

Gametocytic Carriage of *Plasmodium Falciparum* in Asymptomatic Schoolchildren in Côte D'ivoire

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ABSTRACT

Asymptomatic carriers of *Plasmodium falciparum* are considered to be the parasite's reservoir in humans. They may therefore be at the origin of the expansion of chemoresistant plasmodial strains. The aim of this study was to determine the proportion of *P. falciparum* gametocyte carriage in asymptomatic children in Côte d'Ivoire. This cross-sectional study was carried out from May 2015 to April 2016 in 3 rural and urban schools in Grand-Bassam, Abengourou and San Pedro during the rainy and dry seasons. In each school, 11 pupils/class were drawn at random. Each pupil included was asked to complete a questionnaire. A venous blood sample was then taken in an EDTA tube for mixed smears. We obtained 29.5% asymptomatic carriers of *P. falciparum*. The average age was 9.4 ± 2.5 years. Gametocyte density ranged from 8 to 48 gametocytes/ μ l blood. Gametocytes were detected in both the rainy season (23/52) and the dry season (29/52) in San-Pedro and Abengourou health districts. In Grand-Bassam health district, on other hand, they were found only during the rainy season. All age groups were affected by asymptomatic gametocyte carriage. However, the gametocytic rate of *P. falciparum* varied with the age of the asymptomatic carrier ($p=0.010$). The prevalence of asymptomatic *P. falciparum* gametocyte carriage was 8.70%. The high rate of asymptomatic *P. falciparum* gametocyte carriage suggests that asymptomatic carriers constitute a potential reservoir for malaria transmission. Malaria control strategies in rural and urban areas of Côte d'Ivoire are urgently needed to reduce the burden of disease in vulnerable populations.

Keywords: *Plasmodium falciparum*; Asymptomatic; Gametocyte

Abbreviations: NMCP: National Malaria Control Programme; CNER: National Committee for Research Ethics; SPSS: Statistical Package for Social Sciences; QT-NASBA: Quantitative-Nucleic acid Sequence-Based Amplification

Background

By 2020, 95% of malaria cases (228 million) and 95% of deaths will have occurred in sub-Saharan Africa (World Health Organization and Global Malaria Programme, [1]). Substantial reductions in malaria transmission and morbidity have been reported in many parts of East Africa, due to intensified interventions in the region (Golassa et al. [2]). The percentage of malaria infections among the population at risk in sub-Saharan Africa, fell from 17% in 2010 to 13% in 2015. However, malaria remains a public health problem. Children aged 0-5 and pregnant women are the most vulnerable. In Côte d'Ivoire, malaria is a major public health problem, with around 33% of the population at risk. Malaria transmission remains stable year-round

throughout the country, with recrudescences in the rainy season (National Malaria Control Programme (NMCP)). To combat this endemic, two strategies have been adopted: elimination of the vector agent using insecticides, and the use of antimalarial drugs for both curative treatment and prophylaxis. However, current control interventions are mainly aimed at symptomatic patients, yet cases of asymptomatic malaria are common in endemic areas and generally go untreated (Whittaker, et al. [3]). This situation may result in a significant source of gametocyte carriage, which could serve as a reservoir for malaria transmission (Mvumbi, et al. [4-7]) and may even be responsible for the spread of chemoresistant plasmodial strains within populations. The prevalence of symptomatic and asymptomatic infections in malaria-endemic areas has been well documented (Assoumou, et al

[8-21). However, there are few data on gametocyte carriage in these asymptomatic individuals. The aim of this study was to determine the proportion of *P. falciparum* gametocyte carriage in asymptomatic children in Côte d'Ivoire.

Materials and Methods

Study Sites and Sample Collection

This was a cross-sectional study of malaria infestation carried out from May 2015 to April 2016 in schoolchildren aged 4 to 16 years in the rural and urban areas of Grand-Bassam, Abengourou and San Pedro in the rainy and dry seasons. In each zone, 3 elementary school and 11 pupils per class were randomly selected by drawing lots. A questionnaire was administered to each pupil and parent of a pupil after obtaining informed consent and assent for children over 9 years of age, in order to collect socio-demographic and clinical data. A blood sample was then taken from the elbow in an EDTA tube for thick drop and blood smear analysis. The slides were read by two independent laboratory technicians.

Ethical Aspects

This study was approved by the National Committee for Research Ethics (CNER) under Number 020/MSLS/CNER-dkn. It was conducted in accordance with the Helsinki declaration adopted by the 18th World Medical Assembly in 1964 and its amendments, the ICH (International Conference on Harmonisation) recommendations for clinical studies and the national laws and regulations of Côte d'Ivoire.

Statistical Analysis

Data were entered using Epidata Version 3.1 and Microsoft Excel 2013, then transferred to Statistical Package for Social Sciences (SPSS) 18.0 for statistical analysis. Graphical representations were made using Microsoft Excel 2013. The significance level for statistical tests was set at 0.05. A subject was considered asymptomatic if he or she carried asexual forms of Plasmodium, was non febrile (temporal temperature below 37.8°C) and had no other clinical signs of malaria in the 48 hours before and after the medical visit.

Results

Of a total of 2,361 samples, 1,190 or 50.4% were taken during the rainy season and 1,171 or 49.6% during the dry season. The male/female sex ratio in the study population was 0.9, and the mean age of participants was estimated at 9.4 years (SD 2.512 years). The plasmodic index was 39.9% (943/2361). It was 17.7%, 50.3% and 51.9% respectively in Grand-Bassam, Abengourou and San-Pedro. *P. falciparum* was the species most frequently found in monoinfestation, 95.7% (902/943). The prevalence of *P.falciparum* was 97.1%, 93.1% and 97.6% respectively in Grand-Bassam, Abengourou and San-Pedro (Table 1). Parasite density ranged from 15 to 79,520 trophozoites/ μ L blood, with a mean of 1,593 trophozoites/ μ L blood (standard deviation = 4,442 trophozoites/ μ L blood). Microscopic analysis of mixed smears coupled with clinical diagnosis of subjects revealed 409 (17.3%) symptomatic and 576 (24.4%) asymptomatic subjects out of 1952 (82.7%) subjects without clinical signs of malaria in the general population (2361). The percentage of subjects asymptomatic for *P.falciparum* malaria among those without clinical signs of malaria was therefore 29.5% (576/1952) Tables 2 & 3. In asymptomatic subjects, the mean parasite density was 1,243 trophozoites/ μ L blood (standard deviation 2,262 trophozoites/ μ L blood), with extremes of 15 trophozoites/ μ L and 17,120 trophozoites/ μ L blood. A total of 50 *P. falciparum* gametocytes were detected. The gametocyte-specific rate and gametocyte prevalence were 96.2% and 8.70% (50/576) respectively for *P. falciparum* in asymptomatic carriers. Gametocyte density ranged from 8 to 48 gametocytes/ μ L blood, with a mean of 13.6 gametocytes/ μ L blood and a standard deviation of 10.8 gametocytes/ μ L blood. Gametocytes were detected in both the rainy season (44.23% (23/52)) and the dry season (55.77% (29/52)) in the San-Pedro and Abengourou health districts. In the Grand-Bassam health district, on the other hand, they were only detected during the rainy season (Table 4). All age groups are concerned by asymptomatic gametocyte carriage (Table 5). However, the *P. falciparum* gametocytic rate varied with the age of the asymptomatic carrier ($p=0.010$).

Table 1: Prevalence of plasmodial species detected by light microscopy as a function of site and season.

Site	Season	<i>P. fal</i>	<i>P. mal</i>	<i>P. fal+P. mal</i>	<i>P. ova</i>	<i>P. fal+P. ova</i>	<i>P. fal+P. mal+P. ova</i>	Negative	Total
Grand-Bassam	Rainy	37	0	1	0	0	0	356	394
	Dry	98	1	1	1	0	0	292	393
Abengourou	Rainy	192	0	20	0	0	2	183	397
	Dry	174	0	5	0	0	0	206	385
San-Pedro	Rainy	190	0	7	0	2	0	200	399
	Dry	211	0	0	0	1	0	181	393
Total		902	1	34	1	3	2	1418	2361

Note: *P.fal*: *Plasmodium falciparum*; *P.mal*: *Plasmodium malariae*; *P.ova* : *Plasmodium ovale*

Table 2: Distribution of subjects by clinical status.

Site	Season	Symptomatic	Asymptomatic	Other	Total
Grand-Bassam	Rainy	14	24	356	394
	Dry	34	68	291	393
Abengourou	Rainy	70	152	175	397
	Dry	74	114	197	385
San-Pedro	Rainy	128	81	190	399
	Dry	89	137	167	393
Total		409	579	1376	2361

Table 3: Distribution of non-febrile subjects according to season.

Season	Asymptomatic subjects (%)	Other (%)	Total	p-value
Rainy	257 (26.28)	721 (73.72)	978	0.002
Dry	319 (32.75)	655 (67.25)	974	
Total	576 (29.5)	1376 (70.49)	1952	

Table 4: Prevalence of *P. falciparum* gametocytes as a function of season and locality in non-febrile subjects.

Season	Area	<i>P. falciparum</i> gametocytes		Total	p-value
		Presence	Absence		
Rainy	Grand-Bassam	2(8.33%)	22(91.67%)	24	0.555
	Abengourou	9(5.92%)	143(94.08%)	152	
	San-Pedro	11(13.60%)	70(86.40%)	81	
Total rainy season		22 (8.60%)	235 (91.10%)	257	
Dry	Grand-Bassam	0(0%)	68(100%)	68	0.096
	Abengourou	15(13.16%)	99(86.84%)	114	
	San-Pedro	13(9.49%)	124(90.51%)	137	
Total dry season		28 (8.80%)	291 (90.90%)	319	
Total		50 (8.70%)	526 (91.30%)	576	

Table 5: Prevalence of *P. falciparum* gametocytes as a function of age in asymptomatic carriers.

Age range	<i>P. falciparum</i> gametocytes		Total	p-value
	Presence	Absence		
< 5 years	3 (14.3%)	18 (85.7%)	21	0.010
6-8 years	29 (15.3%)	160 (84.7%)	189	
9-11 years	11 (5%)	211 (95%)	222	
> 12 years	7 (4.9%)	137 (95.1%)	144	
Total	50 (8.7%)	526 (91.3%)	576	

Discussion

The overall plasmodic rate was 39.9%. San-Pedro and Abengourou had roughly the same plasmodic rate. Grand-Bassam, on the other hand, had a lower plasmodic rate. Grand-Bassam is a coastal and lagoon area in the south of Côte d'Ivoire, very close to the economic capital Abidjan, while Abengourou is a forested area in the east of Côte d'Ivoire's cocoa belt. As for San-Pedro, in addition to being a for-

est area, it is also a coastal and lagoon area. Moreover, the practice of lowland rice-growing could explain the higher rate of Plasmodium found in San-Pedro and Abengourou than in Grand-Bassam. Rice paddies are an evolving environment where different types of biotopes, more or less favorable to Anopheles and particularly to Anopheles gambiae, succeed one another [Assi, et al. [22-25]]. Our results indicate that malaria transmission in Côte d'Ivoire is also a function of phytogeographical area. Microscopic examinations showed that *P.*

falciparum was the species responsible for most cases of Plasmodium infestation (97.1%) in Côte d'Ivoire in the present study. These data are similar to those of several studies carried out in Côte d'Ivoire (Assi, et al. [6,22,26-28]) (World Health Organization and Global Malaria Programme, 2017). The Plasmodium index of 29.5% in non-febrile subjects is high compared with that obtained by Assoumou et al. in 2001 in Abidjan (Assoumou, et al. [8]) Koukouikila-koussounda et al. in Congo (Koukouikila-Koussounda, et al. [29]) in 2010 (16.3%), Nzobo et al. in 2015 (Nzobo, et al. [30]) in Tanzania (5.4%), Balogun et al. (Balogun et al. [31]) Nigeria (12.7%) and Vafa et al. in 2008 (Vafa et al. [7]) in Senegal (13.7%).

In contrast, Baliraine et al. (Baliraine, et al. [32]) in Kenya and Pinto et al. in São Tomé et Príncipe (Pinto, et al. [33]) respectively obtained 25.8% and 35.7% of subjects asymptomatic for malaria. These results were very similar to our own. In contrast, Owusu-Agyei et al. and Sumari et al. obtained higher plasmodial indices (57.5%) and 82.5% respectively in asymptomatic subjects in Tanzania in 2014 and Ghana in 2002 (Owusu, et al. [6,34]). The plasmodic rate obtained in the dry season (32.75) was significantly higher than that obtained in the rainy season (26.28). During periods of low transmission, most infections are asymptomatic, as population immunity is high during this period (Lindblade et al. [13]). Portugal et al. had obtained 26.3% at the dry-season thick drop in Mali in 2013 (Portugal et al. [15]). The prevalence of sexed forms of *P. falciparum* was determined in order to assess the impact of asymptomatic carriage of *P. falciparum* gametocytes on malaria transmission in Côte d'Ivoire. The gametocyte index was 96.2% for *P. falciparum*. This demonstrates that *P. falciparum* is the endemic species in Côte d'Ivoire.

The prevalence of *P. falciparum* gametocyte carriage was 8.95% and 9.09% in the rainy and dry seasons respectively. There was no significant difference between the gametocyte rate obtained in the dry and rainy seasons ($p=0.555$ and $p=0.096$). However, they reveal that gametocyte carriage is not related to the intensity of *P. falciparum* transmission. These results are in line with those found in 2010 by Balogun et al. in Nigeria (8.6%) in subjects aged 3-12 years (Balogun et al. [31]). The age of the asymptomatic subject was cross-referenced with the gametocytic index to determine its influence on asymptomatic gametocyte carriage. Gametocytes were found in all age groups. However, the proportion of gametocytes found in subjects over 5 years of age was highest ($p=0.010$). Our results concur with those obtained by some authors in Haiti between 2010 and 2013 respectively (Raccurt et al. [35]) in Mali in 2012 (Adomako-Ankomah, et al. [36]) and in Malawi between 2012 and 2013 (Coalson, et al. [37]). However, Ouedraogo et al, in a study carried out in Burkina Faso in 2003 using the QT-NASBA (Quantitative-Nucleic acid Sequence-Based Amplification) technique, found that the prevalence of gametocytes decreased with the age of the subject (Ouedraogo et al. [38]). Natama et al. found that median gametocyte density was significantly higher in asymptomatic infections than in clinical episodes in a study carried out in Nanoro, Burkina faso, between 2014 and 2015 (Natama et al.

[39,40]). The difference observed would be due to the sensitivity of the QT-NASBA technique (more sensitive) for gametocyte detection.

Conclusion

Côte d'Ivoire is a hotbed of asymptomatic *P. falciparum* gametocyte carriage in children. High rates of asymptomatic infection and asymptomatic carriage of *P. falciparum* gametocytes suggest that asymptomatic carriers are a potential reservoir for malaria transmission. Preventive efforts in rural and urban areas of Côte d'Ivoire are needed to reduce morbidity in vulnerable populations.

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Author's Contribution

Akpa Paterne Gnagne wrote the protocol, William Yavo coordinated the protocol ethics approval process, designed and supervised the field data collection. Akpa Paterne Gnagne carried out the statistical analysis and interpretation of the results. Akpa Paterne Gnagne drafted the initial version of the manuscript; Abibatou Konaté-Touré and Akoua Valérie Bédia-Tanoh revised the manuscript.

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