0.75 [0.56-1.01]
Y 2010	4.6%	5.2%	0.193	0.659	0.88

					[0.52-1.50]
Y 2011	3.8%	4.4%	0.057	0.811	0.86 [0.24-3.03]

Table 2. Comparison between the PI of the 2 villages noticed every year (* statistical difference).

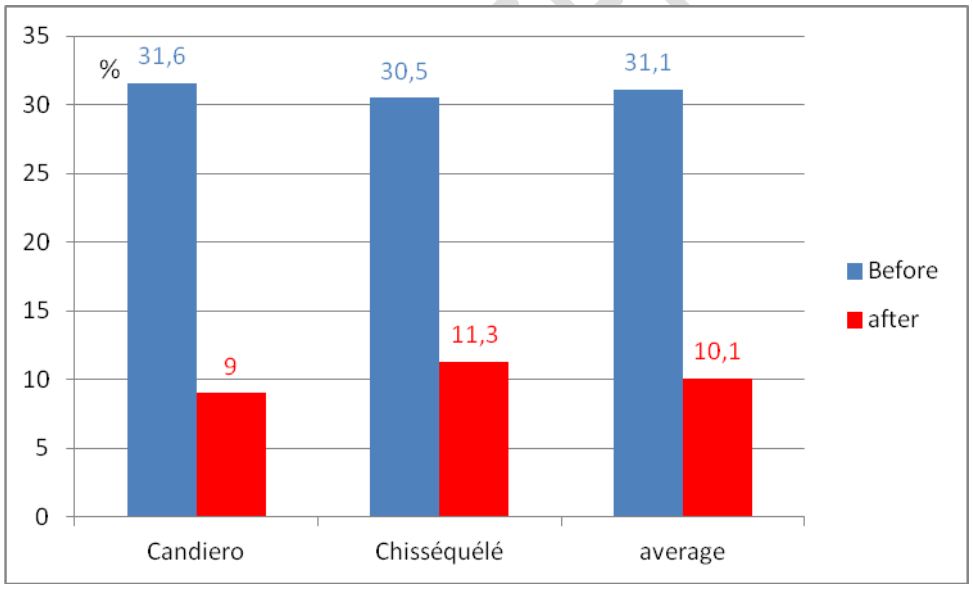
III-1-3. Evolution of overall plasmodial prevalence before/after vector control operations

From table 1 it is possible to sort out positive blood films noticed before vector control implementation (years 2007-2008) and after (years 2009-2010 and 2011) in each village (table 3).

villages	Candiero			Chisséquélé			total		
	n	TBF+	%	n	TBF+	%	n	TBF+	%
before	1098	347	31.6%	1003	306	30.5%	2101	653	31.1%
after	1415	128	9.0%	1276	144	11.3%	2691	272	10.1%
total	2513	475	18.9%	2279	450	19.7%	4792	925	19.3%

Table 3. Plasmodial infections microscopically detected in thick blood films (TBF) done in Candiero and in Chisséquélé before and after implementation of vector control.

With vector control the *Plasmodium* index significantly dropped (by 71.5%) in Candiero (χ^2= 205; OR= 0.21 [0.17-0.29]), by 63% in Chisséquélé (χ^2= 131, OR= 0.29 [0.24-0.36]) and when gathering data of the 2 villages the overall PI dropped by 67.5%, from #31% to # 10%, (χ^2= 333; OR=0.25 [0.21-0.29]) showing the clear impact of vector control of the reduction of plasmodial infections in children ≤15 years old (graph. 3).



Graph. 3. Evolution of overall plasmodial infections before and after implementation of vector control in each village.

Therefore with the plasmodial index (PI) as indicator ITPS appeared as efficient as IRS in reducing the Plasmodial infections prevalence in the ≤15 years old children surveyed in these villages.

III-2. Evolution of plasmodial index according to age group and vector control

III-2.1. Evolution of Plasmodial index in Candiero

Data dealing with the number of thick films prepared and noticed positive in each group are reported table 4.

Years	Age group	n	TBF+	%
Y 2007	0-2 y	95	28	29.5%
	3-5 y	83	31	37.3%
	6-9 y	103	47	45.6%
	10-15 y	69	33	47.8%
Y 2008	0-2 y	193	40	20.7%
	3-5 y	214	63	29.4%
	6-9 y	203	60	29.6%
	10-15 y	138	45	32.6%
Y 2009	0-2y	130	20	15.4%
	3-5 y	216	32	14.8%
	6-9 y	151	23	15.2%
	10-15y	158	19	12.0%
Y 2010	0-2 y	110	4	3.6%
	3-5 y	218	8	3.7%
	6-9 y	156	8	5.1%
	10-15y	145	9	6.2%
Y 2011	0-2y	20	0	0%
	3-5 y	40	2	5%
	6-9 y	52	1	1.9%
	10-15 y	19	2	10.5%
total	0-2 y	548	92	16.8%
	3-5 y	771	136	17.6%
	6-9 y	665	139	20.9%
	10-15 y	529	108	20.4%

Table 4. *Plasmodium* prevalence each year in each age group in Candiero.

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475 of the 2513 thick blood films were noticed positive i.e. an overall prevalence of 18.9% for the 5 years and the general prevalence was similar for the 4 age-groups considered (table 5a): 16.8% (= 548) in 0-2 years old; 17.6% (n= 771) in 3-5 years old; 20.9% (n= 665) in 6-9 years old and 20.4% (n= 529) in 10-15 years old (annex 1).

age	n	BF+	%
≤2 y	548	92	16.8%
3-5 y	771	136	17.6%
6-9 y	665	139	20.9%
10-15 y	529	108	20.4%
total	2513	475	18.9%

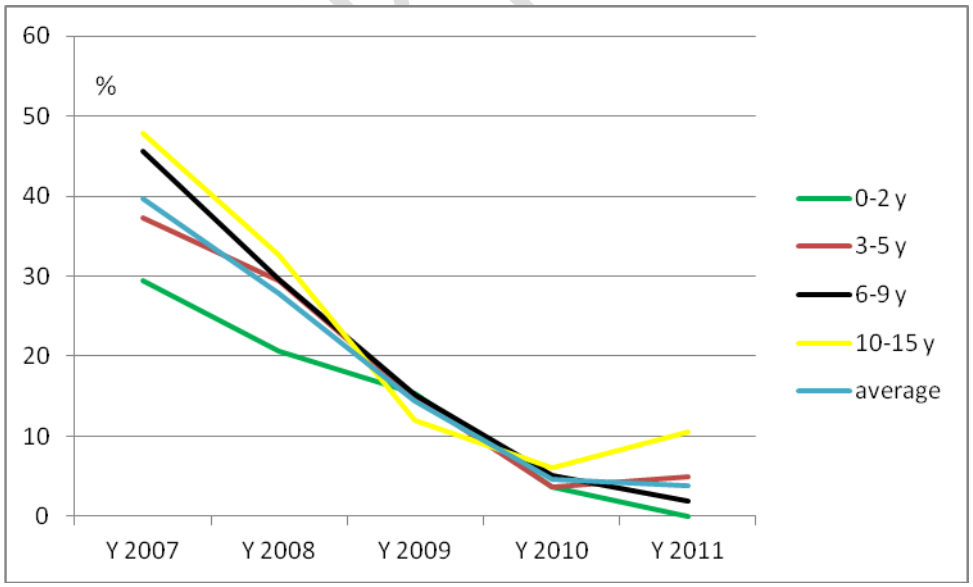
Table 5a. Overall *Plasmodium* index in each age group of Candiero village.

It is interesting to examine the evolution of PI each year for each age group (table 5b)

Candiero	Y 2007	Y 2008	Y 2009	Y 2010	Y 2011
0-2 y	29,5	20,7	15,4	3,6	0
3-5 y	37,3	29,4	14,8	3,7	5.0
6-9 y	45,6	29,6	15,2	5,1	1,9
10-15 y	47,8	32,6	12	6,1	10,5
average	39,7	27,8	14,4	4,6	3,8

Table 5b. Yearly Evolution of the PI according to age groups in Candiero village.

It is worth underlining the similar trends in the evolution of PI whatever the age groups (graph 4a).



Graph. 4a. Evolution of PI in each age groups during the 5 years of the project in Candiero.

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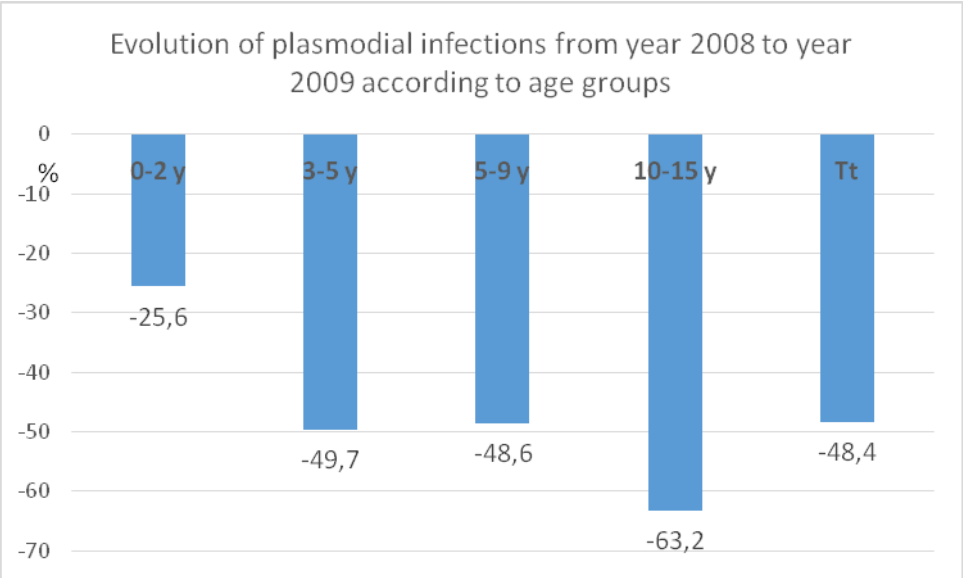
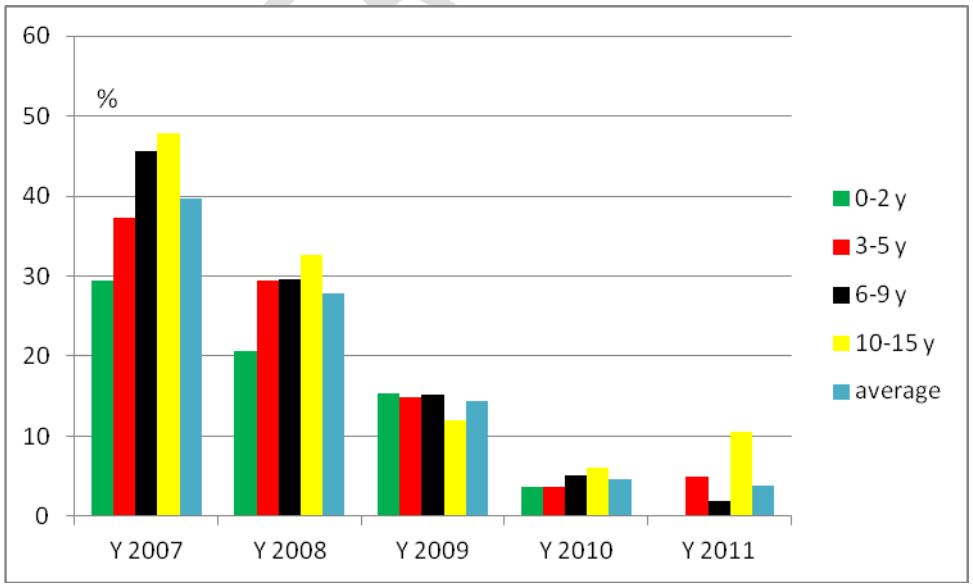
Before VC implementation (years 2007-2008) the PI of ≤ 2 years old babies (23.6%; n=288) was significantly lower than the PI of the 3 other age groups gathered (34.1%; n=810) (X^2= 10.80; P value= 0.0010; OR=0.59 [0.44-0.81]).
The first year following VC the PI dropped by almost 50% compared to the previous year (respectively #28% in y2008 and # 14% in y2009) and this drop was observed in each age group (graph. 4b) even if less important in babies (< 2 years) than in older age group. This is important to keep in mind as an evaluation based only on the 0-2 years old could concluded at a relatively low efficacy of vector control while considering other age groups could lead to a more positive conclusion.



Graph 4b. Drop of *Plasmodium* prevalence in each age group the first year after vector control implementation (Σ= total) in Candiero.

The low level of PI (# 5%) was maintained the following 2 years (graph. 4c).



Graph 4c. Evolution of *Plasmodium* prevalence each year in each age group in Candiero.

From the table 4 it is possible to sort out the change in plasmodial prevalence in each age group before (years 2007-2008) vs after (years 2009-2010-2011) vector control implementation (table 6).

Age groups	Before			After			Difference
	n	TBF+	%	n	TBF+	%	
0-2 y	288	68	23.61	260	24	9.23	-60.9%
3-5 y	297	94	31.65	474	42	8.86	-72%
6-10 y	306	107	34.97	359	32	8.91	-74.5%
11-15 y	207	78	37.68	322	30	9.32	-75.3%

Table 6. Evolution of *Plasmodium* prevalence in each age group after vector control in Candiero.

It thus appeared an overall reduction of 71.4%: from 31.6% (n=1098) before VC to 9.05% (n= 1415) after VC. The reduction was statistically significant for each age group: 0-2 years: $\chi2$= 19.2; 0R= 0.34 [0.20-0.56]; 3-5 y: $\chi2$ = 65.3; OR= 0.21 [0.14-0.35]; 6-9 y: $\chi2$= 67.8; OR= 0.18 [0.12-0.28] and 10-15 y: $\chi2$= 62.4; OR= 0.17 [0.11-0.27] the least reduction was observed in babies (≤ 2 years old).

For the "at risk" group (0-5 years):

- the year after VC *Plasmodium* prevalence significantly dropped by #41% (from 25.3% (n=407) to 15.0% (n=346) ($\chi2$= 12.1; OR= 0.52 [0.36-0.76]).
- After VC the reduction reached 67.5%, from 27.7% (n=585) to 9% (n=734) with an overall PI of 17.3% (n=1319) ($\chi2$= 79.6; OR=0.26 [0.19-0.35]).

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III-2.2. Evolution of Plasmodial index in Chisséquélé.

Data gathering the number of thick films prepared (n) and observed positive (TBF+) in each group are reported table 7.

Years	Age group	n	TBF+	%
Y 2007	0-2 y	114	31	27.2%
	3-5 y	102	48	47.1%
	6-9 y	96	31	32.3%
	10-15 y	45	11	24.4%
Y 2008	0-2 y	194	41	21.1%
	3-5 y	206	60	29.1%
	6-9 y	144	45	31.3%
	10-15 y	102	39	38.2%
Y 2009	0-2y	166	27	16.3%
	3-5 y	202	38	18.8%
	6-9 y	141	22	15.6%
	10-15y	92	23	25.0%
Y 2010	0-2 y	145	1	0.69%
	3-5 y	208	15	7.2%
	6-9 y	108	8	7.4%
	10-15y	101	5	4.95%
Y 2011	0-2y	14	0	0%
	3-5 y	55	2	3.64%
	6-9 y	31	3	9.7%
	10-15 y	13	0	0%
total	0-2 y	633	100	15.8%
	3-5 y	773	163	21.1%
	6-9 y	520	109	21.0%
	10-15 y	353	78	22.1%

Table 7. *Plasmodium* prevalence each year in each age group in Chisséquélé.

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450 of the 2279 thick blood films were noticed positive i.e. a prevalence of 19.7% for the 5 years (table 8a) and the plasmodial index were different for the 4 age-groups considered; it was significantly lower for the 0- 2 years old (15.8%; n= 633) than in 3-5 years old (21.1%; n= 773), in 6-9 years old; (21.0%; n= 520) and in 10-15 years old (22.1%; n= 353) while it was similar between the 3 older age group (annex 2).

age	n	BF+	%
≤2 y	633	100	15.8%
3-5 y	773	163	21.1%
6-9 y	520	109	21.0%
10-15 y	353	78	22.1%
total	2279	450	19.7%

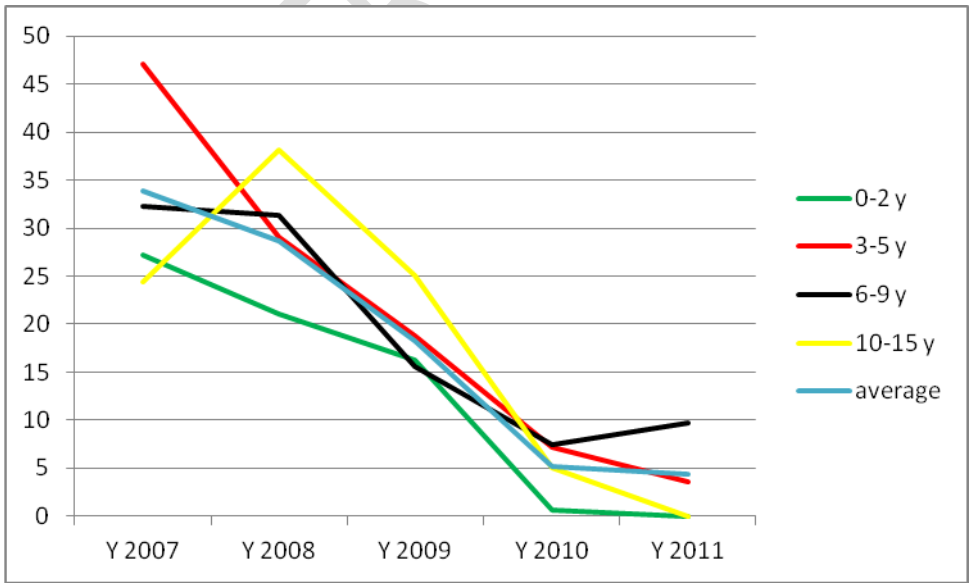
Table 8a. Overall *Plasmodium* index in each age group of Chisséquélé village.

It is interesting to examine the evolution of PI each year in each age group (table 8b)

Chisséq.	Y 2007	Y 2008	Y 2009	Y 2010	Y 2011
0-2 y	27,2	21,1	16,3	0,7	0
3-5 y	47,1	29,1	18,8	7,2	3,6
6-9 y	32,3	31,3	15,6	7,4	9,7
10-15 y	24,4	38,2	25	5	0
average	33,9	28,6	18,3	5,2	4,4

Table 8b. Yearly Evolution of the PI according to age groups in Candiero village

It is worth underlining the trends these PI with age groups (graph…a) where it could be noticed some variations different from Candiero (graph 5a)



Graph. 5a. Evolution of PI in each age groups during the 5 years of the project in Chisséquélé.

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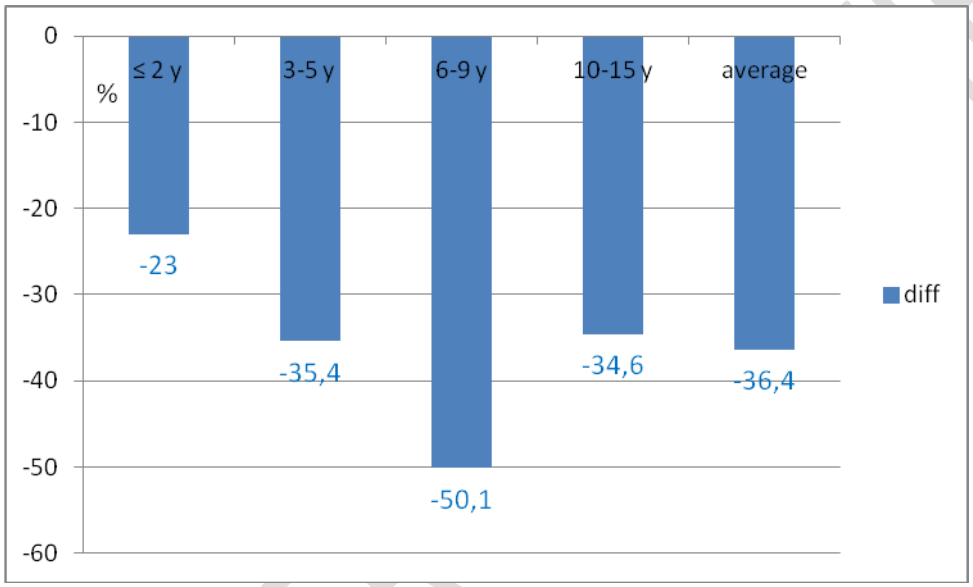
Before VC implementation (years 2007-2008) the PI of ≤ 2 years old babies (23.4%; n=308) was significantly lower than the PI of the 3 other age groups gathered (33.7%; n=695) (X2= 10.66; P value= 0.0010; OR=0.60 [0.44-0.82]).
The 2 years before the implementation of vector control the evolution of *Plasmodium* prevalence differed according to age group (graph a): slightly decreasing in 0-2 years old; sharply decreasing in 3-5 years old; similar in 6-9 years old and slightly increasing in 10-15 years old, without any biological explanation.
After VC the trends in the reduction of PI are similar in the 4 age groups (graph.).
The first year following VC the drop of PI was different according to each group (graph) being lower in babies (≤2 years old) than in other age groups, this was also observed in Candiero (graph 5b) and could lead to the same conclusion of a vector control operation if the indicator is only dealing with Plasmodium prevalence in babies .

It can also be noticed that IRS in Candiero induced an average reduction of 48% while in Chisséquélé installation of ITPS induced a slightly less reduction of 36%



Graph 5b. Drop of *Plasmodium* prevalence in each age group the first year after vector control implementation in Chisséquélé.

After the initial important drop of PI induced by VC implementation it was observed a plateau with a PI of about 5% in 2010 and 2011. (graph. 5c).

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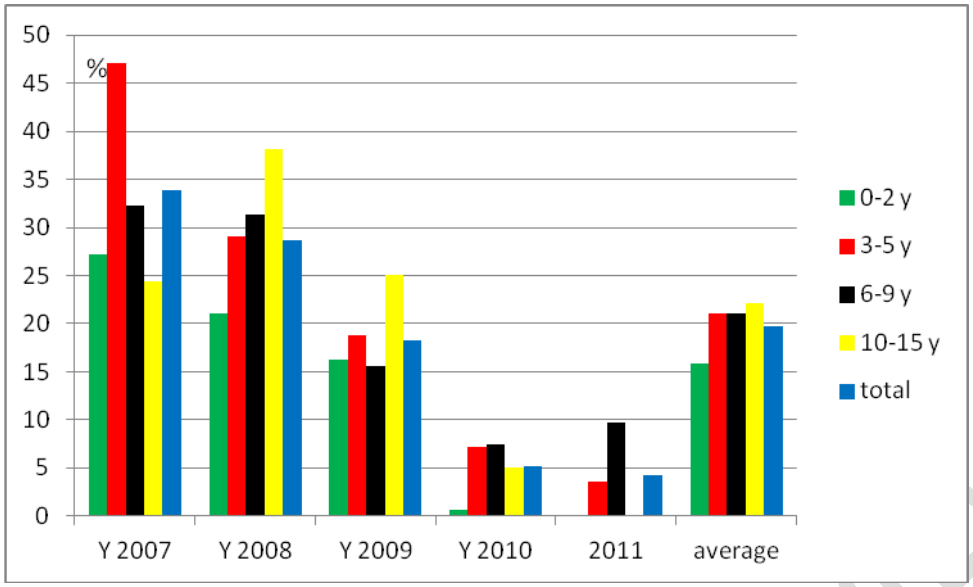

Graph 5c. Evolution of *Plasmodium* prevalence each year in each age group in Chisséquélé.

From the table 6 it is possible to sort out the change in plasmodial prevalence in each age group before (years 2007-2008) vs after (years 2009-2010-2011) vector control implementation (table 9).

Age groups	Before			After			Diff.
	n	TBF+	%	n	TBF+	%	
0-2 y	308	72	23.4	325	28	8.6	-63.1%
3-5 y	308	108	35.1	465	55	11.8	-66.3%
6-10 y	240	76	31.7	280	33	11.8	-60.0%
11-15 y	147	50	34.0	206	28	13.6	-60%

Table 9. Evolution of *Plasmodium* prevalence in each age group after vector control based on ITPS in Chisséquélé (diff.= difference).

It thus appeared a similar reduction of PI in each age group (#60-65%) and an overall reduction of *Plasmodium* prevalence, from 30.5% (n=1003) to 11.3% (n=1276) i.e. 63%, reduction after ITPS implementation.

The reduction was statistically significant for each age group: 0-2 years: $\chi2$= 25.9; OR= 0.31 [0.19-0.49]; 3-5 y: $\chi2$ = 68.1; OR= 0.25 [0.17-0.36]; 6-9 y: $\chi2$= 30.8; OR= 0.29 [0.18-0.45] and 10-15 y: $\chi2$= 20.8; OR= 0.30 [0.18-0.52] and for the total of the 4 age groups considered $\chi2$= 130.9; OR=0.29 [0.23-0.36]).

For the "at risk" age-group (≤ 5 years old):

- The year after installation of ITPS the Plasmodium Index dropped significantly by 30%,from 25.3% (n=400) in Y 2008 to 17.7% (n=368) (X2= 6.51; P value= 0.0107; OR= 0.64 [0.45-0.90]). The drop was #40% with IRS in Candiero.

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- After VC the PI decreased signicantly by 64%, from 29.2% (n= 616) to 10.5% (n= 790) (X2= 79.7; OR= 0.28 [0.21-0.38]), a reduction similar to the one noticed with IRS in Candiero.

It thus appeared that vector control had comparable impact on plasmodial prevalence in each age groups even if almost always slightly better in Candiero than in Chisséquélé with a reduction of # 60% after installation of δITPS in this village and # 70% after the 2 rounds of λIRS followed by installation of δITPS in Candiero.

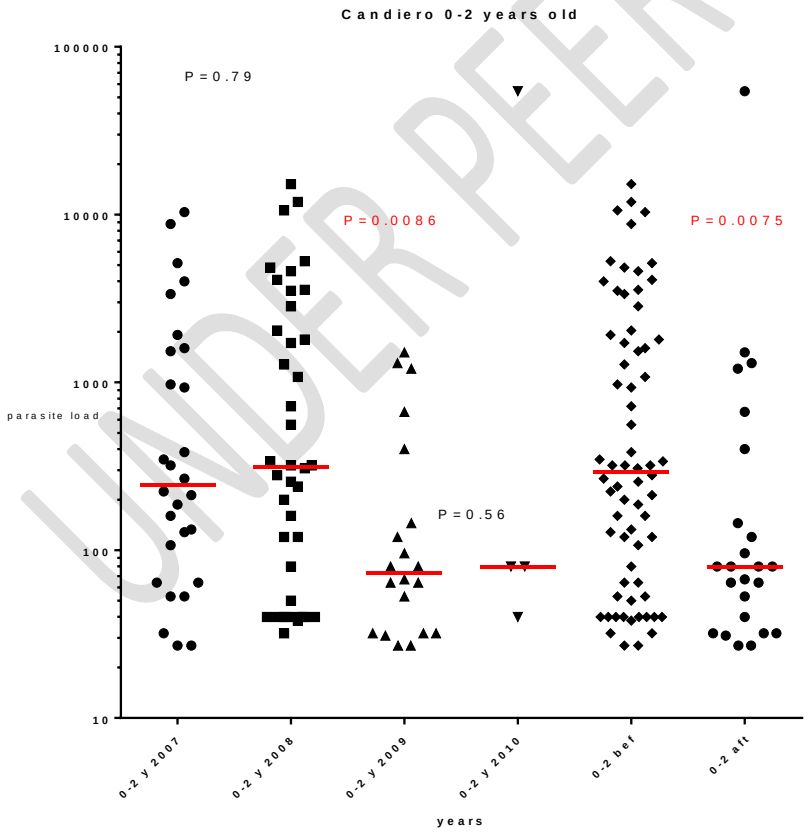
III-3. Evolution of plasmodial parasitaemia ("PL") according to age group and vector control

III-3-1. In Candiero

a) In children 0-2 years old

In children 0-2 years old (graph 6a) analyzes of the parasite load of the 92 thick blood films showed that the parasitaemia were similar in years 2007 and 2008 without vector control (P value= 0.79) then significantly dropped in 2009 (P value= 0.0086) and remained at its low value in 2010 (P value = 0.56).

The parasitaemia of children 0-2 years significantly decreased after vector control (P value= 0.0075).



Graph 6a. Evolution of parasite load in children ≤2 years old according to years and vector control (in red= median significantly different).

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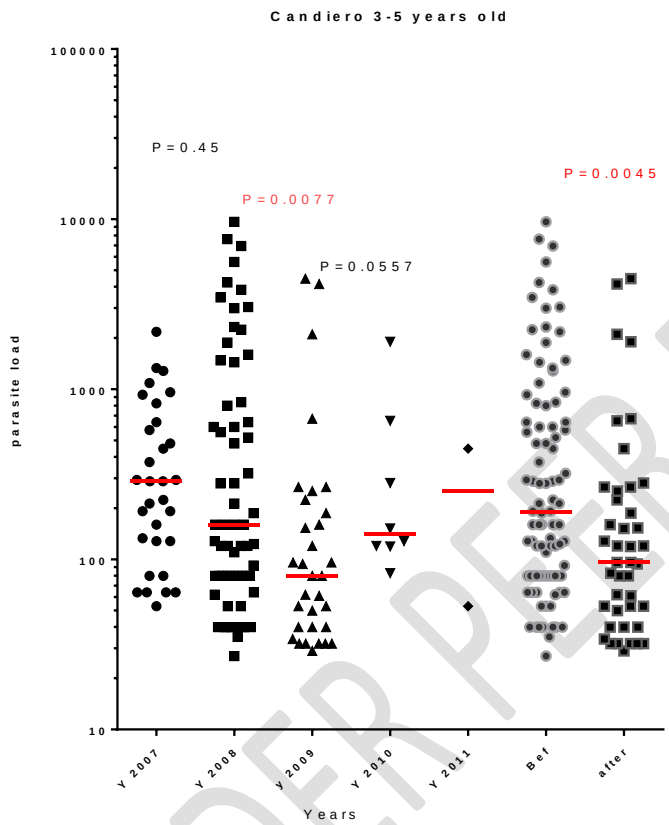
b) In children 3-5 years old

In children 3-5 years old (graph 6b): analyses of the parasite load 136 positive thick blood films showed that the parasitaemia were similar in years 2007 and 2008 (P value= 0.45) before any intervention but significantly dropped in 2009 after IRS implementation (P value= 0.0077) then remained similar (P value= 0.0557).

For children 3-5 years old the parasite load significantly dropped (P value= 0.0045) after IRS vector control implementation.



Graph. 6b. Evolution of parasite load in children 3-5 years old in Candiero before and house spraying.

c) In children 6-9 years old

In children 6-9 years old (graph. 6c) the analyses of the 139 positive thick blood films showed completely different situation as parasite load were similar in years 2007 and 2008 before vector control (P value= 0.0534) but also in years 2008 and 2009 (P= 0.9899) and 2009-2010 (P value= 0.5415). Therefore parasitaemia were similar before and after vector control (P value= 0.6475).

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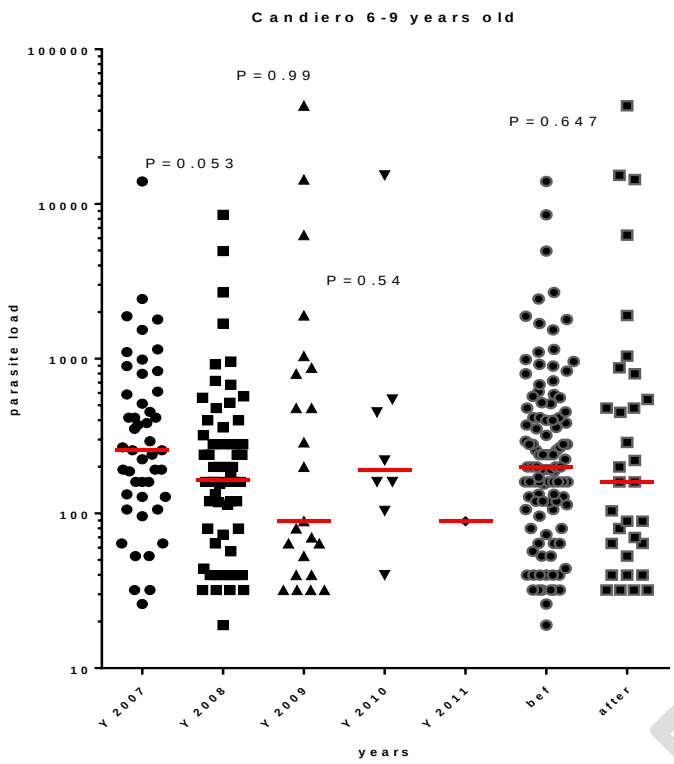

Graph. 6c. Evolution of parasite load in children 6-9 years old in Candiero before and house spraying

d) In children 10-15 years old

In children 10-15 years old (graph 6d) the situation appeared different when analyzing the parasite load of the 108 positive thick films with a significant lower parasitaemia in 2008 than in 2007 (P value = 0.0258) while parasitaemia were similar in 2008 and 2009 (P value= 0.118); 2009 and 2010 (P value= 0.774) but a significant difference occurred when comparing before and after vector control (P value= 0.0033).

Therefore it appeared that the evolution were similar in children ≤2 years old and 3-5 years old but difference occurred with older age groups; nevertheless for 3 age group the parasitaemia were always significantly lower after than before implementation of vector control.

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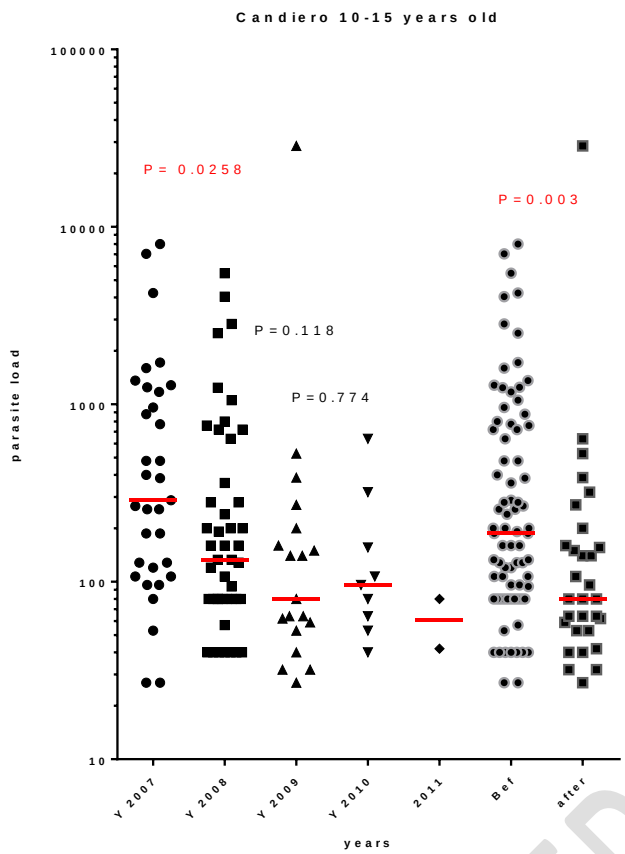

Graph. 6d. Evolution of parasite load in children 10-15 years old in Candiero before and house spraying

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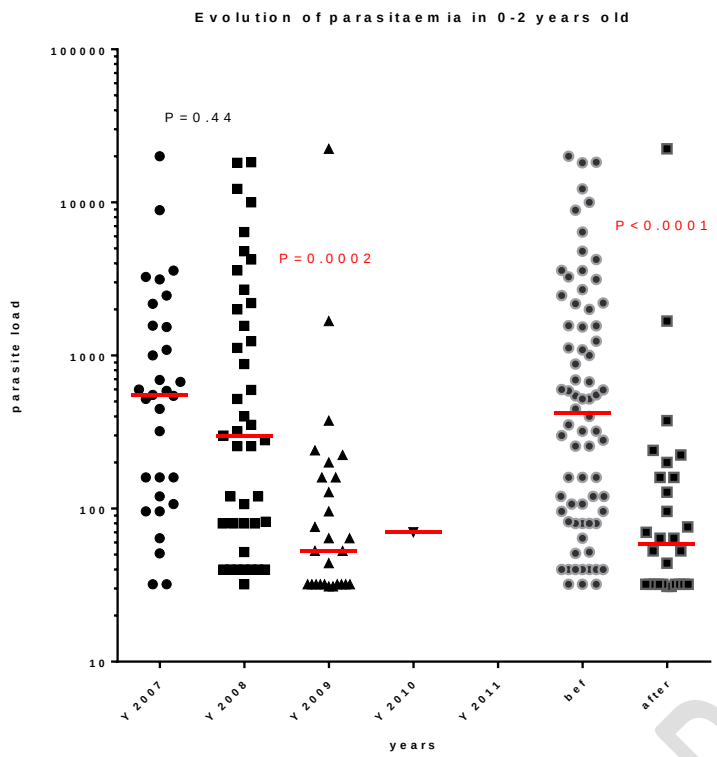
III-3-2. In Chisséquélé

a) In children 0-2 years old

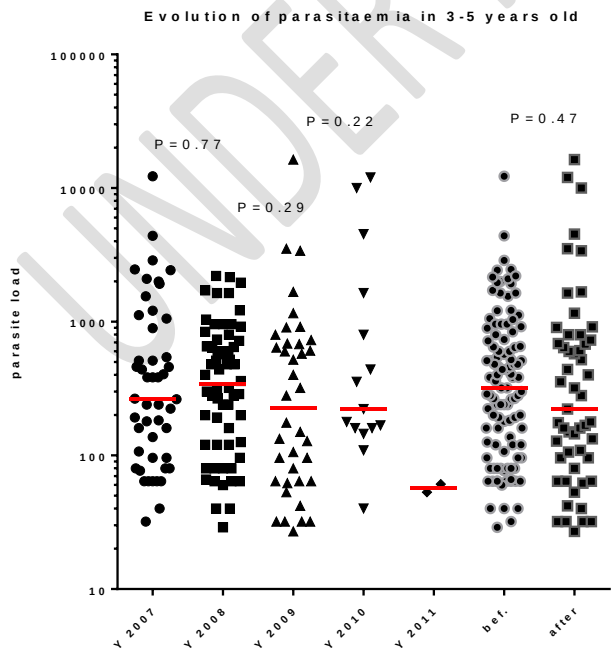
The parasitaemia were similar between years 2007 and 2008 (without vector control) (P value= 0.44; NS) and significantly different between years 2008 and 2009 (= year after installation of ITPS) (P value= 0.0002) and between the period before/after vector control (P value < 0.0001) (graph. 7a)



Graph. 7a. Evolution of the parasite load in children ≤ 2 years old in Chisséquélé according to the years and vector control implementation.

b) In children 3-5 years old

The parasitaemia were similar between years 2007 and 2008 (P value= 0.77; NS) but also between years 2008 and 2009 (P value= 0.298; NS), years 2009 and 2010 (P value= 0.221; NS) and the period before/after vector control (P value= 0.47; NS) (graph 7b).



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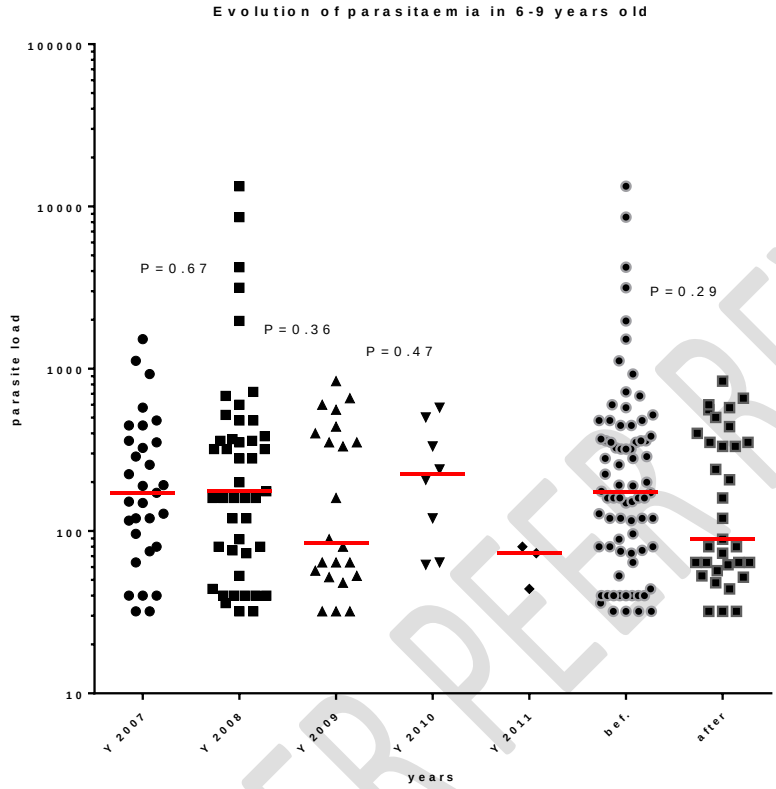
Graph. 7b. Evolution of the parasite load in children 3-5 years old in Chisséquélé according to the years and vector control implementation.

c) In children 6-9 years old

The parasitaemia were similar between years 2007 and 2008 (P value= 0.67; NS), years 2008 and 2009 (P value= 0.46; NS), 2009 and 2010 (P value= 0.47; NS) and the period before/after vector control (P value= 0.29; NS) graph 7c).



Graph. 7c. Evolution of the parasite load in children 6-9 years old in Chisséquélé according to the years and vector control implementation.

d) In children 10-15 years old

The parasitaemia were similar between years 2007 and 2008 (P value= 0.33; NS), years 2008 and 2009 (P value = 0.16; NS), 2009 and 2010 (P value= 0.26) and the period before/after vector control (P value= 0.17; NS) (graph 7d).

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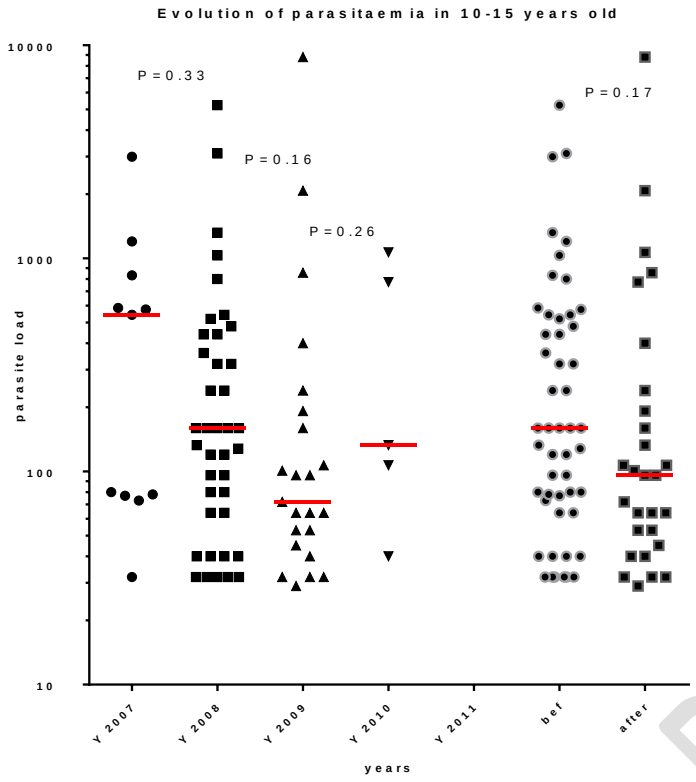

Graph. 7d. Evolution of the parasite load in children 10-15 years old in Chisséquélé according to the years and vector control implementation

Remark: it is worth noticing that in Chisséquélé, after the installation of ITPS, the plasmodial prevalence actually decreased in each age group but the parasitaemia decreased only in the younger age group 0-2 years old while remaining at the same level in the 3 other age groups considered.

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IV. Discussion

The question spraying or insecticide treated nets was raised [55, 56] each method having its advantages and issues but both showed their actual efficacy in strikingly reducing malaria morbidity [9].

After the failure of the Global Malaria Eradication Programme, vector control was almost abandoned (if not forgotten) for case management but drug resistance appeared of great concern. The emergence of insecticide treated nets as a new method of vector control and the clear demonstration of their efficacy in reducing malaria morbidity and overall infant mortality [57] constituted an important steps for vector and malaria control; especially when industrialized treatment increased their long lasting activity and nets insecticide treated nets ("ITNs") become "long lasting insecticide treated nets ("LLIN") which are now the main tool in malaria prevention and control even in some conditions of insecticide resistance [58].

But the actual use of nets, even largely distributed free of charges, is matter of concern [59] as recently noticed in Nigeria "the distribution of free ITNs has resulted in universal household ownership, but the use of the nets is still very poor" as "of the 102 ITNs that were properly deployed, only 27.5% were occupied the night before the survey".

On the other hand the misuse of nets was often reported [60-63], even if it was discussed, considering that misuse is mainly done with worn-out nets no more useful for mosquito protection as recently underlined "we should remember that long-lasting insecticide-treated nets typically wear out after 2–3 years. Therefore, we hypothesize that at least some of the anecdotal reports of nets being used for such things as fishing and weddings may actually be worn-out nets no longer in use for protection against mosquitoes, and thus their use for such purposes would not really constitute misuse of an effective ITN"[64]. As recently reported from Kenya "after those nets are torn, most people use them for other purposes" [65].

Non-used, misused, repurposing, not maintained, relatively short actual efficacy (2-3 years), acceptability (social, cultural issues), moreover insecticide resistance, constituted some worried limitations of LLIN even if they are still the main method of vector control.

It is important to find another, complementary method of vector control at community level and the recently developped long lasting insecticide treated plastic sheeting could represent such new tools [66] as their acceptability and efficacy were clearly demonstrated and this was the objective of the Balombo program developped in 8 villages around Balombo (Angola) [47] while the acceptability of ITPS was already reported from Huambo, a close town [41] and elsewhere in Africa and SE Asia [42].

One of the key point in evaluating the parasitological efficacy of a vector control program is the choice of relevant indicators[67].

The parasite index (PI) is an interesting, easy to get, useful indicator, with one well done survey ("cross sectional survey" "CSS") it is possible to obtain relevant information on the actual presence of the Plasmodium at a given time ("point prevalence"), which species and the level of endemicity ([52, 68] in one area [54, 69]. But several issues could be raised: when to do the survey, dry vs rainy season [70], techniques of detection of the parasite: classical Light Microscopy (LM) which depends greatly on the skills of microscopists [71]; Rapid Detection Test (RDT) [72] or new molecular methods [73-81] which allows a better detection of low parasitaemia in symptomless carriers, even lower than

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2 par./µl of blood while the limit of RDT which is about 50 to 100 par./µl and 20 to 100 with LM according to the amount of blood examined (thick vs thin film).

Nevertheless, even with its limitations, classical parasitological surveys with LM, well standardized and regularly done with representative sample could be a relevant indicators to evaluate the "period prevalence" in an given amount of time, or the incidence rate and the efficacy of vector control operations for eradication [2] or elimination of malaria [82, 83] as recently used in Equatorial Guinea malaria control program [17].

Analyses of PI in the 4 age groups considered, in each village, before and after vector control gave interesting informations. In Candiero the PI of babies before VC were significantly lower than in the 3 older age groups; the first year after implementation of VC the PI decreased by 26% in babies while the drop was # 50% in other age groups which could lead to a relative negative conclusion on the impact of VC if considering only the plasmodial prevalence in babies. But the 2nd and 3rd years after VC the IP were similar in the 4 age groups and the trends in evolution of PI were also similar, the VC induced an overall reduction of # 70% of plasmodic index.

In Chisséquélé, before VC the PI were also significantly lower in babies than in older age groups; the first year after VC the plasmodial prevalence decreased by 23% in babies and by # 40% in older age groups, an overall reduction lower than in Candiero. Such as in Candiero, considering only the age group ≤2 years old in Chisséquélé could lead to a relative negative conclusion on the impact of VC. The 2nd and 3rd years after VC the plasmodial prevalence were almost similar and # 10%. Nevertheless the installation of ITPS induced a reduction of # 60% of plasmodic index

On the other hand parasite load (PL) is an important parameter [84] giving information of the risk of malaria morbidity [85] through the "clinical threshold" or "pyrogenic threshold" [86]. But it was reported that this threshold could be variable with age [87] and underlying the question of how to evaluate malaria morbidity in endemic areas [88, 89] with frequent overdiagnosis [90]. Considering the relation, whatever it could be, between parasitaemia and clinical illness it clearly appeared that reducing parasite load could reduce malaria morbidity and PL is undoubtfully a relevant indicator but it implies the choice of the age group for a right evaluation of the vector control operation. For exemple in the analyze of parasitaemia data from Candiero (with IRS) and Chisséquélé (with ITPS): the PL were similar in both villages in each age group before implementation of VC, but differences appeared after VC according to age group studied.

In Candiero the first year after VC the parasite load significantly decreased in babies, but not in the older 3 age groups; while for the 3 years after VC the PL significantly decreased in babies, 3-5 and 10-15 years old but not in 6-9 years old without any biological obvious reason (considering the drop in plasmodic index).

In Chisséquélé the PL significantly dropped for babies the first year after VC and for the period before/after VC while no significant differences were observed in PL of older age groups.

It is important to underline the risk of opposite conclusion according to the parasitological indicator and the age groups: considering babies it appeared that the reduction induced by VC on the parasite index was lower than on the 3 older age groups thus leading to a "negative" conclusion. But considering the parasite load it appeared that the impact of VC was always better in babies than older age group, this was quite clear in Chisséquélé.

Therefore great care must be given in the choice of indicator and the targeted population, in this study it can be considered that both method of vector control, inside residual spraying or insecticide

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treated plastic sheeting were actually efficient in reducing parasitaemia when checking children of 0-2 years old, or 0-5 (the "at risk" group") of 0-15 years considered elsewhere.

Annex 1

Age-groups	X2	P value	OR
0-2 vs 3-5	0.16	0.687	0.94 [0.70-1.26]
0-2 vs 6-9	3.29	0.069	0.76 [0.57-1.02]
0-2 vs 10-15	2.34	0.126	0.78 [0.58-1.07]
3-5 vs 6-9	2.45	0.117	0.81 [0.62-1.05]
3-5 vs 10-15	1.58	0.208	0.83 [0.63-1.10]
6-9 vs 10-15	0.042	0.837	1.03 [0.78-1.37]

Comparison of *Plasmodium* prevalence according to age-groups in Candiero

Annex 2

Age-groups	X2	P value	OR
0-2 vs 3-5	6.40	0.011	0.70 [0.53-0.92]
0-2 vs 6-9	5.13	0.023	0.71 [0.52-0.96]
0-2 vs 10-15	6.10	0.014	0.66 [0.47-0.92]
3-5 vs 6-9	0.0029	0.96	1.00 [0.77-1.32]
3-5 vs 10-15	0.15	0.70	0.94 [0.69-1.28]
6-9 vs 10-15	0.16	0.69	0.93 [0.67-1.29]

Comparison of *Plasmodium* prevalence according to age-groups in Chisséquélé

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