AUCTORES
Globalize your Research
Clinical Medical Reviews and Reports
P. Carnevale *
Open Access
Research Article

Beware the Plasmodial Infection Indicators: Plasmodium Falciparum Prevalence and Parasitaemia During two Years in Symptomless below 5 years old Children of Two Villages with long-Lasting Insecticide Treated Nets and two Villages without Nets in Angola

P. Carnevale [1*], JC Toto [2], V. Foumane [2], L. Eugenio [3], G. Carnevale [4], R. Ouarou [5], F. Gay [5]

[1] Le Majoral Building 34420 Portiragnes Plage, France.

[2] OCEAC PO 288 Yaoundé Cameroon.

[3] Malaria Control Program, Medical Department, Sonamet Lobito, Angola.

[4] Hygiene, Safety, Environment; Vector Control in Emergency situation; Batuan, Bali, Indonesia.

[5] Pitié-Salpêtrière Hospital & Sorbonne University, 47 Bd Hôpital, 75013 Paris.

***Corresponding Author:** P. Carnevale, Le Majoral Building 34420 Portiragnes Plage, France.

Received Date: August 29, 2023; **Accepted Date:** September 09, 2023; **Published Date:** September 18, 2023

Citation: P. Carnevale, JC Toto, V. Foumane, L. Eugenio, G. Carnevale, R. Ouarou, F. Gay (2023), Beware the Plasmodial Infection Indicators: Plasmodium Falciparum Prevalence and Parasitaemia During two Years in Symptomless below 5 years old Children of Two Villages with long-Lasting Insecticide Treated Nets and two Villages without Nets in Angola, *Clinical Medical Reviews and Reports,* 5(5); **DOI:**10.31579/2690-8794/175

Abstract

Forty parasitological cross-sectional surveys (CSS) were regularly done during two consecutive years in four villages around Balombo town (Benguela Province, Angola) to assess the influence of a full coverage with long lasting insecticide treated net (LLIN) PermaNet© 2.0 on plasmodial infection of under 5 years old symptomless children (“U5”).

Two villages received LLIN following a two steps process: 1rst year 1 LLIN per house; 2nd year: 1 LLIN per sleeping unit (= everything regularly used for sleeping) while two close villages remained as control for these two years.

Overall P. falciparum infections were microscopically observed in 1006 of the 2865 blood thick films made i.e., a plasmodial prevalence of 35.1%.

During these two years the plasmodial prevalence decreased significantly in the two LLIN furnished villages but also in the two control villages.

Therefore, the plasmodic index could not be considered as a relevant indicator to evaluate the parasitological efficacy of LLINs implemented in this study.

The gametocytic index did not significantly changed from year to year neither in LLIN villages nor in control villages and did not appear also as a relevant indicator in this study.

But on the other hand, the analysis of the evolution of the parasite load procured a crucial information with a significant decreased of the parasitaemia of U5 children in LLINs villages while it remained at the same level in control villages without any organized vector control operations.

Parasite load appeared as the relevant indicator showing the actual impact of LLIN in reducing the parasitaemia, and therefore the risk of malaria, in under five children in this area.

Keywords: vector control; parasitological evaluation; long lasting insecticide treated nets; P. falciparum prevalence and parasitaemia; indicator of impact

Introduction

In the WHO Global technical strategy for malaria 2016-2030 [1]. vector control "is an essential component of malaria control and elimination" and "National malaria control programmes need to ensure that all people living in areas where the risk of malaria is high are protected through the provision, use and timely replacement of long-lasting insecticidal nets or, where appropriate, the application of indoor residual spraying".

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

Some groups such as children below 5 years old and pregnant women are « particularly susceptible to malaria illness, infection and death" and are considered as "at risk groups which deserve a high priority of protection**.**

According to the WHO Malaria report (2022) "between 2019 and 2020 estimated malaria cases increased from 218 million cases to 232 million, and deaths from 544,000 to 599,000 in the WHO African Region. In 2021, while cases increased to 234 million, deaths decreased to 593,000. This region accounted for about 95% of cases and 96% of deaths, 78.9% of deaths in this region were among children aged under 5 years in 2021 compared to 91% in 2000."

Universal ITN coverage is recommended, which is expected to provide community protection, and it was estimated that in 2017," about half of the population at risk of malaria in Africa was protected by impregnated mosquito nets"; however, "protection by persistent spray did not exceed 3% in 2017" [3].

To assess the ITN coverage one indicator was at least one impregnated net per house[4]. In the double concept "one net/house" and "universal coverage" we developed a program of distribution of longlasting insecticide treated nets ("LLIN") PermaNet 2.0 in two villages, Caala and Cahata, in a classical two steps process: first year, in February 2007, distribution of nets to obtain "at least one net/house" then in February and December 2008 second distribution to get one net/sleeping units. These operations were part of a general malaria vector project implemented in eight villages around Balombo town (Benguela Province, Angola) to compare the efficacy of four method of vector control: classical LLIN alone, Insecticide treated plastic sheeting (ITPS) alone or associated with LLIN or following two rounds of indoor residual spraying [5].

One of the problems for the epidemiological evaluation of vector control is the choice of indicators considering that the aim of the control is to reduce the "burden" of malaria that can be understood in terms of «malaria-parasite» and/or "malaria-disease".

Several methods can be used for epidemiological surveys [6]. mainly "passive detection case" in Health Center or "active detection case" directly in communities [7]. One of the problems in Health Centre is often the well-known lack of reliability of data, from both clinical and parasitological examinations [8]. with some overdiagnosis of "malaria" reported as high as 85% [9]. and its consequences on drug pressure and the risk of increasing drug resistance.

As early as 1949 it was considered that "when diagnoses are based on symptomatology alone it is likely of any errors will be commonly inclusive rather than exclusive and as a consequence malaria will appear to be of exaggerated importance in the community" [10].

As there is no health center, in the villages studied, the population must go to the Hospital of the nearby city, Balombo, with a Pediatrics department. Active detection case in communities procured reliable information in bed net assessment studies [11,12]. but requires resources that are not always available. "For an extended period the detection of the malaria parasite in blood smears was the only available laboratory aid to diagnosis" [13]. But in endemic malaria areas the classical crosssectional surveys reveal plasmodial prevalence of 50% and more in symptomless people, prevalence which was noticed in studied area [14-19]. And "the opinion is entertained that if in any ambulatory group in whom 50 per cent infection is found on a single examination, the group is actually nearly 100 per cent infected" [10].

An additional criterion was then proposed taking into account the level of parasitaemia [20,21]. as a "pyrogenic threshold" or "parasite threshold" or "pyrogenic level" or "pyrexia level" [10-23]. which is variable according to ages, epidemiological conditions and other factors. It has been the subject of several studies [24-26]. and even some controversies considering that "the determination of this level in *falciparum* infections is practically impossible" [10].

The objective of our study was not to determine this pyrogenic threshold in the targeted populations but to analyse, and compare, the evolution of both the parasite prevalence and parasite density of *P. falciparum* during two consecutive years in the "at risk" group (i.e. children ≤ 5 years) of two villages with insecticide treated nets and two close villages without nets (= "control") but which will received vector control in the framework of the large village-scale vector control "Balombo project" [5].

II-Materials & methods

II-1. First census

A house census was done in each village by the local health worker. Each house received a number and was localized by GPS.

Implementation of deltamethrin treated LLIN PermaNet© 2.0 was done in Caala village (14°47'E ; 12°25'S ; 238 houses ; 792 inhabitants ; 469 sleeping units) and Cahata village (14°48'E ; 12°20' S ; 138 houses ; 484 inhabitants ; 442 sleeping units). A sleeping unit is "anything" used by inhabitants to sleep: mattress, piece of cardboard, loincloth, grass skirt, etc.

Two villages of the same area served as "control" for the first two years: Candiero (14°45'E; 12°22'S; 190 houses; 654 inhabitants) few kilometers West of Caala and Chisséquélé (14°47'E; 12°22'S; 181 houses; 418 inhabitants) few kilometers South of Cahata.

These four villages, with four others, are involved in the long-term large scale Balombo vector control comprehensive program implemented to compare four methods of vector control: LLIN alone; LLIN+ deltamethrin Insecticide Treated Plastic Sheeting (ITPS) pinned on the wall; ITPS alone and lambdacyhalothrin Inside Residual Spraying (IRS), two rounds followed by ITPS implementation to increase the long-lasting efficacy

II-2. LLIN distribution process

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

- February 2007: the objective was 1 LLIN per house (distribution of 360 LLIN in Caala and 310 in Cahata);
- February 2008: the objective was a full coverage with 1 LLIN per sleeping unit (distribution of 277 LLIN in Caala and 195 in Cahata);
- December 2008: complementary distribution of 49 LLIN in Caala and 25 in Cahata

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

II-3. Parasitological evaluation

In November 2006, before any intervention in this area, a cluster-based sampling parasitological survey was done in urban Balombo and some surrounding villages. A plasmodial prevalence of 54.9% (± 4.9%) in children < 15 years old (n=557) and a gametocytic index of 7.5% were reported (14).

From these information cross-sectional parasitological surveys (css) were planned and regularly made every two months, two weeks after the entomological survey, following the protocol implemented in Côte d'Ivoire to assess the efficacy of lambdacyhalothrin-treated nets in area where the main vector, *An. gambiae* was resistant to pyrethroids with a high frequency of kdr gene [12].

Field made blood thick films were Giemsa colored *in situ* then microscopically examined in Lobito in the Medical Department of the Angolan Sonamet© Society which sponsored a «Malaria Control Program" (MCP) including this village-scale vector-control project in Balombo.

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

As the team was well-known and well accepted, people of the villages come spontaneously to have their blood analysed for malaria diagnosis; this was done for everyone and results given to the village health worker for action according to the guidelines of the National Malaria Control Program.

From the initial listing of symptomless patients having had a thick blood film in the field, we extracted data of under-five (≤) children considered as the "at risk" group to analyze the evolution of both prevalence and parasite load for *P. falciparum* according to the two steps implementation of LLIN coverage in Caala and Cahata and comparing to the situation observed in the two control villages.

II-4. Statistical analyses

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

III. Results

III-1 Evolution of plasmodial prevalence in U5 symptomless children

2865 thick blood films were done on symptomless under-five children during the 40 surveys carried in the four villages during the first two years. *Plasmodium* infections (mainly *P. falciparum* with few *P. malariae*, most of them in association with *P. falciparum*) were observed in 1006 i.e. an overall plasmodial prevalence rate of 35.1% ±1.7% (Table 1).

years	indicators	Caala	Cahata	Candiero	Chisséquélé	total
Year 2007	Nb Pf+	161	204	77	79	521
	n	390	406	223	216	1235
	%	41,3%	50,2%	34,5%	36,6%	42,2%
	G+	11	19	6	18	54
	nb css	5	5	3	3	16
Year 2008	Nb Pf+	144	137	103	101	485
	n	447	376	407	400	1630
	%	32,2%	36,4%	25,3%	25,3%	29,8%
	G+	17	26	25	9	77
	nb css	6	6	6	6	24
total	Nb Pf+	305	341	180	180	1006
	n	837	782	630	616	2865
	%	36,4%	43,6%	28,6%	29,6%	35,1%
	G+	28	45	31	27	131
	nb css	11	11	9	9	40

Table 1: Results of parasitological surveys done in 2007 and 2008 in the four villages among the ≤ 5 years old children (nb Pf+= number of *P. falciparum* positives thick films; n= number of thick films made; % = % of Pf positive thick films; G+= number of blood films with gametocytes; Nb surveys= number of cross- sectional surveys made).

Overall, it appeared a significant reduction of plasmodic index in the four villages, from year to year (fig.1), but which was different according to villages.

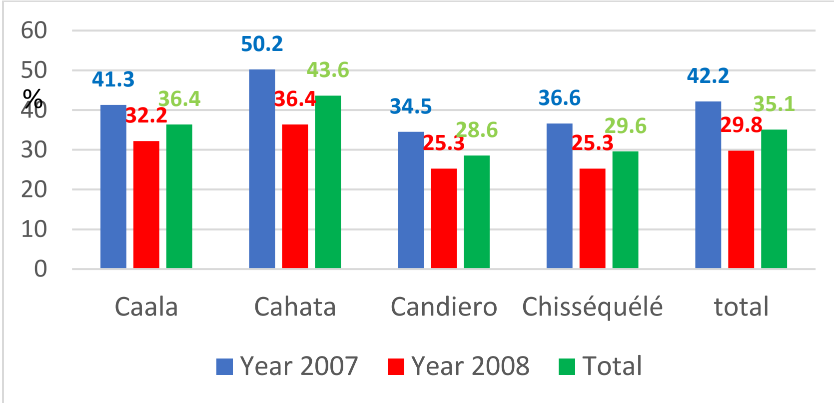

Figure 1: Evolution of *P. falciparum* prevalence in symptomless children ≤ 5 years old during the two years in the four villages surveyed.

In Caala: *Plasmodium* were observed in 305 of the 837 thick blood films prepared during the 11 surveys done in 2007 and 2008 (i.e. plasmodial infection= 36.4%) and the plasmodic prevalence significantly dropped (by 22%) from 41.3% (n= 390) to 32.2% (n= 447) ($\chi2$= 7.39; p-value=< 0.005; OR= 0.67 (0.51-0.89]).

In Cahata: *Plasmodium* were observed in 341 of the 782 thick blood films (i.e. plasmodial infection= 43.6%) with a significant reduction (by 27%) of plasmodial prevalence, from 50.2% (n= 406) to 36.4% (n=376) ($\chi2$= 15.14; p value < 0.0005; OR= 0.57 [0.43-0.76]).

In Candiero: *Plasmodium* were observed in 180 of the 630 thick blood films (i.e. plasmodial infections= 28.6%) with a significant reduction (by 27%) from 34.5% (n=223) to 25.3% (n= 407) ($\chi2$= 6.00; p value= 0.014; OR= 0.64 [0.45-0.92]).

In Chisséquélé: *Plasmodium* were observed in 180 of the 616 thick blood films (i.e. plasmodial infections= 29.6%) with a significant reduction (by 31%) from 36.6% (n= 216) to 25.3% (n= 400) ($\chi2$= 8.69; p value= 0.0032; OR= 0.58 [0.41-0.84]).

From the year 2007 to the year 2008 the plasmodial prevalence significantly dropped by 25.6% in villages with LLIN (from 45.8%; n= 796 to 34.1%, n= 823; $\chi2$= 23.1; OR= 0.61 [0.50-0.75]) and by 28.7% in villages without nets (from 35.5%, n= 439 to 25.3%, n= 807; $\chi2$= 14.6; OR= 0.61 [0.48-0.79]).

As a significant reduction of plasmodial prevalence was observed in LLIN furnished villages and in control villages the plasmodial prevalence itself could not be considered as a relevant indicator in the condition of this field trial.

II-2. Evolution of parasite load in U5 symptomless children

The parasite load significantly decreased from year to year in villages with LLIN (P value= 0.0031 in Caala; < 0.001 in Cahata) but remained at the same level in villages control without LLIN (P value=0.65 in Chisséquélé and 0.51 in Candiero) (fig. 2a).

Adding and comparing the parasitaemia of the two LLIN villages and the two control villages clearly showed the significant reduction of parasite load in villages with LLIN while it remained at the same level in control villages (fig 2b).

Considering the relation between parasite density and the risk of clinical malaria, the parasite load appeared as an interesting relevant indicator of the impact of LLINs installation on the malaria burden in the area studied.

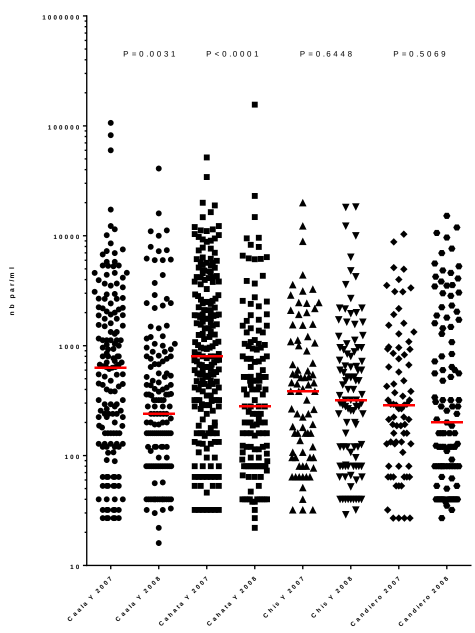

Figure 2a: Evolution of parasite load in each villages, with LLIN (Caala, Cahata) and without (Chisséquélé, Candiero) in under five symptomless children.

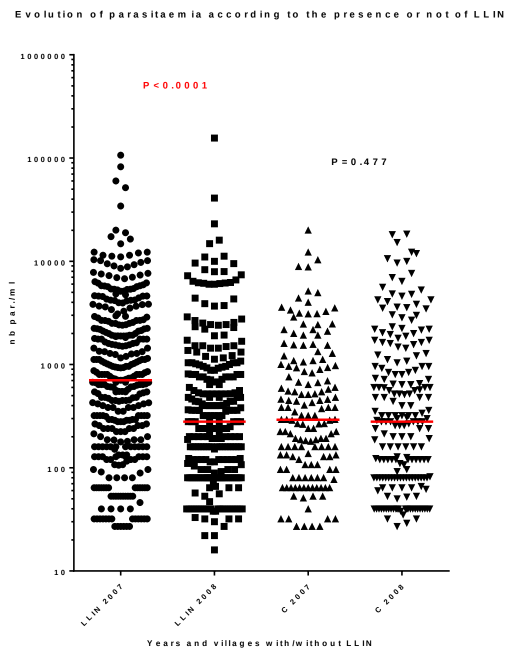

Figure 2b: Comparison of the evolution of *P. falciparum* parasitaemia in symptomless children ≤ 5 years old in the 2 villages with LLIN and in the 2 control villages (C).

III-3. Gametocytic index in U5 symptomless children

From the Table 1 it is possible to calculate the gametocyte index, and its evolution in each village and each year (Table 2).

	GI Caala	GI Cahata	GI Candiero	GI Chisséquélé	GI Total
Year 2007	6,83%	4,68%	2,69%	8,33%	4,37%
Year 2008	3,80%	6,91%	6,14%	2,25%	4,72%
Total	3,35%	5,75%	4,92%	4,38%	4,57%

Table 2: Evolution of the gametocyte index (GI) in symptomless U5 children in each village and for each year.

The evolution, from year to year, appeared different according to village (figure. 3) with a decrease in Caala and Chisséquélé and an increase in Cahata and Candiero. But the overall gametocyte index in 2007 and 2008 were remarkably similar, respectively 4.37% (=1,235) and 4.72% (n=1,006).

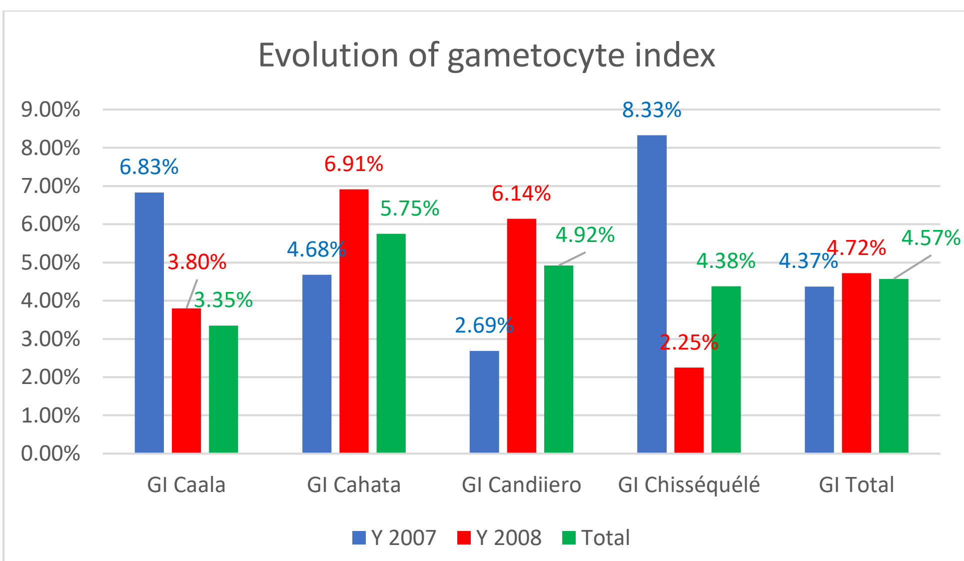

Figure 3: Evolution of gametocyte index in symptomless U5 children in each year for each village.

The gametocyte index in LLIN villages were similar in years 2007 (GI= 3.77%; n= 796) and 2008 (GI=5.22%; n=823) ($\chi2$= 1.99; p=0.16; OR=0.71 [0.44-1.14]).

But they were also similar in control villages, GI= 5.47% (n= 439) in years 2007 and GI= 4.21% (n= 807) in 2008 ($\chi2$= 0.042; p= 0.84; OR=0.94 [0.53-1.67]).

There fore the gametocyte index did not appear as a relevant indicator of the impact of LLIN in this trial.

IV. Discussion

Since the first observations of the entomological and epidemiological efficacy of impregnated mosquito nets [27-31]. numerous studies have demonstrated that large-scale use of impregnated mosquito nets ("ITNs") drastically reduces transmission, malaria morbidity and overall infant and child mortality [11-34].

Vector control can still keep actual efficacy against malaria [35]. even when vectors are resistant to pyrethroid insecticides [12-41]. this issue represents an increasingly worrying problem [42-45]. and new mosquito nets are treated with the association of pyrethroid and other chemical such as PBO [46-48]. or PPF [49,50].

Another problem arises with the assessment of the actual epidemiological impact of impregnated mosquito nets on malaria morbidity given the several "co-factors" [51]. involved in this issue which requires specific protocols [7-52]. The two main problems, among several others, are the challenge of "malaria" diagnosis with the lack of pathognomonic symptoms and the high percentage of symptomless *Plasmodium falciparum* carriers in malaria endemic areas, inducing a lack of reliability of data, often reported [7-26]. with a great overdiagnosis of "malaria" [53,54].
As early as 1949, Boyd [10-13]. underlined "it must be stressed that the present-day diagnosis of malaria should imply the existence of a plasmodial infection, and that such a diagnosis is not adequate unless the species of the causative parasite is identified. This criterion necessitates a microscopic diagnosis…the presence of *Plasmodium* is a necessary but not sufficient condition". According to the results of various malariological surveys, Boyd (*loc.cit*.) underlined "in view of the fluctuations of parasite density above and below the level of microscopic detectability, the opinion 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". A situation regularly observed during "routine" cross sectional malaria surveys in the field [14]. and which questioned the validity of the "plasmodial presence" only for the diagnosis of "malaria" in endemic area and hence for the epidemiological evaluation of a vector control program.

In Balombo area, several cross sectional malaria parasite surveys showed a high frequency of plasmodial infections in symptomless carriers [15]. and it appeared that the plasmodic index significantly decreased in LLIN furnished villages as well as in control villages showing that the classical plasmodic index could not be a relevant indicator in this study.

Another parasitological indicator should therefore be taken into consideration. As Christophers (in Boyd, *loc. cit*.) underlined, "a parasite rate of say 50 per cent may largely be made up of scanty infections representing little or no morbidity or of a relatively heavy infections with all the signs of a community suffering severely from malaria "and further on "we can therefore only adequately describe the parasitaemia in a community by ascertaining the numerical value of the infections encountered. The number of parasites per cu.mm is the PARASITE COUNT. All these are means of expressing the PARASITE DENSITY for the individual or community as the case may be".

A new interest was devoted to this notion of parasite load and the issue of its relationship with malaria morbidity [24-55]. inducing the notion of pyrogenic threshold [25-60]. In a study on malaria morbidity in children under 3 years old in Benin it is reported that "the comparison with a control group demonstrated that children with parasitaemia exceeding 1000 infected red blood cells per microliter of blood had fever significantly more often than children with lower levels of parasitaemia" [56]. For [61]. Plucinski *et al* "Malaria parasites must be present at sufficiently high densities in order to cause symptoms such as fever, especially among individuals living in high-transmission settings who have been exposed multiple times and developed antimalarial adaptive immunity. The *P. falciparum* parasite density necessary to provoke fever, i.e., the pyrogenic threshold, is dependent on factors that influence host responses and immunity and can vary substantially". Numerous studies were done on this notion of pyrogenic threshold, and its estimation [57-62]. but this is not the aim of our study.

On the other hand, this notion of the parasite load appeared relevant in our study to compare the evolution of malaria parasitaemia in the two villages provided with impregnated mosquito nets versus the two villages without any organized vector control operations.

Actually, the statistical analysis of the distributions of the parasite densities showed a significant reduction in parasitaemia with the overall implementation of impregnated mosquito nets while in the two control villages these parasitic loads remained similar during the first two years.

The findings in Balombo studies clearly showed that several parasite indicators are needed to better appreciate the natural, or induced, evolution of situation of malaria parasite and the impact of mosquito nets in reducing the potential of malaria burden in under 5 years children.

V. Conclusion

The choice of indicators is essential to monitor, and evaluate, a malaria control program and take eventual action.

Gaining base line data is a crucial component of any control program, and for having forgotten this point, a recent house spraying operation in Angola was a failure, and loss of funds.

The initial two years studies at the beginning of the village scale long term malaria vector control program around Balombo town (Benguela Province, Angola) clearly showed how conclusions could be different according to the selected indicators.

Plasmodium prevalence, and its evolution from year to year, were the same in symptomless children ≤ 5 years old (= "at risk" age group) of the two villages with treated nets and the two villages without, leading to a conclusion of the inefficiency of treated nets if based upon this indicator only.

But the parasitaemia of children of villages with treated nets was significantly lower than in children of villages without nets leading to the conclusion on the actual efficacy of nets to reduce potential malaria morbidity as there is a link between parasite load and clinical illness a point well in the line of some recent analysis [35].

This two years study shows how the conclusion could be completely different according to the indicator and the Balombo project underlined, once again, the key point of "Beware the plasmodial infection indicators".

Contributions of authors.

This work was carried out in collaboration among all authors. PC elaborated the protocol, participated in field surveys, did analyzing of data and drafted the report. JCT, VF and LE participated in field surveys, VF participated in microscopical examination of blood slides and verification, GC participated in the interpretation data and English version. FG and RO participated in data statistical analysis.

All authors read and approved the final manuscript.

Ethical approved.

Studies done at the request and with the National Malaria Control Program and the Public Health Provincial Department in the context of the ongoing “Malaria Control Program” of the Angolese Society Sonamet.

Competing interest.

Authors have no competing interests.

Acknowledgements.

We would like to thank the managers of the Angolan company Sonamet© and its medical department; as well as the international company SubSea©7 for their permanent support for this work and their implication in malaria control in the region.

We thank Dr Titelman who procured the material for vector control: nets and plastic sheeting.

Our thanks also to the national and provincial authorities for their authorization and participation in these studies as well as to the population of the villages for their positive cooperation.

References

1. WHO. Global Technical Stratey for Malaria 2016-2030. WHO Geneva. 2015;ISBN 978924156499&.
2. WHO. World Malaria Report. WHO Global Malaria Programme. 2015.
3. WHO. World Malaria Report. World Health Organization, Geneva, Switzerland. 2017:196 p.
4. WHO/AFRO. Systèmes d'information pour l'évaluation des programmes de lutte antipaludique. Guide pratique. AFRO/CTD/MAL/943. 1994:73 p.
5. Carnevale P, Foumane Ngane V, Toto J, C., Dos Santos M, Fortes F, Manguin S. The Balombo (Benguela Province, Angola) Project: a village scale malaria vector control programme with a long term comprehensive evaluation. 6th PAMCA Annual Conference and Exhibition Strengthening surveillance systems for vector-borne disease elimination in Africa Yaoundé 23-25 septembre 2019. 2019.
6. Abramson J. Survey Methods in Community Medicine. Epidemiological Studies. Programme Evaluation. Clinical Trials. Fourth Edition. Churchill Livingstone Edinburgh London Melbourne and New York. 1990:339 p.
7. Rogier C, Fusai T, Pradines B, Trape J-F. Comment évaluer la morbidité attribuable au paludisme en zone d'endémie ? Rev Epidém et Santé Publique. 2005;53:299-309.
8. Baudon D, Gazin P, Galaup B, Pellotier-Guinart E, Picq J-J. Fiabilité de l'examen clinique dans le diagnostique des fièvres palustres en zone d'endémie ouest africaine. Méd Trop. 1988;48:123-6.
9. Manguin S, Foumane V, Besnard P, Fortes F, Carnevale P. Malaria overdiagnosis and subsequent overconsumption of antimalarial drugs in Angola: Consequences and effects on human health. Acta Trop. 2017;171:58-63.
10. Boyd MF. Malariology. A Comprehensive Survey of All Aspects of This Group of Diseases from a Global Standpoint. MF Boyd ed The WBSaunders Co, Philadelphia, Pa. 1949:3-25.
11. Lengeler C, Cattani J, De Savigny D. Net gain. A new method for preventing malaria deaths. 1996:189.
12. Henry MC, S.-A. A, Rogier C, Dossou yovo J, Chandre F, Guillet P, et al. Protective efficacy of lambda-cyhalothrin treated nets in Anopheles gambiae pyrethroid resistance areas of Côte d'Ivoire. Am J Trop Med Hyg. 2005;73(5):859-64.
13. Boyd M, Christophers S, Coggeshall L. Laboratory diagnosis of malaria infections. in: Boyd MF (ed) Malariology WB Saounders , Philadelphia. 1949;I(8):155-204.
14. Foumane V, Besnard P, Le Mire J, Foucher J, Soyto A, Fortes F, et al. Enquêtes paludométriques en 2006 et 2007 dans la province de Benguela, Angola Sciences et Médecines d'Afrique. 2009;1: 60-5.
15. Carnevale P, Dos Santos M, Moniz Soyto A, Besnard P, Foumane V, Fortes F, et al. Parasitological Surveys on Malaria in Rural Balombo (Angola) in 2007-2008. Base Line Data for a Malaria Vector Control Project. Int J Trop Dis. 2018;31(3):1-12.
16. Carnevale P, Toto J, Foumane V. "House Plasmodial Prevalence Index" Another relevant indicator of evaluating a malaria vector control operations, Example of Capango village (Benguela Province, Angola). Intern J Trop Dis Hlth. 2020;415:53.
17. Carnevale P, Toto J, Foumane V, Manguin S. Influence of Partial and Full Coverage on Long Lasting Deltamethrin Treated Nets (“Δllin”) on Plasmodium falciparum Parasitaemia in 2 Villages around Balombo Town (Benguela Province, Angola). OAJBS Open Access Journal of Biomedical Sciences. 2021;4(6):ID.000340.
18. Carnevale P, Toto J, Foumane V, Carnevale G, Stahl D. Plasmodial Prevalence with Respect to Gender in 6 villages around Balombo town, Benguela Province, before malaria vector control implementation. Int J Trop Dis & Hlth. 2022;43(7):19-42.
19. Carnevale P, Foumane V, Toto J, Soyto A, Breda M, Gay F. 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. International Journal of Tropical Disease & Health 2023;44(1):1-30.
20. Christophers S. The Mechanism of Immunity against Malaria in Communities Living under Hyperendemic Conditions. Indian J M Research. 1924;12:273.
21. Rice M, Watson R. The Relationship between Malaria Morbidity and Parasitaemia. Am J Hyg. 1943;3:164.
22. Sinton J, Harbhagwan., Singh J. The Numerical Prevalence of Parasites in Relation to Fever in Chronic Benign Tertian Malaria. Indian J M Research. 1931;18:971.
23. WHO. Terminology of Malaria and of Malaria Eradication. Report of a Drafting Committee. Worl Health Organization. 1963:127 pp.
24. Bouvier P, Rougemont A, Breslow N, Doumbo O, Delley V, Dicko A, et al. Seasonality and malaria in a West African village: does high parasite density predict fever incidence? Am J Epidemiol. 1997;145(9):850-7.
25. Rogier C, Commenges D, Trape J. Evidence of an age dependent pyrogenic threshold of Plasmodium falciparum parasitaemia in highly endemic populations. Am J Trop Med Hyg. 1996;54(6):613–9.
26. Rogier C, Henry MC, Spiegel A. Diagnostic des accès palustres en zone d'endémie: bases théoriques et implications pratiques. Méd Trop. 2001;61:27-46.
27. Darriet F, Robert V, Tho Vien N, Carnevale P. Evaluation de l'efficacité sur les vecteurs de paludisme de la permethrine en imprégnation de moustiquaires intactes et trouées. WHO/VBC/84899 & WHO/MAL/841008. 1984.
28. Carnevale P, Robert V, Snow RW, Curtis C, Richard A, Boudin C, et al. L' impact des moustiquaires imprégnées sur la prévalence et la morbidité liée au paludisme en Afrique sub-saharienne. Ann Soc Belge Med Trop. 1991;71(Suppl 1:):127-50.
29. Robert V, Carnevale P. Influence of deltamethrin treatment of bednets on malaria transmission in the Kou Valley, Burkina Faso. Bull Wld Hlth Org. 1991;69:735-40.

30. Robert V. Brève histoire des moustiquaires imprégnées d'insecticide dans la lutte contre le paludisme : un témoignage sur la cruciale décennie 1980 Brief History of Insecticide-Treated Bed Nets in the Fight against Malaria: A Testimony on the Crucial 1980's Decade. Bull Soc Pathol Exot. 2020;113:88-103.
31. Ranque P, Touré Y, Soula G, Du L, Diallo B, Balique H. Etude expérimentale sur l'utilisation de moustiquaires imprégnées de deltaméthrine dans la lutte contre le paludisme. Parassitologia. 1984;26:261-8.
32. Lengeler C. Insecticide-treated bed nets and curtains for malaria control. The Cochrane Library Edition, Issue 3, Oxford. 1998.
33. WHO. Insecticide-treated nets: a WHO position statement. Geneva: World Health Organization. 2007.
34. Carnevale P, Gay F. Insecticide-Treated Mosquito Nets. Methods Mol Biol. 2019;2013:221-32.
35. Bhatt S, Weiss D, Cameron E, Bisanzio D, Mappin B, Dalrymple U, et al. The effect of malaria control on Plasmodium falciparum in Africa between 2000 and 2015. Nature. 2015;526(7572):207-11.
36. Henry MC, Doanio JMC, Darriet F, Nzeyimana I, Carnevale P. Efficacité des moustiquaires pré-imprégnées de permethrine OLYSET(R) Nets en zone de résistance des vecteurs aux pyréthrinoides II. Evaluation parasito-clinique. Med Trop. 1999;59:355 - 7.
37. Cook J, Tomlinson S, Kleinschmidt I, al. e. Implications of insecticide resistance for malaria vector control with long-lasting insecticidal nets: trends in pyrethroid resistance during a WHO-coordinated multi-country prospective study. Parasit Vectors. 2018;11(1):550.
38. Kleinschmidt I, Bradley J, Knox T, et al. Implications of insecticide resistance for malaria vector control with long-lasting insecticidal nets: a WHO-coordinated, prospective, international, observational cohort study. Lancet Infect Dis. 2018;18(6):640-9.
39. Alout H, Labbé P, Chandre F, Cohuet A. Malaria Vector Control Still Matters despite Insecticide Resistance. Trends Parasitol. 2017;33(8):610-8.
40. Churcher T, Lissenden N, Griffin J, Worrall E, Ranson H. The impact of pyrethroid resistance on the efficacy and effectiveness of bednets for malaria control in Africa. Elife. 2016;5:pii: e16090. doi: 10.7554/eLife.
41. Viana M, Hughes A, Matthiopoulos J, Ranson H, Ferguson H. Delayed mortality effects cut the malaria transmission potential of insecticide-resistant mosquitoes. Proc Natl Acad Sci U S A. 2016;113(32):8975-80.
42. Ranson H, N'guessan R, Lines J, Moiroux N, Nkuni Z, Corbel V. Pyrethroid resistance in African anopheline mosquitoes: what are the implications for malaria control? Trends Parasitol 2011;27:91–8.
43. Ranson H, Lissende N. Insecticide resistance in African anopheles mosquitoes: a worsening situation that needs urgent action to maintain malaria control. Trends Parasitol. 2016;32:187–96.
44. Strode C, Donegan S, Garner P, Enayati A, Hemingway J. The impact of pyrethroid resistance on the efficacy of insecticide-treated bed nets against African anopheline mosquitoes: systematic review and meta-analysis. PLoS Med. 2014;11(3):e1001619.
45. Hemingway J, Ranson H, Magill A, Kolaczinski J, Fornadel C, Gimnig J, et al. Averting a malaria disaster: will insecticide resistance derail malaria control? Lancet. 2016;387(10029):1785-8.
46. Tungu P, Magesa S, Maxwell C, Malima R, Masue D, Sudi W, et al. Evaluation of PermaNet 3.0 a deltamethrin-PBO combination net against Anopheles gambiae and pyrethroid resistant Culex quinquefasciatus mosquitoes: an experimental hut trial in Tanzania. Malar J. 2010; 9:21.
47. Gichuki P, Kamau L, Njagi K, Karoki S, Muigai N, Matoke-Muhia D, et al. Bioefficacy and durability of Olyset® Plus, a permethrin and piperonyl butoxide-treated insecticidal net in a 3-year long trial in Kenya. Infect Dis Poverty. 2021;10(1):135.
48. Protopopoff N, Mosha J, Lukole E, Charlwood J, Wright A, Mwalimu C, et al. Effectiveness of a long-lasting piperonyl butoxide-treated insecticidal net and indoor residual spray interventions, separately and together, against malaria transmitted by pyrethroid-resistant mosquitoes: a cluster, randomised controlled, two-by-two factorial design trial. Lancet. 2018;391(10130):1577-88.
49. Djènontin A, Ahoua Alou L, Koffi A, Zogo B, Duarte E, N'Guessan R, et al. Insecticidal and sterilizing effect of Olyset Duo®, a permethrin and pyriproxyfen mixture net against pyrethroid-susceptible and -resistant strains of Anopheles gambiae s.s.: a release-recapture assay in experimental huts. Parasites & Vectors. 2015;22:27.
50. Ngufor C, N'guessan R, Fagbohoun J, Odjo A, Malone D, Akogbeto M, et al. Olyset Duo® (a pyriproxyfen and permethrin mixture net): an experimental hut trial against pyrethroid resistant Anopheles gambiae and Culex quinquefasciatus in Southern Benin. PLoS One. 2014 9(4):e93603.
51. Kleinschmidt I, Torrez M, Schwabe C, Benavente L, Seocharan S, Jituboh D, et al. Factors influencing the effectiveness of malaria control in Bioko Island, Equatorial Guinea. Am J Trop Med Hyg. 2007;76(6):1027–32.
52. Kleinschmidt I, Mnzava A, Kafy H, Mbogo C, Bashir A, Bigoga J, et al. Design of a study to determine the impact of insecticide resistance on malaria vector control: a multi-country investigation. Malar J. 2015;14:282.
53. Gentilini M, Caumes E, Danis M, Mouchet J, Duflo B, Lagardère B, et al. Médecine Tropicale. Médecine-Sciences, Flammarion. 1993:929 pp.
54. Gentilini M, Caumes E, Danis M, Richard-Lenoble D, Bégué P, Touze J, et al. Paludisme. Médecine Tropicale, chap 10. 2012:191-231.
55. Delley V, Bouvier P, Breslow N, Doumbo O, Sagara I, Diakite M, et al. What does a single determination of malaria parasite density mean? A longitudinal survey in Mali. Trop Med Int Health. 2000;5:404-12.
56. Velema J, Alihonou E, Chippaux J, Van Boxel Y, Gbedji E, Adegbini R. Malaria morbidity and mortality in children under three years of age on the coast of Benin, West Africa. Trans R Soc Trop Med Hyg. 1991;85:430-5.
57. Dicko A, Mantel C, Kouriba B, Sagara I, Thera M, Doumbia S, et al. Season, fever prevalence and pyrogenic threshold for malaria disease definition in an endemic area of Mali. Trop Med Int Health. 2005;10:550-6.
58. Dollat M, Talla C, Sokhna C, Diene Sarr F, Trape J, Richard V. Measuring malaria morbidity in an area of seasonal transmission: Pyrogenic parasitemia thresholds based on a 20-year follow-up study. PLoS One. 2019;14(6):e0217903.
59. Gatton M, Cheng Q. Evaluation of the pyrogenic threshold for Plasmodium falciparum malaria in naive individuals. Am J Trop Med Hyg. 2002;66(5):467-73.
60. Smith T, Ross, A., Maire N, Rogier C, Trape J, Molineaux L. An epidemiologic model of the incidence of acute illness in Plasmodium falciparum malaria. Am J Trop Med Hyg. 2006;75(2):56-62.
61. Plucinski M, Rogier E, Dimbu P, Fortes F, Halsey E, Aidoo M, et al. Performance of Antigen Concentration Thresholds for

Attributing Fever to Malaria among Outpatients in Angola. J Clin Microbiol. 2019;57(3):pii: e01901-1918.

62. Boisier P, Jambou R, Raharimalala L, Roux J. Relationship between parasite density and fever risk in a community exposed to a low level of malaria transmission in Madagascar highlands. Am J Trop Med Hyg. 2002;67(2):137-140.

DOI:10.31579/2690-8794/175