Seasonal Depiction of Malariometric Indices in Children under Five Years in a Sudanese Semi-Urban Area of Burkina Faso

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Authors’ contributions

This work was carried out in collaboration among all authors. Authors SBS, ABT, AO, INO and AD designed the study and wrote the protocol. Authors AD and INO managed the analyses of the study. Author BS managed the data. Authors SMO, ABT and AO contributed in data collection. Authors SMO and MV performed the statistical analysis and wrote the first draft of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Malariometric indices are essential for the assessment of both new therapies and control strategies. As part of the characterization of a new malaria clinical trial site, this study was carried out to assess malariometric indices during the two seasons of a Sudanese area of Burkina Faso, in children aged under five years.

Study Design: Two community-based cross-sectional surveys were conducted as follow: the first during the rainy season of 2009 and the second during the following dry season. Socio-
1. INTRODUCTION

Global control efforts have led to a very significant decrease in the burden of malaria in the past decade. According to the World Health Organization (WHO), the number of malaria episodes increased from 233 million in 2000 to 244 million in 2005 but declined to 225 million in 2009. From 2000 to 2009 the number of deaths due to malaria decreased from 985 000 to 781 000 [1,2,3]. About 88% of this global burden was reported from Sub-Saharan Africa [1]. In Burkina Faso, about 5 million RDT (Rapid diagnosis Test) confirmed malaria episodes were reported in 2014 and more than half was concerning the under-five years old [4,5]. In 2012, the national statistics on malaria reported an increase in the cumulative incidence of malaria among the under-fives, going from 657‰ in 2008 to 1125‰ episodes in 2012 [6,4]. This trend is contradicting with the global decrease of malaria but should be taken with caution because of possible over-diagnosis or over-report of malaria by the health practitioners, as reported by some studies in Africa [7,8,9]. To have a better understanding of the national epidemiological level, more community-based malariometric surveys covering all seasons are needed. Malariometric indices are essential measures for malaria control programs or malaria trial sites for forthcoming studies [10,11,12,13,14,15]. It is precisely in the perspective of assessing a malaria vaccine candidate in a new trial site that the present study was conducted, as part of a set of field preparation activities. Malariometric surveys are thus considered as a prerequisite for the establishment of new malaria trial site as they provide useful baseline information and could guide on activities planning. Furthermore, by assessing the same indices during rainy season and dry season, this study allowed to quantify the gap between seasons and highlighted the residual level of the disease during dry season, which is somewhat understudied.

2. METHODS

2.1 Study Setting

The study was conducted in a semi-urban area of the health district of Banfora (latitude 10°37’59” North, longitude 4°46’00” West and altitude 299m above sea level), specifically in Bounouna and Nafona, located at about 465 km south west from the capital Ouagadougou (Burkina Faso). The pattern of the climate is southern Sudanese, characterized by a rainy season from April to October and a dry season from November to March. It is one of the most wet area in the country with an average rainfall between the isohyets 1000-1200 mm per year and a large hydrographic network. The average annual temperature ranges from 17 to 36°C. The vegetation is abundant with a lot of trees and a strong presence of a grassy carpet [16]. In 2010, the population of the health district of Banfora was estimated at 291,895 inhabitants, of which 64.4% lived in rural area and children under-five years accounted for about 19.0%. Like the rest of the country, the inter-census (1996 – 2006) growth rate was estimated at 3.1% per year in 2010 and 44.0% of the population lived below the national poverty line estimated in 2009 at XOF 108,454 (about 165 euros) per year. Most houses have clay walls with thatched roofs and most of the population lives by subsistence...
farming. Malaria is seasonal in the area and is responsible of about 43.0% of consultations. Children under 5 years old bear 42.0% of this morbidity and 70.0% of malaria mortality [17]. *Plasmodium falciparum* (*P.f*) is responsible of some 90% malaria cases and the main malaria vectors are *Anopheles gambiae*, *An. arabiensis* and *An. funestus* [16,18,19].

### 2.2 Study Participants

Participants to this study were children aged up to 5 years, living in households located in the study area (Bounouna or Nafona) and who had not received any malaria chemo-prevention or any presumptive malaria treatment in the past 28 days. Out of the 271 households present in the future malaria vaccine study site, 230 were selected based on their proximity (within an hour of walk) to the nearest health facility and were invited to attend the survey sessions with their under-five children. A sample size of 656 participants was enough to provide a confidence level of 99% with 5% margin of error given that the under-five population size was 55460 in the district and the response distribution (parasitemia prevalence) considered as 50%. All the children from voluntary parents/guardians who attended the survey were screened. The same selection process was applied to each survey (rainy and dry season).

### 2.3 Study Design and Data Collection

We conducted an observational study which involved two cross-sectional surveys of which, one during rainy season (September 2009, 10th-15th) and the other during dry season (March 2010, 16th-20th). The same parameters were assessed during each survey and are described later in this manuscript. Before the beginning of the study, meetings were arranged with the population in the study area to explain the objectives and the procedures of the study and answer resident's questions. During each survey, selected households were visited and all their children under-five year were surveyed. The child's parent/guardian was interviewed to collect socio-demographic data and medical history or current symptoms if any. A brief clinical examination including tympanic temperature and abdominal palpation for spleen assessment was performed by a physician. A finger prick blood sample was collected from each participant to perform malaria blood smears and measure the hemoglobin level. A malaria RDT (Rapid Diagnostic Test, Optimal-ITH DiaMed Basel, Switzerland) was performed in case the child was feverish (Temperature≥38°C or reported history of fever in the last 24 hours) and RDT positive cases were treated with Artemether-lumefantrine. Malaria or any other diagnosed illness was treated in accordance with the local standard of care.

### 2.4 Lab Methods

Thick and thin blood films were prepared on the same slide and air-dried. The thin blood film was fixed with methanol and the slide stained with 3% Giemsa solution. Two competent readers (as confirmed by the external quality control carried out thrice a year by the College of American Pathology Proficiency testing) read independently each slide for parasite density determination and *Plasmodium* species identification. A slide was considered as negative only after reading of a hundred high power fields. For positive slides, the number of parasites and leucocytes were counted to reach 200 leucocytes. The assumed WBC (White Blood Cell) count of 8,000/µl was used to calculate the parasite density. The average of the parasite density of the two first readings was calculated as result if there was no discrepancy (difference of species, positive-negative readings or ratio of the two-parasite densities >1.5 or <0.67 [15]). In case of discrepancy, a third independent reader was required and the two most concordant results were considered. Hemoglobin level was measured using a HemoCue® machine (Angelholm, Sweden).

### 2.5 Definition of Malariometric Indices

A *positive parasitemia* was any blood film reading reporting a result of asexual parasite density ≥ 1 trophozoite / μl of blood, irrespective of the parasite species. The parasitemia prevalence was then defined as the number of people with a positive parasitemia over the total number of participants. The proportion of each species in the total positive parasitemia was also computed.

*Gametocytemia* was considered positive if any sexual stage of malaria parasite was reported in the result, irrespective of the parasite species. The gametocytemia prevalence was then defined as the number of people with a positive gametocytemia over the total number of participants.

*Anemia* was considered as a hemoglobin level of < 11 g/dl. This include all grades anemia, from
mild to severe [20]. The prevalence of anemia was then defined as the number of people with anemia over the total number of participants.

Splenomegaly was any palpable enlarged spleen reported by clinical examination. The prevalence of splenomegaly was then defined as the number of people with splenomegaly over the total number of participants.

2.6 Statistical Methods and Data Management

For analysis purpose, participants were split into 2 age groups. An infant was considered as a child aged >24 – 59 months and nurslings were those aged 0 - ≤24 months. Where possible, mean, standard deviation, median, min and max were evaluated for any continuous variable. Proportions and 95%CIs were evaluated for categorical variables. Hypothesis testing for proportion using a Z-test was performed for the bivariate comparison of the malariometric indices between seasons. A Student t-test was used to compare the mean ages according seasons. The geometric mean of parasite density among infected people was calculated as the exponential of the mean of the log transformed positive parasitemia (considering only asexual forms of P. falciparum). The 95% CI was calculated by using:

$$CI = e^{\frac{GM \pm 1.96 \times sd \times (log(x))}{\ln(\text{mean}(x))}}$$

With GM the geometric mean of x, sd the standard deviation of x. The CI is the exponential of the margin of error computed from the standard deviation of the log transformed positive parasitemia [21]. The comparison of the parasite density between the two seasons was performed using the Wilcoxon Rank-Sum test with a continuity correction as the distribution of parasitemia is not homogeneous with the normal distribution. Medians of the hemoglobin level according to seasons were compared using a Bootstrap distribution. We performed, by repeatedly sampling with replacement from our original sample, 1000 random samples of the same size than the original sample. Then, we calculated a 95% confidence interval for the difference in medians of hemoglobin level according to seasons. Multiple logistic model was used to assess the association of positive parasitemia, gametocytemia and anemia with factors such as age-categories, sex, season and mosquito net usage. Asexual parasitemia in categories was also included as a factor in the model of gametocytemia and anemia. A general linear model of the parasite density, after a log-transformation of individual positive parasite density, was used to assess factors associated with the intensity of parasite density (as a continuous variable) and explain the severity of infection. All tests were two-tailed and the significance level was set at 5%. The data were recorded on paper source, double entered into a Microsoft Access database, controlled and cleaned on Excel and analyzed using the software R version 3.2.3.

3. RESULTS

3.1 Study Population Characteristics

A total of 692 participants (47.7%) during rainy season and 760 (52.3%) during dry season were included. The mean age for the whole study population was 29.9 months (standard deviation (sd) = 15.99); it was 30.4 months (sd = 15.65) in rainy season versus 29.4 months (sd = 16.28) in dry season. The difference in age means between seasons (0.98 month) was not significant (p = 0.2403). The infants (aged >24 – 59 months) were slightly more represented in each survey while their proportions were similar between the seasons (p value = 0.1096). The sex ratio was roughly equal to 1 and comparable between both surveys (1.1 in rainy season vs 1.0 in dry season). The Table 1 summarizes the socio-demographic characteristics of the study population.

3.2 Comparison of the Malariometric Indices According to Season

P. falciparum was identified in all positive blood films and was mixed with P. malariae in 3.1% of the infections. Only one case of P. ovale was diagnosed in mixed infection with P. falciparum. The parasitemia prevalence was 55.2% (95% CI = [51.5, 58.9]) in rainy season against 23.3% (95% CI = [20.2, 26.4]) during dry season (p< 0.0001). The geometric mean of parasite density was 3439 trophozoites falciparum (tf)/ µl of blood (95% CI = [2850, 4148]) during rainy season and 1368 tf / µl in dry season (95% CI = [1094, 1710]). The comparison of the parasite densities between seasons showed a significant difference (p = 2.879 e-10). The gametocytemia prevalence was 21.7% (95% CI = [18.6, 24.8]) during rainy season while it was 6.5% (95% CI = [4.7, 8.3]) in dry season and that difference was significant (p< 0.0001). The splenomegaly prevalence was
Table 1. Socio-demographic characteristics of study population

<table>
<thead>
<tr>
<th>Demographic characteristics</th>
<th>Rainy season (N = 692)</th>
<th>Dry season (N = 760)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>n</td>
<td>%</td>
<td>n</td>
</tr>
<tr>
<td>Male</td>
<td>367</td>
<td>53.0</td>
<td>381</td>
</tr>
<tr>
<td>Female</td>
<td>325</td>
<td>47.0</td>
<td>379</td>
</tr>
<tr>
<td>Age group</td>
<td>n</td>
<td></td>
<td>n</td>
</tr>
<tr>
<td>Nursling</td>
<td>279</td>
<td>40.3</td>
<td>338</td>
</tr>
<tr>
<td>Infant</td>
<td>413</td>
<td>59.7</td>
<td>422</td>
</tr>
<tr>
<td>Usage of Mosquito net</td>
<td>n</td>
<td></td>
<td>n</td>
</tr>
<tr>
<td>Use</td>
<td>380</td>
<td>55.2</td>
<td>503</td>
</tr>
<tr>
<td>Don’t use</td>
<td>309</td>
<td>44.8</td>
<td>245</td>
</tr>
</tbody>
</table>

n = number; % = percentage; 12 missing values for “usage of mosquito net”, 3 in rainy season and 12 in dry season

11.1% (95% CI = [8.8, 13.5]) in rainy season against 4.2% (95% CI = [2.7, 5.6]) in dry season (p< 0.0001). The estimated drop in hemoglobin level in rainy season is about 1 g/dl, considering the median of hemoglobin level in each season (95% CI for the difference between the 2 seasons is [0.7, 1.2] g/dl, in favour of dry season). The anemia prevalence was 90.0% (95% CI = [87.8, 92.3]) during rainy season versus 70.6% (95% CI = [67.3, 73.8]) in dry season (p < 0.0001).

The Fig. 1 shows the hemoglobin level per age group and season.

The age and sex specific malariometric indices are presented in Table 2.

3.3 Factors Associated with Malaria Parasite Carriage

Regression analysis showed that significant risk factors associated to malaria parasite carriage were the rainy season, the nursing age group and the non-usage of insecticide treated net (ITN) (Table 3). Adjusting for age group, usage of insecticide treated net and sex, the odds of carrying malaria parasite was 4 times higher during rainy season compared to dry season (95% CI OR = [3.1, 5.0]).

Considering the seasons separately, the logistic analysis showed that the non-use of ITN was a significant factor for malaria parasite carriage in rainy season (p = 0.0006) but not in dry season (p = 0.6897). In rainy season, the children who were not using ITN were 1.7 times more likely to carry malaria parasite than those who were using ITN (OR 95% CI = [1.2, 2.4]); during dry season, there was no difference between the two categories (OR 95% CI = [0.63, 1.34]).

3.4 Factors Associated with Gametocyte Carriage

Age group, sex and the usage of Gametocyte carriage were not statistically significant predictors of the gametocyte carriage (Table 3). Gametocyte carriage was more prevalent in rainy season (95% CI OR = [1.6, 3.5]) compared to dry season and was associated to asexual parasite carriage (Table 3).
Table 2. Summary of maliometric indices

<table>
<thead>
<tr>
<th>Categories</th>
<th>PP (%(N))</th>
<th>GP (%(N))</th>
<th>AP (%(N))</th>
<th>SP (%(N))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RS</td>
<td>DS</td>
<td>RS</td>
<td>DS</td>
</tr>
<tr>
<td>Male</td>
<td>59.0 (354)</td>
<td>25.0 (359)</td>
<td>23.3 (355)</td>
<td>6.4 (359)</td>
</tr>
<tr>
<td>Female</td>
<td>51.0 (323)</td>
<td>21.6 (361)</td>
<td>19.8 (322)</td>
<td>6.6 (361)</td>
</tr>
<tr>
<td>Nursling</td>
<td>40.3 (268)</td>
<td>17.2 (313)</td>
<td>15.5 (270)</td>
<td>5.7 (313)</td>
</tr>
<tr>
<td>Infant</td>
<td>65.0 (409)</td>
<td>28.0 (407)</td>
<td>25.8 (407)</td>
<td>7.1 (407)</td>
</tr>
<tr>
<td>Use ITN</td>
<td>48.2 (371)</td>
<td>23.6 (478)</td>
<td>19.1 (370)</td>
<td>6.2 (478)</td>
</tr>
<tr>
<td>Don't Use ITN</td>
<td>64.1 (304)</td>
<td>23.3 (231)</td>
<td>24.9 (305)</td>
<td>7.3 (231)</td>
</tr>
<tr>
<td>Total</td>
<td>55.2 (677)</td>
<td>23.3 (720)</td>
<td>21.7 (677)</td>
<td>6.5 (720)</td>
</tr>
</tbody>
</table>

N= total number of computed observations; % = percentage; PP = Parasitemia Prevalence; GP = gametocytaemia Prevalence; AP = Anaemia Prevalence; SP = Splenomegaly Prevalence; RS = High Transmission Season (rainy season); DS = Low Transmission Season (dry season); ITN = Insecticide treated net. Missing values: 55 parasitemia (40 in DS and 15 in RS), 55 gametocytaemia (40 in DS and 15 in RS), 16 haemoglobin level (8 in each season) and 3 values in RS for splenomegaly were missing and not included in this Table2.
3.5 Factors Associated with Anaemia

Malaria parasite carriage, age group, sex and season were significant predictors for anaemia (p<0.001). The odds of being anemic was 2.24 times higher in malaria parasite carriers compared to those who were parasite free, adjusting for season, sex, usage of ITN and age group (OR 95% CI = [1.61, 3.15]). Also, adjusting for other covariates, anemia was 3.11 times more likely to be found in nurslings compared to infants (OR 95% CI = [2.29, 4.29]). The Table 3 summarizes the results of all the logistic analysis.

The Fig. 2 illustrates the Odds ratios of the 3 logistic regressions.

3.6 Factors Associated with Malaria Parasite Density

A general linear model for malaria parasite density showed that only the age group and the season were significantly associated to the variation of parasite density (Table 4). Adjusting for age group, ITN use and sex, the parasite density was significantly higher in rainy season compared to dry season. The Tables 4 and 5 summarizes the relationship of parasite density with these factors and provides the adjusted least square mean of log transformed parasite density for each category.

4. DISCUSSION

Malariometric indices are essential data in a field preparation process for a malaria vaccine candidate trial. Unlike many studies that focused on high transmission period (rainy season) only, this study described the indices in both seasons and added evidence to malaria seasonality in the country. More, this study revealed a residual level of malaria in dry season that was not as negligible as it was often considered. Despite the limit of convenience sample that may suffer this kind of study, our sample size was large enough and our results could be generalized to area with similar environmental conditions.

4.1 Malaria Seasonality

In Burkina Faso, malaria seasonality is unanimously shared and highlighted in many studies [11,15,14,22]. Anyway, the seasonal malaria chemoprevention in children under five, currently applied in the country is based on that assumption [23]. Our findings are adding evidence to malaria seasonality at the country level, by juxtaposing the malariometric indices measured during rainy and dry season. Indeed, all the indices in rainy season were at least twice higher than those in dry season and these differences were statistically significant. Moreover, when adjusting for other covariates (age group, sex and use of ITNs), the odds of parasite carriage for instance was 3 – 5 times higher in rainy season compared to dry season. The rainy season offers ideal environmental conditions for mosquitoes to breed whereby resulting in a high vector density, which widely participate in the transmission of the disease.

4.2 Parasitemia Prevalence and Classification of Endemicity

Parasitemia prevalence at community level is widely considered as the standard marker of malaria endemicity [24,12]. As per the endemicity classification Table used by WHO [25], our result qualifies the study site as a high transmission area with hyperendemic and seasonal malaria. In fact, the parasitemia prevalence in rainy season in our study site was above the 50% required to admit this level of endemicity. Moreover, if we consider only the infant’s group (age > 24 months), which better corresponds to the age range required for the endemicity assessment, the parasitemia prevalence was about 65% in rainy season and largely satisfies the hyperendemic malaria criteria. However, the parasite prevalence in dry season (about 20 – 26%) falls in the range of meso-endemic area and confirms the seasonality of the disease. These results corroborate those of some malariometric studies conducted in similar geographical areas [14,26,27,28].

4.3 Geometric Mean of Parasite Density

The geometric mean of parasite density (3439 tf / µl in rainy season vs 1368 tf / µl in dry season) was relatively high compared to numerous studies in west Africa [29,22,30]. However, these values were comparable to those reported by Ouedraogo et al. in Saponé (BF) [14]. This high geometric mean of parasite density may be explained by the age range of our study population (under-five). In malaria endemic area, immunity against malaria is described to be fully acquired and effective from the fifth year of life and the effect of immunity on parasite density is documented [31]. Other factors like the multiplicity of infections or parasite genetical diversity, as mentioned in several studies [32,33], may also explain our results, given the difference in parasite density between season that we observed in our sample.
### Table 3. Logistic models of malaria parasite carriage, gametocyte carriage and anaemia

<table>
<thead>
<tr>
<th>Analysis description</th>
<th>Variables</th>
<th>Estimate</th>
<th>OR</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Logistic model of</td>
<td>Intercept</td>
<td>- 0.85</td>
<td>0.42</td>
<td>0.33</td>
<td>0.53</td>
</tr>
<tr>
<td>malaria parasite</td>
<td>Season (RS)</td>
<td>1.38</td>
<td>3.98</td>
<td>3.14</td>
<td>5.05</td>
</tr>
<tr>
<td>carriage</td>
<td>Age group (Nursling)</td>
<td>- 0.81</td>
<td>0.44</td>
<td>0.34</td>
<td>0.56</td>
</tr>
<tr>
<td></td>
<td>ITN (Don't Use)</td>
<td>0.29</td>
<td>1.33</td>
<td>1.05</td>
<td>1.69</td>
</tr>
<tr>
<td></td>
<td>Sex (Female)</td>
<td>- 0.22</td>
<td>0.79</td>
<td>0.63</td>
<td>1.01</td>
</tr>
<tr>
<td>Logistic model of</td>
<td>Intercept</td>
<td>- 3.23</td>
<td>0.03</td>
<td>0.02</td>
<td>0.06</td>
</tr>
<tr>
<td>gametocyte carriage</td>
<td>Season (RS)</td>
<td>0.88</td>
<td>2.40</td>
<td>1.66</td>
<td>3.51</td>
</tr>
<tr>
<td></td>
<td>Age group (Nursling)</td>
<td>- 0.18</td>
<td>0.83</td>
<td>0.58</td>
<td>1.18</td>
</tr>
<tr>
<td></td>
<td>Malaria parasite (present)</td>
<td>1.63</td>
<td>5.12</td>
<td>3.53</td>
<td>7.53</td>
</tr>
<tr>
<td></td>
<td>ITN (Don't Use)</td>
<td>0.12</td>
<td>1.12</td>
<td>0.80</td>
<td>1.56</td>
</tr>
<tr>
<td></td>
<td>Sex (Female)</td>
<td>- 0.03</td>
<td>0.96</td>
<td>0.69</td>
<td>1.34</td>
</tr>
<tr>
<td>Logistic model of</td>
<td>Intercept</td>
<td>0.52</td>
<td>1.69</td>
<td>1.27</td>
<td>2.25</td>
</tr>
<tr>
<td>anaemia</td>
<td>Season (RS)</td>
<td>1.14</td>
<td>3.14</td>
<td>2.29</td>
<td>4.34</td>
</tr>
<tr>
<td></td>
<td>Age group (Nursling)</td>
<td>1.13</td>
<td>3.11</td>
<td>2.29</td>
<td>4.29</td>
</tr>
<tr>
<td></td>
<td>Malaria parasite (present)</td>
<td>0.80</td>
<td>2.24</td>
<td>1.61</td>
<td>3.15</td>
</tr>
<tr>
<td></td>
<td>ITN (Don't Use)</td>
<td>0.06</td>
<td>1.06</td>
<td>0.79</td>
<td>1.44</td>
</tr>
<tr>
<td></td>
<td>Sex (Female)</td>
<td>- 0.51</td>
<td>0.59</td>
<td>0.44</td>
<td>0.79</td>
</tr>
</tbody>
</table>

OR: odds ratio, 95%CI: 95% Confidence Interval for the Odds Ratio

**Fig. 2. Odds ratios plot of the logistic models of malaria parasite carriage, gametocyte carriage and anemia**

In the legend:
- Factors name starting by “P_...” are factors included in the model of malaria parasite carriage
- Factors starting by “G_...” are factors included in the model of gametocyte carriage
- Factors starting by “A_...” are factors included in the model of anaemia
### Table 4. General linear model for parasite density

|                      | Estimate | Std. Error | t value | Pr(>|t|) |
|----------------------|----------|------------|---------|----------|
| (Intercept)          | 7.26     | 0.16       | 42.86   | < 2e-16  |
| Age group (Nursling) | -0.40    | 0.16       | -2.42   | 0.0156   |
| Sex (female)         | 0.13     | 0.15       | 0.91    | 0.3632   |
| ITN (Don't use ITNs) | 0.03     | 0.15       | 0.19    | 0.8448   |
| Season (RS)          | 0.91     | 0.16       | 5.55    | 4.34e-08 |

Std. Error: Standard error; lsmean: least square mean; asymp.LCL: asymptotic lower confidence limit; asymp.UCL: asymptotic upper confidence limit; RS: rainy season; DS: dry season

### Table 5. Adjusted least square mean of log transformed parasitemia

| Socio-demographic Categories | lsmean | asymp.LCL | asymp.UCL | Pr(>|t|) |
|-------------------------------|--------|----------|----------|---------|
| Season DS                     | 7.1    | 6.9      | 7.4      | 4.34e-08|
| Sex Male                      | 7.5    | 7.3      | 7.8      | 0.3632  |
| Usage of Use ITNs             | 7.6    | 7.4      | 7.8      | 0.8448  |
| Age group Infant              | 7.8    | 7.6      | 8.0      | 0.0156  |
| ITN Don't Use ITNs            | 7.6    | 7.4      | 7.9      |         |
| Age group Nursling            | 7.4    | 7.1      | 7.7      |         |

Std. Error: Standard error; lsmean: least square mean; asymp.LCL: asymptotic lower confidence limit; asymp.UCL: asymptotic upper confidence limit; RS: rainy season; DS: dry season

### 4.4 Gametocytemia Prevalence

The prevalence of gametocyte carriers was high, mostly in the wet season and marked by a significant seasonal variation (18-25% in rainy season vs 4-8% in dry season). These findings corroborate those of several studies [34,35,14]. The age-range of our study population (<5 years), the prevalence of the asexual forms of the parasite and the high parasite densities could explain this high prevalence that is observed. Moreover, the difference in gametocytemia prevalence between seasons highlights the association of this indices with the season (95% CI OR = [1.6, 3.5]) and carriage of the asexual forms (95% CI OR = [3.5, 7.5]). Although gametogenesis remains a poorly understood phenomenon, several studies seem to agree that parasite density, wet season, age and immune mechanisms are the most crucial factors [36,34]. Given that even sub-microscopic gametocyte reservoir could sustain malaria transmission [37], we could imagine that the gametocytemia prevalence found in our study is just the visible part of a huge transmission level.

### 4.5 Splenomegaly Prevalence

With respect to the high parasitemia prevalence hence the endemicity level observed on this site, the splenomegaly prevalence was relatively low (about 11% in rainy season and 4% in dry season). Similar values have been reported in other studies conducted in the sudano-sahelian band [14,28,38]. Spleen enlargement is mainly due to chronic exposure to malaria infection. In our study area which was semi-urban, the proximity of local health facilities and the availability of effective and affordable antimalarial drugs may have reduced the frequency of chronic parasite carriage, thus explaining our low values. Long before plasmodia were known to cause malaria, the splenomegaly prevalence was used as an indirect measure of the risk of the disease [39]. Some decades ago, it was the reference measure used to determine the level of malaria endemicity [25]. However, the indirect nature and the great variability of this indices according to the stability of malaria, the level of urbanization, the access to antimalarial treatment and the immunity could explain why it is no more reflecting the real endemicity level; it is nowadays abandoned in favor of more direct measures such as the parasitemia prevalence.

### 4.6 Anaemia Prevalence

Our findings highlighted the strong association between malaria and childhood anemia in the area. From dry to rainy season, children loose roughly 1 g/dl in hemoglobin level (95% CI: [0.774, 1.226]). Adjusting for age, season, sex and use of ITNs, anemia was 1.6 - 3.1 times more frequent in parasite carriers compared to
those who were parasite free. The association between anemia and parasite carriage has been demonstrated in several studies [40,41]. Malaria is a major cause of anemia which is common in African children. However, nutritional deficiencies, intestinal helminthiasis, repeated bacterial infections are considerable contributing factors [42]. Reciprocally, an underlying pre-existing anemia is a major worsening factor for malaria and its evolution towards fatal outcome.

5. CONCLUSION

This study provides further evidence in favour of malaria seasonality in the country. Despite the announced decline of its burden, malaria was still hyper-endemic and highly seasonal in the Sudanese semi-urban area of Burkina Faso in 2010. Although the indices showed that the disease is more prevalent in wet season, the estimated magnitude in dry season is significant and constitute unfortunately an important reservoir of parasite for the next wet season. Wherefore, there is a need to develop strategies to target dry season malaria, to break the chain of transmission of the disease.

CONSENT AND ETHICAL APPROVAL

This study was approved by the National Ethical Committee of Burkina Faso (Deliberation N°: 2009-36). Before the study start, community permission was obtained through meetings with the population. Written informed consent have been obtained from parents/guardians of children who participated, before any study procedure.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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