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Parasitological surveys before, and ten years after village-scale malaria vector control operations in Angola

Pierre Carnevale*[1], Jean-Claude Toto[2], Franck Martineaud[3], and Guillaume Carnevale[4]

[1]The George Building, 34170, Castelneau-le-Lez, France
[2]OCEAC (Organization for Coordination of Endemic Diseases Control in Central Africa), Yaoundé Cameroon
[3]Malaria Control Program, Head Medical Department, Sonamet®, Lobito, Angola
[4]Hygiene Safety Environment, Batuan, Bali Indonesia

ABSTRACT

A comprehensive, village-scale long-term malaria vector control was implemented around Balombo town (Benguela Province, Angola), to compare the efficacy of four methods.

***Methods:** Four methods were implemented, each one in two villages: long-lasting insecticide treated nets LLINs PermaNet® P2.0 alone; insecticide-treated plastic sheeting (ITPS) model ZeroVector® (ZV) alone; combination LLIN P2.0 and ITPS model ZeroFly® (ZF) and ITPS after two rounds of inside residual spraying (IRS)*

Two cross-sectional parasitological surveys were done before vector control (February 2008) and ten years after (February 2018) in asymptomatic children ≤15-year-old, in the eight villages.

Two parasitological indicators were analyzed: Plasmodium prevalence and parasitaemia.

***Results:** 1,648 thick blood films (TBF) were prepared. Plasmodium (largely P. falciparum) was observed in 637 TBF, (overall parasite prevalence=38.6%). A significant decrease, by 32%; occurred between before (44.2%; n=1,004) and after vector (29.9%; n= 644).*

The parasite prevalence remained similar in villages furnished in LLIN and decreased with the three other methods.

The evolution of parasite load was opposite, with a significant increase between 2008 and 2018 in the whole sample but not in children ≤5-year-old (at risk group) covered with LLIN.

No parasitological rebound was observed.

***Conclusion:** The diversity of information according to age-group, vector control method and parasitological indicator, highlight the need for long-term, comprehensive, evaluation in several villages before drawing any definitive conclusion.*

The combination LLIN nets and ITPS was not better than ITPS only, which was as efficient as indoor residual spraying, and may constitute an interesting new tool for the national malaria control programme.

***Keywords:** Angola; vector control; four methods; two parasitological surveys; before and 10 years after vector control; no parasitological rebound.*

I. Introduction

Malaria is transmitted throughout Angola. 100 percent of the population (# 40 million) is at risk of the disease. Malaria remains a primary health burden in the country and is the main cause of ill health and death.

For 2023 it was officially reported 10,089 deaths due to malaria, with an estimation of 16,169 deaths; presumed and confirmed cases of malaria: 10,496,880 cases, estimated 8,251,449 cases.

Within the central African region, Angola recorded the second highest (15%) malaria cases. Malaria was responsible for 44% of all reported deaths in 2022, rising from 42% in 2021, making it the leading cause of death. In 2021, 33 percent of all patients seeking health care were diagnosed with malaria. 5.6% of all malaria cases were also reported to be severe malaria (people hospitalized for malaria).

Between 2022 and 2023, the incidence of malaria cases decreased from 236 to 225 per 1000 people at risk, representing a 4.8% decrease in malaria burden in the country. Also, deaths decreased by 18% from 0.54 per 1000 of the population at risk in 2022 to 0.44 per 1000 of the population at risk in 2023. The current estimated mortality rate for children under five years of age is 68 deaths per 1,000 live births, which represents a 42 percent decrease over the past decade.

The situation could be worsened by the resistance of *P. falciparum* drugs [1-6] and malaria cases, confirmed by microscopy or rapid detection test (RDT) should be treated with an artemisin-based combination therapy (ACT) with three alternative first-line ACT treatments: artesunate/amodiaquine (AS/AQ), artemether-lumefanthrin (AL), and

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Corresponding Authors: **Pierre Carnevale**
Email: **pjcarnevale2001@yahoo.fr**

dihydroartemisin/piperaquine [7].« But several deviating practices were reported such as widespread use of artemether (for a prolonged period even when the patient is already eating normally), issue to require calculations for artesunate dilution, insufficient healthcare infrastructures, logistical difficulties; still limited qualified health professionals etc »

These issues in case-management underline the need for vector control.

In Angola, due to operational issues, indoor residual spraying (IRS) was stopped, [8] the National Malaria Control Program (NMCP), focuses on large-scale distribution of long-lasting insecticide treated nets (LLINs) through different channels, such as antenatal care Department in Hospital, to protect pregnant women who are considered, like children below five years old, as a "at risk group."

But one of the key issues is the spreading of pyrethroid resistance in Africa South of the Sahara countries [9-13], which can reduce the efficacy of currently available LLIN and IRS [14], and underlines the needs for new tools for "averting a malaria disaster" [11]

Therefore, new mosquito nets are treated with two products such as pyrethroid +chlorfenapyr [15] or pyriproxyfen [16], [or] piperonyl butoxide PBO [17-18] .

Another recently developed tool, is the insecticide-treated plastic sheeting (ITPS) [19] (also called "wall lining" WL or "durable lining" DL) successfully used in refugees camps [20] and many other places and conditions. To deal with the resistance issues, several approaches were developed such as "Combining Organophosphate Treated Wall Linings and Long-lasting Insecticidal Nets for Improved Control of Pyrethroid Resistant *Anopheles gambiae"* [21] or carbamate treated plastic sheeting combined with pyrethroid treated LLIN [22].

Several studies were devoted to such ITPS [23-30] and it was recently considered that they have "future prospects for control of malaria and other vector-borne diseases" [30].

Another main issue in vector control is the sustainability [31-32] including the actual community acceptability [27] and participation.

On the other hand, "it is essential that long-term surveillance is included as part of LLINs interventions, with particular attention to the age range over which rebound may occur" [33]

Actually, the first conference on malaria in Equatorial Africa was held in Kampala (Uganda) from 27 November to 9 December 1950 to discuss the eradication of malaria through the treatment of houses with DDT. Two "schools" appeared: the interventionists", with a will to act immediately and the conservatives", requesting more scientific information on immunity before launching major vector control programs that could alter the immunity of populations in hyperendemic areas, a still matter of concern recently. [33-39]

It was stated that "the populations of moderate or highly malaria endemic areas gradually acquire some immunity to malaria as a result of repeated exposure to the infection. When this exposure is reduced as a result of effective malaria control measures, subjects who benefitted from the intervention may consequently be at increased risk of malaria if the intervention is withdrawn, especially if this is done abruptly, and an effective malaria vector remains. There have been many examples of this occurring in the past, a phenomenon often termed 'rebound malaria', with the incidence of malaria rebounding to the level present before the intervention was introduced...." [38]

In Zanzibar a program was implemented for malaria elimination which was stopped in 1968.

It was reported a rebound malaria epidemic in the 1970-1980 "when prevalence rates surged higher than they were prior to the WHO's intervention. We show that scientists were aware of the risks of rebound before it happened and recognized the rebound epidemic as it was happening. We argue that many of the challenges facing Zanzibar in the 1960s remain dilemmas today, and many of the ethical questions about rebound malaria remain" [39]

It was reported that "The efficacy and relative cost-effectiveness of insecticide-treated nets (ITNs) for the control of malaria in children under 5 years of age have recently been demonstrated by several large-scale trials. However, it has been suggested that long-term use of ITNs in areas of high transmission could lead to mortality rebound in later childhood, which would reduce the cost-effectiveness of the intervention, and at the extreme could lead to negative overall effects. Rebound was considered over two possible age ranges: 5-9 years and 3-6 years. With mortality and morbidity reductions due to ITNs in children aged 1-59 months and rebound in the 5-9 years age class." [33]

This possible shift of morbidity from age <5 years to 5-9 year, or older, is of concern, and explains why we subdivided our large sample (≤15 years) to specify situation in children ≤5 years, and older age group, and its evolution in term of parasite prevalence and parasite load, (one of the markers of malaria morbidity) long-term after (10 years) after vector control.

Considering the need for new tools, and the fear of some eventual rebound effect, a long-term (eleven years) comprehensive, village-scale, malaria vector control program, was implemented, in eight villages around Balombo town (Benguela Province, Angola) to compare the efficacy of four methods [40-41].

A major element to be considered is the national malaria outbreak which occurred in 2015.

II. Material and Method

II-1. Study area

The villages are in the Balombo Municipality, a city of western Angola (12°S; 14°E), located 600 km southwest of the capital, Luanda (map Fig 1), at an altitude of 1,200 meters, in a tropical savannah, with a rainy season from November to April.

Villages, and the area of the trial, were selected by the National Malaria Control program as the acceptability of insecticide-treated plastic sheeting was already observed in Huambo town, the next town of the Eastern Road, and relatively close to Balombo. [26]

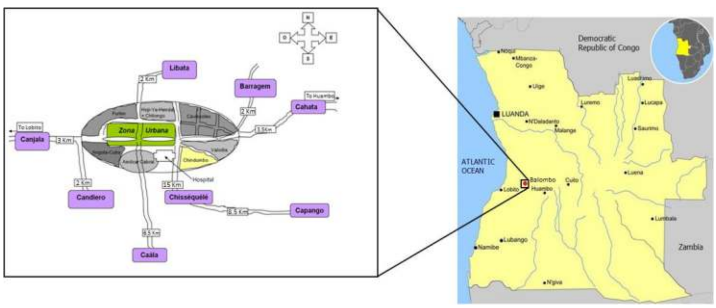

Figure 1. Map of Balombo town and villages studied in Angola. [40]

The malaria transmission season occurred all the yearlong, with a peak between January and May. The main malaria vectors are *Anopheles funestus* and *An. gambiae* [41].

A previous cluster sampling (32 clusters) survey done in the area, in November 2006, before any intervention, focusing of children ≤15-year-old, showed an average parasite prevalence of 54.9% (n= 557).

As already precised « 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 ». [42]

II-2. Vector control operations
According to the National Malaria Control Program the trial was carried out in eight villages to test and compare the four methods of vector control.
A random parasitological survey, done previously in villages of the area to get base line data, identified villages with « high level parasite prevalence » (HPP> 50%) and villages with « low level parasite prevalence » (LPP< 50%). Forming thus two groups of villages.
Villages were randomly paired from the two groups, one village with HPP paired with one village with LPP. Each one of the four vector control methods was randomly implemented in one paired-villages (Table 1)

Table 1. Vector control operations. (LLIN = long-lasting insecticide-treated nets, model PermaNet® 2.0; ZF = insecticide treated plastic sheeting, model ZeroFly®; ITPS ZV = insecticide treated plastic sheeting model ZeroVector®; λIRS 1 = indoor residual spraying with lambdacyhalothrin λ, round 1; λIRS 2 = indoor residual spraying with lambdacyhalothrin, round 2; * =target: 1 LLIN/house; ** = target: 1 LLIN/sleeping unit) (xx = no vector control done; C = control villages: 360 LLIN = 360 long-lasting insecticide treated nets distributed; m^2 = surface covered by ITPS or IRS; 191 houses= number of houses treated)

Village	February 2007	February 2008	December 2008	June 2009	January 2010
Caala (469 sleeping units)	LLIN* 360 LLIN	LLIN** 227 LLIN	LLIN** 49 LLIN	xx	xx
Cahata (442 sleeping units)	LLIN* 310 LLIN	LLIN** 195 LLIN	LLIN** 25 LLIN	xx	xx
Capango	C	C	93 LLIN + 93 ZF	xx	xx
Canjala	C	C	422 LLIN + 621 ZF	xx	xx
Barragem	C	C	5,554 m^2 ITPSZV	xx	xx
Chisséquélé	C	C	5,441 m^2 ITPSZV	xx	xx
Candiero	C	C	λIRS 1 191 houses 9,500 m^2	λIRS 2 176 houses 8,750 m^2	ITPS ZV 4,914 m^2
Libata	C	C	λIRS 1 258 houses 12,825 m^2	λIRS 2 263 houses 13,070 m^2	916 ITPSZF 17,505 m^2

Two villages (Caala and Cahata) received long-lasting insecticide (deltamethrin δ) treated nets, PermaNet® 2.0 only; two villages (Capango and Canjala) received PermaNet® 2.0 in combination with insecticide (deltamethrin) treated plastic sheeting model "ZeroFly"® (picture 1); two villages (Barragem and Chisséquélé) received insecticide (deltamethrin)-treated plastic sheeting alone, model "ZeroVector"® (picture 2) and two villages (Libata and Candiero) received two rounds of lambdacyhalothrin (λ) indoor residual spraying (six-month interval) at the target dosage of 25 mg a.i./m^2 , then insecticide (deltamethrin) treated plastic sheeting.
Complete vector control coverage occurred in December 2008.
Details of these operations (Table 1) were already presented [40-41]
In each village, a census of the number of inhabitants, number of houses, number of sleeping units, was done by the village health worker one (Table 2). Each house received a number (on the door) and location made with GPS.
This information was used to check for a complete coverage of each house, and each sleeping units, with nets in villages where LLINs were given, and to check the place of patients with positive thick film at each field survey to identify any eventual hot spot in the village.

Table 2. Demographic information on the eight villages involved, at the beginning of the project, in February 2007. (* a sleeping unit was defined as any item used for sleeping, including permanent beds, temporary mats, cardboard, loincloths, and other items unfolded at night to sleep.)

Village	Number of inhabitants	Number of houses	Number of sleeping units*
Caala	808	239	469
Cahata	517	154	442
Capango	177	60	89
Canjala	873	401	422
Chisséquélé	418	181	201
Barragem	620	134	168
Libata	1,344	258	513
Candiero	654	190	380
Total	5,411	1,617	2,684

II-3. Protocol
The comprehensive program was planned, and implemented, in these eight villages, to compare the evolution plasmodial infections during eleven years (year 2007-year 2018), two years before and nine years after vector control.
Immunological, entomological and parasitological results of the first five years, (two years before and three years after vector control), dealing with short term impact of vector control, have already been published [40,41]. Results of the long-term parasitological evolution, each year, during the eleven years, in four villages, (one per each vector control method) were submitted for publication.
The present document compares the parasitological data of the cross-sectional surveys, done in the eight villages, in February 2008 (before vector control), and ten years after, in February 2018.
The field sampling was done following classic cross-sectional random surveys method. In each village almost all villagers were volunteered to have their blood examined for malaria infection diagnosis.

A thick blood film (TBF) was prepared from each one, at the same time we noted information on age, genera, location of house etc. This constituted the "field survey list" which were completed by the parasitological results, and analyzed.
Thick blood films were examined with optical microscope, in the medical department of the Angolese Sonamet® Society in Lobito, in the context of its "Malaria Control Program", which is in process for several years. Results were supplied to the Health Care worker of each village for action, according the guidelines of the National Program.
This protocol is in the line of what was done before or elsewhere. According to Boyd [42] "considerations of a practical nature in routine field work commonly necessitate a disregard of the adult population, restricting the observations to infants, children and adolescents, in other words to the population below fifteen years of age". In some situation" examinations have been largely restricted to children in their third to tenth year of life i.e. from ages two to nine"
In Equatorial Guinea the age-group 2-<15-year-old was used to analyze vector control operations [43, 44] and the group 1 to 14 years "in cross-sectional household surveys over a 13-year period of intensive interventions to track the progress of malaria control" [45]
The lists of volunteers sampled in the field were composed of men, women, children, babies of all ages.
From this heterogeneous field sample, we sorted out children ≤15-year-old to get "age homogenous samples" which could be analyzed, and compared.
II-4. Indicators
Two parasitological indicators were analyzed: *Plasmodium* prevalence (parasite prevalence or "PP") and parasite load (or "PL").
For each survey the whole sample of asymptomatic children ≤15-year-old was sub divided in three age-groups: ≤5-year-old (to check the evolution of plasmodial infections in the "at risk group") [46-51] ; 2–9-year-old (to check for the evolution in group used for classification of endemicity level) and ≤15-year-old (=" whole sample") to increase the size of sample allowing thus observation of an eventual small change in the parasite prevalence.

Plasmodium species were diagnosed, and parasites were counted versus 200 white blood cells to get a parasite load per milliliter of blood. Actually "a parasite rate of say 50 per cent may largely be made up of scanty infections representing little of no morbidity or of relatively heavy infections with all the signs of a community suffering severely from malaria. "[42].
Parasite prevalence gathers all *Plasmodium* species as previous, and current, parasitological surveys show the large predominance of *P. falciparum* and the very low number of *Plasmodium malariae, P. vivax* and *P. ovale.*
II-5. Statistical analysis

Data were analysed, and graphs constructed with GraphPad Prism5H software (San Diego, CA, USA). The non-parametric Mann-Whitney test was used for comparison of median of parasite density, before and after, each vector control intervention, in each village, and for each vector control method
The χ^2 test was used for comparison percentages.
All differences were considered significant at $P<0.05$.

III. Results

A total of 1,648 thick blood films (TBF) was prepared from volunteers' asymptomatic children ≤15-year-old during the two parasitological cross-sectional surveys. *Plasmodium* were observed in 637 TBF, i.e., an overall parasite prevalence (PP) of 38.6%.
In February 2008, *Plasmodium* were noticed in 444 of the 1,004 thick blood films, (i.e. PP=44.2%), while in February 2018, *Plasmodium* were diagnosed in 193 of the 644 thick blood films, (i.e. PP=29.9%), a significant 32% reduction (χ^2=92.6; OR=0.35 [0.28-0.43]).
Plasmodium falciparum was largely preponderant; two *P. malariae* were diagnosed in February 2008, while in February 2018, it was observed eight *P. malariae*; seven association *P. falciparum+ P. malariae*; seven *P. vivax*; one association *P. vivax + P. falciparum*; one *P. ovale*.

III-1. Evolution of parasite prevalence
Data of the number (n), and thick positive blood films (TBF+) observed during the two cross-sectional surveys, and their statistical analysis, are gathered in Table 3 for children ≤5-year-old; Table 4 for children 2–9-year-old and Table 5 for children ≤15-year-old.

Table 3. Parasite prevalence (PP) in asymptomatic children ≤5-year-old of the eight villages in February 2008 and February 2018. (TBF+= thick blood film with *Plasmodium*; n= number of thick blood films examined; Stat = statistical conclusion; NS= non-significant difference; OR= Odd Ratio; ↓S= significant decrease)

Village		Feb. 2008			Feb. 2018					
≤ 5 years	TBF+	n	PP %	TBF+	n	PP %	χ^2	*P* value	OR	Stat
Caala	22	81	27.2%	15	44	34.1%	0.66	0.42	1.39 [0.63-3.06]	NS
Cahata	25	53	47.2%	10	34	29.4%	2.71	0.099	0.47 [0.18-1.27]	NS
Capango	8	28	28.6%	18	42	42.9%	1.47	0.22	1.87 [0.67-5.21]	NS
Canjala	33	83	39.8%	8	46	17.4%	6.83	0.0089	0.32 [0.13-0.77]	↓S
Barragem	31	56	55.4%	6	18	33.3%	2.64	0.1040	0.40 [0.13-1.23]	NS
Chisséq.	20	54	37.0%	4	37	10.8%	7.77	0.0053	0.26 [0.06-0.66]	↓S
Candiero	19	66	28.8%	10	40	25%	0.18	0.67	0.82 [0.34-2.01]	NS
Libata	51	99	51.5%	10	32	31.3%	3.99	0.996	0.43 [0.18-0.99]	↓S
Total	209	520	40.2%	81	295	27.5%	13.32	0.0003	0.56 [0.41-0.77]	↓S

Table 4. Parasite prevalence (PP) in asymptomatic children 2–9-year-old of the eight villages in February 2008 and February 2018. (TBF+= thick blood film with *Plasmodium*; n= number of thick blood films examined; Stat = statistical conclusion; NS= non-significant difference; OR= Odd ratio; ↑S= significant increase; ↓S= significant decrease)

Village		Feb. 2008			Feb. 2018					
2-9 years	TBF+	n	PP %	TBF+	n	PP %	χ^2	*P* value	OR	Stat
Caala	34	97	35.1%	27	54	50%	10.0	0.0015	2.85 [1.47-5.52]	↑S
Cahata	38	77	49.4%	18	48	39.1%	1.21	0.270	0.66 [0.31-1.38]	NS
Capango	15	38	39.5%	25	52	41.8%	0.66	0.42	1.42 [0.61-3.31]	NS
Canjala	62	120	51.7%	13	58	22.4%	13.72	0.0002	0.27 [0.13-0.55]	↓S
Barragem	45	79	58.2%	13	35	37.1%	9.84	0.0017	0.34 [0.17-0.67]	↓S
Chisséq.	21	57	36.8%	11	54	20.4%	3.67	0.055	0.44 [0.18-1.03]	NS
Candiero	26	84	31.0%	13	42	30.9%	0	1	1 [0.42-2.41]	NS
Libata	77	127	60.6%	15	45	33.3%	9.95	0.0016	0.32 [0.16-0.66]	↓S
Total	318	679	46.8%	135	386	35.0%	14.16	0.0002	0.61 [0.47-0.79]	↓S

Table 5. Parasite prevalence (PP) in asymptomatic children ≤15-year-old of the eight villages in February 2008 and February 2018. (TBF+= thick blood film with *Plasmodium*; n= number of thick blood films examined; Stat = statistical conclusion; NS= non-significant difference; OD= Odd ratio; ↓S= significant decrease; ↑= significant increase)

Village		Feb. 2008			Feb. 2018					
≤ 15years	TBF+	n	PP %	TBF+	n	PP %	χ^2	*P* value	OR	Stat
Caala	50	144	34.7%	33	82	40.2%	0.68	0.41	1.27 [0.72-2.21]	NS
Cahata	53	107	49.5%	26	79	32.9%	5.14	0.0.23	0.49 [0.27-0.91]	↓S
Capango	21	60	35.0%	36	83	41.8%	1.02	0.31	1.42 [0.72-2.82]	NS
Canjala	83	178	46.6%	20	93	21.5%	16.3	<0.005	0.31 [0.18-0.56]	↓S
Barragem	64	123	52.0%	17	61	37.9%	9.6	0.0019	0.36 [0.18-0.69]	↓S
Chisséq.	35	102	34.3%	18	81	22.2%	3.21	0.073	0.55 [0.28-1.06]	NS
Candiero	41	126	32.5%	19	81	23.5%	1.980	0.159	0.63 [0.34-1.19]	NS
Libata	97	164	59.1%	24	84	28.6%	20.78	<0.005	0.27 [0.17-0.49]	↓S
Total	444	1,004	44.2%	193	644	30.0%	33.62	<0.005	0.54 [0.44-0.66]	↓S

III-1-1. Evolution of parasite prevalence according to age-group and village

a). According to age

The evolution of parasite prevalence (PP), according to village, and age-group, are presented in Fig. 2 (children ≤5-year-old), Fig. 3 (children 2–9-year-old) and Fig. 4 (children ≤15-year-old) which show the overall decreasing trends of *Plasmodium* prevalence in 2018 compared to 10 years before.

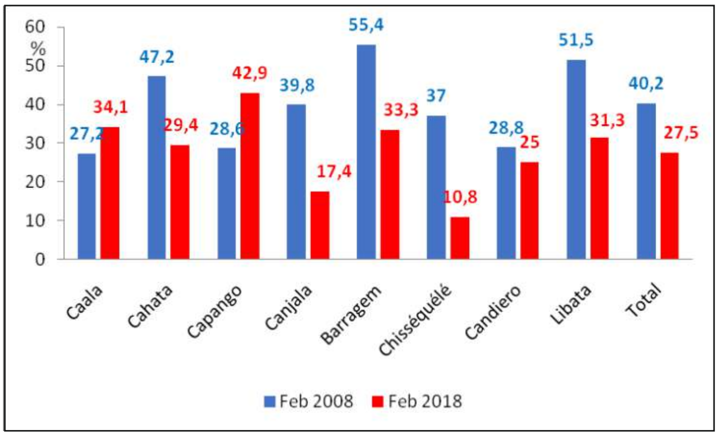

Figure 2. Evolution of the parasite prevalence in asymptomatic children ≤5-year-old in February 2008 (Feb 2008) and February 2018 (Feb 2018)

In the at-risk age-group the overall parasite prevalence decreased significantly by 31.6% between February 2008 and February 2018 (χ^2=13.3; p<0.05; OR= 0.56 [0.41-0.77]).

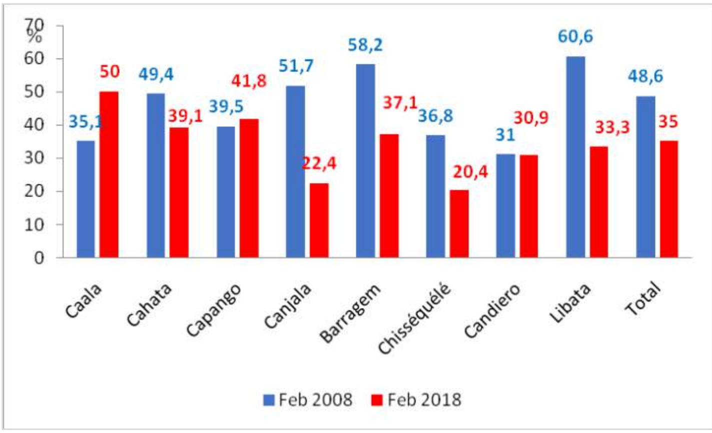

Figure 3. Evolution of the parasite prevalence in asymptomatic children 2–9-year-old in February 2008 (Feb 2008) and February 2018 (Feb 2018)

In the 2-9-year-old the overall parasite prevalence decreased significantly by 28% between February 2008 and 2018 (χ^2= 14.2; p< 0.005; OR= 0.61 [0.47-0.79])

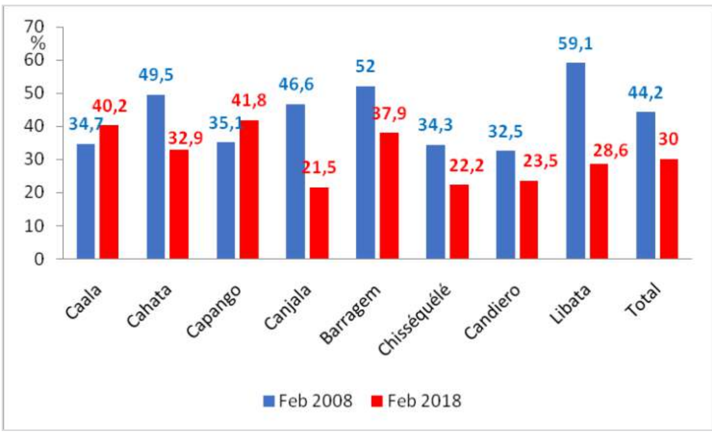

Figure 4. Evolution of the parasite prevalence in asymptomatic children ≤15-year-old in February 2008 (Feb 2008) and February 2018 (Feb 2018)

In the whole sample of children ≤15-year-old the overall parasite prevalence decreased significantly by 32.1%. (χ^2= 33.6; p<0.005; OR= 0.54 [0.44-0.66])

b) According to village

- In Caala village

The examination of the 226 thick blood films (respectively 144 in February 2008 and 82 in February 2018) showed different evolution according to age-group, being not statistically different in children ≤5-year-old (with a small but not significant increase of 25%), and overall sample children ≤15-year-old (#37%), but a statistical increase (from 35% to 50%) occurred in the age group 2–9-year-old (Tables 3, 4 and 5)

- In Cahata village

The examination of the 186 thick blood films (107 in February 2008 and 79 in February 2018) revealed similar parasite prevalence in children ≤5 years and 2-9 years, but a significant decrease for the whole sample (from 49.5% to 32.9%) (Tables 3, 4 and 5)

- In Capango village

In Capango, the parasite prevalence remained at the same level in February 2008 and February 2018, for each age-group considered, with an average of 40% for the whole sample. (Tables 3, 4 and 5)

- In Canjala

The examination of the 271 thick blood films done in Canjala showed a significant decrease of the parasite prevalence, for each age-group, from February 2008 (overall #47%) to February 2018 (overall #21%) (Tables 3, 4 and 5)

- In Barragem

The examination of the 184 thick blood films showed a significant reduction of the parasite prevalence, from February 2008 (PP=52%) to February 2018 (PP=28%) in the whole sample (children ≤15-year-old). The reduction was also observed in the age group 2–9-year-old but not in children ≤5-year-old where the parasite prevalence decreased, but not significantly (Tables 3, 4 and 5).

- In Chisséquélé

The examination of the 183 thick blood films showed a lower, but not statistical, level of parasite prevalence in February 2018 (PP=22%) compared to February 2008 (PP=34%) for the whole sample (≤15 years) and also for the age group 2–9-year-old. But it appeared a significant decrease (from 37% to 11%) in children ≤5-year-old (Tables 3, 4 and 5).

- In Candiero

The examination of the 207 thick blood films showed similar values of parasite prevalence in February 2018 (overall PP=23.5%) as in February 2008 (overall PP= 32.5%); this was observed in each age-group analyzed (Tables 3, 4 and 5).

- In Libata

In Libata, the parasite prevalence significantly decreased from February 2008 (PP=60%) to February 2018 (PP= 30%), and this was observed in each age-group (Tables 3, 4 and 5).

III-1-2. Synthesis of the evolution of parasite prevalence according to villages

Gathering the data of the parasite prevalence of February 2008 and February 2018 (Tables 3, 4 and 5) highlights the diversity of evolution, according to village, and age-group, considered (Table 6) with:

-in the whole age group ≤ 15-year-old the parasite prevalence dropped or remained similar;

-in the 2–9-year-old group, excepted in Caala, parasite prevalence dropped or remained similar;

-in the at-risk group (≤5-year-old) parasite prevalence remained at the same level, or significantly decreased, which represents an interesting epidemiological finding.
The trends in the evolution of parasite prevalence, according to age-groups, were not the same in each village. (Table 6)
In four villages (Capango, Canjala, Candiero, Libata) the trends were similar in each one of the three age-groups; in the four other villages it was observed an increase (Caala) or a decrease (Cahata, Chisséquélé) in one age group; and in Barragem similar values in ≤5 but increased in other age-groups.

Table 6. Comparison of parasite prevalence noticed in February 2008 and February 2018 in the three age-groups considered. (= similar values; D↘ = significant decrease; I↗= significant increase)

Period		February 2008=> February 2018	
Age-group	≤ 5-year-old	2–9-year-old	≤ 15-year-old
Caala	=	I↗	=
Cahata	=	=	↘D
Capango	=	=	=
Canjala	D↘	D↘	D↘
Barragem	=	D↘	D↘
Chisséquélé	D↘	=	=
Candiero	=	=	=
Libata	D↘	D↘	D↘
Total	D↘	D↘	D↘

It will be noteworthy to compare this information on parasite prevalence evolution with the evolution of parasite load for the same age-groups and villages (Table 10).
III-1-3. Evolution of the parasite prevalence (PP) according to age-group and vector control method implemented
Data of cross-sectional surveys done in February 2008, and February 2018, analyzed according to vector control methods, and age-group, are gathered Table 7.

Table 7. Parasite prevalence noticed in February 2008 and 2018, according to vector control method, and age-group. (indic. = indicator; TBF+ = blood film with *Plasmodium*; n= number of thick blood films examined; LLIN alone = only long-lasting insecticide treated nets P 2.0; LLIN + ZF= association long-lasting nets and insecticide treated plastic sheeting model "ZeroFly®"; ITPS ZV= insecticide-treated plastic sheeting alone model ZeroVector®; IRS= indoor residual spraying; "diff." = difference; S= statistically significant; NS= non-significant difference)

Method VC	period		February 2008			February 2018		χ2		
	Age -group	TBF+	n	%	TBF+	n	%	*P* value	OR	Diff
	≤ 5 years	47	134	35.1%	25	78	32.0%	χ2=0.20 P=0.65	0.87 [0.48-1.58]	NS
LLIN alone	2-9 years	72	174	41.4%	45	100	45%	χ2=0.29 P=0.59	1.15 [0.69-1.88]	NS
	≤ 15 years	103	251	41.0%	59	161	36.6%	χ2=0.79 P=0.37	0.86 [0.55-1.25]	NS
	≤ 5 years	41	111	36.9%	26	88	29.5%	χ2=1.20 P=0.27	0.72 [0.39-1.30]	NS
LLIN + ZF	2-9 years	77	158	48.7%	35	110	34.5%	χ2=5.33 P=0.021	0.55 [0.34-0.92]	D↘
	≤ 15 years	104	238	43.7%	56	176	31.8%	χ2= 6.02 P=0.014	0.60 [0.40-0.90]	D↘
	≤ 5 years	51	110	46.4%	10	55	18.2%	χ2=12.5 P=0.0004	0.26 [0.12-0.56]	D↘
ITPS ZV alone	2-9 years	67	136	49.3%	24	89	26.9%	χ2=11.1 P=0.0009	0.38 [0.21-0.67]	D↘
	≤ 15 years	99	225	44.0%	35	142	24.6%	χ2=14.1 P=0.0002	0.42 [0.26-0.66]	D↘
	≤ 5 years	70	165	42.4%	20	72	27.8%	χ2=4.56 P=0.033	0.52 [0.28-0.95]	D↘
IRS then ITPS	2-9 years	103	211	48.8%	28	87	32.2%	χ2= 6.91 P=0.0085	0.49 [0.29-0.84]	D↘
	≤ 15 years	138	290	47.6%	43	165	26.1%	χ2=20.3 P<0.005	0.39 [0.26-0.59]	D↘

a) Long-lasting insecticide-treated nets PermaNet® 2.0 (LLIN P2.0) alone
In Caala and Cahata, the two villages which received nets, the overall parasite prevalence was not significantly different in February 2018 and February 2008, in the three age-groups considered. (# 40%) (Table 7)

b) Association LLIN P2.0 + insecticide-treated plastic sheeting model ZeroFly® ("LLIN+ITPS ZF")
In Capango and Canjala, the two villages which received the association nets P2.0 + ITPS ZF, the parasite prevalence was similar in the "at-risk" group (≤5-year-old), but significantly lower in the group 2–9-year-old (from #49% to # 35%) and for the whole sample of children ≤15-year-old (from # 44% to # 32%) (Table 7)

c) ITPS model ZeroVector® alone
In Barragem and Chisséquélé, the two villages which received ITPS "ZeroVector®" alone, the parasite prevalence was statistically lower in February 2018 (25%) than in February 2008 (44%) in the three age-groups (Table 7).

d) Indoor residual spraying ("IRS") then ITPS

In Candiero and Libata, the two villages which received two rounds of lambdacyhalothrin IRS ("λIRS"), then deltamethrin treated plastic sheeting, the parasite prevalence was significantly lower in February 2018 (PP=# 26%) than in February 2008 (PP=#48%) for the three age-groups (Table 7).

III-1-4. Synthesis of parasite prevalence and vector control method

The results of the analysis of parasite prevalence noticed in February 2008 and February 20018 according to age-group, and vector control method, are synthesized in Table 8 where it is clear that - - parasite prevalence remained at similar values in villages furnished with LLIN and LLIN +ZF (for the "at risk" group) but

- *significantly decreased in villages with the three other methods of vector control.*

Table 8. Evolution of parasite prevalence according to age-group, and vector control method. (NS =non-significant difference, D↘= significant decrease; VC= vector control; LLIN P2.0 = long-lasting insecticide treated nets P2.0; ZF= insecticide treated plastic sheeting model ZeroFly®; ITPS ZV= insecticide treated plastic sheeting model ZeroVector®; IRS= indoor residual spraying)

Period		February 2008 =>	February 2018	
Method of VC	LLIN P2.0	LLIN + ZF	ITPS ZV alone	IRS then ITPS
≤5-year-old	NS	NS	D↘	D↘
2–9-year-old	NS	D↘	D↘	D↘
≤15-year-old	NS	D↘	D↘	D↘

III-2. Evolution of parasite load (PL)

III-2-1. Comparison of parasite load between age-groups according to village

Comparisons, with Mann-Whiney non-parametric test, of parasite loads according to age, in each village, in February 2008 and February 2018 are gathered in the Table 9.

Table 9. Comparison of parasite loads between age-group in each village. (stat= statistical conclusion; NS= non-significant difference; D↘= significant decrease)

Village	Age-group	February 2008		February 2018	
		P value	stat	*P* value	stat
	≤5 y vs 2-9 y	*P*=0.1866	NS	*P*=0.8305	NS
Caala	≤5 y vs ≤15 y	*P*=0.2163	NS	*P*=0.9865	NS
	2-9 y ≤15 y	*P*=0.7691	NS	*P*=0.6332	NS
	≤5 y vs 2-9 y	*P*= 0.5787	NS	*P*= 0.5629	NS
Cahata	≤5 y vs ≤15 y	*P* > 0.99	NS	*P*= 0.4174	NS
	2-9 y ≤15 y	*P*= 0.4740	NS	*P*=0.8454	NS
	≤5 y vs 2-9 y	*P* > 0.99	NS	*P*= 0.7559	NS
Capango	≤5 y vs ≤15 y	*P*= 0.8572	NS	*P*=0.8026	NS
	2-9 y ≤15 y	*P*= 0.7564	NS	*P*= 0.8527	NS
	≤5 y vs 2-9 y	*P*= 0.1033	NS	*P*= 0.6324	NS
Canjala	*≤5 y vs ≤15 y*	*P= 0.0294*	D↘	*P*= 0.5248	NS
	2-9 y ≤15 y	*P*= 0.5891	NS	*P*= 0.8772	NS
	≤5 y vs 2-9 y	*P*= 0,1399	NS	*P*= 0,9793	NS
Barragem	*≤5 y vs ≤15 y*	*P= 0.0366*	D↘	*P*= 0.8517	NS
	2-9 y ≤15 y	*P*= 0.5813	NS	*P*= 0.8447	NS
	≤5 y vs 2-9 y	*P*= 0.5563	NS	*P*= 0.9311	NS
Chisséquélé	≤5 y vs ≤15 y	*P*= 0.4843	NS	*P*=0.9202	NS
	2-9 y ≤15 y	*P*= 0.9565	NS	*P*= 0.6024	NS
	≤5 y vs 2-9 y	*P*= 0.6933	NS	*P*= 0.9637	NS
Candiero	≤5 y vs ≤15 y	*P*= 0.7680	NS	*P*= 0.7434	NS
	2-9 y ≤15 y	*P*= 0.8852	NS	*P*= 0.7630	NS
	≤5 y vs 2-9 y	*P*= 0.2517	NS	*P*= 0.7958	NS
Libata	≤5 y vs ≤15 y	*P*= 0.1813	NS	*P*= 0.9333	NS
	2-9 y ≤15 y	*P*= 0.6923	NS	*P*= 0.6529	NS

Parasite loads were similar among the three age-groups considered, except between ≤5-year-old and ≤15-year-old in Canjala (LLIN + ZF), and in Barragem (ITPS ZV), where the parasite load of the "at risk" group were higher than in the whole sample in February 2008; but not in February 2018 where PL were similar.

III-2-2. Evolution of parasite loads in each village and age-group between February 2008 and February 2018

a) Evolution of parasite load in Caala

In Caala, the parasite loads were similar in children ≤5-year-old (*P* value= 0.5457) but significantly increased in 2–9-year-old children (*P* value = 0.0059) and children ≤15-year-old (*P* value= 0.0047) (Fig. 5).

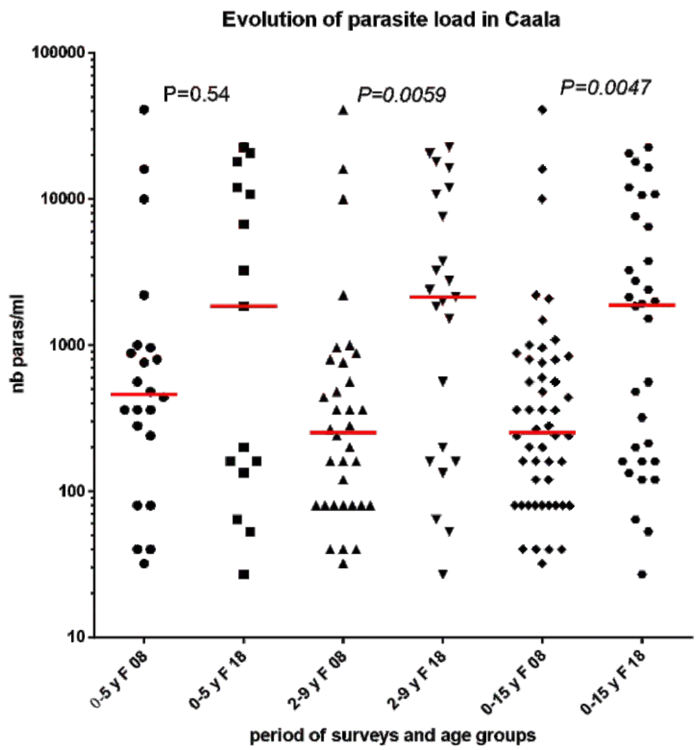

Figure 5. Distribution of the parasite loads in Caala according to age-group, in February 2008, and in February 2018. (horizontal line --- = median) (0-5 y F 08= ≤5-year-old February 2008; 0-5 y F 18= ≤5-year-old February 2018; 2-9 y= 2–9-year-old; 0-15=≤ 15-year-old)

b) Evolution of parasite loads in Cahata

In Cahata, the parasite loads were similar in children ≤5-year-old (*P* value= 0.4331) and 2–9-year-old (*P* value= 0.1741) but significantly increased when considering children ≤15-year-old (*P* value= 0.0075) (Fig. 6).

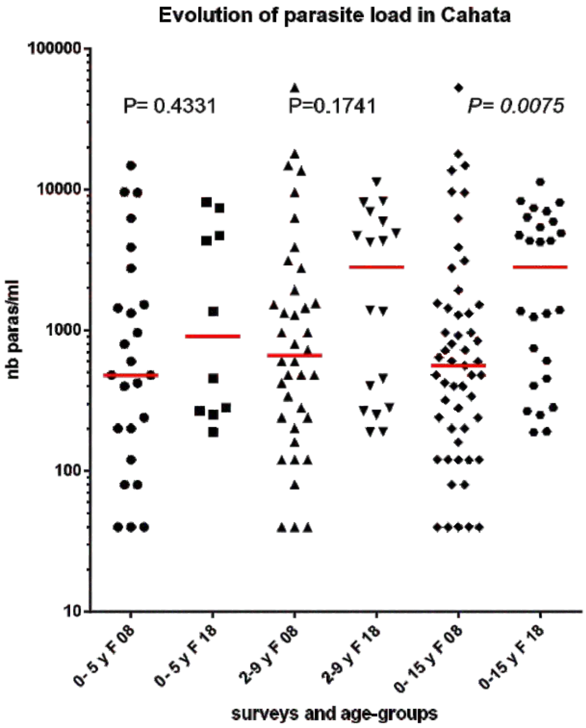

Figure 6. Distribution of the parasite loads in Cahata according to age-group, in February 2008, and in February 2018. (horizontal line --- = median) (0-5 y F 08= ≤5-year-old February 2008; 0-5 y F 18= ≤5-year-old February 2018; 2-9 y= 2–9-year-old; 0-15=≤ 15-year-old)

c) Evolution of parasite loads in Capango

In Capango, the parasite loads increased significantly in the three age-groups (Fig. 7) between February 2008 and February 2018.

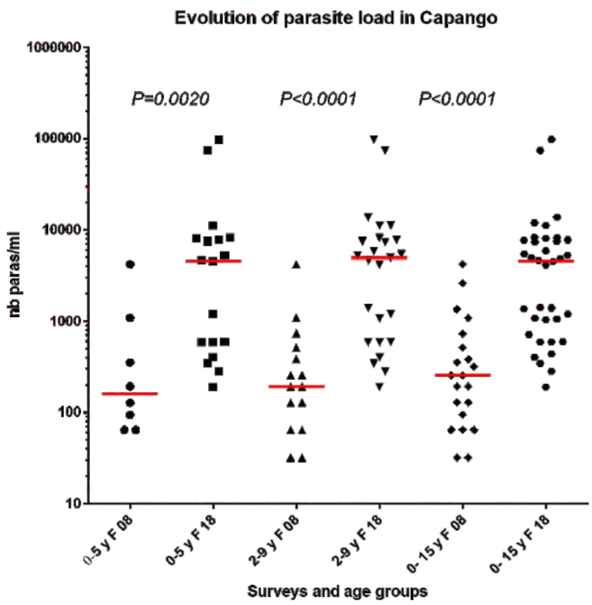

Figure 7. Distribution of the parasite loads in Capango according to age-group, in February 2008, and in February 2018. (horizontal line --- = median) (0-5 y F 08= ≤5-year-old February 2008; 0-5 y F 18= ≤5-year-old February 2018; 2-9 y= 2–9-year-old; 0-15=≤ 15-year-old)

d) Evolution of parasite load in Canjala

In Canjala, the parasite loads remained comparable in children ≤5-year-old but significantly increased in the two other age-groups (Fig. 8).

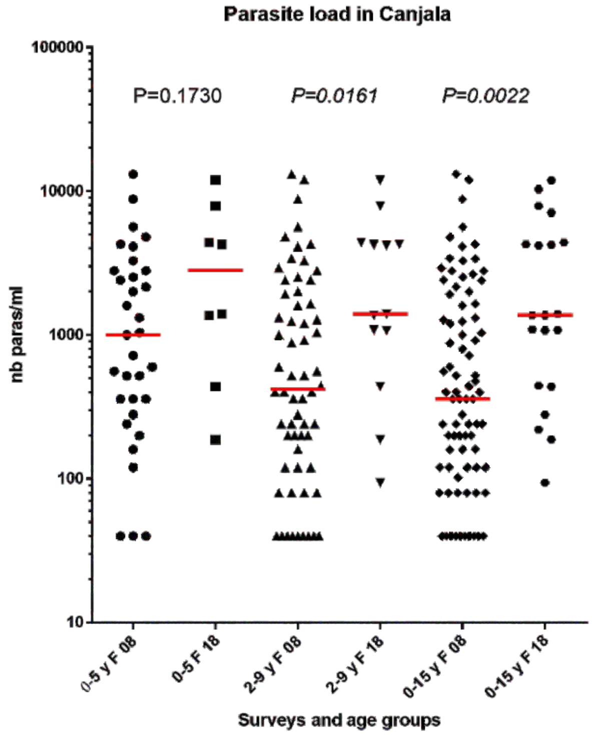

Figure 8. Distribution of the parasite loads in Canjala according to age-group, in February 2008, and in February 2018. (horizontal line --- = median) (0-5 y F 08= ≤5-year-old February 2008; 0-5 y F 18= ≤5-year-old February 2018; 2-9 y= 2–9-year-old; 0-15=≤ 15-year-old)

e) Evolution of parasite loads in Barragem

In Barragem, the parasite loads increased, but not statistically, in children ≤5-year-old (but sample in 2018 was small) and significantly increased in the two other age-groups (Fig. 9).

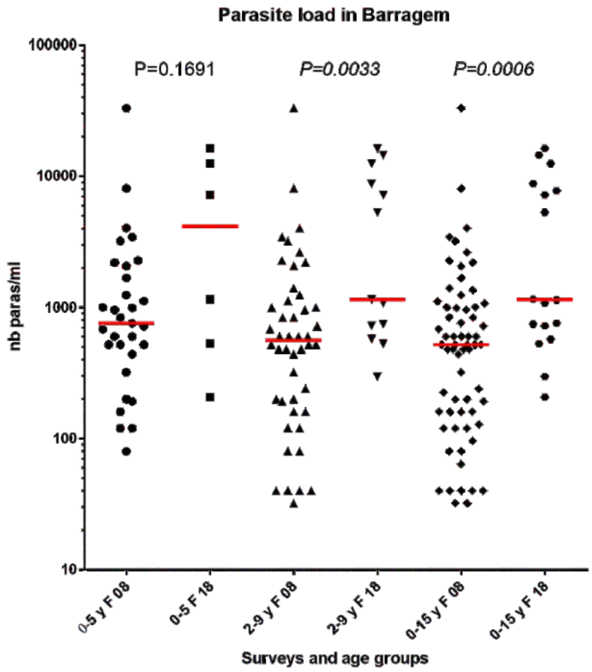

Figure 9. Distribution of the parasite loads in Barragem, according to age-group, in February 2008, and in February 2018. (horizontal line --- = median) (0-5 y F 08= ≤5-year-old February 2008; 0-5 y F 18= ≤5-year-old February 2018; 2-9 y= 2–9-year-old; 0-15=≤ 15-year-old)

f) Evolution of parasite loads in Chisséquélé

In Chisséquélé, the parasite loads increased, but not statistically, in children ≤5-year-old (but the size of the Feb 2018 sample was small), and significantly increased in the two other age-group: 2–9-year-old and of ≤15-year-old (Figure 10).

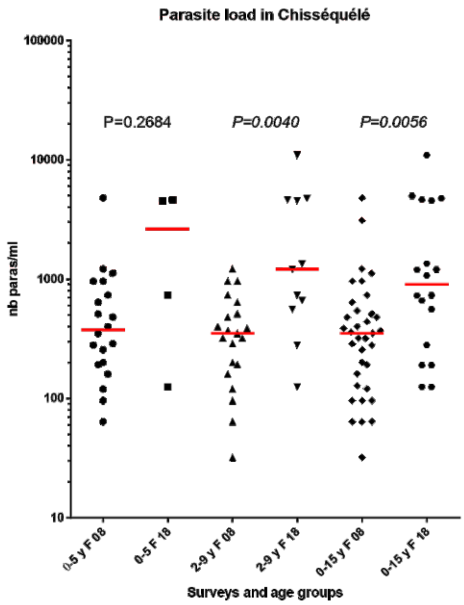

Figure 10. Distribution of the parasite loads in Chisséquélé according to age-group, in February 2008, and in February 2018. (horizontal line --- = median) (0-5 y F 08= ≤5-year-old February 2008; 0-5 y F 18= ≤5-year-old February 2018; 2-9 y= 2–9-year-old; 0-15=≤ 15-year-old)

g) Evolution of parasite loads in Candiero.

In Candiero, parasite loads significantly increased from February 2008 to February 2018 in the three age-groups considered (Figure 11).

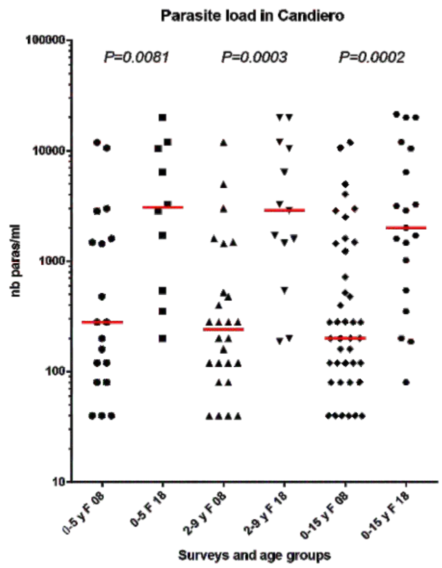

Figure 11. Distribution of the parasite loads in Candiero according to age-group, in February 2008, and in February 2018. (horizontal line --- = median) (0-5 y F 08= ≤5-year-old February 2008; 0-5 y F 18= ≤5-year-old February 2018; 2-9 y= 2–9-year-old; 0-15=≤ 15-year-old)

h) Evolution of parasite loads in Libata.

In Libata, the parasite loads remained similar in ≤5-year-old while they significantly increased in the two other age-groups considered (Fig. 12).

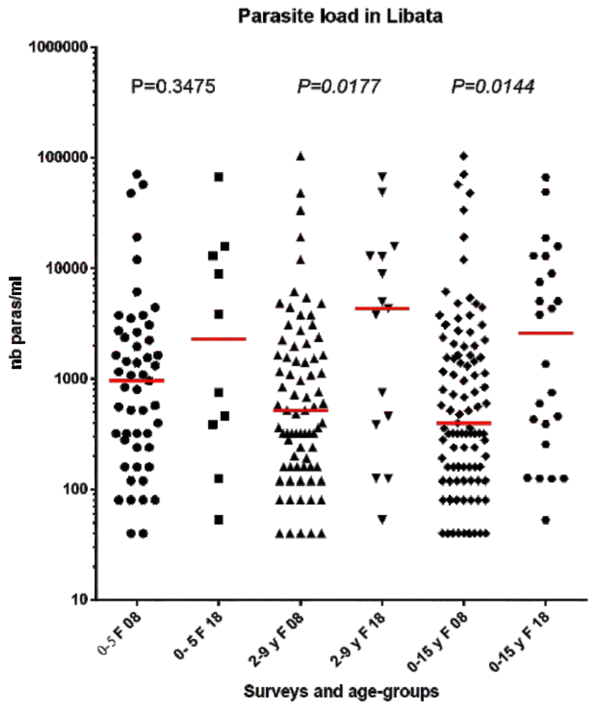

Figure 12. Distribution of the parasite loads in Libata according to age-group in February 2008 and in February 2018. (horizontal line --- = median) (0-5 y F 08= ≤5-year-old February 2008; 0-5 y F 18= ≤5-year-old February 2018; 2-9 y= 2–9-year-old; 0-15=≤ 15-year-old)

i). Synthesis of the evolution of parasite loads according to village and age-group

When gathering the information on the evolution of the parasite load for each age-group considered, and each village, it appeared that the conclusion could be highly different according to the age-group studied.

For the "at-risk" group (below 5-year-old) the parasite load *remained statistically similar* except in two villages, Capango (LLINs+ITPS) and Candiero (IRS then ITPS)

For the whole ≤15-year-old sample *the increase of parasite load was observed everywhere.*

For the age group 2-9-year-old *the increase was also observed in all, except one* village (Cahata, LLINs alone) (Table 10).

Table 10. Evolution of parasite loads, from February 2008 to February 2018, according to age-group in each village. (↗ = significant increase)

Village	≤5-year-old	2–9-year-old	≤15-year-old
Caala	=	↗	↗
Cahata	=	=	↗
Capango	↗	↗	↗
Canjala	=	↗	↗
Barragem	=	↗	↗
Chisséquélé	=	↗	↗
Candiero	↗	↗	↗
Libata	=	↗	↗

This clearly shows how risky it could be to draw any definitive conclusions when dealing with one village only, or one age group only.

III-2-3. Evolution of parasite loads according to vector control method and age-group

a) In the "at-risk" age-group

In children ≤5-year-old the parasite loads of remained similar in villages which received LLIN (*P* value= 0.29) and ITPS ZV alone (*P* value= 0.051), while they significantly increased in villages with LLIN + ZF (*P* value=0.0035) and villages with IRS then ITPS (*P* value= 0.017) (Fig. 13).

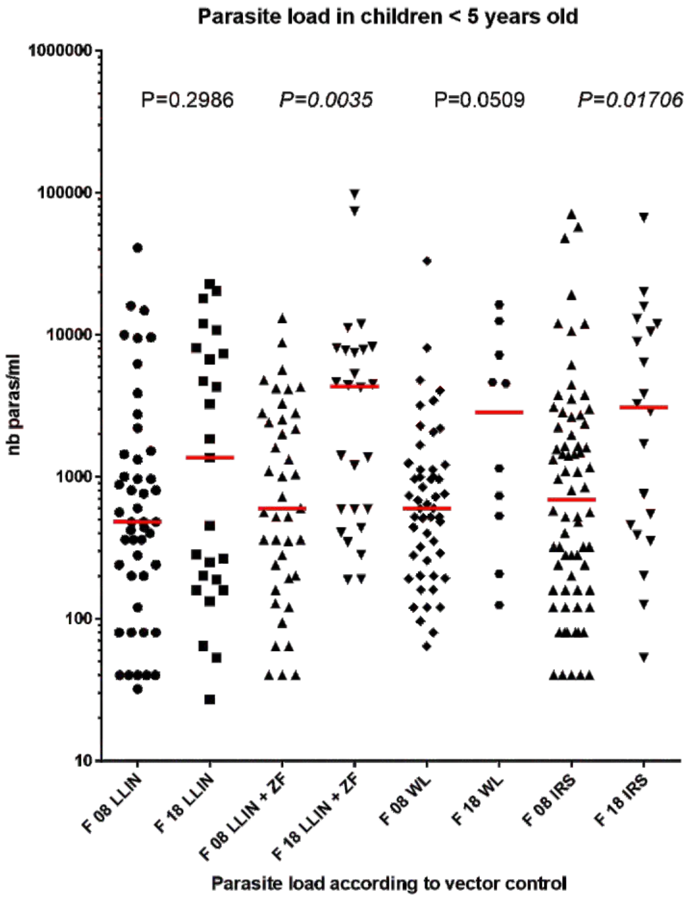

Figure 13. Distribution of the parasite loads in February 2008 (F 08) and February 2018 (F 18) in children ≤5-year-old according to vector control method. (horizontal line --- = median) (LLIN= long-lasting insecticide treated net; ZF= insecticide-treated plastic sheeting model ZeroFly®; WL= insecticide-treated plastic sheeting model ZeroVector®; IRS= inside residual spraying)

It is interesting to notice that the parasite loads remained similar in villages with one method only, LLIN or ITPS, while it increased in villages which received two methods, in combination (LLINs+ITPS) or IRS followed by ITPS.

Considering only this indicator (evolution of parasite loads in the "at risk" groups") the conclusion could be different according to the vector control method implemented.

b) In children 2–9-year-old

In the 2-9-year age the parasite loads were significantly higher in February 2018 than in February 2008, whatever the vector control method used. (Fig. 14)

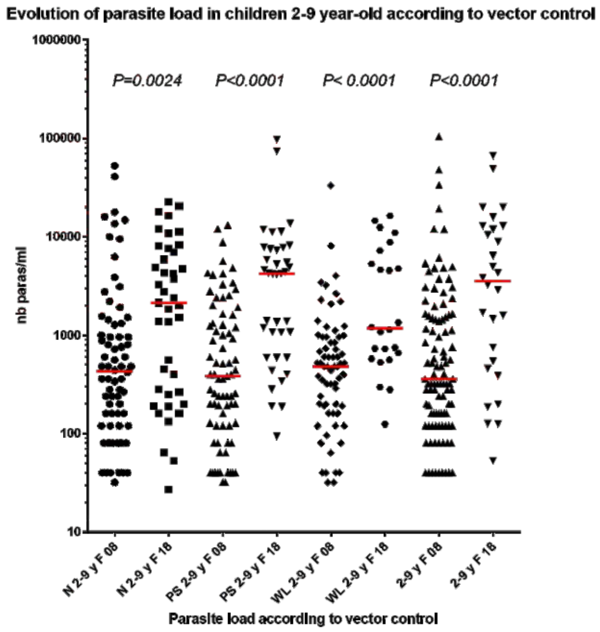

Figure 14. Distribution of the parasite loads in February 2008 (F 08) and February 2018 (F 18) in children 2–9-year-old according to vector control method (horizontal line --- = median) (LLIN= long-lasting insecticide treated net; ZF= insecticide-treated plastic sheeting model ZeroFly®; WL= insecticide-treated plastic sheeting model ZeroVector®; IRS= inside residual spraying)

c) In the ≤15-year-old age group

In children ≤15-year-old, the parasite loads significantly increased (Fig. 15) between February 2008 and February 2018 whatever the vector control method was.

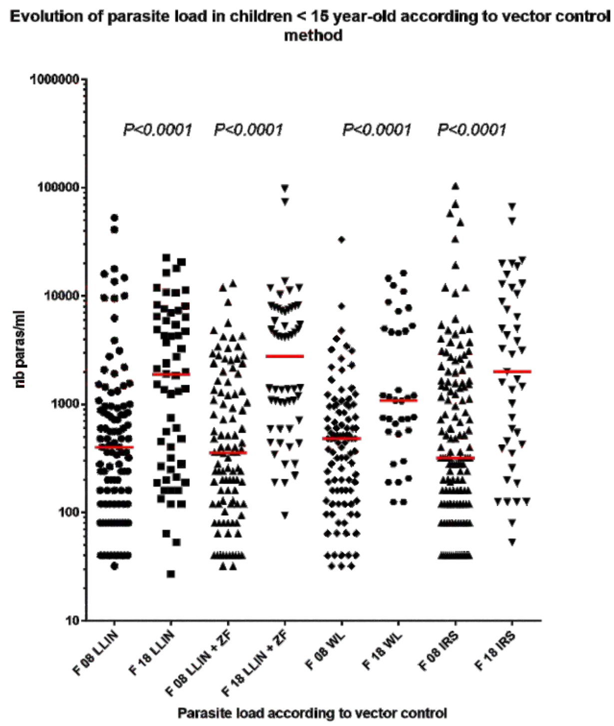

Figure 15. Distribution of the parasite loads in February 2008 (F 08) and February 2018 (F 18) in children ≤15-year-old according to vector control method (horizontal line --- = median) (LLIN= long-lasting insecticide treated net; ZF= insecticide-treated plastic sheeting model ZeroFly®; WL= insecticide-treated plastic sheeting model ZeroVector®; IRS= inside residual spraying)

III-2-4. Synthesis of the evolution of parasite loads in each age-group according to vector control method

From Fig. 10, 11 and 12 it is possible to make a synthesis of the parasite loads unregistered in February 2008 and 2018, in asymptomatic children of the three age-groups considered.

The data highlight *the overall increase of parasite load* whatever the method of vector control, except in children ≤5-year-old in villages furnished with LIN alone, or with treated plastic sheeting alone (Table 11).

Table 11. Evolution of the parasite loads observed in the three age groups, according to vector control method implemented. (LLIN= treated nets PermaNet® 2.0; ZF= insecticide-treated plastic sheeting model ZeroFly®, ITPS ZV= insecticide-treated plastic sheeting model ZeroVector®, IRS = indoor residual spraying; "=" *P* value non-significant; ↗ = significant increase)

		Feb. 2008 => Feb. 2018	
	≤ 5-year	2-9 year	≤ 15 year
LLIN	=	↗	↗
LLIN + ZF	↗	↗	↗
ITPS ZV	=	↗	↗
IRS then ITPS	↗	↗	↗

III-2-5. Overall evolution of parasite loads according to each age-group

When gathering data of parasite loads (PL) of each thick blood film done in February 2008, and in 2018, it appeared that PL increased significantly in the three age-groups considered. (Fig 16)

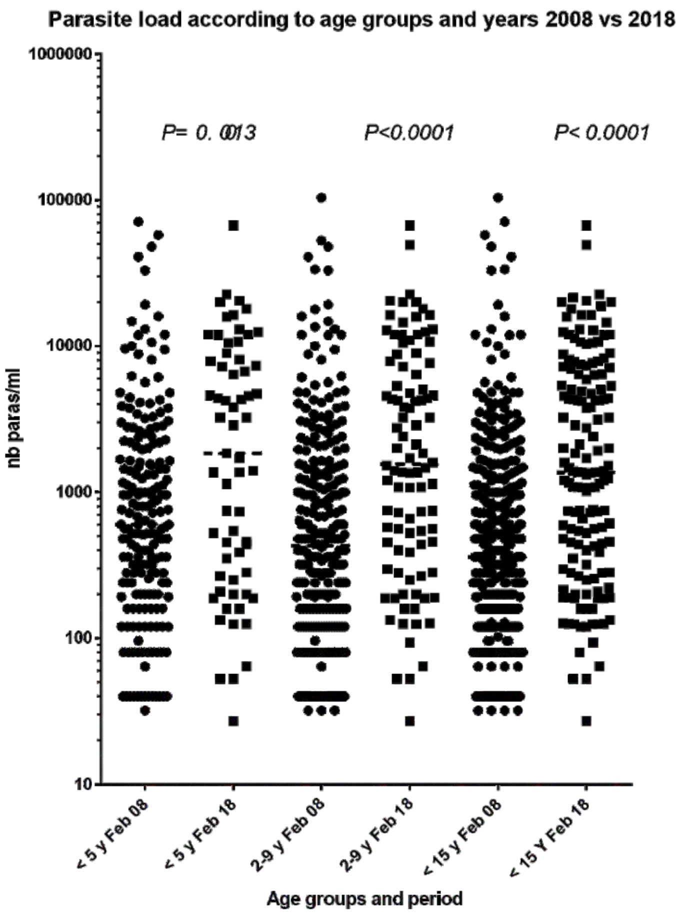

Figure 16. Evolution of the parasite loads noticed in each one of the three age-groups considered for the period February 2008 and February 20018 (P value in italics = significant difference) (horizontal line --- = median). (< 5 y Feb 08= ≤5-year-old February 2008; Feb 18= February 2018)

III-3. Comparison of parasite prevalence and parasite loads according to villages, age-groups and vector control method

III-3-1. Evolution of parasite prevalence and parasite loads in the three age-groups according to village

Combining Table 6 (for parasite prevalence) and 10 (for parasite loads) shows the diversity of the situation observed, and the conclusion which could be drawn. (Table 12)

Table 12. Comparison of the evolution of parasite prevalence (PP) and parasite loads (PL), by village, and age-group, between February 2008 and February 2018. (= similar values; ↑= significant increase; ↓= significant decrease)

Period		Feb 2008	=>	Feb 2018		
Age-group	≤5years		2-9 year		≤15 year	
Indicator	PP	PL	PP	PL	PP	PL
Caala	=	=	↑	↑	=	↑
Cahata	=	=	=	=	↓	↑
Capango	=	↑	=	↑	=	↑
Canjala	↓	=	↓	↑	↓	↑
Barragem	=	=	↓	↑	↓	↑
Chisséquélé	↓	=	=	↑	=	↑
Candiero	=	↑	=	↑	=	↑
Libata	↓	=	↓	↑	↓	↑
Total	↓	↑	↓	↑	↓	↑

In the whole sample (≤15 years) parasite loads always increased while parasite prevalence decreased in four the eight villages, and remained similar in the four others, one village per vector control method implemented.

The discrepancy between the evolution of prevalence and load in some villages is noteworthy.

In the ≤5-year-old group: in Capango and Candiero the prevalence remained similar but parasite load increased; in Canjala, Chisséquélé and Libata prevalence decreased but loads remained similar.

For this age-group the overall trend was a decrease in parasite prevalence but an increase in parasite load. This overall trend was also observed for the 2-9-year-old group and the whole sample.

Summing the data (line "Total") shows complete inverse information on the evolution of parasite prevalence (decreased) and parasite load (increased) ten years after vector control.

III-3-2. Evolution of parasite prevalence and parasite loads in the three age-groups according to vector control method

The trends in the evolution of parasite prevalence (PP) and parasite loads (PL), according to vector control method implemented and age-group are presented in Table 13.

Table 13. Comparison of the evolution of parasite prevalence (PIP) and parasite loads (PL), by vector control method, and age-group, between February 2008 and February 2018. (= similar values; ↑= significant increase; ↓= significant decrease)

Period		February	2008 =>	February	2018s	
Age group	≤ 5 years		2-9 years		≤15 years	
Indicator	PP	PL	PP	PL	PP	PL
LLIN	=	=	=	↑	=	↑
LLIN + ZF	=	↑	↓	↑	↓	↑
ITPS ZV	↑	=	↓	↑	↓	↑
IRS then ITPS	↓	↑	↓	↑	↓	↑

The evolution of the prevalence, and the parasite loads, were heterogeneous.

In the "at-risk" sample ≤5-year-old neither the prevalence nor the parasite loads increased where LLINs were distributed; there appeared a complete opposition between PP and PL with house spraying; a similar parasite prevalence but an increasing parasite load in nets + ZeroFly villages and an increasing prevalence but a similar parasite load in villages with insecticide treated plastic only.

When considering the whole sample (≤15-year-old), there was a clear trend of an opposite evolution of the two indicators, parasite prevalence (which decrease) and parasite loads (which increased), except with nets only where prevalence did not increase.

For the whole sample, the three methods involving the treatment of walls gave the same trends with a decrease of prevalence and an increase of parasite load.

IV. Discussion

According to WHO (2025) "Globally in 2024, there were an estimated 282 million malaria cases, an increase of about 9 million cases (3%) compared with 2023. Since 2015, malaria cases have increased by 22.6%. Most of this increase was observed in the WHO African Region (88%) and the WHO Eastern Mediterranean Region (12%). Globally in 2024, there were an estimated 610 000 malaria deaths, an increase of 12 000 compared with 2023. Between 2015 and 2024, deaths increased by 5.5%. The malaria mortality rate almost halved between 2000 and 2015, from 28.6 to 14.9 per 100 000 population at risk. Since 2015 the decline has slowed, decreasing by a further 7.4% over the past 9 years. The mortality rate remained unchanged in 2024 compared with 2023, at 13.8 per 100 000 population at risk".

The risk of worsening increases with the spread of artemisinin partial resistance, along with the threat of resistance to ACT partner drugs, and, resistance to pyrethroids remains widespread, with resistance confirmed in 55 of the 64 countries where it was monitored between 2018 and 2023.

WHO published recommendations on new types of insecticide-treated mosquito nets (ITNs): pyrethroid–chlorfenapyr nets which combine a pyrethroid and a pyrrole insecticide to enhance the killing effect of the net; pyrethroid–pyriproxyfen nets which combine a pyrethroid with an insect growth regulator, and pyrethroid + PBO to increase the efficacy of the insecticide.

According to a recent evaluation *Plasmodium falciparum* infection prevalence in endemic Africa halved and the incidence of clinical disease fell by 40% between 2000 and 2015." Authors "estimate that interventions have averted 663 (542-753 credible interval) million clinical cases since 2000. Insecticide-treated nets, the most widespread intervention, were by far the largest contributor (68% of cases averted)". "Increasing access to these interventions, and maintaining their effectiveness in the face of insecticide and drug resistance, should form a cornerstone of post-2015 control strategies. » [53]

It clearly appeared that long-lasting insecticide treated nets are the main tool for vector control but sustainability remains a key point [54, 55, 56] and it was observed that in one, or two years, more than 50% of nets are torn and removed, with an increase of parasite prevalence [57]

Therefore "There is an urgent need for the development of novel insecticide delivery mechanisms to sustain and consolidate gains in disease reduction and to transition towards malaria elimination and eradication.

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. » [19, 20, 58]

At the request of the National Malaria Control Program a comprehensive, long-term, village scale, malaria vector control program was implemented around Balombo town to evaluate, and compare, insecticide-treated plastic (ITPS) alone, or associated with long-lasting insecticide treated nets (LLIN), or following two rounds of inside residual spraying (IRS). LLINs alone were considered as golden standard. Each method of vector control was randomly implemented in two (paired) villages. Pairing was based on parasite prevalence observed during preliminary parasitological survey in Balombo villages where it appeared villages with « high point prevalence » (HPP) (say HPP> 50%) and villages with « low point prevalence » « LPP » (say LPP < 50%); then one village of HPP was randomly paired with one village of LPP. The choice of method of each vector control method was randomly made in these paired villages.

Both entomological, and parasitological evaluation were done during the first five years, two years before and three years after vector control. [41]

Entomological observations reported «after implementation of vector control, the densities of main vectors per trap dropped by 70%, similarly with all four methods. Inoculation rates decreased similarly by some 80% after the implementation of each one the four vector control methods. »

First parasitological surveys, on asymptomatic children ≤15-year-old, reported that the observed overall reduction of parasite prevalence (PP) for the eight villages was 71% for the three years after vector control (PP=11.9%; n=10,397), compared to the two years before (PP=41.7%; n=9,791). Comparing the *Plasmodium* prevalence before, and after, implementation of each method of vector control, showed a similar, and significant, reduction of some 70% (± 5%) of the prevalence of *Plasmodium* in the asymptomatic children examined. [41]

Immunological evaluation was based on the evolution of « Human antibody (Ab) response to *Anopheles* whole saliva, used as biomarker of *Anopheles* exposure ». This was investigated « over a period of two years (2008–2009), in asymptomatic children between 2 to 9 years old, before and after, the introduction of malaria vector control ». It was concluded that "the number of *Anopheles*, positive blood smears, and the levels of anti-saliva IgG Ab were most reduced when LLIN and ITPS-ZF were used in combination, compared to the use of one vector control method alone, either ITPS-DL or IRS. Therefore, as a combination of two vector control methods is significantly more effective than one control method only, this control strategy should be further developed at a more global scale. » [40]

These studies showed the actual short-term efficacy of the four-vector control method implemented, with different conclusions according to the indicator, the number of years analysed before, and after vector control, and age-group studied.

Long-term evolution analysis was based on parasitological indicators only, parasite prevalence and parasite load, gained with two cross-sectional surveys done at a ten years interval, on samples of asymptomatic children ≤15-year-old.

To analyse the plasmodial infections with age this sample was divided in children ≤5-year-old considered as the « at-risk » group; in 2-9-year-old, (already studied) [40] and used for malaria endemicity classification [59] and whole sample to increase the size of the sample and the power of the study. This is a classical method for malaria control evaluation.

In Bioko Island, Equatorial Guinea "malaria infection and haemoglobin were measured annually in children (1 to 14 years) in cross-sectional household surveys over a 13 years period of intensive interventions to track the progress of vector and malaria control" [45]

In Balombo program the evolution of parasite prevalence, and parasite load, in February 2008 and February 2018, according to villages, and vector control methods, show the diversity of information, and conclusion, from the same original data. For the whole sample the overall trends showed a significant reduction of parasite prevalence, and a significant increase of parasite load. But some differences were noted according to age-group, or village, or vector control method. For example, in villages with LLIN the parasite prevalence remained similar while it decreased with other methods. For the "at-risk" group the parasite prevalence remained similar in villages with LLIN alone, or combined with insecticide-treated plastic sheeting, while it decreased in villages with insecticide-treated plastic sheeting alone or indoor residual spraying then ITPS.

During the parasitological studies done each year, during ten years, in four villages, with one village per vector control method, it was observed a strong "immediate" effect (2-3 years) of vector control on parasite prevalence. Then, for over five years, the parasite prevalence plateaued at a very low level (# 5%) until it increased at the time of the national malaria outbreak which occurred in Angola. But, even in these situations, the parasite prevalence remained lower than before vector control, and even lower than in other villages not involved in the Balombo project.

On the other hand, the increase in parasite load, observed in 2018, in the eight Balombo villages, could, obviously, have been linked to this malaria outbreak.

With data gained in February 2008 and 2018, it appeared what could be considered as some epidemiological paradox with a decrease of *Plasmodium* prevalence but an increase of parasite load ten years after vector control implementation in these villages.

This induces reflections on the risk of drawing any definitive conclusion from observations in one village only, or with one indicator only, in natural conditions such as those occurring during this Balombo project, where, as elsewhere, several cofactors were involved [60, 61] and it is not possible to identify exactly which "cofactors" had (or could have) some influence, except particular events (rain, dry, social events etc).

Whatever the reasons could be, the diversity of information, with this discrepancy between prevalence which decreased, and parasite load which increased 10 years after implementation of various method of vector, underscore the need for long term surveys. It also underscores the risks of drawing definitive conclusions after considering only one village, or one vector control method, or one parasitological indicator, when working in a completely natural situation in the context of all of the events, social, ecological, and financial, which occurred in the region

V. Conclusion

In eight villages around Balombo town (Benguela Province, Angola) a parasitological cross-sectional survey was done in February 2018, on asymptomatic children ≤15-year-old, 10 years after implementation of four methods of vector control.

This survey was compared to the survey done in February 2008, before vector control, to compare the long-term evolution of plasmodial infections and to check for any rebound [33], or other effect.

The microscopic analysis of more than one thousand thick blood films made in the same villages, by the same team, with the same protocol, produced what might appear to be heterogeneous information. Actually, conclusions could be different depending on the method of vector control implemented, the age-group considered and the parasitological indicator, parasite prevalence or parasite load.

The overall trends were a *significant decrease in the parasite prevalence,* indicating a long-term effect of treated material, often discarded in the field within each village, and a *significant increase of the parasite load* which could be due to the national malaria outbreak which occurred in 2015. Less asymptomatic children were *Plasmodium* carriers but with more plasmodial infections.

This variability of interpretation underscored the importance of the choice of indicator which could lead to contradictory conclusions.

It was considered as "essential that long-term surveillance is included as part of ITN interventions, with particular attention to age range over which rebound may occur." [33]

Our long-term parasitological survey was devoted to this issue and showed that, in the condition of the surveys, in completely natural situation, no rebound of *Plasmodium* prevalence was noticed in asymptomatic children <5-year-old ("at risk" group), or 2-9 year-old or all <15 year-old sample 10 years after implementation of four method of vector control, and the parasite load of the at-risk group did not increase even during the National Malaria outbreak.

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Author's Contributions

PC designed the protocol, participated in field surveys, data analysis and writing the manuscript.; JCT participated in field surveys; FM was the head of the medical department of the Sonamet® Company in Lobito and responsible of MCP program and its agents; GC was involved in data analysis, writing and English editing the document

Declaration of competing interest: The authors declare that they have no competing interest.

Ethics Statement: This study was conducted in accordance with the Edinburgh revision of the Helsinki Declaration and was approved by the National Malaria Control Program of the Ministry of Health of Angola, the Ethical authority in charge of approving studies on malaria research in Angola.

Written consent (signed by the head of each household) was obtained for all individuals enrolled in the study by the SONAMET® Company - Malaria Control Program (MCP) which is responsible for malaria surveillance and control amongst company employees and their families » (Brosseau et al., 2012).
In line with Brosseau work, this study was a part of the on-going Malaria Control Program of the Sonamet® Angolese Company and was implemented at the request and with National Authorities: National Malaria Control Program and the Public Health Department of Benguela Province.

Authors assured not use of a.i. for any part of the document**.**

Consent for publication
GC I do agree.
JCT. I do agree to be among the authors of this article
Dr FM I do consent

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Pictures (P. Carnevale)

Picture 1: Long-lasting insecticide-treated nets in combination with insecticide-treated plastic sheeting ZeroFly® on the wall in a house

Picture 2: Insecticide-treated plastic sheeting ZeroVector® on the wall.

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