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Variation of prevalence of malaria, parasite density and the multiplicity of Plasmodium falciparum infection throughout the year at three different health centers in Brazzaville, Republic of Congo

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Abstract

Background: In the Republic of Congo, hot temperature and seasons distortions observed may impact the development of malaria parasites. We investigate the variation of malaria cases, parasite density and the multiplicity of Plasmodium falciparum infection throughout the year in Brazzaville. Methods: From May 2015 to May 2016, suspected patients with uncomplicated malaria were enrolled at the Hôpital de Mfilou, CSI « Maman Mboualé», and the Laboratoire National de Santé Publique. For each patient, thick blood was examined and parasite density was calculated. After DNA isolation, MSP1 and MSP2 genes were genotyped. Results: A total of 416, 259 and 131 patients with suspected malaria were enrolled at the CSI «Maman Mboualé», Hôpital de Mfilou and the Laboratoire National de Santé Publique respectively. Proportion of malaria cases and geometric mean parasite density were higher at the CSI «Maman Mboualé» compared to over sites (P-value 0.001). However the multiplicity of infection was higher at the Hôpital de Mfilou (P-value 0.001). At the Laboratoire National de Santé Publique, malaria cases and multiplicity of infection were not influenced by different seasons. However, variation of the mean parasite density was statistically significant (P-value 0.01). Higher proportions of malaria cases were found at the end of main rainy season either the beginning of the main dry season at the Hôpital de Mfilou and the CSI «Maman Mboualé»; while, lowest proportions were observed in September and January and in September and March respectively. Higher mean parasite densities were found at the end of rainy seasons with persistence at the beginning of dry seasons. The lowest mean parasite densities were found during dry seasons, with persistence at the beginning of rainy seasons. Fluctuation of the multiplicity of infection throughout the year was observed without significance between seasons.
Conclusion: The current study suggests that malaria transmission is still variable between the north and south parts of Brazzaville. Seasonal fluctuations of malaria cases and mean parasite densities were observed with some extension to different seasons. Thus, both meteorological and entomological studies are needed to update the season's periods as well as malaria transmission intensity in Brazzaville.

Introduction

Malaria is still one of the major health problems worldwide. The global incidence of the disease in 2017 has been estimated at 219 million of cases with 435,000 deaths [1]. The sub-Saharan Africa continues to experience considerable burden of the disease with approximately 92% of malaria cases and 93% of deaths occurred in the World Health Organization (WHO) African Region [1].

Climate change has been noticed worldwide with impact on rainfall, temperature and humidity; three factors that are known to affect malaria seasonality as well as, transmission intensity [2-4]. Several studies have demonstrated the influence of these factors on the development of malaria parasites into the mosquitos [4; 5-9] with immediate consequences on the parasite transmission to human host.

In areas with seasonal and intense malaria transmission, the human parasite reservoir declines through the dry season until the beginning of the wet season at which time vector numbers begin to rise [10]. Thus understanding impact of seasonal variations on the development of malaria parasites is crucial to achieve different intervention programs such as malaria prevention, in the perspective of malaria elimination. Entomological surveys are encouraged for this purpose. However, parasitological data such as parasite density should be able to supplement entomological data for better understanding of local seasonality and heterogeneity of exposure [11].

To predict the effect of intervention outcomes in malaria seasonal settings, it is also necessary to understand the dynamic of natural acquired immunity or premunition at the seasonal time scale [11].The multiplicity of *Plasmodium falciparum* infection (MOI), defined as the minimum number of *Plasmodium falciparum* genotypes per infected subject, is thought to be a useful parasitological indicator of transmission or host acquired immunity level [12, 13]. Merozoite surface proteins (MSP) are involved in erythrocyte invasion and affect parasites density [14, 15]. Thus genotyping of MSP1 and MSP2 is a standard method for assessing MOI [16-18]. However several studies have shown an inverse association between MOI and parasite densities [19, 20] while others have shown the positive correlation between MOI and parasite density in clinical *Plasmodium falciparum* infection [21, 22].

In the Republic of Congo, malaria is still the leading cause of attendance in health facilities with 52, 8% outpatient consultation, 44, 1% hospitalization and 28% of deaths due to malaria; and the most vulnerable are pregnant women and children under 5 years old [23]. Study conducted in 1987 has demonstrated that the Republic of Congo is an area of intense and perennial malaria transmission with an entomological inoculation rate of 200–1000 infective bites/person/year [24]. Since then, there has been no update with more recent entomological studies. In these last years, the climate change has also been observed in the Republic of Congo with hot temperature and seasons distortions. Thus, to better control malaria intervention by predicting the optimal times at which to deploy vector control and drug-based interventions in this area in the perspective of malaria elimination, the actual profile of malaria variation is needed.

This study investigated the seasonality of *Plasmodium falciparum* malaria cases, parasite density and the MOI in Brazzaville, the Republic of Congo.

Methods

Study areas The study was conducted in Brazzaville, the political capital hosting 38% (1 642 105 inhabitants) of the total population of the Republic of Congo, estimated at 4 312 715 inhabitants as described elsewhere [25, 26]. Due to the fluctuation of malaria transmission in Brazzaville, which varies from low, moderate to intense with meso-, hyper- to perennial endemicity, three different centers were considered for patients recruitment: Centre de Santé Intégré (CSI) « Maman Mboualé» located in the north part of city (4°13'S, 15°17'E); Hôpital de Mfilou located in the south part of the city (4°15'S, 15°13'E) and the Laboratoire National de Santé Publique (LNSP) located in the center part of city (4°16'S, 15°15'E). Malaria infection is primarily due to Plasmodium falciparum and Anopheles gambiae s.s. is the predominant vector. Two rainy seasons are observed each year with the main one during the months of February to May, and a short one from October to November [27-29].The dry seasons are from June to September and from December to January. Study population, blood samples and data collection From May 2015 to May 2016, patients with clinical signs of uncomplicated malaria, presenting at the laboratory of one of the three study sites were invited to participate in this study. Exclusion criteria comprised pregnancy, severe malaria or other severe illness as judged by the attending physician. The number of representative patients to be included in each site has been estimated by the statistician taking into account the proportion of malaria reported in each health center, one year before starting the study as described elsewhere [25, 26].Thus, 310, 200 and 100 were a minimum number of patients to be recruited at the CSI « Maman Mboualé», Hôpital de Mfilou and the LNSP, respectively. After informed consent was obtained, records were made on patient demographics, fever or history of fever in the last 48 hours, other signs of malaria, provenance, previous antimalarial drugs intake used of bed net treated. The axillary temperature was taken for fever confirmation. At each study site, two thick blood smears were prepared for each patient, with one being read immediately to inform the patient of the respective result. Finger prick blood from each patient was blotted on the Whatman filter paper (3MM CHR) while preparing the thick blood smears, dried and transferred to the LNSP, where isolation of deoxyribonucleic acid (DNA) and polymerase chain reaction (PCR) were performed. Before reading, thick blood smears were dried and stained with 10% Giemsa solution (Sigma Chemical, Sigma Aldrich ChemieGmbh, Taufkirchen, Germany) in pH 7.2, for approximately 10 min. The stain was gently washed away by adding drops of clean water and the slide was completely dried before examination. Thick blood smears were assessed by experienced micrsocopists until 200 leucocytes had been counted. Parasite density was calculated for each patient assuming an average of 8000 leucocytes per µl of blood using the proposed method of the WHO [30]. Individual diagnostic result was given to each patient and advised to meet the prescribers for possible antimalarial chemotherapy. The second uncolored thick blood smear was transferred to the Centre Hospitalier Universitaire de Brazzaville, which is the big referral hospital with a reference laboratory in Brazzaville for microscopy quality control as described by Mayengue et al. [25]. Extraction of parasite DNA Genomic DNA was extracted from samples collected on the Watman filter paper using QIAamp DNA mini Kit (Qiagen, Hilden, Germany) according to the manufacturer’s instruction. Extracted DNA was stored at -20°C until use. Parasites genotyping Samples genotyping of Plasmodium falciparum was performed using the nested PCRs technique. The MSP1 and MSP2 genes in their highly polymorphic loci, namely

MSP1 block 2 and MSP2 central region were used as markers for this genotyping as described previously [17, 18]. PCR amplification was performed following a 2-step amplification procedure, in which the initial amplifications were followed by individual nested PCR reactions using specific primers for K1, Mad20 and RO33 allelic families for MSP1, and FC27 and 3D7 allelic families for MSP2. Allelic specific and DNA free negative controls were included in each step of the reaction. Five microliters of each of the PCR products were loaded on 2% agarose gel (PeqLab, Erlangen, Germany), stained with ethidium bromide, separated by electrophoresis and visualized under ultraviolet trans-illumination. The number of products, corresponding to number of infecting MAD20, RO33 and K1 clones for MSP1 gene as well as FC27 and 3D7 clones for MSP2 gene was counted after visualization. Data analysis Data collected were summarized with average and standard deviation for age and compared using Student's t-test for two independent samples or the Bonferroni method for one-way analysis of variance (ANOVA) for multiple comparisons. Categorical responses were expressed as a percentage, and comparisons were made using Pearson's $\chi 2$ test (or Fisher's exact test if appropriate). The effect of seasonality on malaria infection was assessed using logistic regression method but no relationship was found, because some subgroups had few patients with malaria. So, the non-parametric Kruskal-Wallis test was used for evaluating the influence of the seasons on malaria infection. All analyses were done with SPSS statistical software for Windows, Version 24.0 (Chicago: SPSS Inc.). All tests were two tailed and P-values ≤ 0.05 were considered as statistically significant.

Results

Sociodemographic and clinical characteristics of patients

A total of 416, 259 and 131 patients with suspected malaria were enrolled at the CSI «Maman Mbouale», Hôpital de Mfilou and the LNSP respectively. Sociodemographic as well as clinical characteristics of these patients are summarized in Table1.

Out of 259 patients enrolled at the Hôpital de Mfilou, gender and age were recorded for 257 of them, while with regards to the CSI « Maman Mbouale», out of the 416 recruited patients, 410 had records on gender. Both proportion of malaria cases and geometric mean parasite density were higher at the CSI «Maman Mbouale» compared to over sites *(P-value* 0.001).

Table 1: Characteristics of patients

Characteristics	CSI « Maman Mboualé»	Hôpital de Mfilou	LNSP	*P-value*
Total number	416	259	131	
Gender (F/M)	207/203	131/126	68/63	
Groups of age (n, %)				
5 years	99(23.8)	38(14.8)	0(0.0)	
≥5 years	317(76.2)	219(85.2)	131(100.0)	
Mean age ± ET	14.5±13.49	28.6±19.82	42.31±14.84	
Malaria cases (n, %)	173(41.6)	62(23.9)	12(9.2)	0.001
Geometric mean parasite density (Min-Max)	2399.30 (16-164800)	1905.10 (16-100000)	189.46 (16-8720)	0.001
Multiplicity of infection				
MSP1	1.70	2.05	1.13	0.001
MSP2	1.33	1.51	1.00	0.001
MSP1+2	2.55	3.23	1.89	0.001

Relationship between the multiplicity of infection, parasite density and age

Regardless the molecular marker used, the MOI was higher at the Hôpital de Mfilou compared to the CSI « Maman Mboualé», and the LNSP (Table 1), with the overall MOI of 3.23, 2.55 and 1.89 respectively (*P-value* 0.001). While the MOI was not influenced by age, patients with less than 5 years had significantly higher parasite densities compare to those with the age more than 5 years old (Table 2). Moreover, the MOI was not associated with parasite density.

Table 2: **Relationship between the multiplicity of infection, parasite density and age**

Center	Characteristics	Mean parasite densities	*P-value*	MOI	*P-value*
Hôpital de Mfilou	Age groups				
	5 years	22167.18	0.037	3.25	0.956
	≥ 5 years	9847		3.15	
CSI « Maman Mboualé»	Age groups				
	5 years	32109.11	0.012	2.18	0.469
	≥ 5 years	15816.28		2.63	
LNSP	Age groups				
	5 years	--	--	--	--
	≥ 5 years	1320.33		1.89	

Variation of proportion of malaria cases and parasites density throughout the year

During the year, *Plasmodium falciparum* was the only specie identified in all positive slides confirmed by the quality control expert.

A particular profile has been found at the LNSP with very low malaria cases without impact of different seasons (*P-value* =0.477). However, the difference of the mean parasite density was statistically significant (*P-value* 0,01) with the highest pick in November, corresponding to the rainy season.

At the Hôpital de Mfilou, highest proportions of malaria cases have been found at the beginning of the study in May and June (50%), November (40%), February (38.9%) and April (33.3%) corresponding to the months of rainy seasons and the beginning of dry season for June (Table 3). However, lowest proportions of cases were noticed in September (3.4%), and January (18.8%) corresponding to the peak of dry seasons. The variation of these proportions within the year was statistically significant (*P-value* 0.004). A contrasting profile was observed at the CSI « Maman Mbouale», where the highest proportion of malaria cases were obtained during the main rainy season, in April, May including the beginning of dry season in June. Progressive diminution of malaria cases has been noticed from July to March, reaching the lowest proportion in December and March corresponding to the beginning of dry season and main rainy season respectively (*P-value* 0.01). When tacking all tree sites together the highest proportions of malaria cases were confirmed during the rainy seasons, while lowest proportions were registered in September, December and March (*P-value* 0.01).

With the regards to mean asexual parasite densities, a clear seasonality has been noticed at the Hôpital de Mfilou, with the highest peaks mainly observed at the beginning of dry seasons in June and December as well as in May (corresponding to the end of rainy season) at the end of the study (Figure 1). However the only one malaria case registered in September had also a high parasite density. Moreover, it is obvious from the result that the periods of low mean asexual parasite densities were observed at the peak of the dry season corresponding to the month of August with persistence during of rainy seasons (Figure1) corresponding to October and November as well as, February March and April for the short and main rainy seasons, respectively.

By considering the CSI «Maman Mboualé», tree high picks of mean parasite density have been identified in July, December and May; thereafter, persistence decrease has been noticed during the dry season, including the rainy seasons (Figure 1) corresponding to October and November as well as from February to April (*P-value* 0.043). When tacking all tree sites together, similar profile with the CSI «Maman Mboualé», has been observed (*P-value* 0.01).

Table 3: **Relationship between different seasons and proportion of malaria cases**

Seasons	Months	Hôpital de Mfilou		CSI «Maman Mboualé»		LNSP		All	
		N	n (%)	N	n (%)	N	n (%)	N	n (%)
Rainy	May-15	4	2(50,0)	8	6(75,0)	4	0(0,0)	16	8(50,0)
Dry	June-15	24	12(50,0)	36	29(80,6)	14	1(7,1)	74	42(56,8)
	July-15	27	7(25,9)	36	17(47,2)	14	0(0,0)	77	24(31,2)
	August-15	32	3(9,4)	34	15(44,1)	5	0(0,0)	71	18(25,4)
	September-15	29	1(3,4)	36	11(30,6)	18	4(22,2)	83	16(19,3)
Rainy	October-15	25	6(24,0)	34	9(26,5)	7	0(0,0)	66	15(22,7)
	November-15	15	6(40,0)	34	10(29,4)	6	1(16,7)	55	17(30,9)
Dry	December-15	23	6(26,1)	31	6(19,4)	14	2(14,3)	68	14(20,6)
	January-16	16	3(18,8)	32	10(31,3)	11	1(9,1)	59	14(23,7)
Rainy	February-16	18	7(38,9)	34	14(41,2)	13	1(7,7)	65	22(33,8)
	March-16	16	2(12,5)	37	9(24,3)	4	1(25,0)	57	12(21,1)
	April-16	9	3(33,3)	32	16(50,0)	7	0(0,0)	48	19(39,6)
	May-16	21	4(19,0)	32	21(65,6)	14	1(7,1)	67	26(38,8)
P-value		--	0,004	--	0,01	--	0,477	--	0,01

N: number of enrolled patients per month; n: number of malaria cases per month

Variation of multiplicity of *Plasmodium falciparum* infection throughout the year

Particular profile has also been found at the LNSP regarding the MOI with no infection in some alternative months (May, July, August, October and April), and the MOI did not vary significantly over the year (*P-value* =0,853).

At the Hôpital de Mfilou, significant variation of MOI was found over the year (*P-value* 0.01). From the beginning of the study in May, the MOI was increased reaching a pick in July, thereafter persistence of decrease was observed, with the lowest MOI in September (Figure 2). There was a permanent increase of MOI from October, reaching the highest picks in February and April, regardless the short dry season in December and January.

Concerning the CSI «Maman Mbouale», tree different picks were observed, with highest MOI registered at the beginning of the study in May; follow by those in October and the lowest in December (*P-value* 0.01). Persistence of decrease of MOI was observed in July and August, while the MOI stability was noticed from January to April. When taking all tree sites together, significant variation of MOI was also observed over the year (*P-value* 0.01) with similar profile with the CSI «Maman Mbouale» from May at the beginning of the study to November, but slightly increase of MOI from December to May at the end of the study.

Discussion

The understanding of malaria dynamic in the area is crucial by targeting the peak malaria transmission for malaria control intervention at both vector and drug-based level. In the Republic of Congo, climate change has been observed with hot temperature and seasons distortions shifting obviously the beginning and the end periods of different seasons, with impact on rainfall, temperature and humidity. Thus, it is urgent to evaluate malaria seasonality as well as, transmission intensity. To our knowledge, this is a first study to evaluate seasonality of the malaria parasitaemia and the MOI over the year in Brazzaville.

With the regard to the variability of malaria transmission level in the different parts of Brazzaville [24], three different health facilities according to their location were considered, notably the CSI «Maman Mbouale» in the north, the LNSP in the center and the Hôpital de Mfilou in the south of Brazzaville.

The results indicate a particular profile at the LNSP with very low proportion of malaria cases being almost adults, low mean parasite density, as well as low MOI. No influence of seasons on the MOI and the proportion of malaria cases was found at this study site.

Although, the variability of the mean parasite density was noticed over the year with the highest pick in November, it is obviously difficult to draw any conclusion due to small number of malaria cases. In addition, the majority of patients recruited at the LNSP came from the distant districts of the LNSP. Thus the type of transmission as well as the impact of seasons on malaria should be discussed with caution while low level of malaria transmission was expected at this site which is more urbanized [24].

Annually, the proportion of malaria cases and the mean parasite density were higher at the CSI «Maman Mboualé» compared to the Hôpital de Mfilou. Inversely, the MOI has been found to be high at the Hôpital de Mfilou compared to the CSI «Maman Mboualé». Despite the lack of recent entomological data from Brazzaville, the number of clones coinfecting a single host can be used as an indicator of the level of malaria transmission or the level of host acquired immunity [12, 31]. Therefore, the discrepancies on the MOI may suggest the different level of malaria transmission between the north and the south parts of Brazzaville; with CSI «Maman Mboualé» being more urbanized compared to the Hôpital de Mfilou. However the MOI was influenced neither by age nor by parasite density regardless the study site, concordant with the studies conducted in Brazzaville and Pointe Noire [32, 33]. Therefore, regardless of the parasite densities, and the fact that the sample collection was done from symptomatic infection, the prevalence of multi clonal infections affected all the two age groups.

Both proportion of malaria cases and mean parasite density were influenced by the seasonality in these two study sites, but with some particularities. The higher proportions of malaria cases were found mainly at the end of main rainy season including the month of June (which is the beginning of the main dry season). While, clear impact of dry season has been observed at the Hôpital de Mfilou with lowest proportions in September and January, at the CSI «Maman Mboualé», lowest proportions of malaria cases were found in September and March. Higher mean parasite densities were found meanly at the end of rainy seasons with persistence at the beginning of dry seasons. Inversely to the lowest mean parasite densities founded during dry seasons but with persistence at the beginning of rainy seasons. This could be due to environmental particularities including the humidity relative to the presence of swampy areas around the Hôpital de Mfilou as well as the “Fleuve

Congo" river, surrounding the CSI «Maman Mbouale», which may maintain the multiplication of mosquitos until the beginning of dry seasons, while low level of humidity may influence mosquitos multiplication at the beginning of the rainy season. The outcome of this study is in agreement with those in Nigeria [34-36]. Additionally, persistence of high proportions of malaria cases and mean parasite densities at the beginning of dry seasons and their lower values at the beginning of rainy seasons may also be due to climate change observe in Brazzaville. It has been suggested that weather variation may diminished malaria seasonality [37]. Thus, both meteorological data and entomological studies are needed to update the season's periods as well as malaria transmission intensity.

Fluctuation of the MOI throughout the year was observed without any clear pattern and devoid of seasonality at the Hôpital de Mfilou and the CSI «Maman Mbouale». The findings presented in this study disagree with the results of previous study in Senegal and Ghana [15, 38]. Therefore, the discrepancies may be due to the difference of population groups with the current study being conducted in symptomatic population. Further studies are needed including asymptomatic population to better evaluate the impact of seasonality on the MOI in the Republic of Congo. Interestingly, alternated fluctuation of MOI was observed between these two study sites throughout the year. This observation supports the different level of malaria transmission which may exist between the north and the south parts of Brazzaville.

Conclusion

With the lack of recent entomological data in Brazzaville, this study conducted throughout the year on Plasmodium falciparum symptomatic population suggests that malaria transmission is still variable between the north and the south of Brazzaville. Seasonal fluctuation of proportion of malaria cases and mean parasite density was observed. However, persistence of high proportions of cases and mean parasite densities at the beginning of dry seasons and their lower values at the beginning of rainy seasons may be due climate change observe in Brazzaville. Thus, both meteorological data and entomological studies are needed to update the season's periods as well as malaria transmission intensity.

Abbreviations

WHO: World Health Organization

MOI: multiplicity of *Plasmodium falciparum* infection

MSP: Merozoite surface proteins

CSI: Centre de Santé Intégré

LNSP : Laboratoire National de Santé Publique

DNA: deoxyribonucleic acid

PCR: polymerase chain reaction

CERSSA : Comité d'Ethique de la Recherche en Sciences de la Santé

Declarations

Ethics approval and consent to participate

The study was approved by the institutional "Comité d'Ethique de la Recherche en Sciences de la Santé" (CERSSA) (N° 032/CERSSA-2015). Before the recruitment, the project objectives, methodology and expected results have been explained to patients and/or theirs parents/guardians. Written and signed informed consent was obtained from all study participants or their parents or guardians.

Consent for publication

Not applicable.

Availability of data and materials

The data generated and analyzed in this study are not publicly available for ethical reasons. However, they may be available from the corresponding author upon request.

Competing interests

The authors declare that they have no competing interests.

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Authors’ contributions

PIM designed and coordinated field study, analyzed the data and wrote the draft of the article. RFN, GA, SCK, HJP supervised field samples and data collection; DKB, RIO, AMM, GPUFF analyzed samples. All authors read and approved the final version and the final manuscript.

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Dear Professor Henri Joseph Parra, you have left this world suddenly before the submission of this article. Your name and your scientific character will remain engraved in our hearts and our literature. May the earth be light to you.

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Figures

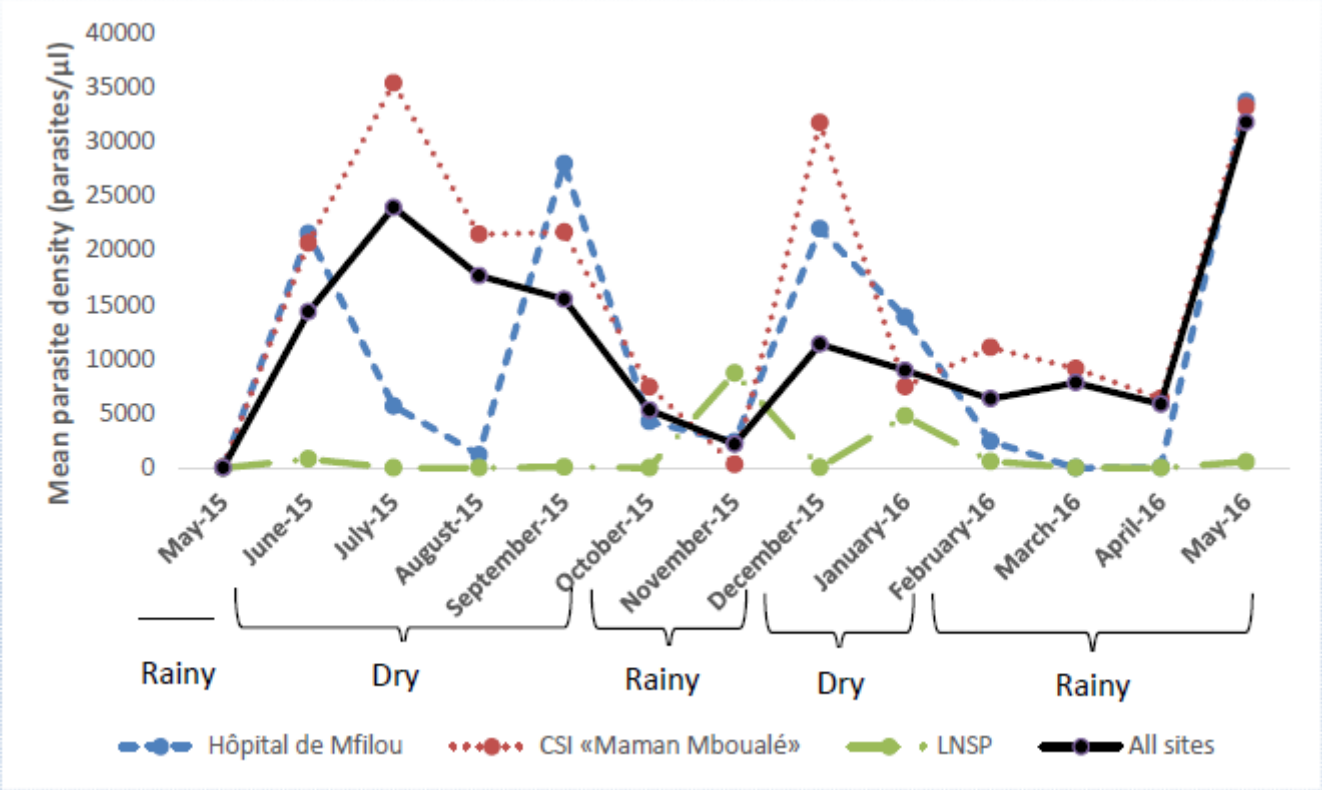


Figure 1

Variation of mean parasite density throughout the year

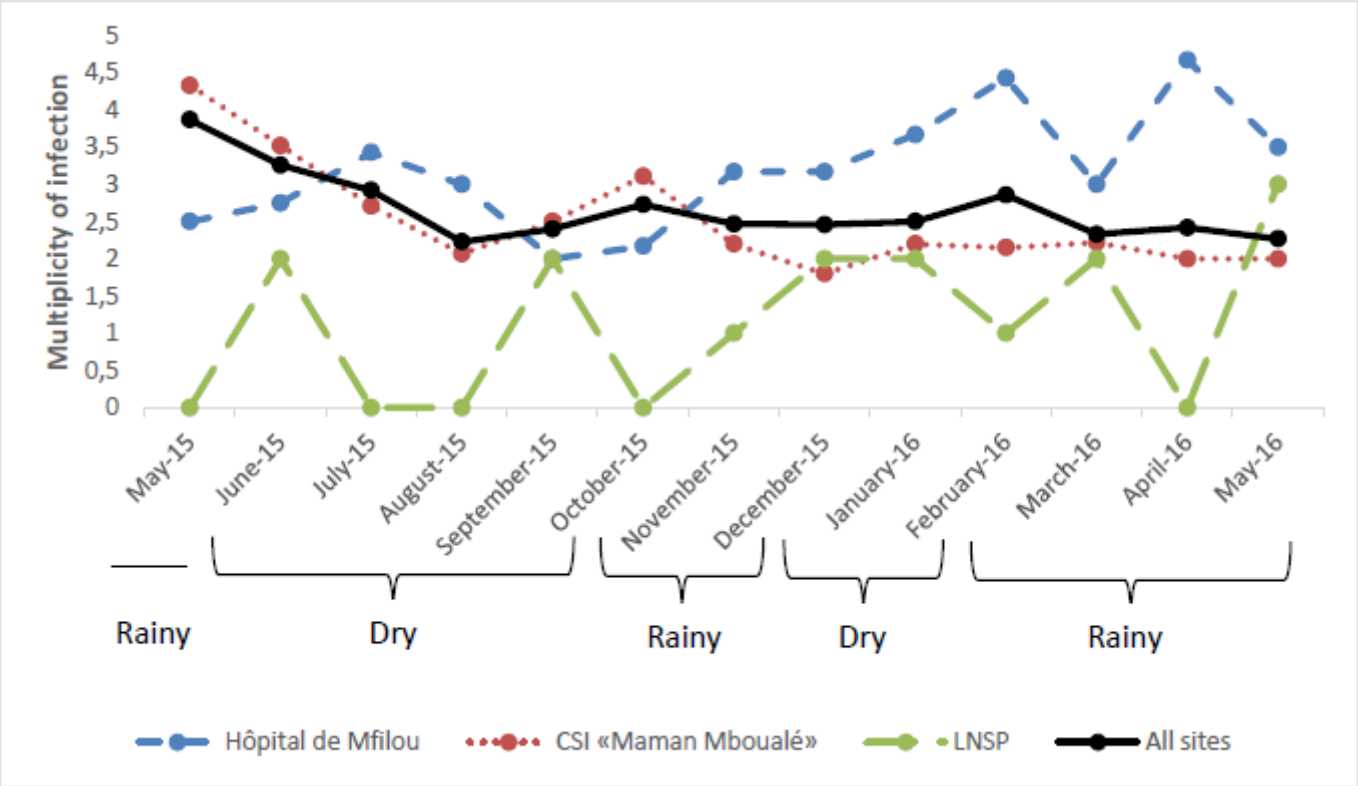


Figure 2

Variation of multiplicity of Plasmodium falciparum infection throughout the year