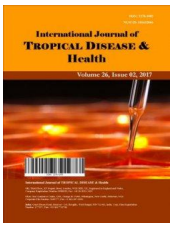

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Parasitological Surveys on Malaria in Rural Balombo (Angola) in 2007-2008: Base Line Data for a Malaria Vector Control Project

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Authors' contributions

This work was carried out in collaboration between all authors. Author CP designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors DSM, AMS, BP, FV and FF managed the analyses of the study. Author TB finalized the manuscript. Author MS checked data and finalized the text. All authors read and approved the final manuscript.

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ABSTRACT

Study Design: Balombo area (Angola) f from each other to avoid the possibility of active flights of the mosquitoes and “contamination” of treated or control villages.
Methodology: Classical Cross Sectional Surveys (CSS) were symptomless children <15 years. During data analysis this sample was stratified into the 3 conventional age groups: <5 years (often considered as “at risk group"); 2-9 years (often used for classical endemicity index) and <15 years already used in other vector control studies. The 3 classical parasitological indicators: plasmodial prevalence, parasite load, gametocyte prevalence were compared between these 3 age-groups to select the most relevant for further analysis and evaluation of the efficacy of vector control implemented. Blood thick films prepared in the field were colored and microscopically examined at the Malaria Control Program's laboratory of the medical department of the Angolan oil company Sonamet (Lobito).
Results: 4625 thick drops were made during the 38 regularly carried out field surveys. *Plasmodium falciparum* was the predominant species with few mixed *P. falciparum* + *P. malariae* infection and a single *P. malariae* one. The overall Plasmodic index was 42.7%, gametocyte index was 4.4% and high parasite load (> 10.000 par./ml) were noticed in children, even very young, without clinical symptoms. Classical seasonal variations of plasmodic index and some variations according to age group were observed. Gametocytic indices showed relatively stable levels with age group.
Conclusion: Statistical analysis showed that “under 15 years” age group could be a relevant indicator to evaluate the efficacy of a vector control programme and it increases the sample size allowing to perceive even small variations induced by vector control. Variability of parasitological index according to age groups, villages, season, confirmed the importance of regularly surveys to know precisely the situation before the implementation of control operations for reliable further evaluation. Missing such base line data induced the failure of a former vector control project in Angola.

Keywords: Malaria; Plasmodium; parasite density; plasmodic index; gametocytic index; prevalence.

1. INTRODUCTION

In Angola, malaria is present all over the country but, for meteorological, ecological, entomological, economic and socio-cultural reasons, epidemic outbreaks occurred recently and required the implementation of emergency and long term control measures.

The 2016 break out of malaria induced, officially, 3,974,253 cases and 15,994 deaths reported by health services (while they were estimated at 8,000 in 2015 and 5,500 in 2014). Since 2017, there is an outbreak in the Huambo region, reaching the towns in the province of Benguela and emergency measures (space spraying) have been implemented. The National Malaria Control Program (NMCP) has adopted the WHO recommended strategy for the elimination of malaria by combining diagnosis (based on rapid detection tests), case management (based on artemisinin-based therapies (ACTs), intermittent presumptive therapies (based on sulfadoxin-pyrimethamin "SP") during pregnancy, vector control based on long-lasting insecticide-treated bed nets, insecticide residual spraying , etc.

Evaluation of the *efficacy* of vector control operations requires a precise knowledge of the epidemiological situation before their implementation to avoid some of the problems recently encountered during the large scale inside residual spraying (IRS) conducted from December 2005 to March 2006 in the southern area of the country at the initiative and support of President Malaria Initiative (PMI) [1]. Somandjinga and col [1] stressed their "Vigorous objections to the lack of reliable data on mosquitoes and malaria" before implementation of vector control that staff from the US Centers for Disease Control (CDC) was supposed to collect in due time but it wasn’t. It is also interesting to note that the treated area had experienced a drought accentuated for 5 years and the author rightly considers that "Implementation of this large-scale malaria control effort despite the lack of malaria transmission in southern Angola during 2005- 2006 was unique and unfortunate. One fault of this project was the rush to spray”.

The objective of the "Balombo Project" was to evaluate the effectiveness of 4 methods of Vector Control (VC): classical Long Lasting Insecticide Treated Nets (LLIN) or Inside Residual Spraying (IRS) versus the newly developed Insecticide Treated Plastic Sheeting (ITPS) used alone or simultaneously with LLIN or after 2 rounds of IRS to increase the long lasting action. Reliable parasitological base line data are of crucial needs to allow an accurate evaluation of the impact of VC implemented.

2. METHODOLOGY

2.1 Presentation of the Study Area and Villages

Surveys were *conducted* in four villages near the town of Balombo (12 ° 21'S, 14 ° 46 'E), in the province of Benguela: Capango (12°23' S; 14°50' E), Canjala (12°21' S; 14°44' E), Candiero (12°22' S; 14°45' E) and Libata (12°20' S; 14°45' E).

These villages are separated by several kilometers preventing any "contamination" of mosquitoes going from one to the other one village *as it is usually considered that the active flight of Anopheles is around one mile.*

According to a census carried out at the beginning of the surveys, the demographic situations of the four villages were: Capango: 177 inhabitants (60 houses); Canjala: 873 inhabitants (401 houses); Candiero: 654 inhabitants, (190 houses); Libata: 1344 inhabitants (457 houses) representing a total of 2948 people in 1108 houses. Each entrance door of these houses received a number and were localized (GPS) to allow the scheduled random sampling for parasitological evaluation and mapping for further follow-upof"*P. falciparum* positive children".

Balombo is located at an altitude of 1183 m in a mountainous area of ancient volcanoes with a hot spring at the western entrance to the city. The vegetation is of the shrub meadow type; the original forest has been severely degraded for crop development and domestic use. Temperatures vary between 17 and 28°C, with an average of 22°C with a "cold season" in May-June-July. The rains are well marked with a great rainy season that lasts from September to the end of April.

2.2 Protocol of Surveys

A preliminary malaria parasite prevalence cluster sampling survey, conducted in November 2006 (rainy season) in Balombo and seven villages around the town, indicated a general *Plasmodium* parasite index of 75% (± 7%) (n = 181) (VF unpublished observations). Considering a Plasmodic Index of # 50% in the area (with a 95% confidence level and the usual margin error of 5%) the size of the sample to be studied could be estimated at 384 to get significant data.

Parasitological studies were field performed according to the classical method of cross-sectional surveys (CSS) on randomized samples. For parasitological evaluation of VC the issue of the choice of the most relevant "age group indicator" in the targeted epidemiological conditions has to be solved and therefore plasmodial infestations were analyzed in the 3 classical age groups:

- Children <5 years of age (Under 5 of "U5"), considered as the "at risk group" [2], by WHO and therefore specially targeted for example with the first large scale distribution, and its evaluation, of impregnated mosquito nets [3];
- Children 2-9 years of age ("2- 9 y"), formerly and largely used to evaluate the traditional endemic index [4,5,6];
- Children <15 years old (Under 15 or "U 15"), already used to evaluate the effectiveness of vector control operations in others programmes [7,8].

Three classical parasitological indicators were analyzed: the plasmodic index, the gametocytic index and the parasite load (estimated in number of parasites / µl by counting the number of parasites versus 200 leucocytes and considering 8000 leucocytes / µl).

The blood thick films performed in the field were stained (classical Giemsa) and microscopically examined at the MCP laboratory / medical service of the Angolan oil company SONAMET in Lobito; 10% of slides were double checked in Yaoundé.

The results were provided to the "activista" of each village for information and action according to the guidelines of the NMCP.

During the first surveys (March and May 2007), for operational reasons, studies were conducted in 2 Capango and Canjala only, but from August 2007 to December 2008 the 4 villages were regularly surveyed every 2 months. No one of these 4 villages had a health center. The hospital is located in Balombo city center, with a pediatric department and a laboratory of biological analyzes.

Plasmodic index were compared by the usual percentage analysis (Epi Info); parasitaemia were analyzed with the non-parametric Mann-Whitney test with graph pad software.

3. RESULTS AND DISCUSSION

3.1 Overall Situation

4625 thick drops were prepared with children below 15 years old during the 38 surveys *conducted* in 2007 and 2008; their microscopic examination revealed the presence of asexual elements of *Plasmodium* in 1974 blood films, giving a general *Plasmodium* prevalence of 42.7%. This shows that the initial assumption of a plasmodial prevalence of # 50% used for sampling size was right.

Gametocytes were observed in 205 thick drops, i.e. a gametocytic index (IG) of 4.43%. It is interesting to underline this value of the GI about 10 times less than that of the PI, often found in the different surveys.

These parasitological index varied according to age and season in each village (Fig. 1), showing the importance of repeated surveys.

Plasmodic Index showed classical variations with highest level during the rainy season and lowest during the dry season and which were well marked in Capango (with clear drop in both August 2007 and 2008 and peaks in March 2007; April, October, December 2008) and less in other 3 villages.

When gathering parasitological data for these 2 years in each village it appeared that "Yearly Plasmodic prevalence" (total positive thick films/total thick films prepared for each year) changed according to villages and age groups (Table 1); 2 villages, Canjala and Libata, had Plasmodic Index higher than the 2 others but in each village PI were similar in the 3 age groups considered (Fig. 2).

3.2 Capango Village

3.2.1 Plasmodic index

During the 10 surveys conducted from March 2007 to December 2008, 649 thick films were prepared from children <15 years of age; *Plasmodium* was observed in 229 blood slides, i. e a general parasite index (PI) of 35.3%.

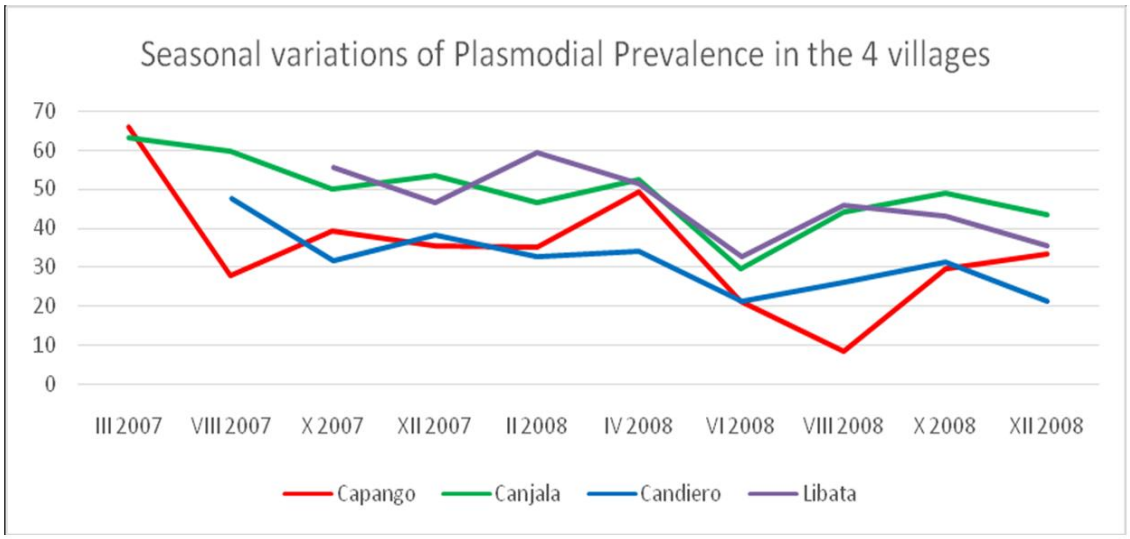

Fig. 1. Seasonal variations of Plasmodial prevalence in children below 15 years (U 15) in the 4 studied villages

Table 1. Plasmodic indices recorded in the 4 villages according to age groups

Villages age	Capango (10 surveys)	Canjala	Candiero	Libata
< 5 years	34.0% (n= 332)	44.5 % (n= 695)	28.6 % (n=630)	45.9 % (n=749)
2-9 years	37.5% (n=456)	52.9% (n=1016	33.6 % (n=792)	51.7 % (n= 940)
< 15 years	35.3% (n= 649)	50.2 % (n=1455)	32.7 % (n= 1188)	47.0 % (n=1333)

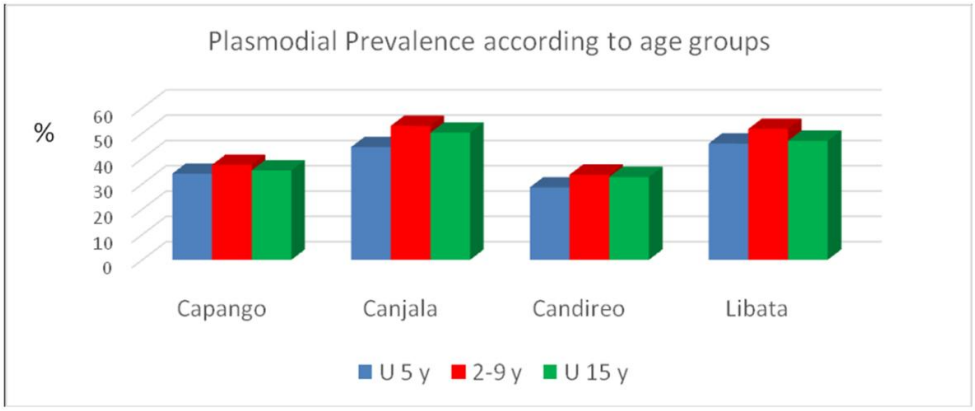

Fig. 2. Plasmodial prevalence according to villages and age groups

All plasmodial infections were due to *P. falciparum* with the exception of a single mixed *P. falciparum* + *P. malariae* infection, observed in April 2008 in a 4-years-old child.

The presence of sexual forms was noted in 29 samples, a gametocytic index (GI) of 4.5%.

All of these children were symptomless, although some thick films contained several thousands of *P. falciparum* trophozoites / µl of blood.

The following parasite index were observed: children <5 years: PI = 34.0% (n = 332), children 2-9 years: PI = 37.5% (n = 456) and children <15 years: PI = 35.3% (n = 649) (Table 1).

It is worth underlining that these plasmodic index are statistically similar in the 3 age groups considered: children <5 years versus 2-9 years: $\chi 2 = 1.00$ (p = 0.32; OR = 0.86 [0.64-1.16]; children <5 years versus <15 years: $\chi 2 = 0.15$ (p = 0.69; OR = 1.06 [0.80-1.39]; children 2-9 years versus <15 years: $\chi 2 = 0.57$ (p = 0.45; OR = 0.91 [0.71-1.16].

3.2.2 Parasitic densities

The comparison of the median of the parasite load (Fig. 3) shows that parasitaemia were similar in <5 years versus 2-9 years (P = 0.949); < 5 years versus <15 years (P = 0.351) and 2-9 years versus <15 years (P = 0.248) even if it could seem slightly lower in under 15 y age group.

3.2.3 Gametocytic index

Gametocytic index were comparable in the 3 age groups: 4.2% (n = 332) for children <5 years; 5.7% (n = 456) for children 2-9 years and 4.5% (n = 649) for children <15 years old. Therefore about one in 25 children is a "*Plasmodium* reservoir potentially infective for *Anopheles* vectors".

Therefore the three parasitological parameters analyzed, Plasmodic index, gametocyte index and parasitaemia were similar in the three age groups and the larger one, “U 15” can be used for reliable evaluation of any changes after vector control implementation.

3.3 Canjala Village

During the 10 surveys carried out between March 2007 and December 2008, 1455 thick films involving children <15 years were obtained in the field then microscopically examined and the presence of *Plasmodium* trophozoites was observed in 731 blood slides,

i.e. a parasite rate of 50.2 %, (Table 1). *P. falciparum*. was largely predominant as only 11 mixed *P. falciparum* + *P. malariae* infections and 3 *P. malariae* alone infections (children aged 2, 7 and 10) were determined. Gametocytes were observed in 76 thick drops, i.e. a gametocytic index of 5.2%.

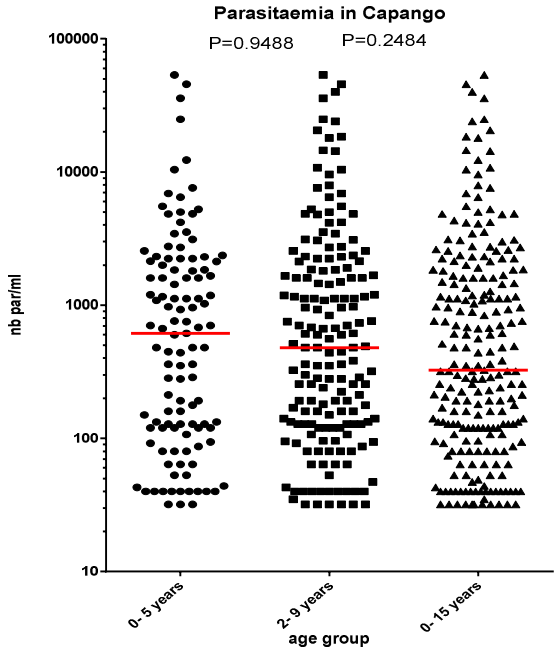

Fig. 3. Parasitic (and median) loads in the 3 age groups at Capango village

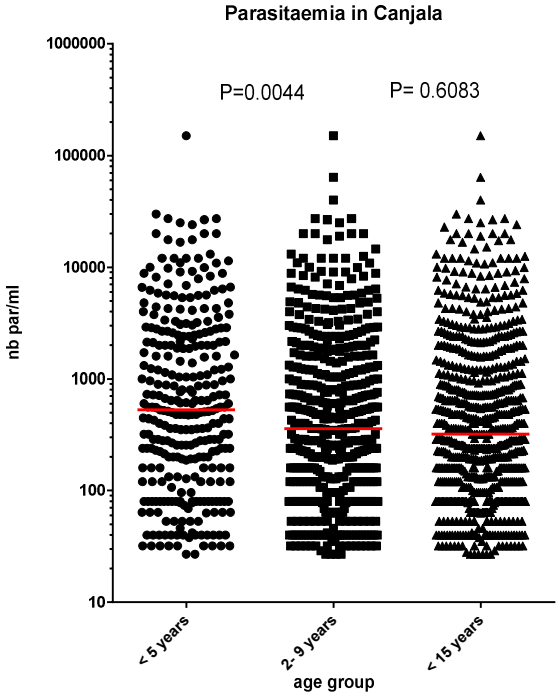

Fig. 4. Distribution of plasmodial parasitaemia according to Canjala age classes (each "point", round, square or triangle, represents a parasite load)

3.3.1 Plasmodic index

The following parasite index were observed in the 3 age groups: children <5 years: PI = 44.5% (n =695); children 2-9 years: PI = 52.9% (n = 1016) and children <15 years: PI = 50.2% (n = 1455) (Table 1).

These index varied according to age group: the PI of children <5 years was significantly lower than the PI of 2-9 years ($\chi2$ = 11.90; p <0.05, OR = 0.71 [0.58-0.86]) and of <15 years ($\chi2$ = 6.29; p =0.012, OR = 0.79 [0.66-0.95]); while the PI of children 2-9 years and <15 years were similar ($\chi2$ =1.76; p = 0.18, OR = 1.11 [0.95-1.31]).

3.3.2 Parasitic densities

Parasitaemia was significantly higher in <5 years than in 2-9 years (P = 0.004) and <15 years (P = 0.007), but comparable in 2-9 years and <15 years (P = 0.608) (Fig. 4).

3.3.3 Gametocytic index

The gametocytic indices are remarkably similar in the 3 age groups considered (Table 2), with a value of the order of 5%. One in 20 children is potentially "infective" for vectors.

It is worth underlining that in Canjala “U5” had Plasmodic Index significantly lower but parasitaemia significantly higher than “2-9” and “U15” while gametocyte index were similar. On the other hand these three parasitological parameters were comparable for the classical “2-9 years old” and “below 15 y.” age groups.

3.4 Candiero Village

During the 9 surveys conducted from August 2007 to December 2008, 1188 thick films were prepared from children <15 years in the field and their microscopical examination revealed the presence of *Plasmodium* in 388 blood slides i.e. a PI of 32.7% (Table 1) all were due to *P. falciparum*. The presence of sexual forms was noticed in 46 thick films i.e. a gametocytic index of 3.9%.

Such as in other villages, all these children were symptomless, although some thick films showed ten thousands of *P. falciparum* trophozoites / µl of blood.

3.4.1 Plasmodic index

The following Plasmodic Index (PI) were observed: children <5 years: PI = 28.6% (n = 630), children 2-9 years: PI = 33.6% (n = 792) and children <15 years: PI = 32.7% (n = 1188) (Table 1). These indices were different in the 3 age groups considered:

- the PI of children <5 years was significantly lower than that of children 2-9 years ($\chi2$ = 4.09; p =0.043, OR = 0.79 [0.63-0.99]) and similar to that children <15 years ($\chi2$ = 3.20; p = 0.073, OR = 0.82 [0.67-1.02]);
- the PI of children 2-9 years was similar to that of children under 15: $\chi2$ = 0.18; p = 0.67; OR = 1.04 [0.86- 1.26].

3.4.2 Parasitic densities

Medians of parasite densities were similar in the three age groups (Fig. 5): <5 years versus 2-9 years (P = 0.2782), <5 years versus < 15 years (P = 0.4370) and 2-9 years versus <15 years (P = 0.65).

3.4.3 Gametocytic index

Gametocytic index were statistically comparable in the 3 age groups: 5.24% (n = 630) for children <5 years, 4.17% (n = 792) for children 2-9 years and 3, 87% (n = 1188) for children <15 years old. About one in every 30 children is a "Plasmodium reservoir infecting anopheline vectors" in the village of Candiero, in the absence of any vector control measures.

Therefore in Candiero the three parasitological analyzed were similar in the three age groups except Plasmodic Index of U5 which appeared lower than PI of 2-9 years old and the age group” U 1” can thus be used for further analysis of parasitological situation in this village after vector control implementation.

Table 2. Gametocyte index according to the 3 age-classes in Canjala (Nb Y + = number of thick blood films where gametocytes* were observed)

Gamet./age	< 5 years	2-9 years	< 15 years
Nb Y*+	38	56	76
N	695	1016	1455
%	5,47	5,51	5,22

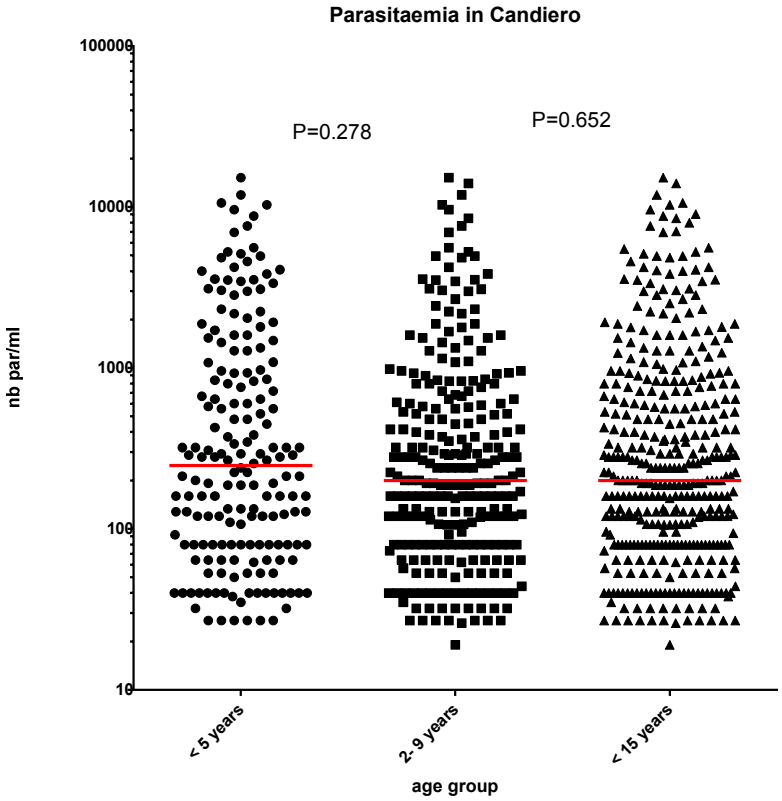

Fig. 5. Parasitic (and median) loads in Candiero / Parasitic age classes (and median) according to age-classes in Candiero

3.5 Libata Village

During the 9 surveys conducted between March 2007 and December 2008, 1333 thick films were made from children <15 and 626 were diagnosed positives, i.e. a PI of 47.0%.

Plasmodial infections were mainly due to *P. falciparum*, with 7 mixed *P. falciparum* + *P. malariae* infections and a single *P. malariae* only infection.

Gametocytes were observed in 54 blood slides, a gametocytic index of 4.1%.

3.5.1 Plasmodic index

For the 3 age groups, the parasite index were: children <5 years: PI = 45.9% (n = 749), children 2-9 years: PI = 51.7% (n = 940) and children < 15 years: IP = 47.0% (n = 1333) (Table 1).

Plasmodial prevalence differed by age group:

- The Plasmodial index of children <5 years was significantly lower than that of 2-9 years ($\chi 2$ = 5.59; p= 0.02, OR = 0.79 [0.65-0.96]), but comparable to <15 years ($\chi 2$ = 0.21; p = 0.65, OR = 0.96 [0.80-1.15]);
- The Plasmodial index of < 15 was significantly lower than the one of 2-9 years old ($\chi 2$ = 4.96; p =0.026, OR = 0.83 [0.69-0.98]).

3.5.2 Parasitic densities

Very high *P. falciparum* densities were noticed in symptomless children, for example in February 2008 it was noticed the following *P. falciparum* infections (Table 3) and such high parasitaemia in babies of few months old is of great concern.

Parasitic loads were comparable (Fig. 6) between <5 years versus 2-9 years (P = 0.139) and 2-9 years versus <15 years (P = 0.353), but significantly higher in <5 years versus < 15 years (P = 0.021).

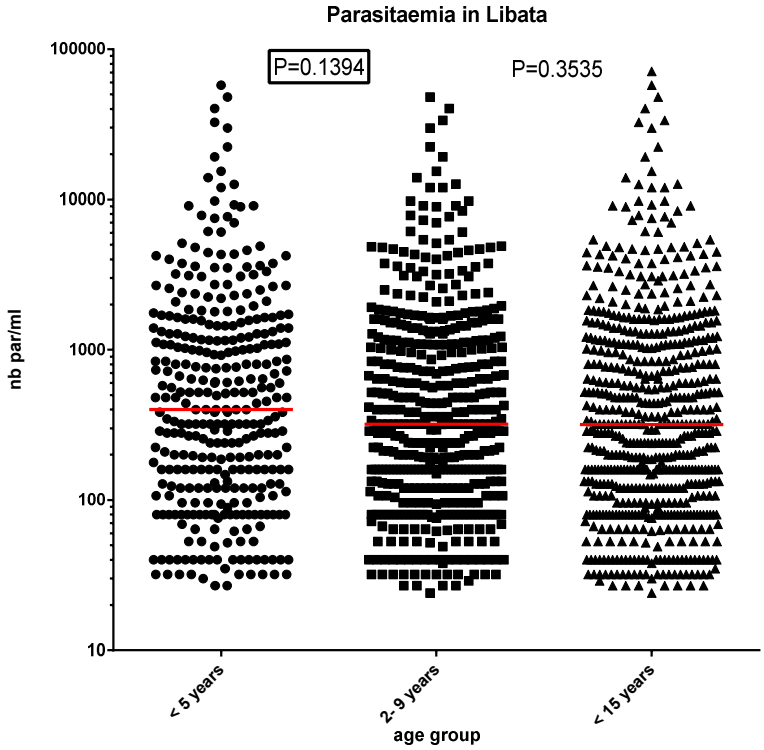

Fig. 6. Distribution of parasitaemia according to the age-classes in Libata

Table 3. Some *Plasmodium* Hyperinfections observed in Libata

Age	Parasitaemia/µl
7 years	104 000
4 months	71 000
3 months	58 000
3 years	48 000
6 years	34 000
3 years	19 000
5 years	12 000

3.5.3 Gametocytic index (GI)

Sexual forms of *P. falciparum* were observed in 54 of 1333 blood smears, i.e. a gametocytic index of 4.1% with values which look variable according to age group: <5 years: IG = 5.34% (n = 749), 2-9 years: IG = 4.15% (n = 940) and <15 years: IG = 4.05% (n = 1333).

In fact GIs for children <5 years were comparable to 2-9 years ($\chi 2 = 1.37$; p = 0.24, OR = 1.30 [0.83-2.06]) and <15 years ($\chi 2 = 1.90$; p = 0.17, OR = 1.34 [0.88- 2.04]) while GIs in the age groups 2-9 and <15 years were also similar ($\chi 2 = 0.0135$; p = 0.91, OR = 1.02 [0.67-1.56]).

It is interesting to notice that in Libata Plasmodic Index in U5 and U 15 were similar still considering that parasitaemia were higher in younger age group; gametocyte index had the same level in the three age groups analyzed.

4. CONCLUSION

Parasitological surveys are a crucial need to evaluate the effectiveness of vector control operations along with the classical entomological, immunological, morbidity and mortality index [9].

Actually the lack of such base line data induced the failure of the recent IRS based vector control program in Angola [1].

On the other hand the lack of reliability of "malaria" diagnosis in several health center of Lobito and surrounding area was recently underlined [10,11] and it clearly appeared that their data cannot be used to evaluate the evolution of malaria following vector control implementation.

As early as 1941, [12] Viswanathan underlined "the utility of malaria parasite indices in Infants in the study of malaria".

Parasitological studies have recently been conducted in The Gambia [13] with age-stratified cross- sectional surveys. In Cameroon, in Bamiléké area, [14] it was observed that the parasite prevalence and parasite density decreased with age with a peak in the 0-5 age group in some villages (low area), but in altitude with low transmission, both prevalence and parasite density were similar. The importance of altitude has also recently been highlighted in Tanzania [15].

Higher prevalence among young children has also been noted in Kenya [16], Tanzania [17,18] and many other epidemiological facies.

Several parasitological parameters can be used, prevalence, incidence, parasite density, gametocytic index depending on the objectives of the study and available resources. Prevalence in children 2-9 years old was often used to classify the different degrees of endemicity of the study area (hypo-meso- hyper-holoendemic) [19]. [2] However, it varies over time (rainy season, dry season), and space (more or less close to larval breeding place), etc. Hence the importance of repeated regular surveys to monitor these natural seasonal changes and environment.

"Rapid Detection Tests" (RDT) have been developed [20] and recommended for malaria diagnosis before any antimalaria treatment [21]. Several tests have been deeply studied and compared, demonstrating their usefulness but also some of their limits particularly for the evaluation of the parasite density; which is a "key indicator", at the population level, to reflect the risks of malaria morbidity.

The problem of parasite *P. falciparum* densities has been raised for long time [22]. The highest density appeared to have been observed by Springall 1943 [23] who reported a case with 44.2% of red blood cells containing one (or more) trophozoites. Earle et al. [24] counted up to 206,000 parasites / µl of peripheral blood.

A *P. falciparum* parasitaemia of 1,200,000/µl was noticed in a young symptomless child ("BM") during routine surveys conducted formerly in Djoumouna village (fish-breeding area 25 km west of Brazzaville (Carnevale, unpub.obs.).

For Rogier et al. [25], the "pyrogenic density" varies according to the age in endemic zone and this notion of threshold must be considered in "epidemiological" term and not at individual level to induce, or not, an antimalarial treatment.

For malariological surveys prior to vector control operations, it is crucial to precisely know the actual current situation and to select the most relevant indicators for the further evaluation of its efficacy.

This was the objectives of these parasitological surveys in Balombo villages where Vector Control with various methods (classical and new) was scheduled for December 2008.

Regularly made field cross sectional surveys on randomly selected children showed that around 40-50% of children harbored *P. falciparum* infections (even at really high level) without obvious anyclinical symptoms and the classical parasitological variations in time and space, from village to village and from year to year were clearly noticed. They confirmed that one village cannot be considered as "control" for the plasmodial evolution in another village and that each village was its own "control".

It is important to consider such high level of plasmodial infections which prove that transmission, due to *An. funestus* in these villages, occurred permanently, even at this altitude (1200 m) and the lack of vector protection in these villages at the time of the study.

Statistical analysis of 4625 blood slide microscopical examination and three classical parasitological parameters showed that the age groups <15 years or 2-9 year old reliably reflect the situation in these villages and they could be considered for the evolution of parasitological changes induced by vector control implementation. The U15 has the advantage of being larger and thus could "catch" even a low impact of vector control when using new tools.

It also appeared that about one child out of 20 was carrying parasite loads above a threshold often considered critical while one in 25 children was potentially infective for *Anopheles* vectors and this obviously participate to such permanent malaria transmission.

The methodologies developed during these first parasitological field surveys and the information gained will be of great importance to correctly evaluate the scheduled VC operations and to

avoid the issues already highlighted for the vector control programme in Angola [1].

CONSENT

Surveys were conducted after and with the chief of each village and with the participation of the "activista" (health care) of each village. Inhabitants participate actively having free blood examination and drugs if needed according to the policy of the National malaria Control Programme.

ETHICAL APPROVAL

The study was conducted at the request and with the Head of the National Malaria Control Programme of the Ministry of Health.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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