

Parasitological surveys on malaria in rural Balombo (Angola) in 2007-2008: base line data for a malaria vector control project

ABSTRACT

Study design: Field surveys were carried out in 4 remote villages far enough from each other to avoid the possibility of active flights of the mosquitoes and "contamination" of treated or control villages.

Methodology: Cross sectional surveys were done on randomly selected symptomless children <15 years. During data analysis this sample was stratified into 3 conventional age groups: <5 years (often considered as "at risk group"); 2-9 years (used for classical endemicity index) and <15 years already used in other vector control studies, to compare the 3 classical indicators: plasmodial prevalence, parasite load, gametocyte prevalence. Blood thick films made 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 prepared during the 38 regularly done field surveys. *Plasmodium falciparum* was predominant with few *P. falciparum* + *P. malariae* and a single *P. malariae* infections. 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 pertinent indicators to increase sample size and to get even small variations induced by vector control. Variability of parasitological index according to age groups, villages, season, confirmed the importance of regularly done cross-sectional surveys to know the situation before the implementation of control operations for reliable evaluation.

Keywords: Malaria, Plasmodium, Parasite density, Plasmodic index, Gametocytic index, Prevalence, Angola, Africa

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 effectiveness 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 indoor 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]. The authors of the article 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 Sheetting (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 done 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 by active flight.

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 and mapping.

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% ($\pm$ 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 done according to the classical cross-sectional surveys (CSS) of randomized samples. For parasitological evaluation of VC the issue of the choice of the best "age group indicator" has to be solved and therefore plasmodial infestations were analyzed in the 3 classical age groups:

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

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

The blood thick films done 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 done in 2 Capango and Canjala only, but from August 2007 to December 2008 the 4 villages were regularly surveyed every 2 months. None 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 made on 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 general plasmodial prevalence of # 50% used for sampling size was right. Gametocytes were observed in 205 thick drops, i.e. a general 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), demonstrating the importance of repeated surveys.

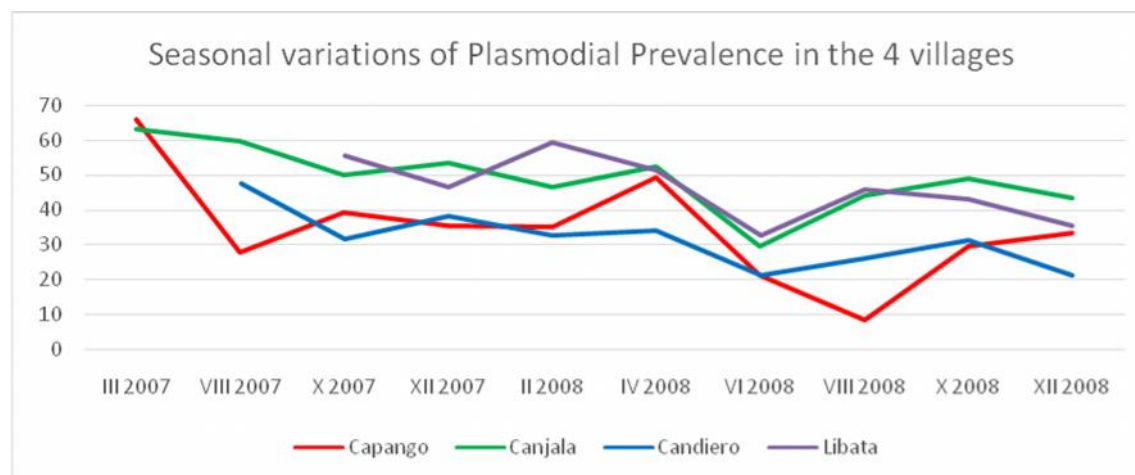


Fig. 1. Seasonal variations of Plasmodial prevalence in children below 15 years (U 15) in the 4 studied villages.

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 less in other 3 villages.

Yearly Plasmodic prevalence changed according to villages and age groups (Table 1); 2 villages, Canjala and Libata, appeared having Plasmodic Index higher than the 2 others but in each village PI appeared similar in the 3 age groups considered (Fig. 2).

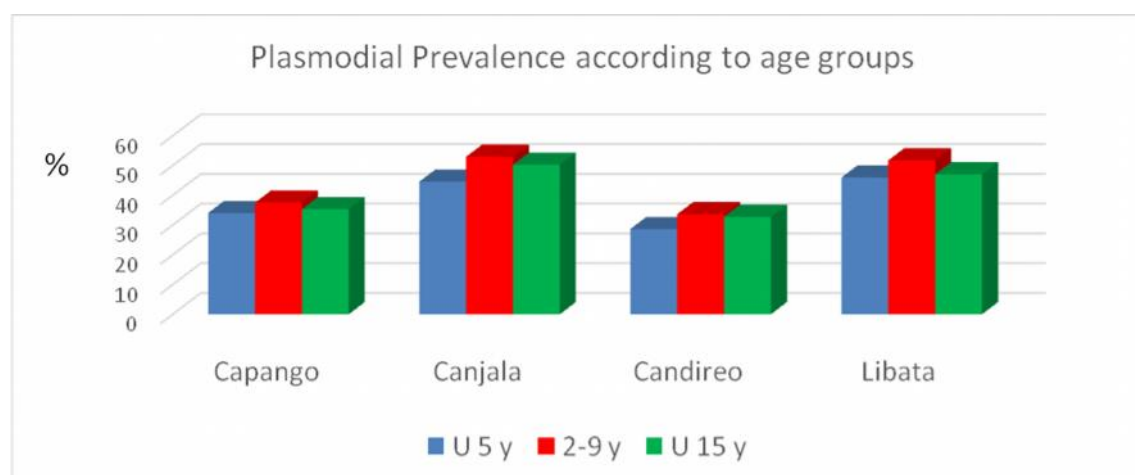


Fig. 2. Plasmodial prevalence according to villages and age groups

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)

3.2. Capango village

3.2.1. Plasmodic index

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

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 tens of thousands of *P. falciparum* trophozoites / μ l 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).

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 comparable parasitaemia 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.

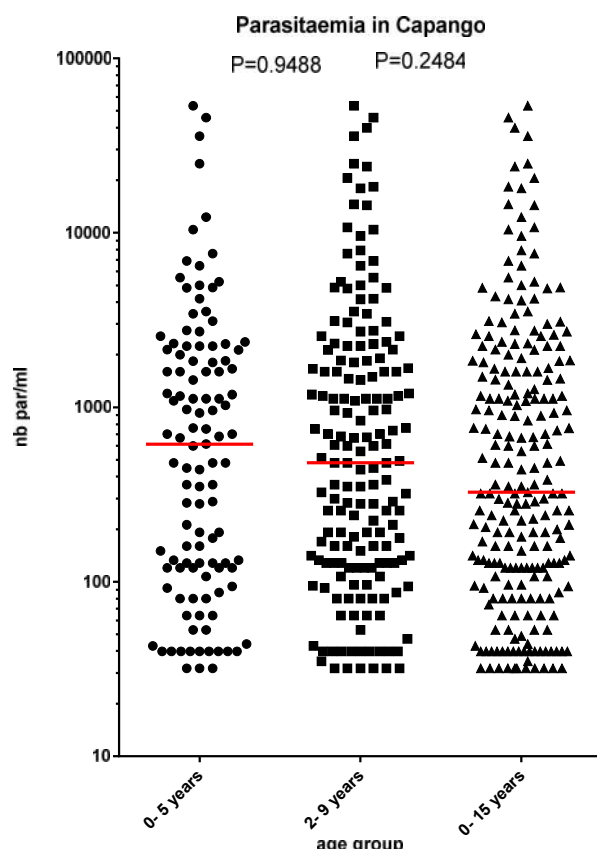


Fig. 3. Parasitic (and median) loads in the 3 age groups at Capango village

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".

3.3. Canjala village

During the 10 surveys carried out between March 2007 and December 2008, 1455 thick films involving children <15 years were examined and the presence of *Plasmodium* trophozoites was observed in 731 slides, i.e. a parasite rate of 50.2%, (Table 1); predominantly *P. falciparum*. Only 11 mixed *P. falciparum* + *P. malaria* infections and only 3 *P. malariae* infections (children aged 2, 7 and 10) were determined.

Gametocytes were observed in 76 thick drops, a gametocytic index of 5.2%.

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 vary by age group: the PI of children <5 years is significantly lower than that of 2-9 years ($\chi^2 = 11.90$; $p < 0.05$, OR = 0.71 [0.58-0.86]) and of <15 years ($\chi^2 = 6.29$; $p = 0.012$, OR = 0.79 [0.66-0.95]); while the PI of children 2-9 years and <15 years are comparable ($\chi^2 = 1.76$; $p = 0.18$, 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).

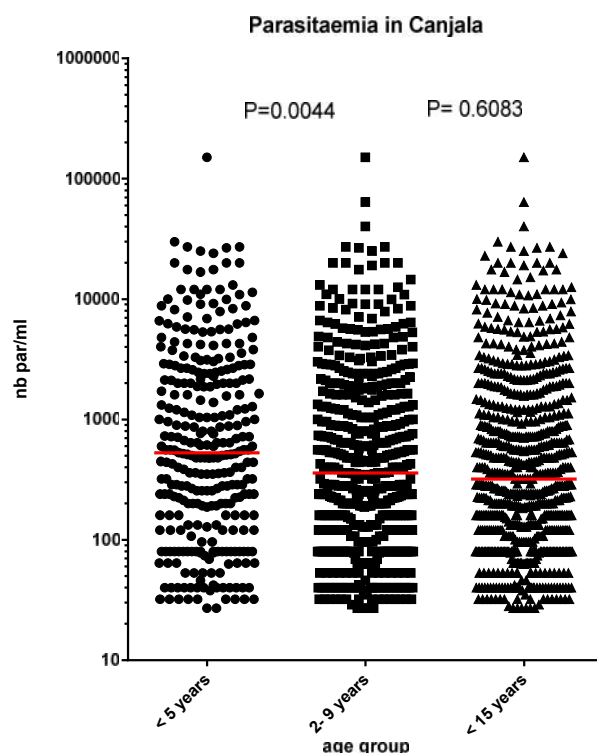


Fig. 4. Distribution of plasmodial parasitaemia according to Canjala age classes (each "point", round, square or triangle, represents a parasite load).

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.

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

3.4. Candiero village

During the 9 surveys conducted from August 2007 to December 2008, 1188 thick films were done in children <15 years with *Plasmodium* observed in 388 slides i.e.a PI of 32.7% (Table 1) all due to *P.falciparum*. The presence of sexual forms was noticed in 46 samples, a gametocytic index of 3.9%. As in the other villages, all these children were symptomless, although some thick films showed tens of thousands of *P.falciparum* trophozoites / μ l 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 are not similar in the 3 age groups considered:

- the PI of children <5 years is significantly lower than that of children 2-9 years ($\chi^2 = 4.09$; $p = 0.043$, OR = 0.79 [0.63-0.99]) and similar to that children <15 years ($\chi^2 = 3.20$; $p = 0.073$, OR = 0.82 [0.67-1.02]);

- the PI of children 2-9 years is similar to that of children under 15: $\chi^2 = 0.18$; $p = 0.67$; OR = 1.04 [0.86- 1.26].

3.4.2. Parasitic densities

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

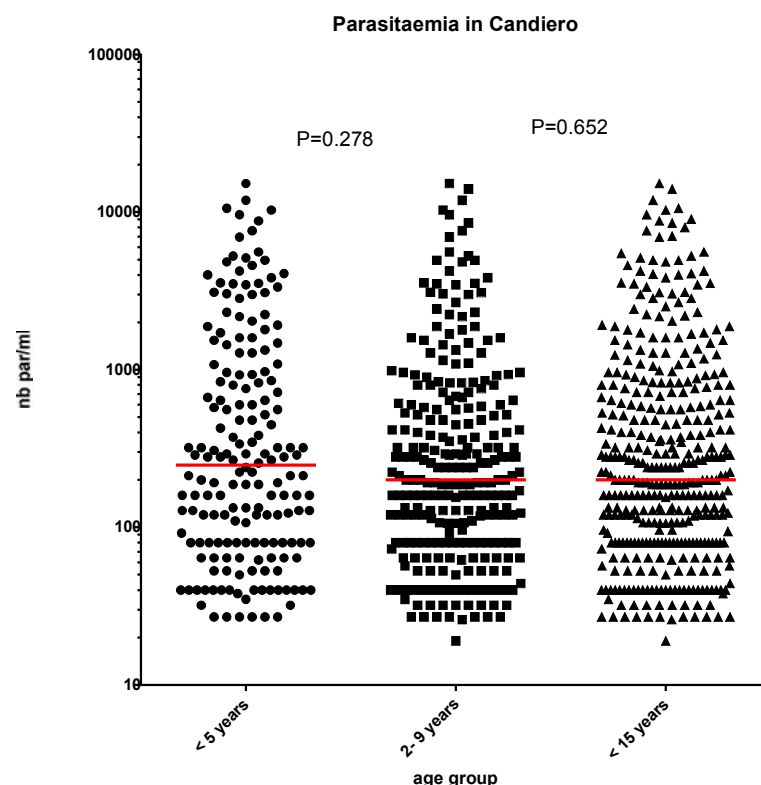


Fig 5. Parasitic (and median) loads in Candiero / Parasitic age classes (and median) according to age-classes in Candiero

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.

3.5. Libata village

During the 9 surveys done between March 2007 and December 2008, 1333 thick films were made with children <15 with 626 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 samples, a gametocytic index of 4.1%.

3.5.1. Plasmodic index

For the 3 age groups, the parasite indices 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.

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

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 ($P = 0.021$).

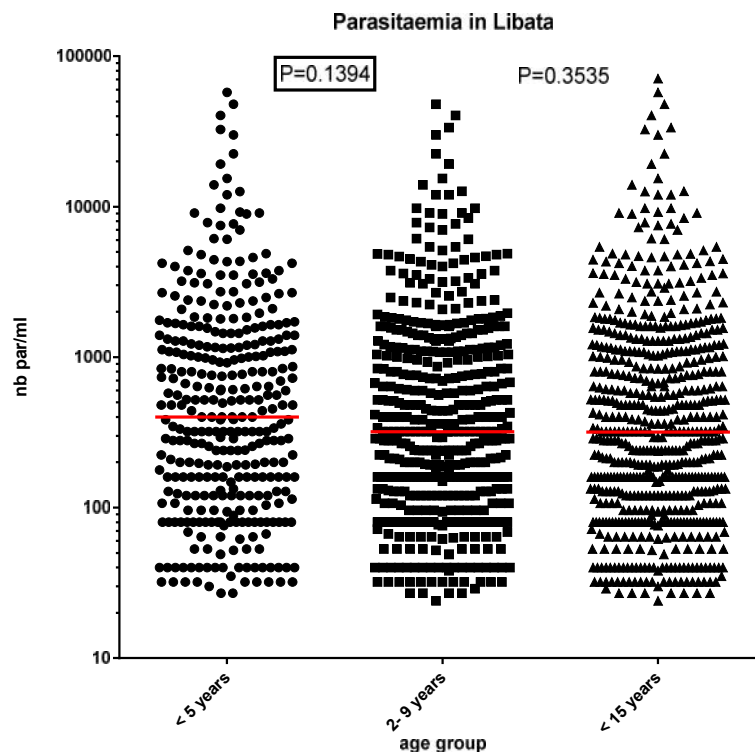


Fig 6. Distribution of parasitaemia according to the age-classes in Libata

3.5.3. Gametocytic index (GI)

Sexual forms of *P. falciparum* were observed in 54 of 1333 blood smears, a gametocytic index of 4.1% with variable values 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).
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]).

4. CONCLUSION

Parasitological surveys are a crucial need to evaluate the effectiveness of vector control operations. as well as entomological, immunological, morbidity and mortality indices [9]).

Actually the lack of 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, as such, cannot be used to evaluate the current malaria situation and its evolution 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 decrease 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 are 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 indices can be taken into consideration, prevalence, incidence, parasite density, gametocytic index depending on the objectives of the study and available resources. Prevalence was 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, according to more or less close larval sites, within the population studied (children, adolescents, adults), etc. Hence the importance of repeated regular surveys to monitor these seasonal changes and with age and environment.

"Rapid tests of detection" (RDT) have been developed [20] with the recommendation to use them systematically for malaria diagnosis prior to the antimalaria treatment [21]. Numerous tests have thus been studied, demonstrating their usefulness but also some of their limits particularly for the evaluation of parasite density; which is a "key indicator", at the population level, to reflect the risks of malaria morbidity.

The problem of parasitic *P. falciparum* densities has been raised for long time [22]. The highest density appears 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., (1939) [24] counted up to 206,000 parasites / μ l of peripheral blood.

A *P. falciparum* parasitaemia of 1,200,000/ μ l was noticed in a young symptomless child ("BM") during routine surveys in Djoumouna (fish-breeding area 25 km west of Brazzaville (Carnevale, unpub.obs.) For Rogier et al.(1996) [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.

In the case of malariological surveys as a prelude to vector control operations, it is important to precisely know the actual situations and to select relevant indicators for the evaluation of its efficacy.

This was the objectives of these parasitological surveys in Balombo villages where Vector Control with various methods was scheduled. Regularly done cross sectional surveys showed the classical parasitological variations in time and space, from village to village and from year to year, confirming that one village cannot be considered as "control" of the plasmodial evolution in another village and that each one was its own "control".

It is important to note the presence of plasmodial infections in all young children, even at a few months old, indicating the relatively high and permanent transmission even at this altitude and the lack of vector protection in these villages at the time of the study.

Examination of the data shows that the age group <15 years could be selected, as well as the classical 2-9 year old group, that almost 50% of children are symptomless carriers of *P. falciparum*

and 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. The methodologies developed during these parasitological surveys and the information obtained will be used, and will be useful to evaluate the scheduled VC operations implemented to compare classical tools (IRS, LLIN) and newly developed one (Insecticide Treated Plastic Sheetting ITPS) avoiding thus the errors already highlighted for the vector control programme in Angola [1].

CONSENT

Surveys were done 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 done at the request and with the Head of the National Malaria Control Programme of the Ministry of Health.

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