Assessment of the Relationship Between Gametocyte Density, Multiplicity of Infection and Mosquito Infectivity in Plasmodium Falciparum Infections

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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 infection prevalence.

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. Microscopy positive gametocyte carriers were selected 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 29.11% (1421/4881). A significantly higher prevalence of *P. falciparum* infection was found among the male gender 31.54% (764/2422) (p-value < 0.001) compared to the females 26.72% (657/2459). The microscopy gametocyte prevalence among the study population was 2% (84/4881). Children (5-9 years) have a higher risk of gametocyte carriage (Odd Ratios = 2.1 [95% CI = 1.3–3.4], P = 0.002) as compared to those between the ages of 10-15 years. Our results indicate that, about 68.1% of the variation in mosquito infection prevalence is accounted for by the gametocytes density and MOI (R-SQR. = 0.681, p < 0.001).

Conclusion: The study reports a *P. falciparum* infection prevalence of 29.11% with a gametocyte prevalence of 2% among the study population as determined by microscopy. Age was a significant risk factor for gametocyte carriage as indicated by the higher risk of gametocyte carriage among the younger children (5-9 years). The gametocyte density and multiplicity of infection statistically significantly predicted mosquito infection prevalence. Both of the variables added significantly to the prediction, (p < 0.05).

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 and its densities are often used as surrogate indicators for the disease transmission potential [11, 12]. The advent of highly sensitive molecular tools has enabled us to understand that every malaria parasite positive individual is a current or prospective gametocytes producer and therefore, have some transmission potential. 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]. Some studies have reported a positive association between mosquito infection rates of *P. falciparum* and gametocyte density particularly at high gametocyte concentrations [25]. However, at low gametocyte concentrations, a varying and less strong association is reported [9]. It is now shown that the transmission potential is influenced by the parasite sex ratio and can be estimated based on (male and female) gametocyte density [26, 27]. However, the proportion of variation in mosquito infection prevalence that can be explained by gametocyte density and multiplicity of infection is yet to be extensively studied.

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, 28, 29]. 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 parasite carriers will be of immense importance [14, 15]. Understanding the association between gametocyte density, MOI and mosquito infectivity will enhance proper identification 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 infection prevalence (percentage of infected mosquitoes).

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^{o} 21’ and 0^{o} 32’ South and longitudes 34^{o} 04’ and 34^{o} 24’ East. The area of the district is about 163.28 km^2 with a population of 124,938 (Fig. 1). A perennial malaria transmission is reported in the region. The peak transmission occurs in July and a relatively mild transmission from November to January [30].

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. The samples analysed in this study were collected from December 2016 to December 2018.

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 [31].

Microsatellite genotyping

Genomic DNA (gDNA) was extracted from the DBS samples using the QIAamp DNA Mini Kit (51304, QIAGEN, Hilden, Germany) based on the manufacturer's protocol. gDNA quality and concentration of each sample was determined using a Nanodrop 2000C (Thermo Fisher Scientific, Waltham, MA, USA) and samples were stored at -20 ^{o}C until used. The microsatellite amplification, fragment analysis and MOI determination method used in this study is the same as described by Mobegi *et al.* 2012 [32]. The samples analysed here are part of those used in our previous study [33].

Data storage and Analysis

Age, gender, weight and *Plasmodium* parasiteamia of each study participant together with mosquito infection prevalence and microsatellite genotyping data collected were carefully verified. Descriptive statistics and Pearson Chi-Square test for significance between groups were determined. Risk factors analysis was done using a binary logistic regression model and multiple correlation and regression analysis was used to determine the regression coefficients, statistical significance of regression model (*t* value), and proportion of mosquito infection prevalence (dependent) contributed by independent variables (gametocyte density and MOI) derived from the multiple coefficient of determination (R^2). The mosquito infection prevalence was determined as the percentage of mosquitoes infected with *P. falciparum* parasite after successfully feeding on the naturally infected human blood. Statistical analysis were conducted in IBM SPSS Statistics for Windows, version 25 (IBM Corp., Armonk, N.Y., USA). Schools were mapped using geographical information system (GIS) and the map generated using QGIS software version 2.4.0.

Results

Demographic and Parasitological characteristics of the study participants

In this study, a total of 4881 schoolchildren (age 5–15 years) were screened using RDT and the parasite status confirmed by microscopy. The total number of the female and male participants 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 [Male: 32% (764/2422); Female: 27% (657/2457); *p*-value < 0.001]. There was no statistically significant difference in *P. falciparum* parasite carriage between the age groups [5–9 yrs.: 28% (712/2545); 10–15 yrs.: 30.4% (709/2336); *p*-value = 0.068]. The total number of mixed infections (*P. falciparum* plus *P. ovale* and/or *P. malariae*) detected in the study population was 204 with a non-significant difference between the age groups 5–9 years 15.73% (112/712) and 10–15 years 12.98% (92/709); (*p*-value = 0.139), while the single infections were 1217. Most of the mixed infections were found among the female gender 16.74% (110/204) in comparison to the males 12.30% (94/764); (*p*-value = 0.017) (Table 1 and Additional file 1).

Table 1
Parasitological characteristics of the study participants.

Variables	Age group (years)		Gender	
	5–9	10–15	Female	Male
Positive	28% (712/2545)	30.4% (709/2336)	27% (657/2459)	32% (764/2422)
Negative	72% (1833/2545)	69.6% (1627/2336)	73% (1802/2459)	68% (1658/2422)
χ2 (p-value)	3.328 (0.068)		13.770 (< 0.001)*	
Mixed Infection	15.73% (112/712)	12.98% (92/709)	16.74% (110/657)	12.30% (94/764)
Single Infection	84.27% (600/712)	87.02% (617/709)	83.26% (547/657)	87.70% (670/764)
χ2 (p-value)	2.192 (0.139)		5.661 (0.017)*	
Negative	72% (1833/2545)	69.6% (1627/2336)	73.3% (1802/2459	68.5% (1658/2422)
Asexual	25.7% (655/2545)	29.2% (682/2336)	25.4% (625/2459)	29.4% (712/2422)
Gametocyte	2.2% (57/2545)	1.2% (27/2336)	1.3% (32/2459)	2.1% (52/2422)
χ2 (p-value)	14.602 (0.001)*		16.137 (< 0.001)*	
Asexual	91.99% (655/712)	96.19% (682/709)	95.13% (625/657)	93.19% (712/764)
Gametocyte	8% (57/712)	3.81% (27/709)	4.87% (32/657)	6.80% (52/764)
χ2 (p-value)	11.253 (0.001)*		2.380 (0.123)	
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 29.11% (1421/4881). 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) (Figs. 1 and 2).

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 = 0.001). There was 2% (84/4881) 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% (84/1421). 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 1.3% (32/2459) and male gender 2.1% (52/2422) (*p*-value < 0.001). The gametocyte prevalence was relatively stable with no statistically significant difference throughout the sampling periods (Fig. 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 prevalence

The total number of samples used in assessing the relationship between gametocyte density, MOI and mosquito infection prevalence 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 prevalence (0.682, *p*-value < 0.0001). In addition, a positive correlation between multiplicity of *P. falciparum* infection (MOI) and mosquito infection prevalence was reported (0.451, *p*-value = 0.035). However, the correlation between MOI and gametocyte density was not statistically significant (0.167, *p*-value = 0.459). The mosquito infection prevalence is defined as the percentage of infected mosquitoes after day 10th of the membrane-feeding assay (Table 3 and Fig. 3).

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

Parameters	Infection rate (*P*-value)	Gametocyte density (*P*-value)	MOI (*P*-value)
Infection prevalence	1 (*Ref*)	0.682 (< 0.0001)*	0.451 (0.035)*
Gametocyte density	0.682 (< 0.0001)*	1 (*Ref*)	0.167 (0.459)
MOI	0.451 (0.035)*	0.167 (0.459)	1 (*Ref*)

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

In Table 4, a multiple regression was run to predict mosquito infection prevalence from gametocyte density (gametocyte/µl) and multiplicity of infection (MOI). These variables statistically significantly predicted mosquito infection prevalence, $F(2, 19) = 20.235$, $p < 0.0001$, $R^2 = 0.681$. All the two variables added statistically significantly to the prediction, $p < 0.05$.

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	**< 0.001**
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 prevalence is accounted for by the gametocytes density and MOI. Thus, the formulated equation for mosquito infection prevalence in this study is:

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

Where $\hat{Y}$ is the expected Mosquito Infection prevalence, X_1 and X_2 are the Gametocyte 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 prevalence 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 [34, 35]. 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 [36]. 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 53.76% (764/1421) and gametocyte carriers 61.9% (52/84) as compared to the females [asexual carriage; 46.23% (657/1421), gametocyte carriage; 38.1% (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 [37]. 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 [38]. 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 [39].

A significant positive association was found between gametocyte density and mosquito infection prevalence (correlation coefficient = 0.682, *p*-value < 0.001). High infection prevalence were observed among mosquitoes that fed on carriers with high gametocyte densities. This result corroborates with the findings of other studies [21, 40]. A plausible explanation to our finding was given by Bradley *et al.* 2018, which states, prior to plateauing at high female gametocyte density, an increase in gametocytaemia corresponds with a rapid increase in the proportion of infected mosquitoes indicating that female gametocyte density accounts for most of the variability in mosquito infection [26, 27]. The total gametocyte density is inversely proportional to the proportion of male gametocytes [27, 41]. That is, in low-density infections, the parasites increase their male gametocyte production in order to ensure that all the female gametocytes are fertilised thereby increasing their chances of transmission (a strategy known as fertility assurance). Therefore, in low gametocyte density infections, transmission may be hindered due to a lack of male gametocytes [41, 42].

The relationship between multiplicity of *P. falciparum* infection and mosquito infection prevalence is not well documented. This study reports a significant positive correlation between MOI and mosquito infection prevalence (correlation coefficient = 0.451, *p*-value = 0.035). *P. falciparum* isolates harbouring multiple distinct clones positively influence the mosquito infection prevalence. In contrast, a negative association between MOI and mosquito infection prevalence and intensity was reported elsewhere [43]. However, the interaction between MOI and gametocyte density was not statistically significant. This is in line with another study [43].

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 [44]. Therefore, understanding the association between gametocyte density and other parasite parameters like MOI with mosquito infection prevalence will improve our understanding of the dynamics of *P. falciparum* transmission. Our result indicates a significant and positive combined effect of the explanatory variables (gametocyte density and multiplicity of *P. falciparum* infection) on the mosquito infection prevalence $F(2, 19) = 20.235$, $p < 0.0001$,

R^2 = 0.681. The result showed that, MOI and gametocyte density account for about 68.1% of the variations in mosquito infection prevalence. 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 [45, 46, 47]. Another plausible explanation for the association between MOI, gametocyte density and mosquito infection prevalence found in this study may be due to a strategic investment by the parasite to maximise either in-host survival or between-host transmission [42, 48, 49, 50] At relatively low MOI, the level of intra-host competition is relatively low and the *P. falciparum* parasites reduce the conversion rates to enhance asexual replication and in-host survival through a reproductive restraint process. However, at high MOI, the intra-host competition is too intense for reproductive restraint to efficiently help the parasites. As a result, the parasites tend to increase the conversion rate to facilitate between-host transmission [48, 49]. The high mosquito infection prevalence observed at high MOI can be explained by the maximised gametocyte production to increase the chances of between-host transmission.

These findings 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. In addition, the results should be validated using a larger data set.

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 statistically significant and positive combined effect of the explanatory variables on the mosquito infection prevalence will help in determining the human infectious reservoirs in different malaria endemic settings. Malaria control interventions that are highly efficient in reducing multiple clone parasite carriage and gametocyte density could aid in disrupting the transmission of the parasite thereby will facilitating the ultimate elimination of the disease in the region.

Declarations

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.

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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.

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Figures

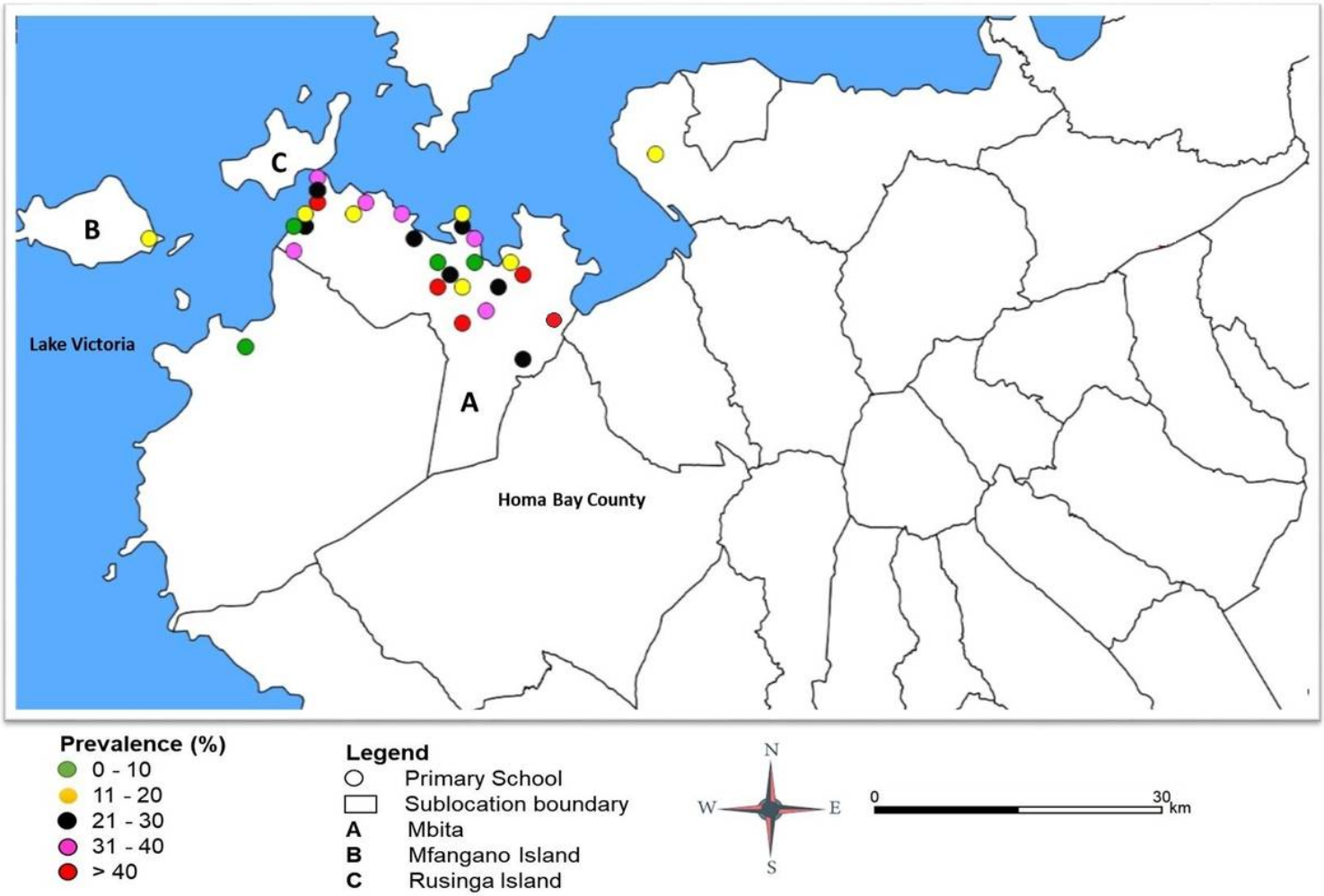

Figure 1

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.

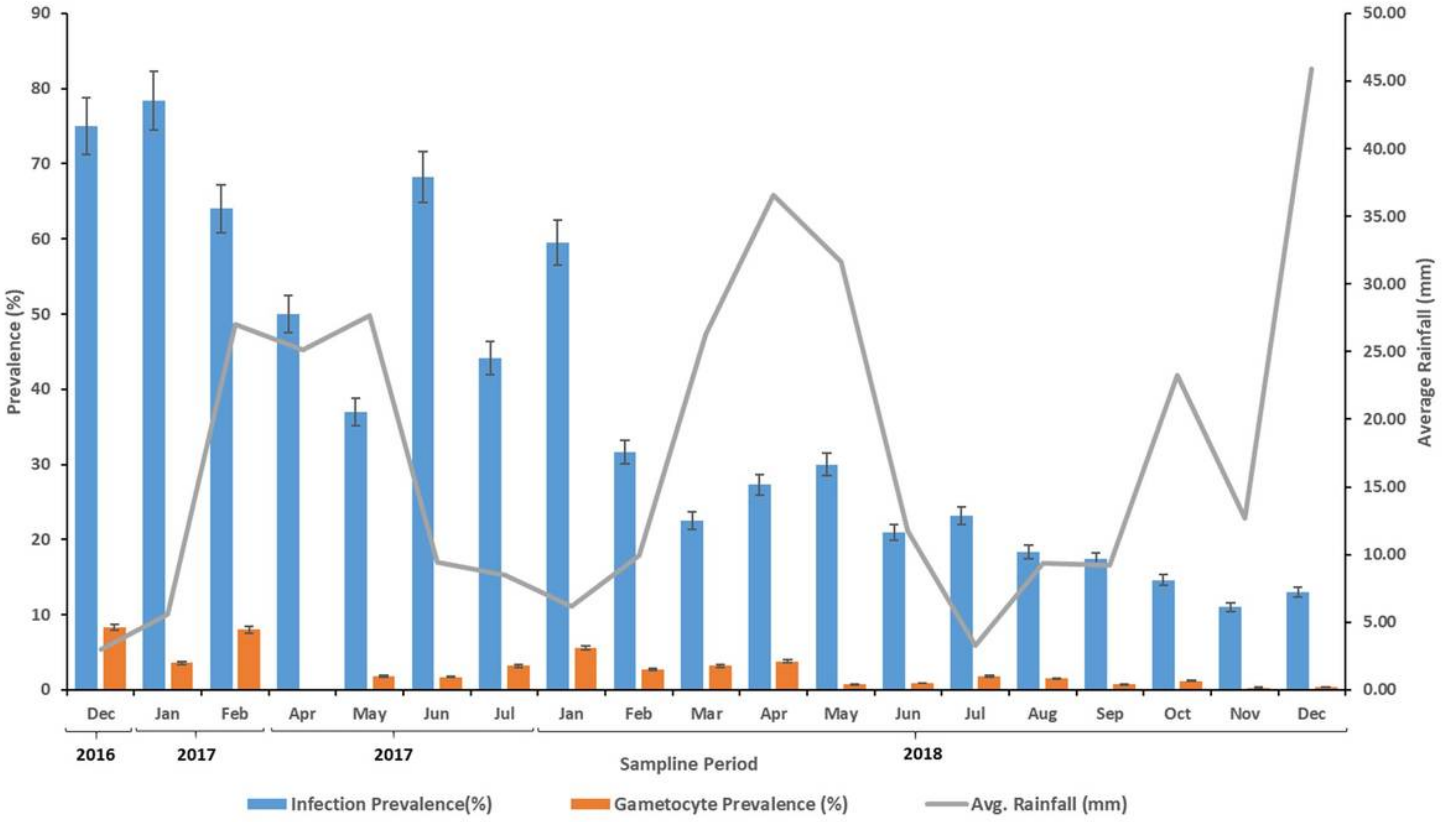

Figure 2

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

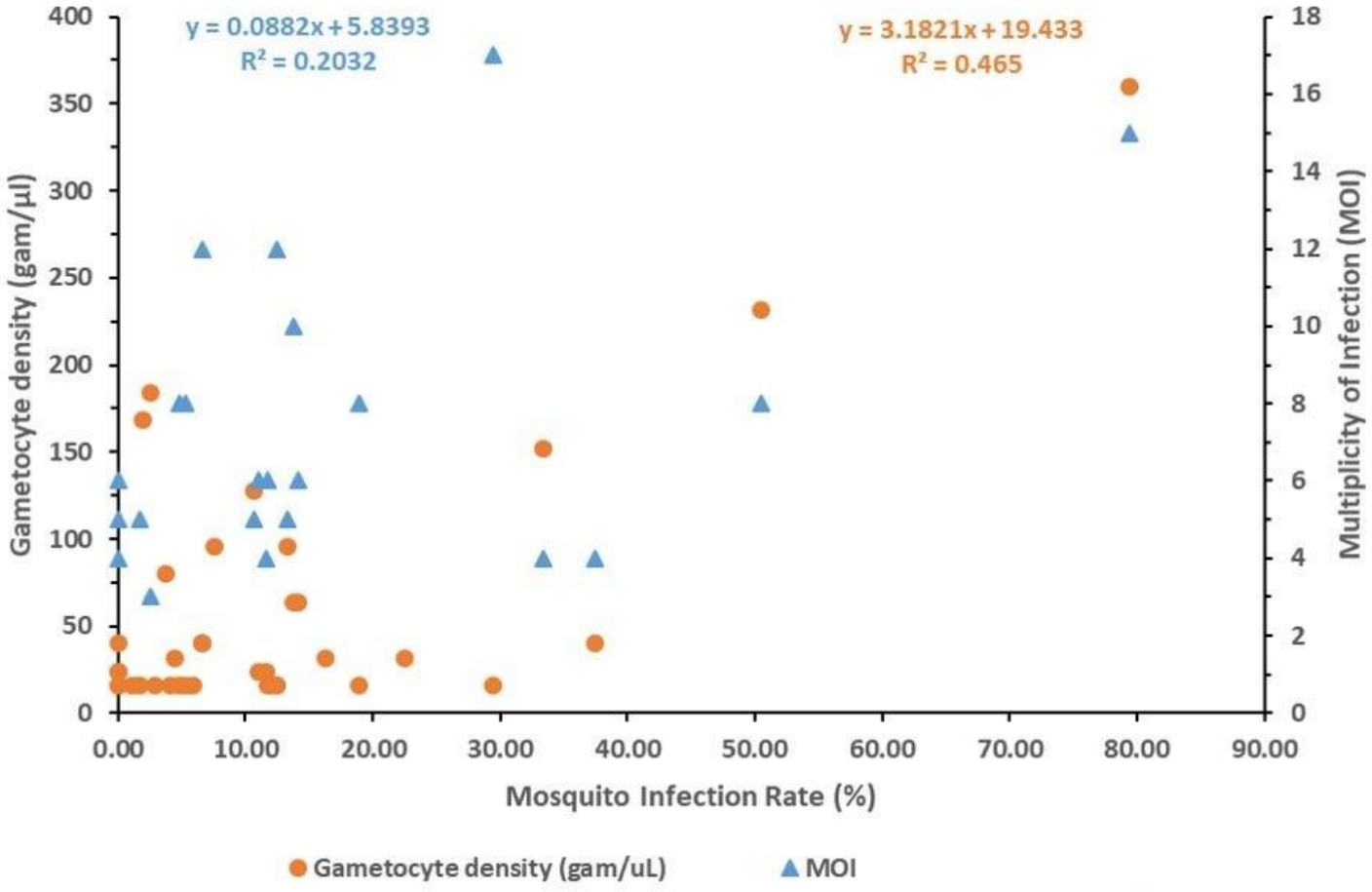

Figure 3

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

Supplementary Files

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