Assessment of the relationship between gametocyte density, multiplicity of infection and mosquito infectivity in Plasmodium falciparum infections

Abdoulie Touray
PAUSTI

Victor Atunga Mobegi (vmobegi@gmail.com)
University of Nairobi https://orcid.org/0000-0002-1962-5583

Fred Wamunyokoli
Jomo Kenyatta University of Agriculture and Technology

Hellen Butungi
International Centre for Insect Physiology and Ecology

Jeremy K. Herren
International Centre for Insect Physiology and Ecology

Research

Keywords: P. falciparum, asymptomatic, gametocyte density, MOI, mosquito infection rates, Mbita

Posted Date: June 9th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-33602/v1

Assessment of the relationship between gametocyte density, multiplicity of infection and mosquito infectivity in *Plasmodium falciparum* infections

Abdoulie O. Touray[*1,4] , Victor A. Mobegi[*2] , Fred Wamunyokoli[3], Hellen Butungi[4,5], and Jeremy K. Herren[*4]

Address: [1]Department of Molecular Biology and Biotechnology, Institute of Basic Sciences, Technology and Innovation, Pan African University (PAUSTI), Nairobi, Kenya, [2]Department of Biochemistry, School of Medicine, University of Nairobi, Nairobi, Kenya, [3]Department of Biochemistry, Jomo Kenyatta University of Agriculture and Technology (JKUAT), Nairobi, Kenya, [4]International Centre of Insect Physiology and Ecology (*icipe*), Nairobi, Kenya and [5]Wits Research Institute for Malaria, School of Pathology, Faculty of Health Sciences, University of the Witswatersrand, Johannesburg, South Africa

Email: Abdoulie O. Touray* - abdoulietouray79@gmail.com; Victor A. Mobegi* - vatunga@uonbi.ac.ke; Fred Wamunyokoli - fwamunyokoli@jkuat.ac.ke; Hellen Butungi - hbutungi@gmail.com; Jeremy K. Herren* - jherren@icipe.org

* Corresponding authors.

Abstract

Background: Malaria is a major public health threat in sub-Saharan Africa. Asymptomatic *P. falciparum* gametocyte carriers are potential infectious reservoirs for sustaining transmission in many malaria endemic regions. The aim of the study was to assess the prevalence of gametocyte carriage and some of its associated risk factors among asymptomatic schoolchildren (age 5-15 years) in Mbita, western Kenya and further analyse the association between gametocyte density, multiplicity of infection (MOI) and mosquito infectivity.

Methods: Rapid diagnostic test (RDT) was used to screen for *P. falciparum* parasite infection among asymptomatic schoolchildren (5-15 years old) residing in Mbita, Western Kenya and the results were further verified using microscopy. Participants positive for *P. falciparum* infection were further screened for gametocyte carriage and those positive were used to feed laboratory

reared *An. gambiae s.s.* mosquitoes using membrane feeding method. Genomic DNA was extracted from dry blood spots (DBS) samples and *P. falciparum* populations were genotyped using 10 polymorphic microsatellite markers. Assessment of the association between MOI and gametocyte density and mosquito infection rates was conducted.

Results: The prevalence of *Plasmodium falciparum* infection among the study population was 29.1%. A significantly higher *P. falciparum* infection was found among the male gender (n=764, p-value < 0.05) compared to the females (n=657). Gametocyte prevalence among the study population was 2%. Children (5-9 years) have a higher risk of gametocyte carriage (Odd Ratios = 2.1 [95% CI = 1.3–3.4], P = 0.002). Our results indicate a significant and positive combined effect of gametocyte density and MOI on mosquito infection rate (R = 0.825, p < 0.0001).

Conclusion: The study reports a relatively stable year-round gametocyte carriage among the study population. This pattern of gametocyte carriage signals the role of schoolchildren in maintaining malaria transmission in the study area. A strong positive correlation was found between gametocyte density, multiplicity of infection and mosquito infectivity.

Keywords: *P. falciparum*, asymptomatic, gametocyte density, MOI, mosquito infection rates, Mbita

Background

The intensification of global and local malaria control measures have led to remarkable reductions in disease burden in many regions including sub-Saharan Africa. The incidence of *P. falciparum* clinical cases and prevalence have respectively declined by 40% and 50% within the African continent between 2000 and 2015 [1]. However, recent data indicates this trend might be reversing, an estimated 213 million malaria cases and 380,700 related deaths in the World Health

Organisation (WHO) African Region between 2017 and 2018, an increase relative to previous years [2]. Clearly, malaria continues to be a serious public health problem in the continent threatening the lives of many people particularly children and pregnant women. In Kenya, like many other African countries, *P. falciparum* is the dominant parasite species with about 70.2% of the population at risk of the disease [3]. Malaria is one of the leading causes of hospital admissions and death in the country accounting for about 30% and 19% outpatient and inpatient cases respectively, with an estimated inpatient death of 3-5% [2, 4].

The Kenyan government through the implementation of a national strategic malaria control plan and subsequently, the launching of the next iteration of its national malaria strategy (KMS) 2019—2023, has intensified its fight against the disease in a bid to attain a **"malaria free Kenya"**. This involved the introduction and scaling up of interventions such as long-lasting insecticide net (LLIN), rapid diagnostic test (RDT), and artemisinin-based combination therapy (ACT) [5, 6]. The implementation of these interventions have resulted to a decline in malaria transmission in many parts of the country [7]. Nevertheless, the coastal part of the country and areas along the shores of Lake Victoria continue to face high malaria transmission [8].

Malaria parasite transmission from humans to the mosquito vectors requires the presence of infectious mature gametocytes in the peripheral blood of the human host [9, 10]. Based on the central role of gametocytes in propagating and sustaining malaria transmission, the prevalence of gametocytes is largely used as a parameter of the disease transmission [11, 12]. Studies in malaria endemic and high transmission areas have reported high asexual parasite and gametocyte prevalence and densities in children relative to adults [13, 14]. In such high malaria transmission settings, due to repeated parasite exposure, older children and adults develop immunity against the parasite [15, 16]. As a result, this category of people are most likely to experience

asymptomatic infections harbouring gametocytes at microscopic and sub-microscopic densities thereby serving as efficient parasite reservoirs for sustaining malaria transmission [12, 14, 17]. Reports about high prevalence of asymptomatic infections and gametocyte densities in schoolchildren have be documented in some malaria endemic areas [17, 18]. Asymptomatic malaria infections in schoolchildren mostly remain undiagnosed and are not treated due to the lack of clinical manifestation. Therefore, this group of people are largely neglected by most of the currently implemented malaria interventions and control programs [18, 17]. In addition, following the decline in malaria burden in many endemic areas, information on the prevalence of asymptomatic *P. falciparum* infections and gametocyte carriage in schoolchildren particularly in remote settings in sub-Saharan Africa remains patchy [19]. Since asymptomatic infections and prevalence of gametocyte carriage in schoolchildren may significantly hamper the attainment of malaria control and elimination goals in sub-Saharan Africa [18, 20], it will be important to further investigate dynamics and infectivity of asymptomatic carriers.

The presence of gametocytes in the peripheral blood of the human host does not necessarily translate into mosquito infectivity [9, 21]. Some of the major factors that influence the successful transmission of *P. falciparum* gametocytes to the mosquito vectors include, human attractiveness and exposure to the mosquito vectors, host and vector immune responses, seasonality, gametocyte maturity and densities, and multiplicity of infection (MOI) [21, 22]. MOI is the number of distinct parasite clones concurrently infecting a host. The link between MOI and gametocytemia of *P. falciparum* is still not fully elucidated [21], however, studies have reported a positive association between MOI and gametocyte carriage [14, 23]. The presence of genetically diverse multiple *P. falciparum* clones is reported to increase the chances of some parasite clones to evade the host anti-parasite immune responses thereby promoting gametocyte

development and persistence [14, 24]. Mosquito infection rates of *P. falciparum* are reported to be positively associated with gametocyte density particularly at high gametocyte concentrations [25]. However, at low gametocyte concentrations, a varying and less strong association is reported [9]. The nature of the tri-partite interaction between gametocyte density, MOI and mosquito infectivity remains poorly documented.

Two common characteristics of asymptomatic malaria infections in endemic settings are the prevalence of varying levels of gametocyte carriage among different age categories due to anti-parasite immunity and high rates of polyclonal infections [15, 26, 27]. In order to ultimately eliminate malaria, interventions geared towards interrupting the disease transmission through efficient and effective identification and treatment of both asymptomatic and symptomatic carriers will be of immense importance [14, 15]. Understanding the association between gametocyte density, MOI and mosquito infectivity will enhance proper identification and targeted treatment of parasite reservoirs responsible for sustaining the ongoing malaria transmission in the region [14]. Here, we report on the prevalence of gametocyte carriage and some of its associated risk factors among asymptomatic schoolchildren (age 5-15 years) in Mbita, western Kenya and further assesses the association between gametocyte density, MOI and mosquito infectivity.

Methods

Study Site

The study was carried out in Mbita sub-county (Suba North), in the Homa Bay County of Western Kenya. Study participants were recruited from primary schools within Mbita sub-county (within 50 km radius of Mbita town). The sub-county is situated on the shores of Lake Victoria

and located between latitudes 0° 21' and 0° 32' South and longitudes 34° 04' and 34° 24' East. The area of the district is about 163.28 km^2 with a population of 124,938 (Figure 1).

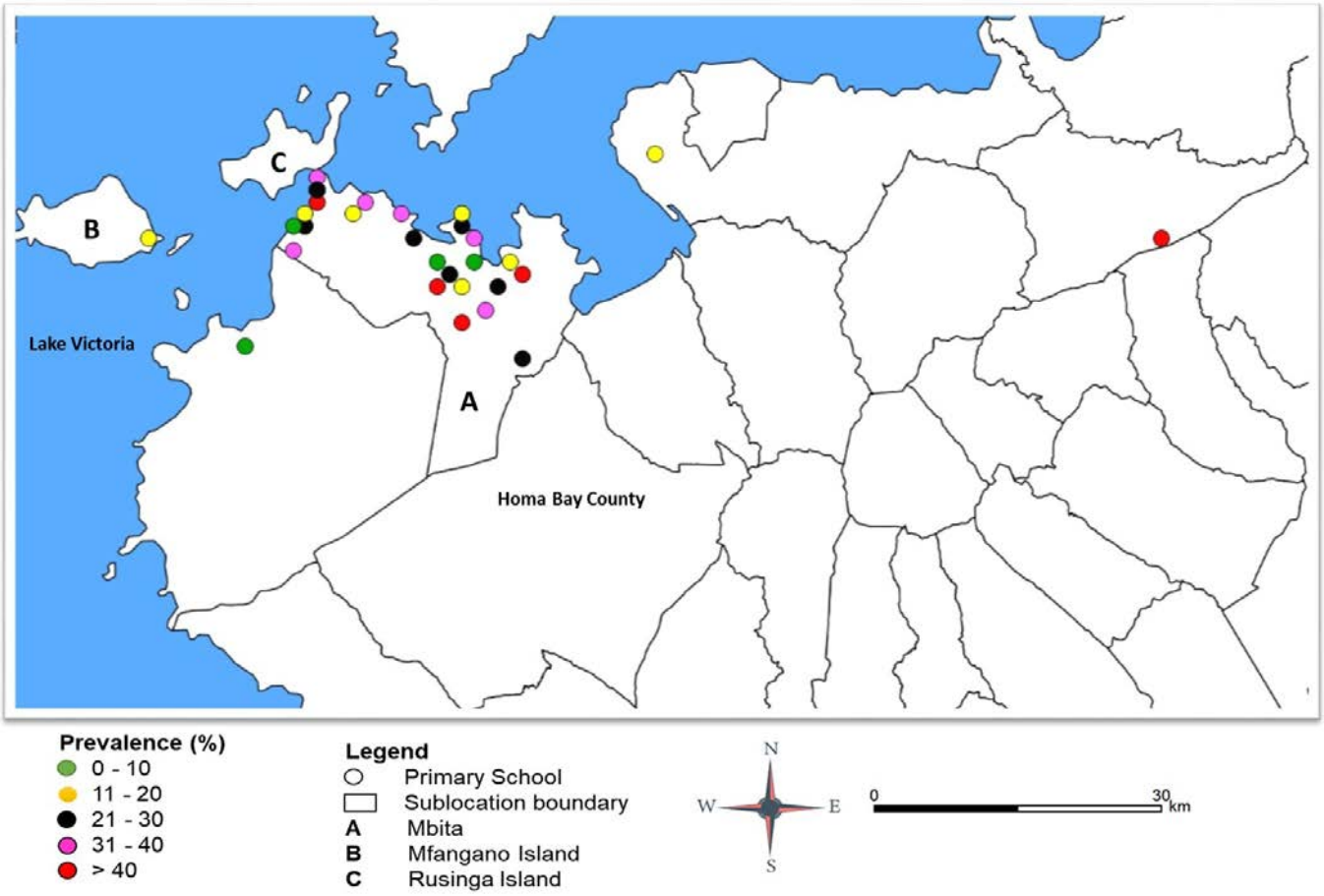

Figure 1

Map of Homa Bay County indicating the prevalence of *Plasmodium falciparum* infection among the schools in the study site. The site-specific prevalence (%) was calculated as the percentage of *P. falciparum* positive infections within each school.

Study subjects and Sample collection

Primary school children between the ages 5 and 15 years residing in Mbita sub-county, Western Kenya were recruited and screened for *P. falciparum* malaria infection using Rapid Diagnostic Test (RDT) (SD Bioline Malaria Ag P.f/Pan HRP-II/pLDH) and microscopy. Schoolchildren

from the various primary schools around Mbita were enrolled in a study that commenced in December 2016 to evaluate the effects of symbiotic microbes and mosquito vector competence.

Blood samples were collected from each participant for RDT and 10% Giemsa stained thin and thick blood films preparation for microscopy diagnosis of *P. falciparum* malaria infection and also collected on a filter paper (Whatman 3MM; Whatman, Maidstone, United Kingdom) for DNA extraction. The filter paper dried blood spots (DBS) were stored at -20 ^{o}C. An additional 4 mL of venous blood was collected from participants with *P. falciparum* gametocytes as detected by microscopy for use in the membrane feeding assays. Four thousand eight hundred eighty-eight (4881) participants were screened in this study.

Experimental infection of mosquitoes

Venous blood samples (4 mL) collected from individuals who tested positive for *P. falciparum* carriage using RDT and subsequently found to be gametocyte-positive from the microscopy diagnosis, were immediately fed to the mosquitoes. Experimental feeds were carried out in batches of 100 (per feeding cup) locally reared 3 to 5 day-old female *An. gambiae s.s.* mosquitoes via an artificial membrane attached to a water-jacketed glass feeder maintained at 37 °C. A total of 37 gametocyte-positive venous blood samples collected from different individuals was used to feed the mosquitoes. After 15-20 minutes, fully fed mosquitoes are selected and kept on glucose for 7 days at 27°C–29°C. On the 10th day post-infection, the mosquitoes that were alive were then collected and stored at -20 ^{o}C in Eppendorf tubes. The proportion of infected mosquitoes was determined by detecting the *P. falciparum* circumsporozoite protein (CSP) in the stored mosquito samples using CSP ELISA as described elsewhere [28].

Microsatellite genotyping

155 Genomic DNA (gDNA) was extracted from the DBS samples using the QIAamp DNA Mini Kit
156 (51304**,** QIAGEN, Hilden, Germany) based on the manufacturer's protocol. gDNA quality and
157 concentration of each sample was determined using a Nanodrop 2000C (Thermo Fisher
158 Scientific, Waltham, MA, USA) and samples were stored at -20 ^{o}C until used. The microsatellite
159 amplification, fragment analysis and MOI determination method used in this study is the same as
160 described by Mobegi *et al.* 2012 [29]. The samples analysed here are part of those used in our
161 previous study [30].

162 **Data storage and Analysis**

163 Age, gender, weight and *Plasmodium* parasiteamia of each study participant together with
164 mosquito infectivity and microsatellite genotyping data collected were carefully verified.
165 Descriptive statistics and Pearson Chi-Square test for significance between groups were
166 determined. Risk factors analysis was done using a binary logistic regression model and multiple
167 correlation and regression analysis was used to test for possible relationship between gametocyte
168 density, MOI and mosquito infection rates. Statistical analysis were conducted in IBM SPSS
169 Statistics for Windows, version 25 (IBM Corp., Armonk, N.Y., USA). Schools were mapped
170 using geographical information system (GIS) and the map generated using QGIS software
171 version 2.4.0.

172 **Results**

173 **Demographic and Parasitological characteristics of the study participants**

174 In this study, a total of 4881 schoolchildren (age 5-15 years) were screened using RDT and the
175 parasite status confirmed by microscopy. The total number of the female and male participants
176 were 2459 and 2422, respectively. Regarding the parasitological characteristics of the study

participants, significant differences were observed among the male and female gender with higher *P. falciparum* prevalence among the male gender (764/1421, *p*-value < 0.05). There was no statistically significant difference in *P. falciparum* parasite carriage between the age groups (5-9 and 10-15 years). The total number of mixed infections (*P. falciparum* plus *P. ovale* and/or *P. malariae*) detected in the study population was 204 with a significantly higher prevalence among the children between the ages 5-9 years (112/204, *p*-value < 0.05) while the single infections were 1217. Most of the mixed infections were found among the female gender (110/204, *p*-value < 0.05) (Table 1 and Additional file 1).

Table 1: Parasitological characteristics of the study participants.

Variables		**RDT**		
Age (years)	**Positive**	**Negative**	**Total**	**χ^2 (*p*-value)**
5-9	712	1833	2545	
10-15	709	1627	2336	3.328 **(0.068)**
Total	1421	3460	4881	
Gender				
Female	657	1802	2459	
Male	764	1658	2422	13.770 **(<0.05)***
Total	1421	3460	4881	
Age (years)	**Mixed Infection**	**Single Infection**	**Total**	**χ^2 (*p*-value)**
5-9	112	600	712	
10-15	92	617	709	4.639 **(<0.05)***
Total	204	1217	1421	
Gender				
Female	110	547	657	
Male	94	670	764	5.661 **(<0.05)***
Total	204	1217	1421	
		MICROSCOPY		
Age (years)	**Asexual**	**Gametocyte**	**Total**	**χ^2 (*p*-value)**

5-9	655	57	712		
10-15	682	27	709	11.253 **(<0.05)***	
Total	1337	84	1421		
Gender					
Female	625	32	657		
Male	712	52	764	2.380 **(0.123)**	
Total	1337	84	1421		

Age (years)	**Negative**	**Asexual**	**Gametocyte**	**Total**	**χ^2 (*p*-value)**
5-9	1833	655	57	2545	
10-15	1627	682	27	2336	14.602 **(<0.05)***
Total	3460	1337	84	4881	
Gender					
Female	1802	625	32	2459	
Male	1658	712	52	2422	16.137 **(<0.05)***
Total	3460	1337	84	4881	

Population gametocyte prevalence	2% (84/4881)
Gametocyte prevalence (*P. falciparum* positives)	6% (84/1421)

Population gametocyte prevalence is the percentage of gametocyte carriers among the total study population (*P. falciparum* positive and negative samples together) while the gametocyte prevalence among the *P. falciparum* positive samples is the percentage of gametocyte carriers among the *P. falciparum* positive samples only (excluding P. falciparum negatives). χ^2 = Pearson's chi-squared test and (*) indicates statistical significance.

The population *P. falciparum* prevalence in this study calculated as the percentage of *P. falciparum* infections within the study sample was 29.1%. The level of *P. falciparum* carriage varies among study sites (range: 0-65.2%, *p*-value < 0.001) and within sampling periods (range: 11-78.4%, *p*-value < 0.001) (Figure 1 and 2).

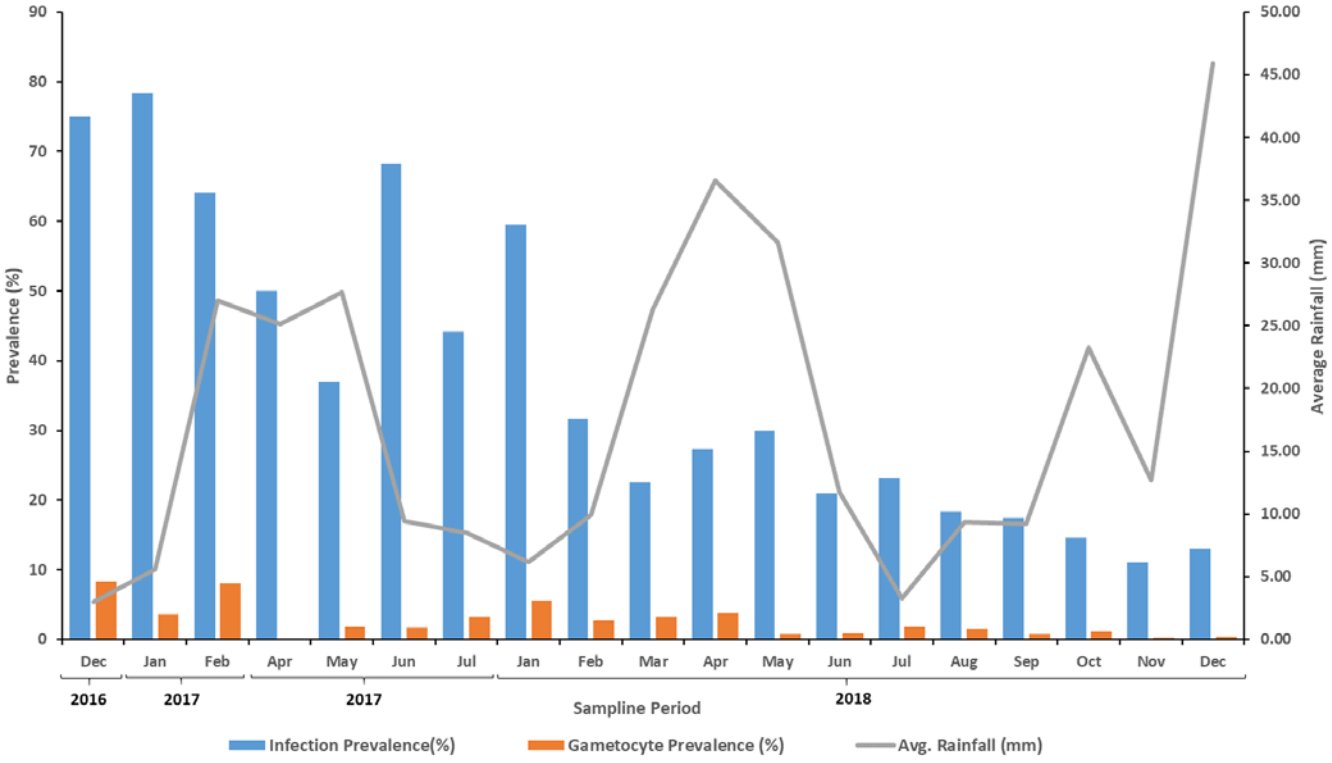

Figure 2

P. falciparum **infection (blue) and gametocyte (brown) prevalence among the study participants and average rainfall (gray) during the various sampling periods.**

Prevalence of gametocyte carriage and associated risk factors in the study population

In Table 1, the total number of gametocyte carriers was 84 with 57 of the carriers found within the age group 5-9 years as compared to 27 in the age-group 10-15 years (p-value < 0.05). There was 2% population gametocyte prevalence in the study population. While the prevalence of gametocyte carriage among the *P. falciparum* malaria carriers (only *P. falciparum* positive individuals) was found to be 6%. These represent a minimum gametocyte prevalence level since the sensitivity of microscopy is relatively low. There was significant difference in the population gametocyte carriage between the female (32) and male gender (52) (p-value < 0.05). The

gametocyte prevalence was relatively stable with no statistically significant difference throughout the sampling periods (Figure 2).

The analysis showed that risk of *P. falciparum* infection was highest among the male gender as compared to the females (OR = 0.8 [95% CI = 0.7–0.9], $P < 0.001$) while the age of an individual was not an independent risk factor. However, children between the ages of 5-9 years have a higher risk of gametocyte carriage when infected with *P. falciparum* as compared to those between the ages 10-15 years (OR = 2.1 [95% CI = 1.3–3.4], $P = 0.002$) (Table 2).

Table 2: Risk factors of *P. falciparum* infection and gametocyte carriage.

	***P. falciparum* parasite carriage**			**Gametocyte carriage**		
Variable	**OR**	**95% CI**	***P*-value**	**OR**	**95% CI**	***P*-value**
Age (Years)						
5 - 9	0.9	0.80, 1.02	**0.101**	2.14	1.34, 3.43	**0.002***
10 - 15	1			1		
Gender						
Female	0.79	0.70, 0.90	**< 0.001***	0.71	0.45, 1.12	**0.136**
Male	1			1		

Risk factors analysis of *P. falciparum* infection and gametocyte carriage among the study population using binary logistic regression model. OR, odds ratio; CI, confidence interval. (*) indicates statistical significance.

Relationship between gametocyte density and multiplicity of Plasmodium falciparum infections (MOI) and Mosquito infection rates

The total number of samples used in assessing the relationship between gametocyte density and MOI was 37. However, 15 of the 37 samples failed to amplify during the microsatellite amplification PCR and are recorded as missing data. The mean mosquito infection rate was 12.71 (SE: 2.63, SD: 16.1) and mean gametocyte density was 59.89 (SE: 12.28, SD: 74.71), respectively while the mean number of distinct alleles per isolate was 7.30 (SE: 0.80, SD: 3.76)

(See Additional file 2). In this study, a significant positive correlation was found between *P. falciparum* gametocyte densities in the patient blood samples and mosquito infection rates (0.756, *p*-value < 0.001). In addition, a positive correlation between multiplicity of *P. falciparum* infection (MOI) and mosquito infection rates was reported (0.451, *p*-value = 0.018). However, the correlation between MOI and gametocyte density was not statistically significant. The mosquito infection rate is defined as the percentage of infected mosquitoes after day 10^{th} of the membrane-feeding assay (Table 3 and Figure 3).

Table 3: Multiple correlation analysis of gametocyte density and multiplicity of *P. falciparum* infection with the infection rate in the mosquitoes.

Parameters	Infection rate (*P*-value)	Gametocyte density (*P*-value)	MOI (*P*-value)
Infection rate	1 (*Ref*)	0.756 **(< 0.001)***	0.451 **(0.018)***
Gametocyte density	0.756 **(< 0.001)***	1 (*Ref*)	0.167 **(0.229)**
MOI	0.451 **(0.018)***	0.167 **(0.229)**	1 (*Ref*)

The dependent variable in this analysis is the infection rate. *Ref* represents the reference (*) denotes statistical significance.

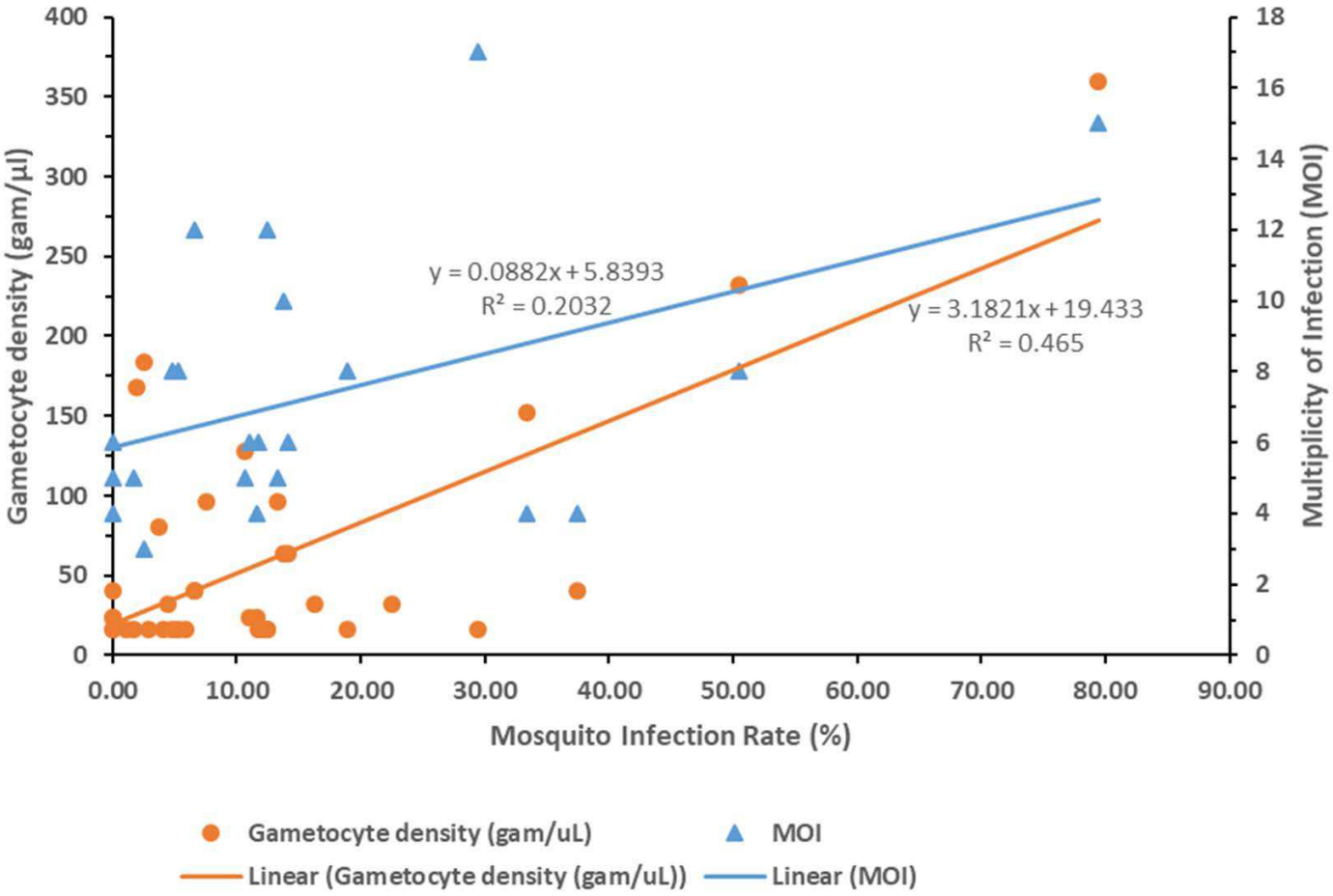

Figure 3

Relationship between gametocyte density (gametocyte/µl) and multiplicity of infection (MOI) with mosquito infection rate.

In Table 4, the multiple linear regression analysis showed that the collective effect of gametocyte density and MOI has a strong positive correlation with mosquito infection rate (R = 0.825, $p < 0.0001$).

Table 4: Parameter of multiple linear Regressions Analysis.

Parameters	Coefficients	Std. Error	t-statistic	*P*-value
(Constant)	-6.644	5.564	-1.194	**0.247**
Gametocyte density	0.151	0.028	5.328	**< 0.010**
MOI	1.707	0.672	2.54	**0.020**

R = 0.825, R-SQR. = 0.681, Adj. R-SQR = 0.647, SE = 11.418. ***R*** is the multiple correlation coefficient, ***R-SQR.*** (R-square) is the multiple coefficient of determination, ***Adj. R-SQR*** represents the adjusted R-square, and ***SE*** is the standard error.

The multiple coefficient of determination (R-SQR. = 0.681) indicated that, about 68.1% of the variation in mosquito infection rate is accounted for by the gametocytes density and MOI. Thus, the formulated equation for mosquito infection rate in this study is:

$$\hat{Y} = -6.644 + 0.151X_1 + 1.707\,X_2$$

Where $\hat{Y}$ is the expected Mosquito Infection rate, X_1 and X_2 are the Gametocytes density and MOI, respectively.

Discussion

This study assessed the prevalence of gametocyte carriers and some of the associated risk factors among asymptomatic schoolchildren (age 5-15 years) residing in Mbita, Western Kenya. In addition, an assessment of the relationship between gametocyte density, MOI and mosquito infection rates was also carried out. In this study, gametocyte prevalence among the *P. falciparum* positive individuals was 6% (84/1421) while the population *P. falciparum* gametocyte prevalence was 2% (84/4881). This corroborates with the finding of another study in the region [31, 32]. The intensification of the fight against malaria in the region by the Kenyan government may be the contributing factor for this relatively low gametocyte carriage reported in our study [33]. However, the changes in gametocyte prevalence in the study population needs further investigation using highly sensitive molecular tools in order to accurately estimate both the microscopic and submicroscopic gametocyte levels in the area. Gametocyte prevalence was higher among the younger age group (5-9 years) and accounted for 67.86% (57/84) of the total

gametocyte carriers in the study population. Similar pattern of gametocyte carriage was reported by other studies [21, 31]. This can be explained by age-dependent development of anti-parasite immunity due to repeated exposure in endemic settings [21, 31]. The high prevalence of gametocyte carriage among the younger age group (5-9 years) pinpoints the potential role of this age group in sustaining malaria transmission in the region. Children were reported to be important contributors to the malaria infectious reservoir in many other settings [21]. Among the *P. falciparum* malaria positive individuals, the male gender have higher proportion of both asexual (764/1421) and gametocyte carriers (52/84) as compared to the females (asexual carriage; 657/1421, gametocyte carriage; 32/84). However, the difference is not statistically significant (*P*-value = 0.123). The *P. falciparum* prevalence was much lower in 2018 when compared to the 2017 season. This is due to the 2018 indoor residual spraying (IRS) campaign conducted by Africa Indoor Residual Spraying (AIRS) Kenya, in the region [34]. Nonetheless, the gametocyte prevalence was relatively stable during all the sampling periods indicating a year-round gametocyte carriage in the study population irrespective of the rainfall levels and pattern. In malaria endemic settings, asymptomatic carriers are known to harbour gametocytes even during the non-transmission season and are reported to be responsible for the resurgence of malaria infections during the subsequent transmission season [32]. With a persistent relatively high prevalence of mosquito vectors and asymptomatic *P. falciparum* gametocyte carriage, there is a perennial transmission of malaria in the region with intense and relatively mild transmission from April to August and November to January, respectively.

The only independent risk factor associated with *P. falciparum* infection found in this study was gender. The male gender have higher odds of *P. falciparum* infection in the study area as compared to the females. Gender was reported as a risk factor in other studies in the region [31].

This finding is in line with the reports that female children are biologically less susceptible to infectious diseases as compared to the male children [35]. However, age was not found to be a risk factor for contracting *P. falciparum* malaria infection in this study but was linked with gametocyte carriage when infected with *P. falciparum.* Younger children (5-9 years) have higher risk of gametocyte carriage when infected with *P. falciparum*. A study in Tanzania has also reported similar association of age with increased gametocyte prevalence [36].

A positive correlation was found between gametocyte density and mosquito infection rates (*p*-value < 0.001). High infection rates were observed among mosquitoes that fed on carriers with high gametocyte densities. This result corroborates with the findings of other studies [21, 37]. However, Churcher *et al.* 2013 have reported a negative association between gametocyte density and mosquito infection rates [38]. The relationship between multiplicity of *P. falciparum* infection and mosquito infection rates is not well documented. This study reports a significant correlation between MOI and mosquito infection rates (*p*-value = 0.018). Gametocyte isolates harbouring multiple distinct clones positively influence the mosquito infection rate. In contrast, a negative association between MOI and mosquito infection prevalence and intensity was reported elsewhere [39].

Gametocyte density is an important factor in predicting the success of *P. falciparum* transmission to the mosquito vector. Nonetheless, other studies have stressed that gametocyte presence or density alone in blood samples does not equal their infectiousness to mosquitoes [38]. Therefore, understanding the relationship between the combined effects of gametocyte density and other gametocyte factors like MOI with mosquito infection rate will improve our understanding of the dynamics of *P. falciparum* transmission. Our results indicate a significant and positive combined

effect of gametocyte density and multiplicity of *P. falciparum* infection on mosquito infection rate. The results showed that about 68.1% of the variations in mosquito infection rate is accounted for by the combined effect of MOI and gametocyte density. These results can be explained by the emergence of highly virulent and infectious parasite strains due to intense intra-host competition and high recombination rates among the distinct infecting clones [40, 41]. However, Morlais *et al.* 2015 reported a non-significant interaction of the combined effect of MOI and gametocyte density on mosquito infection rate [39]. This difference may be due to the fewer number of microsatellite markers (6 markers) used in their study as compared to 10 microsatellite markers in our study. In addition, the difference in mosquito species (*An. coluzzii*) used in their studies might be another contributing factor since *An. gambiae* was reported to have a higher blood-feeding rate compared to *An. Coluzzii* [42]. This finding will help in assessing the infectious reservoirs in different malaria endemic regions thereby guiding the implementation of targeted malaria control interventions.

The results presented here represents minimum level of gametocyte carriage, as some of the microscopic and submicroscopic gametocytes might have been undetected.

Conclusions

Malaria prevalence and gametocyte carriage is high among the asymptomatic schoolchildren particularly the younger age group (5-9 years) in the region. The relatively stable and year-round prevalence of gametocyte carriage among the study participants in this study signals the role of schoolchildren in maintaining malaria transmission in the study area. The strong positive correlation between the combined effect of gametocyte density and MOI with mosquito infection rates will help in determining the human infectious reservoirs in different malaria endemic

settings. Therefore, this will help in identifying and treatment of asymptomatic malaria parasite carriers in the community. Malaria control interventions that are targeted towards asymptomatic gametocyte carriers and reduction in multiple clone parasite carriage could aid in the ultimate elimination of the disease in the region.

Ethics approval and consent to participate

Parents or guardians of the children signed an informed consent form. The Kenya Medical Research Institute (KEMRI) Scientific and Ethics Review Unit (SERU) granted approval for the original study (KEMRI/RES/7/3/1). All experiments were performed in accordance with the relevant guidelines and regulations.

Consent for publication

Not applicable

Availability of data and materials

The datasets used and analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

Funding

This study was funded by Pan African University, Institute of Basic Sciences, Technology and Innovation (PAUSTI) under the postgraduate training program awarded to AOT.

Authors' contributions

AOT participated in data collection, analysis, interpretation and manuscript preparation. HB participated in data collection and manuscript preparation. VAM**,** FW and JKH participated in data interpretation and manuscript preparation. All authors read and approved the final manuscript.

Acknowledgement

We are indebted to all volunteers who partook in this study as well as their parents/guardians for granting consent. The authors are very much grateful to the entire research team of Dr. Jeremy K. Herren and the management of International Centre of Insect Physiology and Ecology (ICIPE) for permitting us to use their samples and giving us access to use their research facilities to conduct the study. Special thanks to Azumah Karim for his valuable assistance and advice in analysing the data.

References

1. Bhatt S, Weiss DJ, Cameron E, Bisanzio D, Mappin B, Dalrymple U, et al. The effect of malaria control on *Plasmodium falciparum* in Africa between 2000 and 2015. Nature. 2015;526:207–11.

2. World Health Organization. WHO. World Malar. Rep. 2019.

3. Kenya National Bureau of Statistics and ICF Macro. Kenya Demographic and Health Survey 2008–2009. Kenya National Bureau of Statistics and ICF Macro. 2010. http://apps.who.int/medicinedocs/documents/s17116e/s17116e.pdf%0Ahttps://dhsprogram.com/pubs/pdf/FR260/FR260.pdf

4. Division of Malaria Control,Kenya Ministry of Public Health and Sanitation. Malaria Control Strategy. 2009.

5. Division of Malaria Control, Kenya Ministry of Public Health K. The National Malaria Strategy 2001–2010. 2001.

6. Kenya Ministry of Health. Kenya Malaria Indicator Survey 2015. Minist. Heal. Kenya. 2016.

7. Macharia PM, Giorgi E, Noor AM, Waqo E, Kiptui R, Okiro EA, et al. Spatio-temporal

analysis of *Plasmodium falciparum* prevalence to understand the past and chart the future of malaria control in Kenya. Malar J. 2018;17:1–13. https://doi.org/10.1186/s12936-018-2489-9

8. Noor AM, Gething PW, Alegana VA, Patil AP, Hay SI, Muchiri E, et al. The risks of malaria infection in Kenya in 2009. BMC Infect Dis. 2009;9:1–14.

9. Ouédraogo AL, Guelbéogo WM, Cohuet A, Morlais I, King JG, Gonçalves BP, et al. A protocol for membrane feeding assays to determine the infectiousness of *P . falciparum* naturally infected individuals to *Anopheles gambiae*. MWJ. 2013;4:17–20.

10. Baker DA. Malaria gametocytogenesis. Mol Biochem Parasitol. Elsevier B.V.; 2010;172:57–65. http://dx.doi.org/10.1016/j.molbiopara.2010.03.019

11. Wampfler R, Timinao L, Beck HP, Soulama I, Tiono AB, Siba P, et al. Novel genotyping tools for investigating transmission dynamics of *Plasmodium falciparum*. J Infect Dis. 2014;210:1188–97.

12. Zeynudin A, Degefa T, Zeynudin A, Zemene E, Emana D, Yewhalaw D. High Prevalence of Gametocyte Carriage among Individuals with Asymptomatic Malaria : Implications for Sustaining Malaria Control and Elimination Efforts in Ethiopia. Hum Parasit Dis. 2015;2016:17–25.

13. Bousema JT, Meutstege AM, Sauerwein RW, Gouagna LC, Githure JI, Drakeley CJ, et al. *Plasmodium falciparum* gametocyte carriage in asymptomatic children in western Kenya. Malar J. 2004;3:1–6.

14. Lamptey H, Ofori MF, Kusi KA, Adu B, Yeboa EO, Baafour EK, et al. The prevalence of submicroscopic *Plasmodium falciparum* gametocyte carriage and multiplicity of infection in children , pregnant women and adults in a low malaria transmission area in Southern Ghana. Malar J. 2018;17:1–12. https://doi.org/10.1186/s12936-018-2479-y

15. Lindblade KA, Steinhardt L, Samuels A, Kachur SP, Slutsker L. The silent threat: Asymptomatic parasitemia and malaria transmission. Expert Rev. 2013;11:623–39.

16. Stone WJR, Dantzler KW, Nilsson SK, Drakeley CJ, Marti M, Bousema T, et al.

Naturally acquired immunity to sexual stage *P. falciparum* parasites. Parasitology. 2016;143:187–98.

17. Coalson JE, Walldorf JA, Cohee LM, Ismail MD, Mathanga D, Cordy RJ, et al. High prevalence of *Plasmodium falciparum* gametocyte infections in school-age children using molecular detection: patterns and predictors of risk from a cross-sectional study in southern Malawi. Malar J. 2016;15:1–17.

18. Cohee L, Laufer M. Tackling malaria transmission in sub-Saharan Africa. Lancet Glob Heal. 2018;6:e598–9.

19. Brooker S, Clarke S, Snow RW, Bundy DAP. Malaria in African schoolchildren: options for control. Trans R Soc Trop Med Hyg. 2008;102:304–5.

20. Kanwugu ON, Helegbe GK, Aryee PA, Abdul-Karim A, Anaba F, Ziblim Z, et al. Prevalence of Asymptomatic Malaria among Children in the Tamale Metropolis: How Does the PfHRP2 CareStart^{TM} RDT Perform against Microscopy? J Trop Med. 2019;2019:7.

21. Bousema T, Drakeley C. Epidemiology and infectivity of *Plasmodium falciparum* and *Plasmodium vivax* gametocytes in relation to malaria control and elimination. Clin Microbiol Rev. 2011;24:377–410.

22. Nilsson SK, Childs LM, Buckee C, Marti M. Targeting Human Transmission Biology for Malaria Elimination. PLoS Pathog. 2015;11:1–17.

23. Nwakanma D, Kheir A, Sowa M, Dunyo S, Jawara M, Pinder M, et al. High gametocyte complexity and mosquito infectivity of *Plasmodium falciparum* in the Gambia. Int J Parasitol. 2008;38:219–27.

24. Nassir E, Abdel-Muhsin AMA, Suliaman S, Kenyon F, Kheir A, Geha H, et al. Impact of genetic complexity on longevity and gametocytogenesis of *Plasmodium falciparum* during the dry and transmission-free season of eastern Sudan. Int J Parasitol. 2005;35:49–55.

25. Schneider P, Bousema T, Omar S, Gouagna L, Sawa P, Schallig H, et al.

(Sub)microscopic *Plasmodium falciparum* gametocytaemia in Kenyan children after treatment with sulphadoxine-pyrimethamine monotherapy or in combination with artesunate. Int J Parasitol. 2006;36:403–8.

26. Grignard L, Gonçalves BP, Early AM, Daniels RF, Tiono AB, Guelbéogo WM, et al. Transmission of molecularly undetectable circulating parasite clones leads to high infection complexity in mosquitoes post feeding. Int J Parasitol. 2018;48:671–7. https://doi.org/10.1016/j.ijpara.2018.02.005

27. Greenwood BM. Asymptomatic malaria infections - Do they matter? Parasitol Today. 1987;3:206–14.

28. Stone W, Grabias B, Lanke K, Zheng H, Locke E, Diallo D, et al. A comparison of *Plasmodium falciparum* circumsporozoite protein-based slot blot and ELISA immuno-assays for oocyst detection in mosquito homogenates. Malar J. 2015;14:1–9.

29. Mobegi VA, Loua KM, Ahouidi AD, Satoguina J, Nwakanma DC, Amambua-Ngwa A, et al. Population genetic structure of *Plasmodum falciparum* across a region of diverse endemicity in West Africa. Malar J. 2012;11:1–9.

30. Touray AO, Mobegi VA, Wamunyokoli F, Herren JK. Diversity and Multiplicity of *P. falciparum* infections among asymptomatic school children in Mbita, Western Kenya. Sci Rep. 2020;10:1–8. http://dx.doi.org/10.1038/s41598-020-62819-w

31. Omondi P, Burugu M, Matoke-Muhia D, Too E, Nambati EA, Chege W, et al. Gametocyte clearance in children, from western Kenya, with uncomplicated *Plasmodium falciparum* malaria after artemether-lumefantrine or dihydroartemisinin-piperaquine treatment. Malar J. 2019;18:1–9. https://doi.org/10.1186/s12936-019-3032-3

32. Idris ZM, Chan CW, Kongere J, Gitaka J, Logedi J, Omar A, et al. High and Heterogeneous Prevalence of Asymptomatic and Sub-microscopic Malaria Infections on Islands in Lake Victoria, Kenya. Sci Rep. 2016;6:1–13.

33. Muthui MK, Mogeni P, Mwai K, Nyundo C, Macharia A, Williams TN, et al. Gametocyte carriage in an era of changing malaria epidemiology: A 19-year analysis of a malaria longitudinal cohort. Wellcome Open Res. 2019;4:1–31.

34. Kenya End of Spray Report 2018. Rockville MD. The PMI Africa IRS (AIRS) Project, Abt Associates Inc. 2018.

35. Gadalla AAH, Schneider P, Churcher TS, Nassir E, Abdel-Muhsin AMA, Ranford-Cartwright LC, et al. Associations between season and gametocyte dynamics in chronic *Plasmodium falciparum* infections. PLoS One. 2016;11:1–15.

36. Nyarko SH, Cobblah A. Sociodemographic determinants of malaria among under-five children in Ghana. Malar Res Treat. 2014;2014.

37. Akim NIJ, Drakeley C, Kingo T, Simon B, Senkoro K, Sauerwein RW. Dynamics of *P. falciparum* gametocytemia in symptomatic patients in an area of intense perennial transmission in Tanzania. Am J Trop Med Hyg. 2000;63:199–203.

38. Churcher TS, Bousema T, Walker M, Drakeley C, Schneider P, Ouédraogo AL, et al. Predicting mosquito infection from *Plasmodium falciparum* gametocyte density and estimating the reservoir of infection. elife. 2013;2:1–12.

39. Morlais I, Nsango SE, Toussile W, Abate L, Annan Z, Tchioffo MT, et al. *Plasmodium falciparum* mating patterns and mosquito infectivity of natural isolates of gametocytes. PLoS One. 2015;10:1–14.

40. Alizon S, de Roode JC, Michalakis Y. Multiple infections and the evolution of virulence. Ecol Lett. 2013;16:556–67.

41. Wargo AR, De Roode JC, Huijben S, Drew DR, Read AF. Transmission stage investment of malaria parasites in response to in-host competition. Proc R Soc B Biol Sci. 2007;274:2629–38.

42. Akogbéto MC, Salako AS, Dagnon F, Aïkpon R, Kouletio M, Sovi A, et al. Blood feeding behaviour comparison and contribution of *Anopheles coluzzii* and *Anopheles gambiae*, two sibling species living in sympatry, to malaria transmission in Alibori and Donga region, northern Benin, West Africa. Malar J. 2018;17:1–12. https://doi.org/10.1186/s12936-018-2452-9

Figures

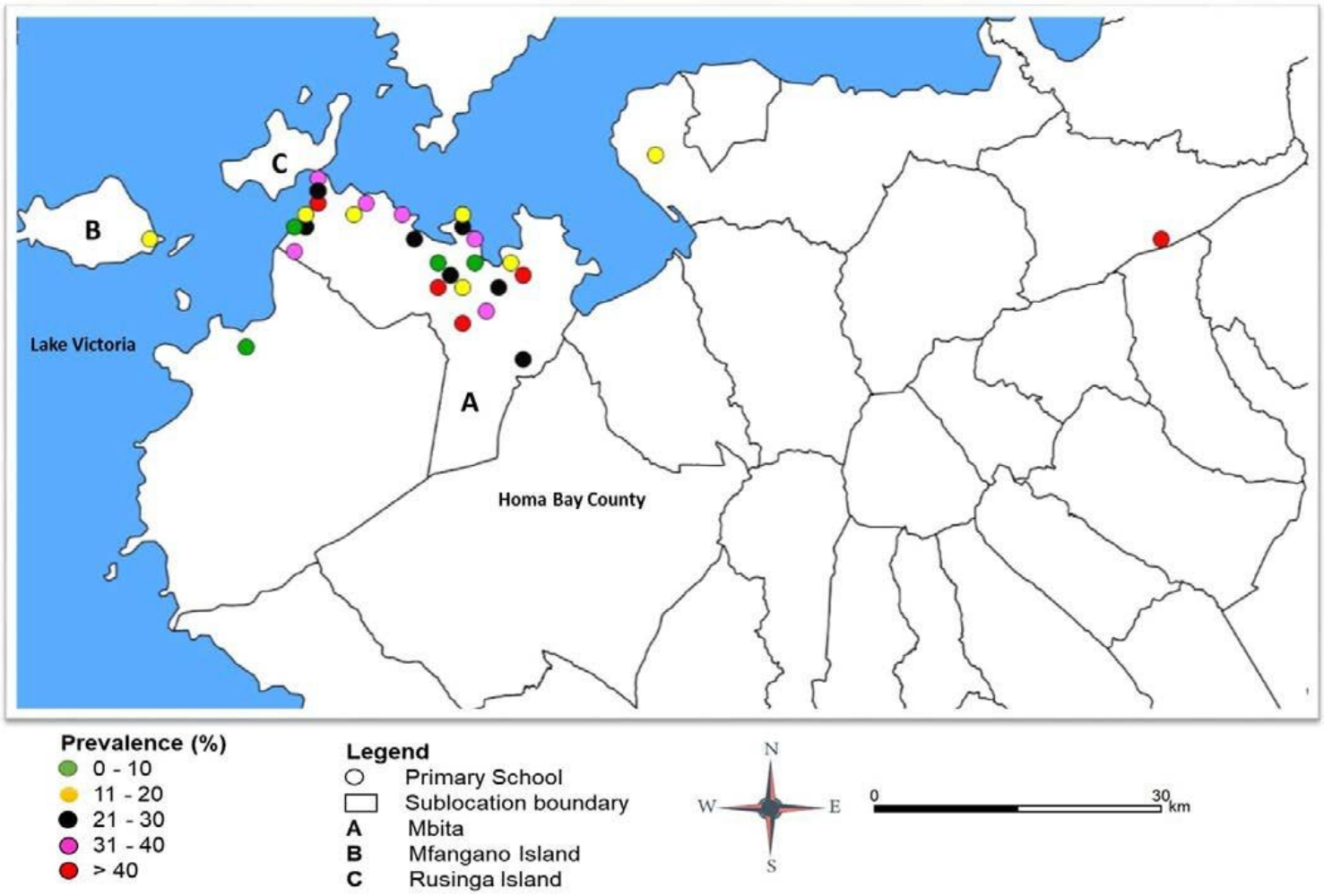

Figure 1

Map of Homa Bay County indicating the prevalence of Plasmodium falciparum infection among the schools in the study site. The site-specific prevalence (%) was calculated as the percentage of P. falciparum positive infections within each school.

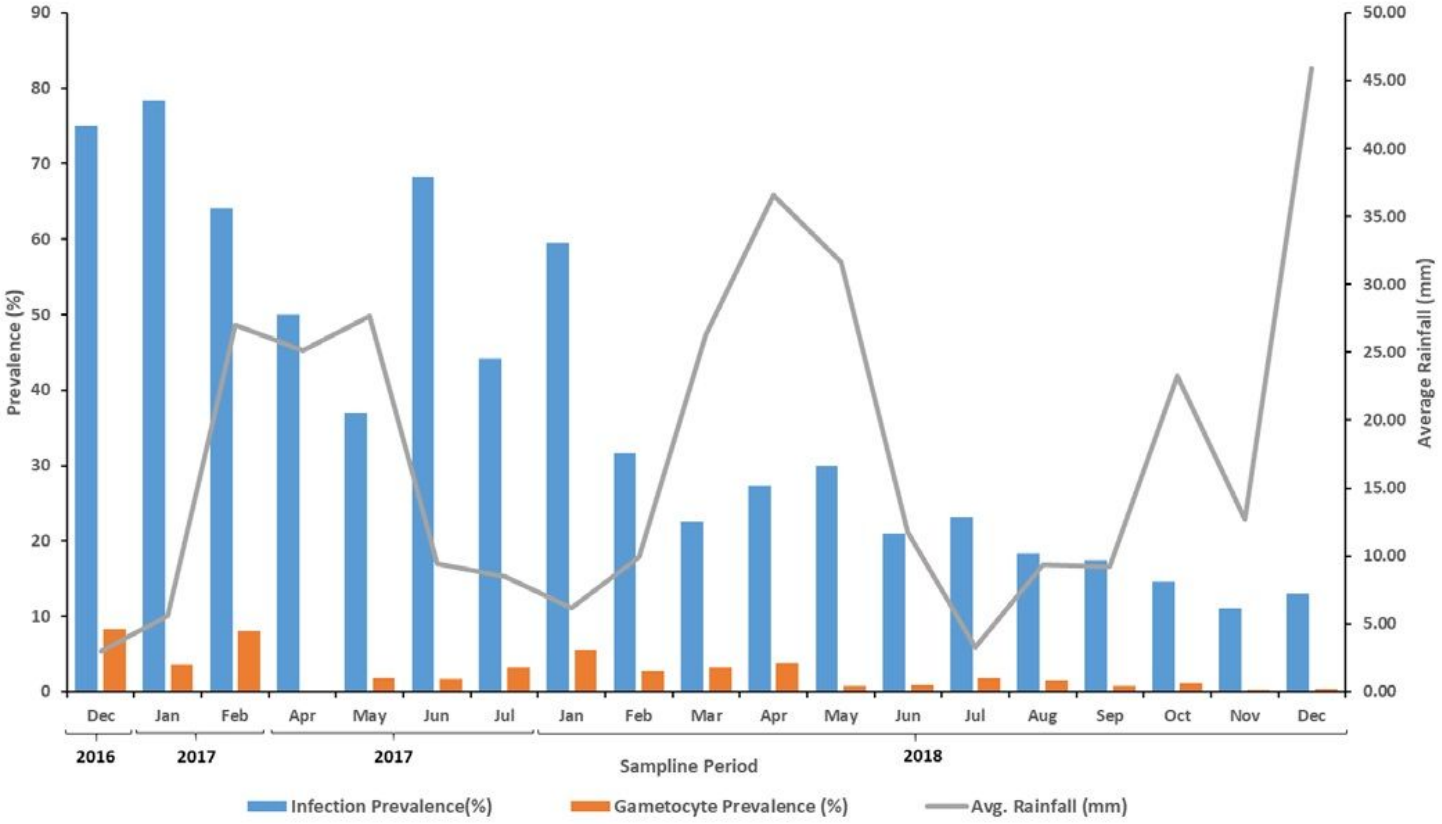

Figure 2

P. falciparum infection (blue) and gametocyte (brown) prevalence among the study participants and average rainfall (gray) during the various sampling periods.

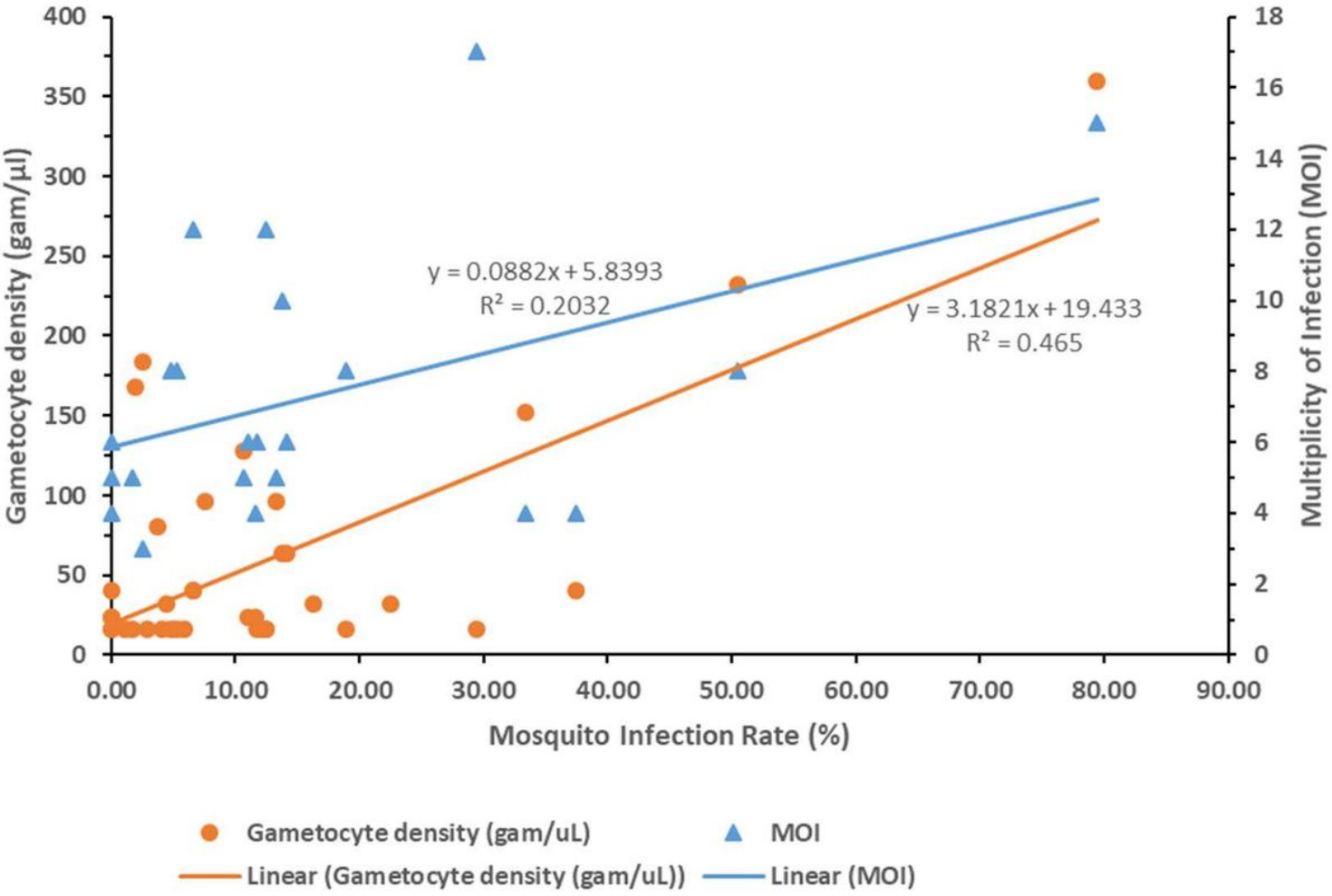

Figure 3

Relationship between gametocyte density (gametocyte/µl) and multiplicity of infection (MOI) with mosquito infection rate.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Additionalfile1.docx
- Additionalfile2.docx