Plasmodial prevalence with respect to gender in 6 villages around Balombo town, Benguela Province, Angola, before malaria vector control implementation.

Abstract

Background. The different susceptibility of gender to infectious diseases is still a matter of concern and several studies were devoted to these issues; for malaria, recent studies reported a comparable "Force of Infection" in both gender while women could have a faster elimination of parasite than men.

Study area and duration period of the research. The study was done in villages around Balombo town, Benguela Municipality, Angola and initially scheduled for 5 years since year 2007 with 2 years before vector control implementation and 3 years after.

Objectives of the research. The village scale malaria vector control program was planned and implemented to compare 4 methods of vector control with comprehensive entomological and parasitological evaluation, focusing on symptomless randomly selected patients, and noticing age and gender during each field survey for further analysis.

Study design. To check an eventual gender difference, we decided to analyze the parasitological data gained during the 56 cross sectional surveys (CSS) done during 2 consecutive years in the 6 villages used as "control" i.e. before implementation of vector control operations (VCO) considering the gender of symptomless patients (≤15 years old) surveyed. We considered 2 indicators: Plasmodial Prevalence (PP) and Parasite Load (PL).

Main results. 6727 thick blood smears (TBS) were prepared during these 56 CSS, and microscopically examined; the sex ratio of this total sample was well balanced with 3406 TBS (=50.6%) from men ("M") and 3321 TBS (49.4%) from women (W"). The overall Plasmodial prevalence (all *Plasmodium* sp gathered because *P. falciparum* infections, as already noticed, are largely preponderant) were similar 41.02% for M and 40.1% for W.

The overall plasmodial prevalence significantly decreased from Year 2007 to Year 2008: respectively 47.8% (n= 2686) and 35.7% (n= 4041); but the plasmodial prevalence were similar each year between gender: in year 2007: PP= 48.9% (n=1338) for M and 46.7% (n= 1348) for W and in year 2008: 35.9% (n=2068) for Men and 35.6% (n=1973) for Women.

In Year 2007 the PP of Men and Women were similar in each village; in Year 2008 the PP were similar in 5 villages, but in Libata PP was significantly higher in Men than in Women.

The trends of change from year to year appeared similar for gender excepted in Capango where the PP remained similar in W and significantly decreased for M.

In term of Parasite Load the comparison of median of parasitaemia with the non-parametric Mann-Whitney test showed similar distribution between gender each year in each village and their evolution from year to year appeared also similar in each gender excepted for Men in Libata.

Conclusion. These possibilities of changes of plasmodial infections according to gender with time and villages must be taken into consideration when planning, implementing and analyzing parasitological data of symptomless carriers before a vector control program, and after which must be implemented on a long term basis. They show that an "only one" CSS could lead to wrong information and

conclusion of malaria and gender and the recommendation to carry on longitudinal regular field studies for relevant conclusion on malaria infections among communities.

The next step will be to analyze the plasmodial infection in both gender after vector control implementation.

Key word: Plasmodium, malaria, sexual dimorphism, vector control

I. Introduction

According to World malaria report 2021 of WHO « In 2020, there were an estimated 241 million cases of malaria worldwide. The estimated number of malaria deaths stood at 627 000 in 2020. The WHO African Region carries a disproportionately high share of the global malaria burden. In 2020, the region was home to 95% of malaria cases and 96% of malaria deaths".

Still according to WHO "Angola is among the ten countries with the highest number of malaria and deaths (3% of the global cases and death. Between 2016 and 2019 there was a 14.4% increase in malaria cases, from 205 to 235 cases per 1000 of the at risk population estimated at 32 866 268 in year 2020. The same year it was reported 2336 deaths (estimated 15 989) and "presumed and confirmed cases": 7 156 110 (estimated cases: 8 268 572).

With the National Malaria Control Program a long term village scale malaria vector control program was implemented since 2007 in several villages around Balombo town (Angola) to compare the efficacy of the classical Long Lasting Insecticide treated Nets ("LLIN") and Indoor Residual Spraying ("IRS") with the newly developped Insecticide Treated Plastic Sheeting ("ITPS", also called Durable Lining "DL") on the walls inside human house [1, 2]. Former parasitological surveys done in this area showed high Plasmodic Index [3, 4] inducing the needs for malaria control including vector control operations. But due to the failure of the Inside Residual spraying project in another Angola Province [5] or other countries [6], other methods had to be implemented such as LLIN, scheduled for large scale distribution at the National level and the ITPS which were proven to be efficient [7, 8] and well accepted in Huambo, a close town [9, 10]. A comprehensive evaluation of this "Balombo Project" was planned, and implemented, including entomology, parasitology and immunology based on the evaluation of the human antibodies biomarkers, such as the gSG6-P1 peptide of *Anopheles* salivary, already used for another evaluation of vector control in Lobito town [11, 12].

On the other hand the eventual different susceptibility to infectious diseases according to gender deserved special attentions and studies [13-25].

As it was recently well underlined by Briggs et al [26] "multiple studies have reported a male bias in incidence and/or prevalence of malaria infection in males compared to females" with "the hypothesis that sex-based differences in host parasite interactions" which could " affect the epidemiology of malaria". They intensively followed *Plasmodium falciparum* infections in a cohort in a malaria endemic area of eastern Uganda and estimated both force of infection (FOI) and rate of clearance and found "no evidence of differences in behavioral risk factors, incidence of malaria, or FOI by sex. In contrast, females cleared asymptomatic infections at a faster rate than males in multivariate models adjusted for age, timing of infection onset, and parasite density. These findings implicate biological sex-based differences as an important factor in the host response to this globally important pathogen".

It was also already reported from Congo that main vectors such as *Anopheles gambiae* or *An.nili* bite men as well as women and boys as well as girls [27]. Therefore it could seem that neither the human behaviour nor the mosquito behaviour could be relevant parameter to explain the parasitological difference, if any, according to gender of *Plasmodium* symptomless carriers.

Zhong et al [28] used a novel method to evaluate *Plasmodium* MOI (Multiplicity of Infection) and molecular epidemiological patterns in symptomatic and asymptomatic *Plasmodium vivax* samples collected from health centers/hospitals and schools, in Ethiopia. Similarly, both symptomatic and asymptomatic *Plasmodium falciparum* samples were collected, in Kenya. They reported that "in this study, no significant difference was found in *P. vivax* MOI between the symptomatic and asymptomatic infections, adults and children, as well as between male and female groups" and "the complexity of infections were similar among age groups, symptoms, genders, transmission settings (spatial heterogeneity), as well as over years (pre- vs. post-scale-up interventions)".

Therefore we decided to analyze the plasmodial prevalence and parasite load in the symptomless randomized samples of children (≤15 years old) men and women in the 6 control villages of the Balombo Project during 2 consecutive years before the implementation of planned vector control. In a second step, we will analyze the same indicators the 3 years following vector control operations. The situation of Plasmodial prevalence in the 2 other villages (Caala and Cahata) where LLIN were distributed in February 2007 is presented in another document [29].

II. Materials and Methods

II-1. The study area

The localization and description of the area and the demographic situation were already presented [1, 4].

Balombo (12°21'S; 14°46'E; # 1200 m altitude), 150 km east of Lobito town and 600 km south-east of Luanda the capital of Angola, is a town and municipality in Benguela Province of Angola on the way to Huambo. The municipality had a population of some 110.000 people and the town itself of about 40.000 inhabitants in 2014 but greatly increasing these last years with the improvement of the road.

Limits to North with the municipality of Cassongue, to the east with the municipality of Londuimbali, to the south with the municipalities of Ukuma, Tchinjenje and Ganda and to the west with the municipality of Bocoio.

It is a humid mountainous region with former volcanoes and a hot spring so that Balombo was a spa town before the long term civil war, the original forest was greatly degraded for cultivation (corn, cassavas etc) and a permanent river flows at the entrance of the village. The weather is characterized by a long rainy season (October to May) and a short dry season (June to September).

II-2. The study design.

A long term vector control project was implemented since 2007 in eight villages selected by the National Malaria Control Program (NMCP) around the Balombo town.
4 methods of vector control (VC) were implemented: classical Long Lasting Insecticide deltamethrin treated Nets ("δLLIN") PermaNet© 2.0 in 2 villages (Caala and Cahata) distributed since the beginning of the trial (February 2007); association δLLIN + deltamethrin Insecticide treated plastic sheeting ("δITPS") in 2 villages (Capango and Canjala); δITPS alone in 2 villages (Chisséquélé and Barragem) and lambdacyhalothrin Indoor Residual Spraying ("λIRS") in 2 villages (Candiero and Libata) with 2 rounds followed by δITPS.
The first 2 years (2007-2008) 6 villages were used as "control" to get base line data before Vector Control ("VC") implementation and full VC was done in December 2008 with a first step of 3 years of regular follow up of entomological, parasitological and immunological indicators [1].
Parasitological evaluation was based upon regularly done cross sectional surveys ("CSS") following the protocol already developped in Côte d'Ivoire for the evaluation of lambdacyhalothrin treated nets in areas where *An.gambiae* population was resistant to pyrethroids with a high kdr level [30].
A previous parasitological survey was done in 4 villages (Capango, Canjala, Candiero and Libata) to compare the *Plasmodium* prevalence in 3 main age-groups: < 5 years (= classical "at risk" group); 2-9 years old (used for malaria classification as meso-hyper endemic etc.) and ≤ 15 years old to increase the size of the samples and the power of statistical analyses. It appeared that Plasmodial prevalence were similar in these three age group and it was concluded that under 15 years age class could be a relevant indicator to evaluate the efficacy of a vector control programme [4].

It was decided to focus the study of malaria situation on this age group ≤ 15 years old following Global Burden of Disease Study [31] which decompose probabilities of death from birth to exact age 15 years, from exact age 15 years to exact age 50 years, and from exact age 50 years to exact age 75 years.

The prevalence of malaria infection in two to 14 year-olds was also used for the evaluation of the risks of malaria after protection by combined use of nets and indoor residual spraying on Bioko Islands [32] to track the progress of malaria control in this island, over a 13 years period of intensive interventions. Malaria infection and hemoglobin were measured annually in children (1 to 14 years) in cross-sectional household surveys from 2004 to 2016 and the impact of the vector control interventions (net use and spray coverage) was thus evaluated [33].

During the Balombo studies the CSS were implemented on randomized samples of the population based on the numbered of houses for their localization by GPS mapping looking for any eventual cluster of malaria cases or equal distribution in the village [34]. Classical thick blood smears(TBS) were done directly in the villages, and Giemsa colored in Balombo, then microscopically examined in Lobito, in the Medical Department of the Private Angolese Company Sonamet© which supported this project through its "Malaria Control Program". *Plasmodium* species were determined and parasites counted against 200 leucokytes for the evaluation of the density/ml of blood assuming 8000 White Blood Cells/ml. For quality control 10% of these TBS were double checked in Yaoundé. During the field surveys first name, name, age and gender of each patient were collected.

Comparison of percentages was done with the classical Chi^2 test (EpiInfo 7.2) and distribution of parasitaemia were analyzed by the non-parametric Mann-Whitney tests to identify and compare the median.

III. Result

56 cross sectional surveys (CSS) were regularly done in these 6 villages during the first 2 years of the Balombo project.

6727 thick blood smears (TBS) were prepared from symptomless patients and 2730 (= 40.6%) were microscopically diagnosed positive with *Plasmodium*. (table 1a and 1b).We do consider here all *Plasmodium* species as it was already observed that *P.falciparum* is, by far, the most abundant species, with few *P.malariae* alone and some mixed infections [4].

This whole sample was composed of 3406 TBS done with from men (= 50.6%) and 3321 from women (= 49.4%) a well-balanced sex-ratio.
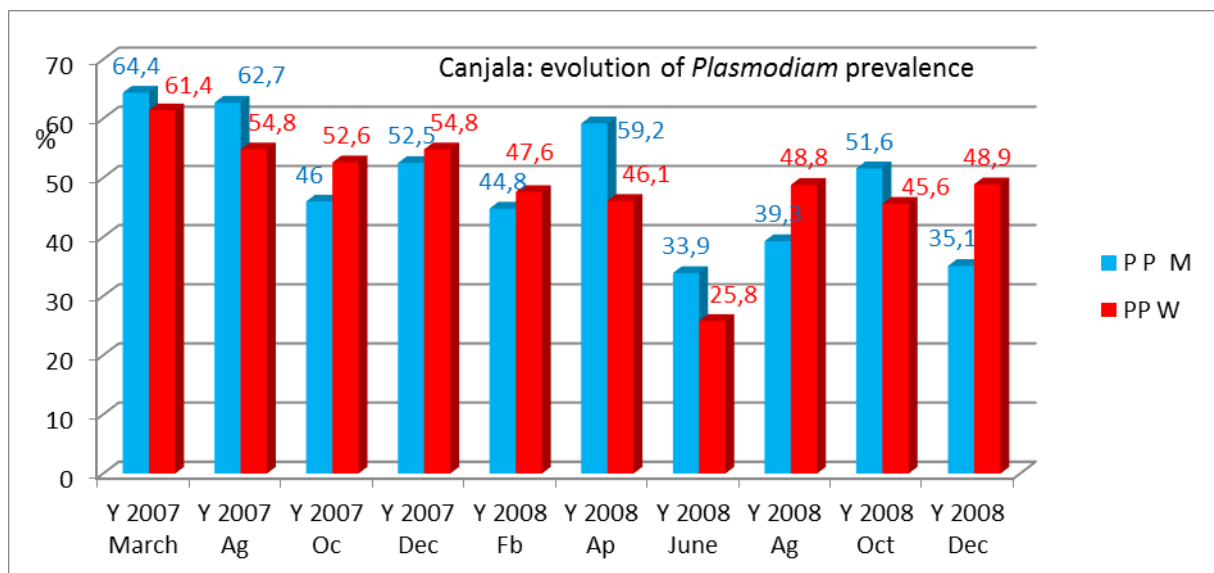
III-1. Plasmodial prevalence

The overall Plasmodial prevalence (PP) was remarkably similar according to gender: for men PP = 41.02% (n= 3406) and for women PP= 40.14% (n=3321) (χ2= 0.54; P value= 0.46; OR= 1.04 [0.94-1.14]).

III-1-1. Evolution according to surveys

The data of each survey for each village during these 2 years are presented in graph 1a, 1b, 1c, 1d, 1e and 1f where the classical seasonal variations with the drop during the dry season is cleared noticeable. The general level of Plasmodial prevalence are different according to villages but the level remained similar between genders.
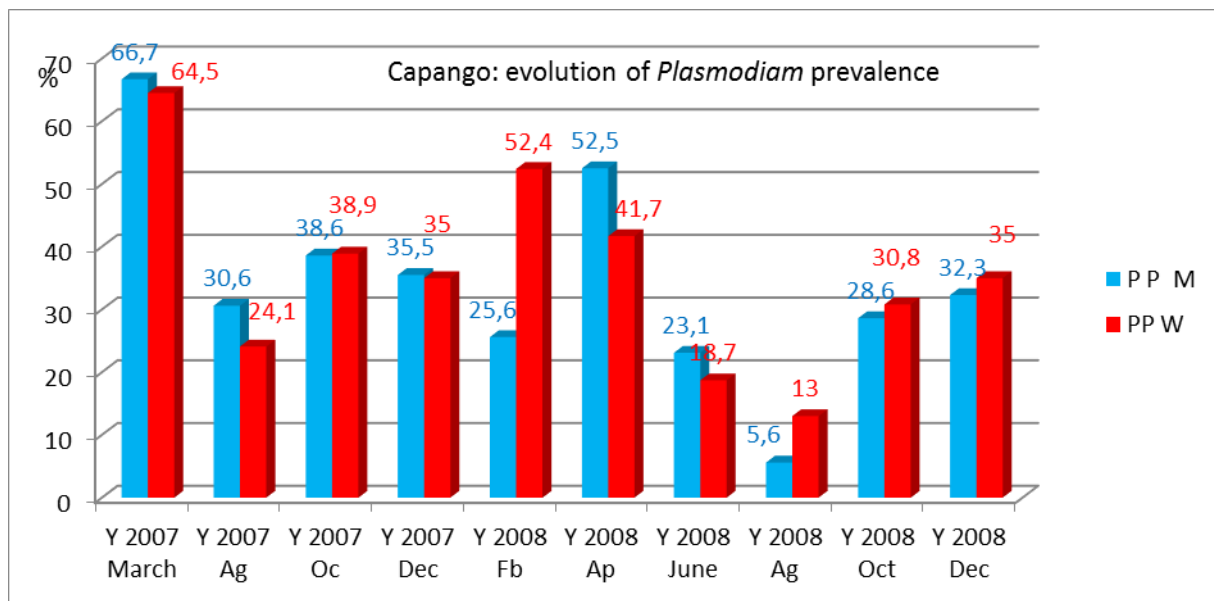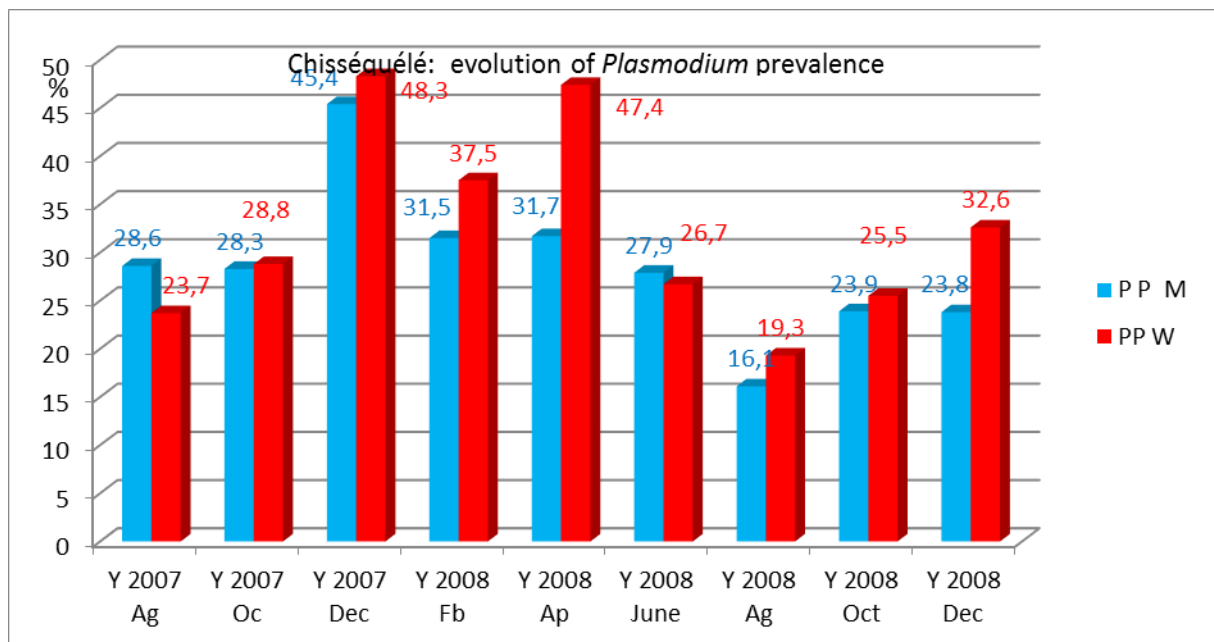
Graph. 1a. Evolution of Plasmodial prevalence in Capango (PP M= plasmodial prevalence in Men; PP W= plasmodial prevalence in Women) in each of the 10 CSS done in 2007 and 2008.
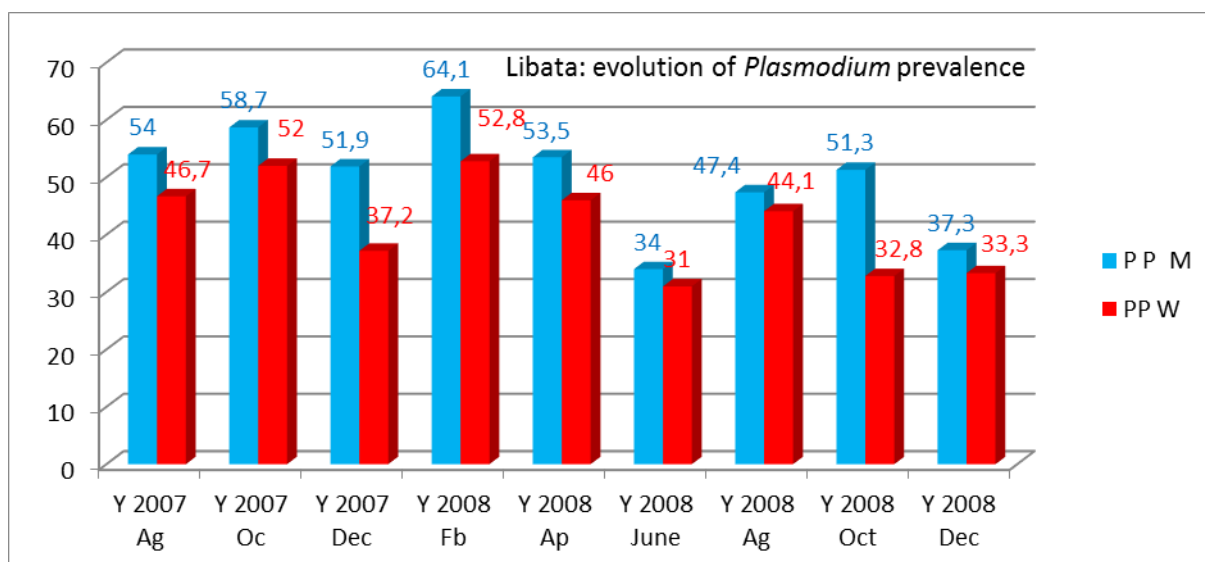

Graph. 1b. Evolution of Plasmodial prevalence in Canjala in each of the 10 CSS done in 2007 and 2008.
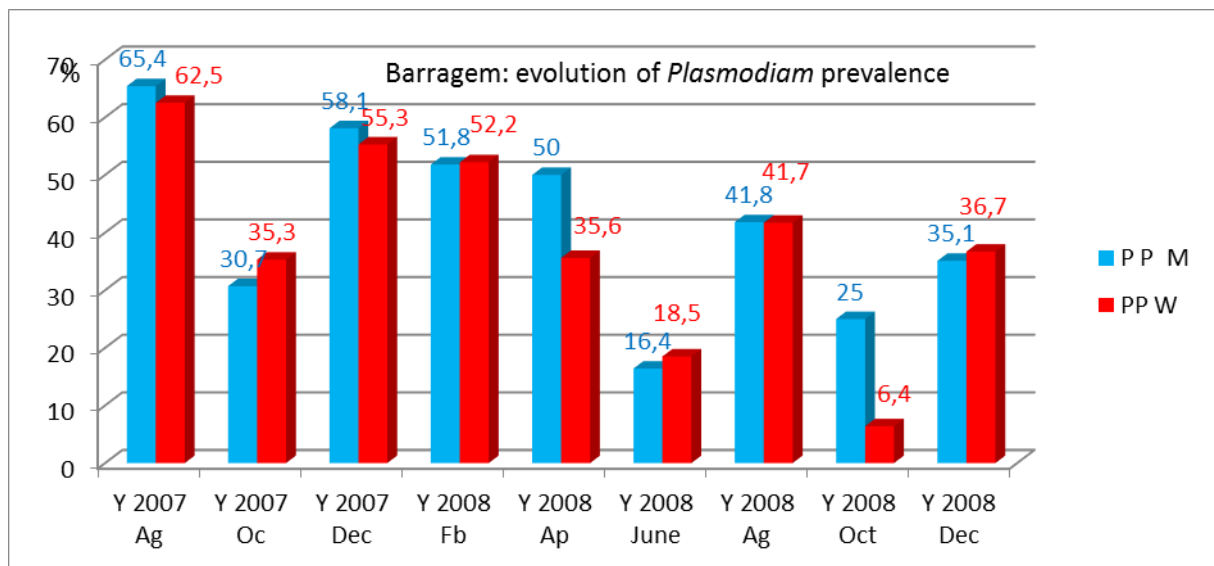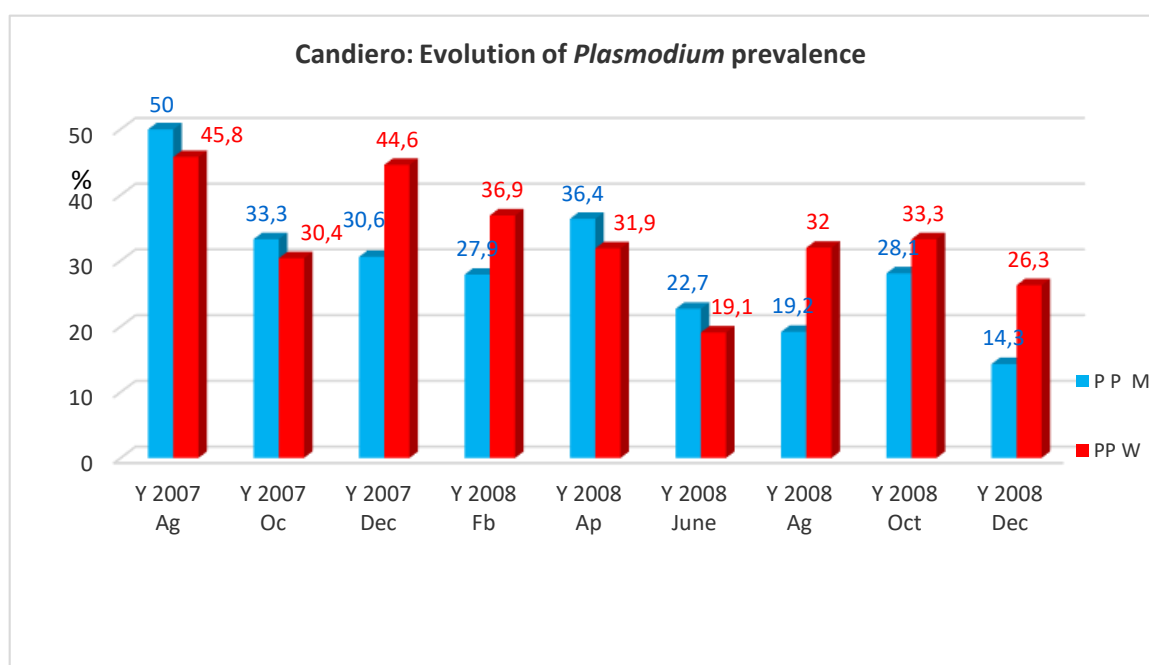
Graph. 1c. Evolution of Plasmodial prevalence in Barragem in each of the 9 CSS done in 2007 and 2008.

Graph. 1d. Evolution of Plasmodial prevalence in Chisséquélé in each of the 9 CSS surveys done in 2007 and 2008.

Graph. 1e. Evolution of Plasmodial prevalence in Libata in each of the 9 CSS done in 2007 and 2008.

Graph. 1f. Evolution of Plasmodial prevalence in Candiero in the 9 CSS done in 2007 and 2008.

III-1.2. Comparison according to years

The first year *Plasmodium* were observed in 1285 of the 2686 TBS (=47.8%) and the second year *Plasmodium* were noticed in 1445 of the 4041 TBS prepared (=35.7%) a significant drop ($\chi2= 97.7$; OR= 0.61 [0.55-0.67]) while no organized vector control operations were implemented.

Nevertheless the overall plasmodial prevalence remained similar according to gender: 41.0% (n=3406) for men and 40.1% (n= 3321) for women ($\chi2= 0.54$; P value= 0.46; OR = 1.04 [0.94-1.14]).

The first year *Plasmodium* prevalence were similar in men and women being diagnosed in 655 of the 1338 TBS dealing with men (=48.9%) and 630 of the 1348 TBS prepared from women (=46.7%) (table 1a) ($\chi2=1.32$; P value= 0.25; OR= 1.09 [0.94-1.27]).

| year	villages	M P+	n	W P+	n
Y 2007	Capango	71	159	48	116
	Canjala	189	325	190	336
	Barragem	110	215	127	250
	Chisséq.	60	178	60	178
	Libata	145	265	105	225
	Candiero	80	196	100	243
	total	655	1338	630	1348

Table 1a. *Plasmodium* prevalence in thick blood smears of men ("M") and women ("W") surveyed the first year (P+= thick blood smears with *Plasmodium*; n= number of thick blood smears done and examined; Chisséq. = Chisséquélé).

The second year *Plasmodium* prevalence were also similar being observed in 742 of the 2068 prepared from men (=35.9%) and 703 of the 1973 prepared from women (=35.6%) (table 1b) ($\chi 2$= 0.027; P value= 0.87; OR= 1.01 [0.89-1.15]).

year 2008	villages	M P+	n	W P+	0 n
	Capango	65	230	45	146
	Canjala	167	381	184	418
	Barragem	104	289	109	333
	Chisséq.	86	329	100	317
	Libata	232	485	145	361
	Candiero	88	354	120	398
	total	742	2068	703	1973

Table 1b. *Plasmodium* prevalence in thick blood smears of men ("M") and women ("W") surveyed the second year (P+= thick blood smears with Plasmodium; n= number of thick blood smears done and examined).

Therefore the overall Plasmodial prevalence were similar every years, in symptomless men and women patients, in our surveys in the 6 villages (graph. 2).
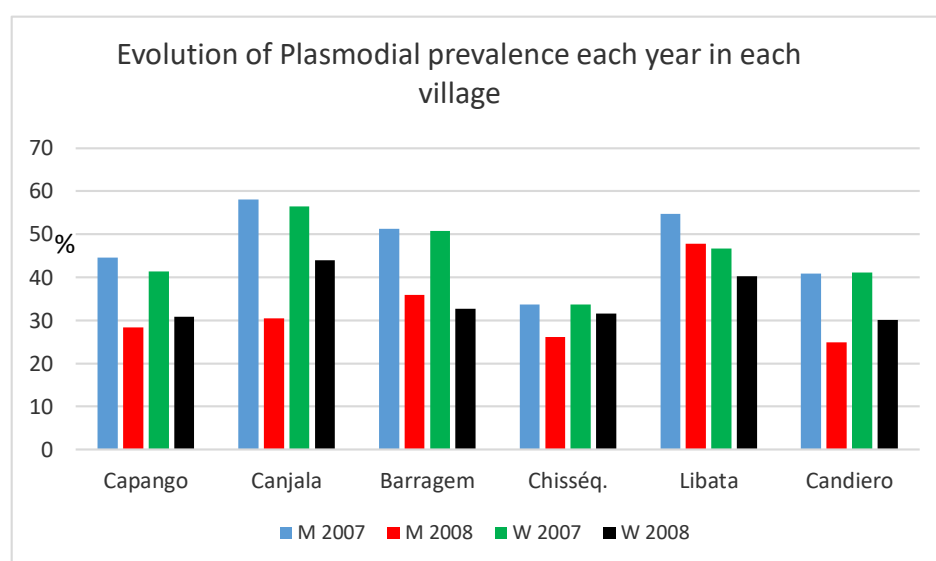
Graph. 2. Evolution of *Plasmodium* prevalence according to gender, years and villages.

III-1-2. Comparison on Plasmodial prevalence between gender according to villages and years

Data dealing with Plasmodial prevalence with gender, each year in each village, with statistical analysis, are gathered in table 2.

villages	years	PP M	n=	PP W	n=	χ2	P value	OR	[CI]
Capango	2007	44,6%	159	41,4%	116	0,29	0,59	0,87	0,54-1,42
	2008	28,3%	230	30,8%	146	0,28	0,59	1.13	0,72-1,78
	total	34,9%	389	35,5%	262	0,02	0,88	1.02	0,74-1,42
Canjala	2007	58,1%	325	56,5%	336	0,17	0,68	1,07	0,78-1,45
	2008	43.8%	381	44,0%	418	0.0028	0.96	1.01	0,76-1.33
	total	50,4%	706	49,6%	754	0,10	0,75	1,03	0,84-1,27
Barragem	2007	51,2%	215	50,8%	250	0,0061	0,94	1,01	0,71-1,46
	2008	35,9%	289	32,7%	333	0,73	0,39	1,16	0,83-1,61
	total	42,5%	504	40,5%	583	0,44	0,51	1,08	0,85-1,38
Chisséq.	2007	33,7%	178	33,7%	178				
	2008	26,1%	329	31,6%	317	2,30	0,13	0,77	0,55-1,08
	total	28,8%	507	32,3%	495	1,47	0,22	0,85	0,65-1,11
Libata	2007	54,7%	265	46,7%	225	3,16	0,075	1,38	0,97-1,97
	*2008**	*47,8%*	*485*	*40,2%*	*361*	*4,93*	*0,026*	*1.37*	*1.04-1.79*
	*total**	*50,3%*	*750*	*42,7%*	*586*	*7,64*	*0,0057*	*1.36*	*1.09-1.69*
Candiero	2007	40,8%	196	41,1%	243	0,0051	0,94	0,98	0,67-1,44
	2008	24,9%	354	30,1%	398	2,62	0,105	0,77	0,55-1,06
	total	30,6%	550	34,3%	641	1,92	0,16	0,84	0,66-1,07

Table 2. Comparison of Plasmodial prevalence (PP) according to gender (% and number of blood thick smears examined) with statistical analysis (Chi2; P value and Odds Ratio; x = statistically significant, in red).

It appeared that plasmodial prevalence of men and women were almost always similar except in 2008 in Libata, where PP of men were significantly higher than in women. This shows that careful attention must be given to gender when doing parasitological surveys and their analysis.

III-1-3. Evolution of Plasmodial according to years, villages and gender

a) In Women

The overall plasmodial prevalence of women was significantly lower in 2008 (35.6%; n= 1973) than in 2007 (46.7%; n=1348) (χ2= 41.10; p< 0.0005; OR= 0.63 [0.55-0.73]).

The plasmodial prevalence remained similar in 3 villages (Capango; Chisséquélé and Libata) while it significantly decreased in the 3 other villages (Canjala, Barragem and Candiero) (table 3a).

villages	years	PP (%)	n	χ2	P value	OR [CI]	Diff.
Capango	2007	41.4%	116	3.15	0.076	1.58 [0.95-2.64]	NS
	2008	30.8%	146				
Canjala	2007	56.5%	336	11.69	0.0006	0.60 [0.45-0.81]-	DS
	2008	44.0%	418				
Chisséq	2007	33.7%	178	0.24	0.62	0.90 [0.61-1.34]	NS
	2008	31.5%	317				
Barragem	2007	50.8%	250	19.35	< 0.0005	0.47 [0.34-0.66]	DS
	2008	32.7%	333				
Candiero	2007	41.1%	243	8.10	0.0044	0.62 [0.44-0.86]	DS
	2008	30.1%	398				
Libata	2007	46.7%	225	2.39	0.12	1.30 [0.93-1.82]	NS
	2008	40.2%	361				

Table 3a Evolution of Plasmodial prevalence in ≤15 years old women surveyed during 2 years in each villages (PP % = % thick blood smears positive; n= number of thick blood smears) (in red Significate Difference).

b)   In Men

In the samples of men the overall plasmodial prevalence significantly decreased from 48.9% (n= 1338) in year 2007 to 35.9% (n=2068) ($\chi2$= 57.4; p<0.005; OR= 0.58 [0.50-0.67]) in year 2008 even if no organized vector control was implemented.

Plasmodial prevalence significantly decreased in Capango, Canjala, Barragem and Candiero while they remained similar in Chisséquélé and Libata (table 3b).

villages	years	PP (%)	n	χ2	P value	OR [CI]	Diff.
Capango	2007	44.6%	159	11.11	<0.005	0.49 [0.32-0.75]	DS
	2008	28.3%	230				
Canjala	2007	58.1%	325	14.39	<0.005	0.56 [0.42-0.76]-	DS
	2008	43.8%	381				
Chisséq	2007	33.7%	178	3.22	0.072	0.69 [0.47-1.03]	NS
	2008	26.1%	329				
Barragem	2007	51.2%	215	11.62	<0.005	0.54 [0.37-0.77]	DS
	2008	36.9%	289				
Candiero	2007	40.8%	196	15.14	<0.005	0.48 [0.33-0.69]	DS
	2008	24.9%	354				
Libata	2007	54.7%	265	3.24	0.071	1.32 [0.98-1.78]	NS
	2008	47.8%	485				

Table 3b. Evolution of Plasmodial prevalence in ≤15 years old men surveyed during 2 years in each villages (PP % = % thick blood smears positive; n= number of thick blood smears; DS= significantly difference; NS= non-significant difference).

Compared to women the trends were similar excepted in Capango where Plasmodial prevalence remained at the same value in women while it slightly but significantly decreased in men.

The fact that variations of plasmodial prevalence from year to year could have the same trends or a different one according to gender must be underlined and they demonstrated how important it is to have a balanced sex ratio in the sample and surveyed several villages and not only one to avoid some bias and mistake in interpretation.

III-1-4. Synthesis about Parasite Prevalence indicator.

Synthesis of the analyses of the Plasmodial prevalence according to gender and villages, and their evolution from year to year, is done in the table 4 which clearly shows the different conclusion which can be drawn according to comparison done Men vs Women, years, villages.

For example, plasmodial prevalence between men and women were almost always similar except in Libata in 2008; in Capango the Plasmodial prevalence were similar in women from year to year but different in men; in Chisséquélé plasmodial prevalence were always similar etc.

villages	Year 2007 M vs W	Year 2008 M vs W	Σ years M vs W	Years 2007 vs 2008 W	Years 2007 vs 2008 M
Capango	NS	NS	NS	NS	DS
χ2	0.29	0.28	0.019	3.15	11.11
Canjala	NS	NS	NS	DS	DS
χ2	0.17	0.0028	0.099	11.69	14.39
Chisséq.	NS	NS	NS	NS	NS
χ2		2.30	1.47	0.24	3.22
Barragem	NS	NS	NS	DS	DS
χ2	0.0061	0.73	0.44	19.35	11.62
Candiero	NS	NS	NS	DS	DS
χ2	0.0051	2.62	1.92	8.10	15.14
Libata	NS	DS	DS	NS	NS
χ2	3.16	4.93	7.38	2.39	3.24

Table 4. Synthesis of statistical analyses of Plasmodial prevalence compared according to gender, villages, years (NS= non-statistically significant; DS= significant difference.

III-2. Plasmodial parasitaemia and gender.

The parasite load is a well-known relevant indicator of the "potential morbidity", even in symptomless patients, and it therefore appeared worth analyzing, and compare, parasitaemia according to gender in each village and for each year.

III-2-1. Capango: Parasitaemia and gender.

Parasitaemia in women and men were similar in year 2007 (P value= 0.0924; ns); and in year 2008 (P value= 0.5428; ns) and for the two years (P value= 0.1724; ns) (graph 3a).

The parasitaemia significantly decreased from year 2007 to 2008 for both women (P value= 0.0006) and men (P value < 0.0001).
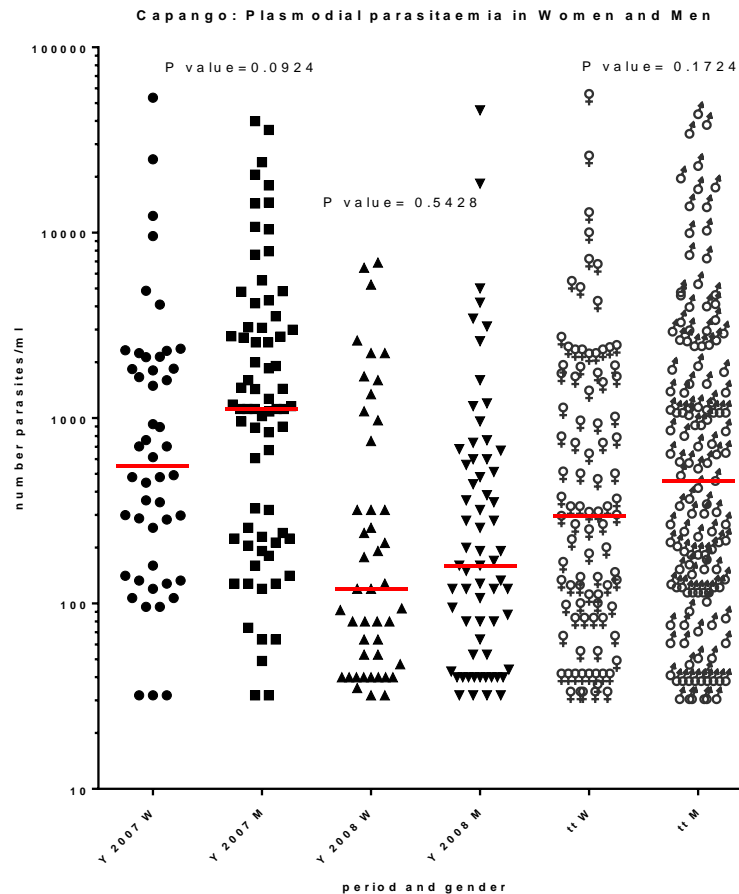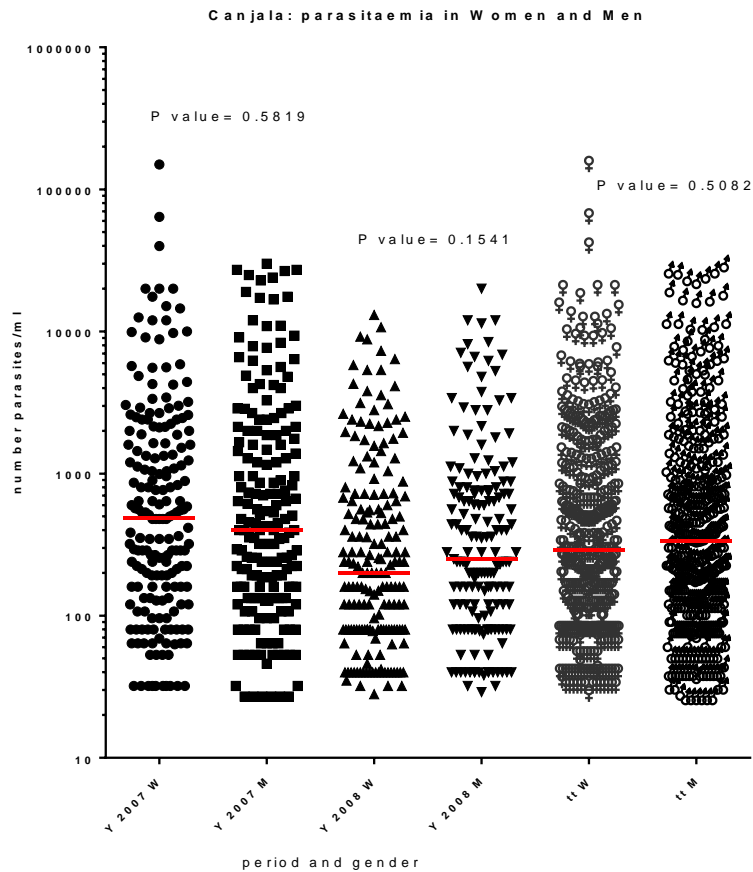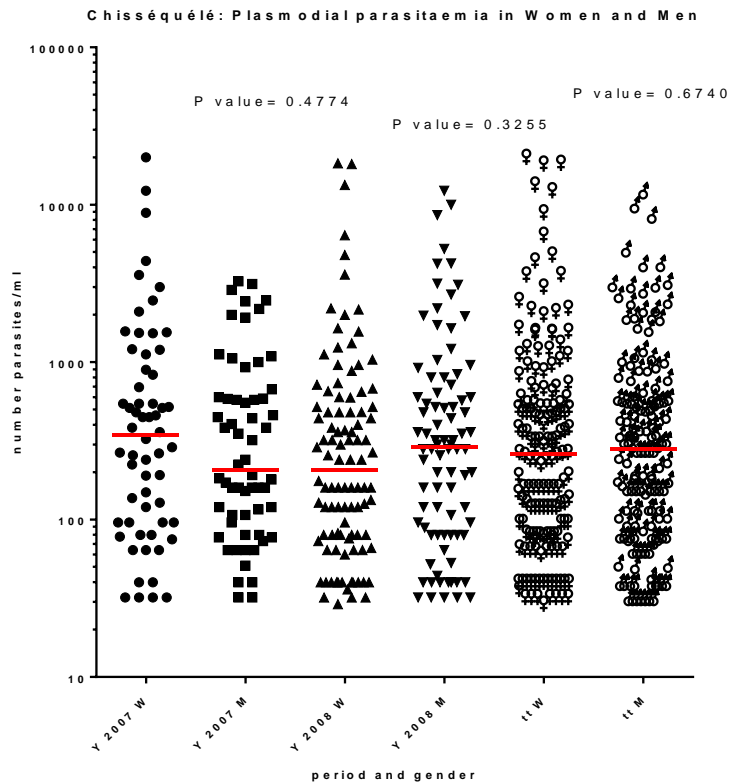
Graph. 3a. Capango: distribution of parasitaemia in men (M) and women (W) in years 2007 and 2008 (with median value in red; tt= total).

III-2-2. Canjala: parasitaemia and gender.

Parasitaemia in women and men were similar in year 2007 (P value= 0.5819; ns); and in year 2008 (P value= 0.1541; ns) and for the two years (P value= 0.5082; ns) (graph. 3b).

The parasitaemia significantly decreased from year 2007 to 2008 for both women (P value < 0.0001) and men (P value= 0.0272).

Graph. 3b. Canjala: distribution of parasitaemia in men (M) and women (W) in years 2007 and 2008 (with median value in red; tt= total).

III-2-3. Chisséquélé: parasitaemia and gender.

Parasitaemia in women and men were similar in year 2007 (P value= 0.4774; ns); and in year 2008 (P value= 0.3255; ns) and for the two years (P value= 0.6740; ns) (graph. 3c).

The parasitaemia were also similar from year 2007 to year 2008 for both women (P value= 0.1313; ns) and men (P value= 0.8576; ns).
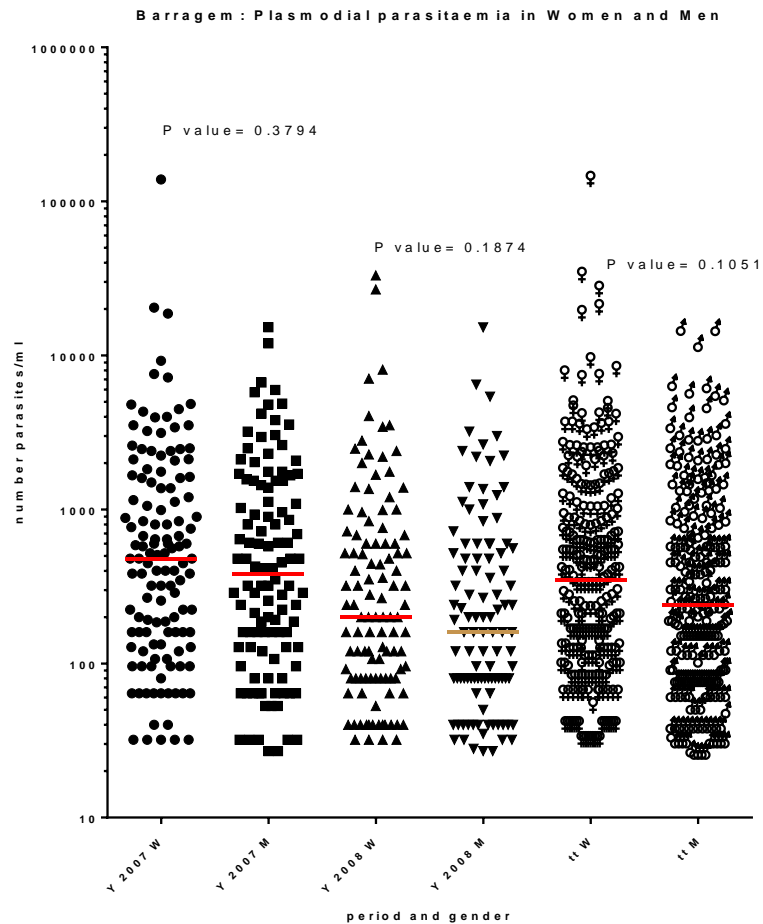
Graph. 3c. Chisséquélé: distribution of parasitaemia in men (M) and women (W) in years 2007 and 2008 (with median value in red; tt= total).

III-2-4. Barragem parasitaemia and gender.

Parasitaemia in women and men were similar in year 2007 (P value= 0.3794; ns); and in year 2008 (P value= 0.1874; ns) and for the two years (P value= 0.1051; ns) (graph. 3d).

The parasitaemia significantly decreased from year 2007 to 2008 for both women (P value= 0.020) and men (P value= 0.0010).

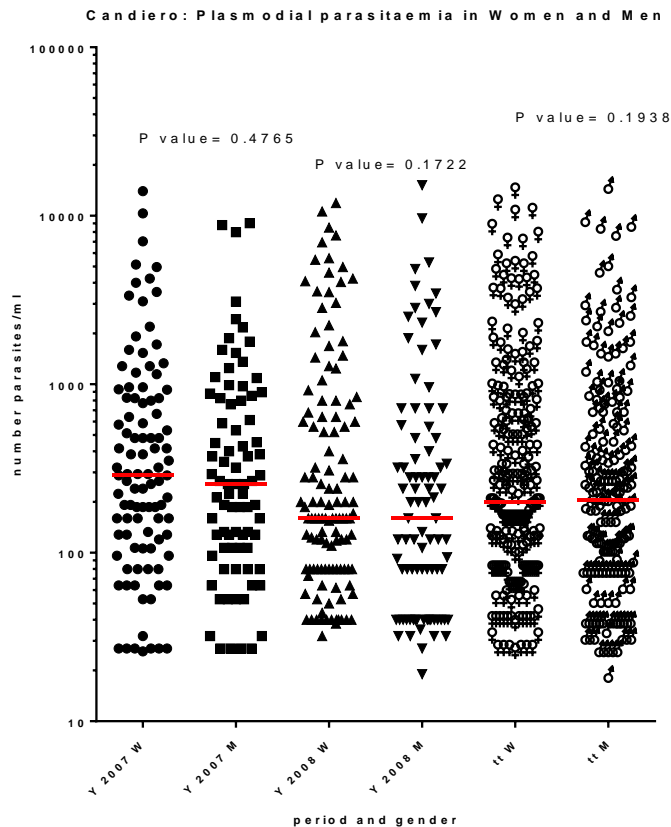**Barragem: Plasmodial parasitaemia in Women and Men**

Graph 3d. Barragem: distribution of parasitaemia in men (M) and women (W) in years 2007 and 2008 (with median value in red; tt= total).

III-2-5. Candiero parasitaemia and gender.

Parasitaemia in women and men were similar in year 2007 (P value= 0.4765; ns); and in year 2008 (P value= 0.1722; ns) and for the two years (P value= 0.1938; ns) (graph 3e).

The parasitaemia were similar from year 2007 to 2008 for women (P value= 0.1461; ns) and for men (P value= 0.0941; ns).
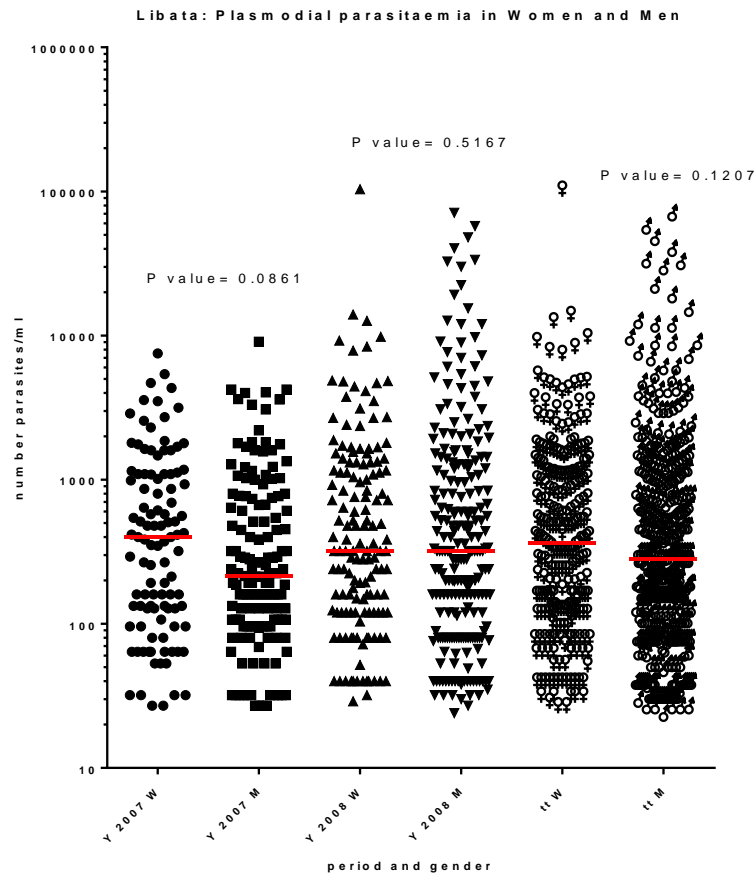
Graph 3e. Candiero: distribution of parasitaemia in men (M) and women (W) in years 2007 and 2008 (with median value in red; tt= total).

III-2-6. Libata: parasitaemia and gender

Parasitaemia in women and men were similar in year 2007 (P value= 0.0861; ns); and in year 2008 (P value= 0.5167; ns) and for the two years (P value= 0.1207; ns) (graph. 3f).

On the other hand it is interesting to notice that in 2008 the plasmodial prevalence in Men was significantly higher than in women (table 2) while parasitaemia were similar.

The parasitaemia were similar from year 2007 to 2008 for women (P value= 0.5790; ns) but with a significantly increase for men (P value= 0.0471; DS yes).

Graph. 3f. Libata distribution of parasitaemia in men (M) and women (W) in years 2007 and 2008 (with median value in red; tt= total).

III-3. Overall synthesis of parasitaemia and gender in the 6 villages.

The statistical analyses by the Mann-Whitney test of the distribution of parasitaemia according to gender in each village are gathered in table 5 where it always appeared a non-significant (ns) difference between gender in both years 2007 and 2008.

villages	Y 2007 W vs M	Y 2008 W vs M	Sum Years W vs M	Y 2007 => 2008: W	Y 2007 => 2008: M
Capango	NS	NS	NS	DS 2007↓2008	DS 2007↓2008
Canjala	NS	NS	NS	DS 2007↓2008	DS 2007↓2008
Chisséq.	NS	NS	NS	NS =	N=
Barragem	NS	NS	NS	DS 2007>2008	DS 2007↓2008
Candiero	NS	NS	NS	NS =	NS =
Libata	NS	NS	NS	NS =	DS 2007↑2008

Table 5. Analyses of the distribution of parasitaemia per villages and years according to gender (NS = non-significant difference; DS= significant difference).

On the other hand it was noticed some differences (DS) between year 2007 and 2008 with an decrease in Capango; Canjala and Barragem; an increase in Libata (for men only), and similar values in Chisséquélé and Candiero underlining the importance of implementing surveys in different villages during several years to avoid some mistake in the conclusion.

When gathering all data of Plasmodial distribution in women and in men for each village for the two years and the total distribution it clearly appeared similar values between gender with P values always non-significant (graph 4).

For the total data it appeared a remarkable similar values of the median for women (med. = 288; n= 1333) and men (med. = 280; n= 1397) (graph. 4) and the quite similar level of median is noteworthy.
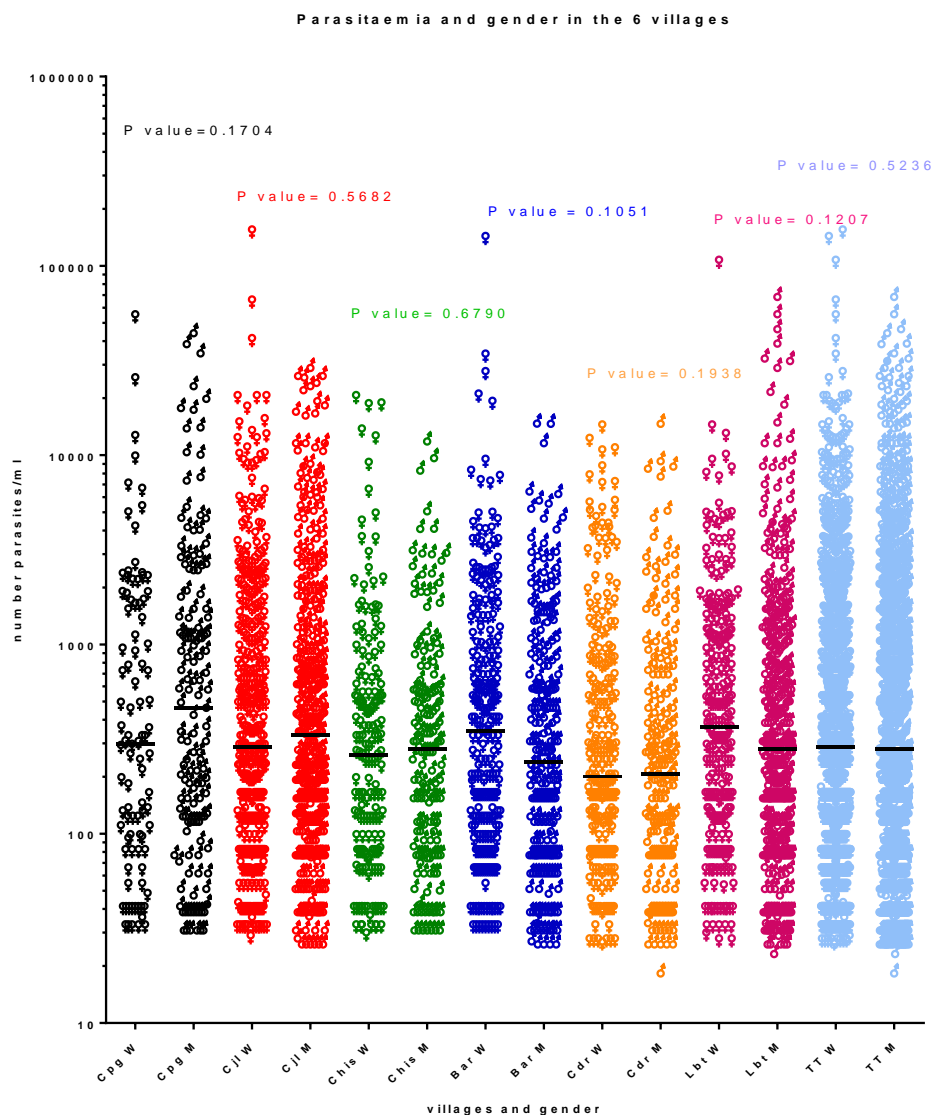
Graph. 4. Distribution of Plasmodial parasitaemia according to gender (Men, Women) in each village (Tt = total of parasitaemia).

III-4. Information according to indicator: Parasite Prevalence (PP) or Parasite Load (PL)

The main evolution of Plasmodial infection from year to year in each village for Men and Women considering the Parasite Prevalence (PP) or the Parasite Load (PL) are gathered in the table 6.

years	Y 2007 => 2008	Men	Y 2007 =>2008	Women
Indicator/ villages	PP	PL	PP	PL
Capango	↓	↓	=	↓
Canjala	↓	↓	↓	↓
Chisséquélé	=	=	=	=
Barragem	↓	↓	↓	↓
Candiero	↓	=	↓	=
Libata	=	↑	=	=

Table 6. Evolution from year to year of Parasite Prevalence (PP) and Parasite Load (PD) according to village and gender.

These similar or different trends of these data clearly showed the importance of using different indicators before drawing definitive conclusion on the different susceptibility to Plasmodial infections according to gender.

IV. Discussion

In the framework of a large scale malaria vector control program implemented since 2007 around Balombo town (Angola) 56 parasitological cross sectional surveys were done the first 2 years in 6 control villages to get base line data before any intervention.
The whole sample of 6727 thick blood smears, prepared from randomized children $\leq$ 15 years old was well balanced with 50.6% from men and 49.4% from women. 40.6% of the total thick blood smears were found positive by classical microscopic observation; with similar plasmodial infection in men (41.2%) and women (40.1%). From year to year the overall positivity decreased significantly but the percentage of positive smears remained similar between gender.
This could be expected when knowing that the main vectors, such as *Anopheles gambiae,* bite men as well as women [27].
But some differences were reported elsewhere.
For Briggs et al [26] "multiple studies have reported a male bias in incidence and/or prevalence of malaria infection in males compared to females". In their cohort study in eastern Uganda, they noted "higher prevalence of malaria infection in males compared to females" and "found that lower prevalence in females did not appear to be due to lower rates of infection but rather due to faster clearance of asymptomatic infections". For these authors "though there are some conflicting reports in the literature, the majority of studies of malaria incidence and/or prevalence that evaluated associations with sex in late childhood, adolescence and adulthood have found a male bias in the observed measure of burden [21] [23] [19, 35-38]. They added that "the inclusion of parasite density in our multivariate models did not meaning fully alter associations between sex and duration of infection, providing evidence that the sex-based differences in duration were not mediated primarily by differences in parasite density in our cohort".

Age-specific immunity to malaria in hyperendemic areas is well characterized, less attention has been paid to the possibility of a sex bias in malarial susceptibility despite evidence for a male bias in malaria infections in non-human animals and a male bias in the prevalence of other human parasitic infections [16, 24, 25].

Several studies have reported a "male bias" in malaria prevalence or incidence in school-children and adults in both hypo and hyperendemic regions [36, 37].

It has often been postulated that these differences in malaria incidence or prevalence cold be related to socio-behavioral factors [23, 35, 39].

Quaresima et al [40] raised the question "are Malaria Risk Factors Based on Gender"? From their observations in a Medical center in Kumasi (Ghana) they concluded that "even though more women than men presented to hospital with malaria infection during the study period, neither parasite density nor clinical manifestations suggested gendered differences". The difference could be related to different malaria exposure behaviors, formal education, occupation and preventive measures between males and females". While in a study done in Tanzania it was noticed that the men's habit of spending time outdoors drinking alcohol and watching television was linked to malarial risk [41].

However, because biological sex itself has been demonstrated to affect responses to other pathogens, an alternative hypothesis is that the sexes may have different responses to the malaria parasite once infected [13-15, 22].

Actually a link between behavior and differential gender/risks of malaria could be done for adults, men/women (with the well-known increasing risk in pregnant women) but not for below 15 years old children.

Several indicators were studied: prevalence, incidence, duration of infection, parasite load in asymptomatic and symptomatic patients with different parasitological technic to detect even few number of parasite/ml of blood.

For Briggs et al (loc.cit.) there was no evidence of a difference in incidence of symptomatic malaria by sex overall but "females had a lower prevalence of infection than men" in spite of a "similar rate of acquiring infection (Force of Infection or FOI) compared to males". The answer could be in the difference in the rate «females cleared their asymptomatic infections more rapidly than males, implicating biological sex-based differences….It seems that "there was no evidence for a significant difference in Force of Infection (FOI) by sex", women having the same FOI as men but lower prevalence of infection, the key should be a sexual differences in the clearance of the parasite, asymptomatic infections cleared naturally at nearly twice the rate in females vs. males".

The concept of "incidence rate" (from *Plasmodium* negative ("P-") to *Plasmodium* positive ("P+") and "recovery rate" (from P+ to P-) was mathematically developped from the Muench model [21, 42-44].

For Briggs et al (loc.cit.) there is a "faster clearance in females vs. males" and thus "males had a longer duration of infection across all age categories. Children aged 5-15 years had the longest duration of infection…males aged 5-15 years had the longest estimated duration of infection by either clone or infection events". The question of immunological differences between males and females in their response to the malaria parasite is actually of great concern when large scale vaccination is strongly promoted. Actually it is reported that "RTS,S vaccination is associated with higher all-cause mortality in girls compared to boys, and a trend toward higher risk of fatal malaria has been noted in vaccinated girls compared to boys, suggesting possible sexual dimorphism in immunological responses to malaria"[17]. Hormonal differences have been incriminated in this "sex-based" immunological differences to malaria infection [45] but several other mechanisms should be involved and "more studies are needed to elucidate the relationship between sex-based biological differences between males and females and their impact on the development of effective antimalaria immunity in humans" concluded Briggs et al. (loc.cit.).

In fact we noticed a gender difference in some villages (but not in the same trends), but that the overall parasite prevalence and parasite load were similar between boys and girls before any intervention is an important information to be considered when analyzing the impact of vector control operations scheduled, and implemented, in this 6 villages [1] such as the situation studied by Briggs et al. (loc.cit.). It should be analyzed after vector control operation in Balombo area but also in other eco-epidemiological and socio-cultural situations to adapt analysis and further plan of action (PoA) to actual local conditions to improve control for the targeted malaria elimination.

V. Conclusion

Forty years ago it was clearly demonstrated that *An.gambiae* bite men as well women and babies less than adults [27] but susceptibility to *Plasmodium* is still matter of concern for eventual prioritization of a risk group to be protected such as pregnant women. On the other hand the choice of method of vector control is of crucial importance while considering cost/efficacy, operational issues etc. For exemple Indoor Residual Spraying was stopped in Angola after the failure of recent operations [5] while the newly developped tools call Insecticide Treated Plastic Sheeting which represent another method of treating the walls inside human houses, showed their efficacy and acceptability [7, 8, 9, 10]. One of the question remains the parasitological evaluation of a malaria situation, and the efficacy of vector control operation, where the use of longitudinal surveys is recommended [43]. This is the protocol developped during a village scale malaria vector control program [1] and the current analyses focused on the *Plasmodium* prevalence and parasite load, in $\leq$ 15 years old symptomless patients, according to gender before implementation of vector control, the same will be done after such implementation. It appeared that conclusion could be different according to the indicator and the method of analysis and great care is needed before drawing definitive conclusion. Generally speaking it appeared that Plasmodial infections were similar in young men and women, therefore such recent parasitological evaluation confirmed the previous entomological evaluation.

ETHICAL APPROVAL

This analysis is a part of the comprehensive evaluation of a vector control program done with the Angola National Malaria Control Programme and Provincial Public Health Authorities.

CONSENT

As per international standard or university standard, respondents' written consent has been collected and preserved by the author(s).

Contribution. PC prepared the protocol, was involved in field surveys, analyzed data and wrote the draft document. JCT and VF were involved in field surveys and parasitological examination. GC was

involved in the writing and English version.DS was one of the B2B physician responsible of the Medical Department of Sonamet Lobito Angola.

All authors read and agreed upon the document.

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