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Prevalence and diversity of *Plasmodium* species in pregnant women attending antenatal clinics in selected health centers of Kakamega County, Western Kenya

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ABSTRACT

Malaria remains a major public health concern around the world with tropical and sub-tropical regions bearing the greatest brunt despite up-scaling of control strategies. Despite the provisioning of insecticidal nets to expectant women attending antenatal care facilities, *Plasmodium* infections was a recurrent problem in Kakamega County. We hypothesized that socioeconomic factors were risk factors for the high prevalence levels of asymptomatic *Plasmodium* infections (API) among pregnant women in the County. A prospective cohort of 304 asymptomatic pregnant women aged 18 years and older was identified to assess the association between patient characteristics and risk of API using a cross-sectional study design. The study was conducted across four representative antenatal clinics (ANC) in the region. A pre-structured and pre-tested questionnaire was used to obtain the socio-demographic characteristics, residence, and knowledge of malaria. The questionnaire was followed by a face-to-face interview. Each participant provided a fingerprick blood sample for a thick and thion blood smear for parasite studies at Masinde Muliro University Science and Technology. The slides were examined at $\times 100$ oil immersion. *Plasmodium* and the developmental stages were checked, quantified, and recorded. The data were analyzed using SPSS ver 16. Differences in parasite densities for various parameters was assessed using Mann Whitney U and Kruskal Wallis non-parametric tests. Ordinary linear squares (OLS) regression was used to uncover any significant associations at 95% CI and p-value ≤ 0.05. The majority of the women had a post-primary education (75%), were married (60.9%), multigravidae (50%), in their second trimester (41.4%), residing in rural areas (62.2%), used ITNs (77%), and did not practice IRS (67.8%) as a malaria prevention method. *Plasmodium* species infection prevalence was (24.34%; 95% CI, 19.52–29.16) *P. falciparum* (82.4%; 95% CI 73.72–91.08) accounting for the majority of infections. The parity and gestation status of the pregnant women were found to have a significant association with API. This study showed that a significant number of women in Kakamega County attending ANCs were asymptomatic for *Plasmodium* malaria. We propose that malaria screening through microscopy and treatment should be incorporated into maternal health within the county.

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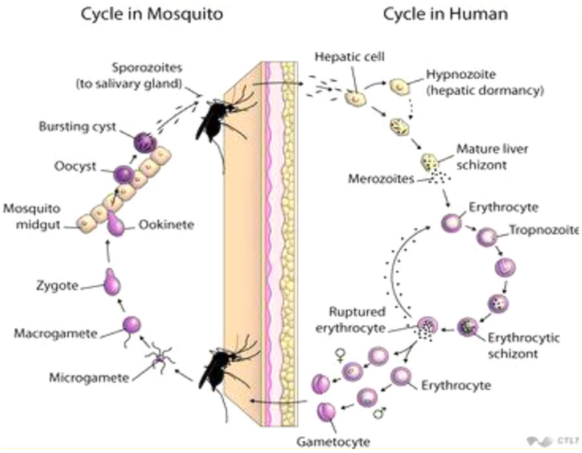

Fig. 1. Life cycle of *Plasmodium* [Source: http://ocw.jhsph.edu/].

Introduction

Malaria remains a major public health concern around the world with tropical and sub-tropical regions bearing the greatest brunt despite up-scaling of control strategies. Of the five malaria parasite species, *Plasmodium falciparum* poses the most serious threat. The prevalence of the female *Anopheles* mosquito maintains the transmission of *P. falciparum* in the human population [27]. As illustrated in Fig. 1, the malaria parasite must infect successively two types of hosts, humans and female *Anopheles* mosquitoes. The infected female *Anopheles* mosquito during a bloodmeal on a human injects sporozoites into the bloodstream which swim to the liver. Here they mature into schizonts that multiply asexually in the hepatocytes (exo-erythrocytic schizogony) to release merozoites which attack erythrocytes. Within the erythrocytes, the merozoites asexually multiply rapidly until the cell bursts and are released into the bloodstream to affect more erythrocytes (erythrocytic schizogony). The infection and bursting of hepatocytes and erythrocytes is what is associated with malaria signs and symptoms. Some of the merozoites differentiate into sexual forms of the parasite to form gametocytes (microgametes and macrogametes), which circulate in the bloodstream. And when a female *Anopheles* picks the gametocytes during a bloodmeal, they develop into gametes. In the mosquito stomach fertilized female gametes (zygotes) develop into ookinetes that burrow through the mosquitoes midgut wall to form oocysts. In the oocysts sporozoites develop and the oocysts burst eventually to release sporozoites into the body cavity of the mosquitoes. The sporozoites travel to the salivary glands of the mosquito and eventually released into a healthy human being during bloodmeal by the mosquitoes to start the infection again (Adopted from Centers for Disease Control and Prevention website, www.cdc.gov >malaria>biology)

The Sub-Saharan Africa (SSA) region had a reduction of 44% of malaria deaths from 680,000 to 386,000 from the period 2000 to 2019. This was attributed to sustainable improvement in health systems, leadership and governance, health financing, essential medicines, service delivery, and empowering communities [2]. However, in as much as there was a marked decrease in malaria cases, the intended goals aimed at malaria control and eventual elimination in endemic countries have not been very successful [36]. The stalling of the intended millennium development goal of curtailing malaria transmission in the SSA region as envisaged by the African Union (AU) Agenda 2063, was because SSA was associated with poor surveillance and response systems, lack of sufficient disease control programs, insufficient or non-existent medical infrastructure resulting to medical tourism [1], collapsed healthcare due to insecurity and poor coordination among humanitarian agencies [10]. Various diseases such as multi-drug resistant tuberculosis and other chest infections [38], Lassa haemorrhagic fever [19], anaemia and malnutrition [29] have contributed to the hindrance of the realization of Africa's economic potential. This calls for multisectoral approach to prevention of these diseases and possibly their elimination. Integrated vector management that gets rid of the malaria vector, removes other mosquito transmitted such as chikungunya from the population.

The government of Kenya (GoK) through its Big 4 Agenda has identified improving health for its citizens as a key plank aimed at sustaining socio-economic growth for the country. However, 25 million people are at risk of malaria infection and the disease accounts for 3.5 million clinical cases accounting for up to 50% of outpatient attendance and results in 10,700 deaths annually. Malaria is endemic to Lake Region and the coastal strip. However, recently fifteen counties including Kakamega have been put on the alert because of a resurgence of the disease [5]. Kakamega County is in western Kenya highlands bordering the Lake Region and immense efforts have been put in place to control the transmission of malaria and

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other mosquito-transmitted infections. However, malaria still ravages the human population particularly pregnant women and children under five years [4]. In 2019, 40% of pregnant women attending antenatal clinics in Kakamega County were infected with malaria (Unpublished data courtesy of KCTRH). However, this data lacks asymptomatic *Plasmodium* infection (API) cases. With the provision of insecticidal treated nets (ITNs) particularly to pregnant women attending antenatal care facilities, *Plasmodium* infections should not be a recurring problem. On the contrary, this has not been the case in the neighboring county of Bungoma [35] and other counties, Kakamega included [37].

It is estimated that up to 125 million expectant mothers annually stand the risk of malaria infection. Sub-Saharan Africa (SSA) accounts for the greatest disease prevalence in this risk group [8]. In Kenya, API prevalence in pregnant women was about 15% and is still responsible for many side effects on the mother and the fetus [26]. Risk factors pointed out as contributing to this API include gravidity, gestational period, and age. Kakamega County reported unstable malaria that commonly caused epidemics mainly during the rainy seasons. Most studies have implicated *P. falciparum* to be the sole cause of API in expectant women [34] and the only report available for western Kenya counties is for Bungoma County [35]. To control and possibly eliminate malaria, it is imperative to assess the impact of risk factors such as reduced funding, socio-economic status of households, education levels of household heads, and residence [5]. So this study sought to associate age, residence, education, pregnancy status, ITN, and IRS use as risk factors for the prevalence of API among pregnant women in Kakamega County.

During pregnancy, API has been associated with numerous health risks to both the mother and the unborn baby and causes a lot of maternal and infant deaths. Approximately 10,000 women and over 200,000 infants die annually in SSA due to malaria [32], and 6,000 women and 35,000 infants [18]. Maternal deaths were caused by anemia [35], placental infections resulting in the sequestration of infected red blood cells in the placenta's intervillous space. This caused placental inflammation and immune reaction [25]. The placenta fails to perform its role in the exchange of materials between the mother and the fetus. The gross effect is prematurity, low birth weight, abortions, and fetal mortality [22]. A study showed that pregnant women act as reservoirs of malaria transmission [6]. This is confirmatory that in the face of declining malaria episodes, more surveys need to be done to provide evidence-based information for program and policy managers.

Malaria disease burden is most challenging to pregnant women, their families, and the health systems. The debility effect reduces productivity due to lost man-hours causing households to lose livelihoods [32], sick pregnant women can not effectively care for their children which may result in labor reallocation, and puts pressure on the available medical facilities [33]. Economic constraints aggravate the situation because pregnant women may not get the requisite diagnosis and treatment of the disease. The incorporation of infectious diseases such as malaria in reproductive health could be a step in the right direction in combating malaria in pregnancy [32].

Governments and world health bodies have collaborated immensely to improve the health status of pregnant women and the general populace. Kenya and other East African countries, governments have come together to ensure maternal protection through freely giving ITNs [26], emphasizing indoor residual spraying (IRS) [23], prompt diagnosis and treatment with artemether combined therapy (ACT) which are readily available in local health centers and over the counter in drug stores [17] However, the effectiveness of antenatal clinics to improve health outcomes of pregnant through availing the net has been put to question in studies done in western Kenya [13]. To this, the Kenya government waived the maternal fee for its pregnant women which has not yielded a significant change in stillbirth outcomes. However, the extent to which this has decreased malaria in pregnancy is still not quantified in many counties [18].

Asymptomatic malaria infection in women has been hampered by low parasite count [26] which evade the diagnostic procedures such as patient history and fever available in most public health centers [32]. Additionally, poor health-seeking behavior by pregnant women infected with API was a major concern to health practitioners [3]. This caused complications in the mother and increased infant mortality [11]. This study purposed to assess the *Plasmodium* species prevalence and diversity in expectant women attending ante-natal clinics in selected health centers in Kakamega County, western Kenya highlands.

Materials and methods

Study sites

This was a facility-based study conducted in selected health centers in Kakamega County of Western Kenya highlands. Kakamega County is located on latitude 0°16'29N, longitude 34°45'31E, and 1,535 m above sea level (asl). Kakamega County has twelve administrative Sub-counties. This study was conducted in Sub-county health centers in four Sub-counties: Navakholo hospital – Navakholo (0°24'49N, 34°49'40E), Malava hospital – Malava (0°26'55N, 34°51'14E), Iguhu hospital – Ikolomani (0°9'48N, 34°44'45E) and County Referral and Teaching hospital (KCRTH) – Lurambi (0°16'29N, 34°45'31E). Kakamega County receives high rainfall of between 1280 and 2214 mm annually which is evenly distributed all year round. The long rainy season is from March – July and the short rainy season is from September – November with dry seasons in between. The temperature ranges from 18°C to 29°C with November to February being the hottest months (Kakamega meteorological Station). Kakamega County is predominated by Luhya ethnic tribe with several sub-tribes. The county's economic activities include mixed farming and livestock rearing, trade, mining, quarrying, and sand harvesting. Sugarcane and maize are the major cash and staple crops farmed. Kakamega County is one of the 15 counties in the Lake Victoria basin where malaria transmission has remained high despite up-scaled control strategies particularly the provision of the LLINs.

Navakholo inhabited by Banyala people is a sugarcane growing area and other farming practices. Malava is surrounded by a remnant of tropical rainforest and a rise in population growth has seen part of this forest reclaimed for human settlement. Sugarcane farming is a major source of income for the Kabras people who inhabit the Sub-county. Iguhu area is inhabited by Idakho people. It is surrounded by evergreen farmlands as well as other subsistence farming. Its proximity to River Yala and its mosaic of land-use practices, makes it have distinct malaria epidemiology through harboring breeding grounds for the principal malaria vectors [24]. While the health facilities in Navakholo, Malava, and Iguhu may be considered to be in rural settings, KCRTH is in Kakamega County headquarter, so urban. The shift in plantation sugarcane farming and forest clearing in Navakholo and Malava could have increased the anopheles abundance. On the other hand, KCRTH receives referral health cases from the county and neighboring counties and countries such as Uganda, Rwanda, and Burundi. Unpublished data from KCRTH indicate that malaria prevalence in pregnant women was 40% in 2019.

Study design

Target population

A prospective cohort of 304 asymptomatic pregnant women age 18 and older was identified to assess the association between patient characteristics and risk of API using a cross-sectional study design. The study was conducted across four antenatal clinics (ANC). This was to enable indicating associations among variables and therefore useful in formulating a hypothesis for future research.

Sample size estimation

The Fisher's formula for sample size determination for cross-sectional studies was used in line with the objective of the study. The formula is suitable when a simple random sampling technique is used since it takes into account the estimated prevalence from most recent previous studies [15]. This study used an estimated prevalence (P) of 27% as derived from the 2015 WHO estimation of the prevalence of malaria parasites in the Lake Victoria basin malaria-endemic zone. Therefore, the assumption was that the prevalence of the *Plasmodium* in Kakamega County was near equal to that of the Lake Victoria basin malaria-endemic region due to its proximity and similarity in socio-economic activities but different climatic conditions.

$$n = \frac{Z^2 PQ}{L^2}$$

Where n = Sample size being calculated
Z = Z-statistic at an alpha of 0.05
P = Estimated prevalence from the most recent previous study
Q = 1 – P
L = Allowable margin of error

Therefore, the 304 participants were calculated as shown

$$n = \frac{1.96^2 \times 0.27 \times 0.73}{0.05^2} = 304$$

To take into consideration eco-epidemiological considerations, an equal number of participants ($n = 76$) were recruited in the four study sites.

Safety procedures

Sample collectors observed all safety protocols by wearing protective clothing such as gowns, gloves to prevent contamination due to contact with blood. Sterile techniques were followed to minimize contamination and harming the participant.

Data collection

Data was collected from March – November 2019: Navakholo – July, September, and October; Malava – March, July, and August; Iguhu – April, May, and June and KCRTH September, October, and November. Asymptomatic pregnant women attending antenatal care in the four hospitals aged 18 years and over were recruited to participate in the study. Eighteen years is the legal adult age in Kenya while 45 years is assumed to be the highest age before a woman transitions into menopause. A 20 - question structured questionnaire was used to obtain the socio-demographic characteristics, residence, and knowledge of malaria. The questionnaire was both in English and Kiswahili. The questionnaire was filled by a face-to-face interview in which some of the questions were translated into the local Luhya language.

A volume of about 2 ml of venous blood samples was collected by venipuncture by a qualified phlebotomist. The blood was collected by hypodermic needle and vacuum tubes (BD company, US) in vacuette tubes with lithium heparin to prevent blood from clotting. The samples tubes were labeled with random codes of the participants. The collected samples were placed in a cool box and carefully carried to Masinde Muliro University of Science and Technology (MMUST) for microscopic smear preparation and examination.

For malaria parasite examination a drop of blood on two grease-free slides for each sample were prepared, one for a thin smear and the second for a thick smear. The code number of the participant corresponding to the register from the centers was inscribed on the slide using an HB pencil. Two microscopists examined each slide and a slide was declared positive or negative after reading fields containing 300 WBCs [28].

Parasite species identification

A thin smear for each participant was prepared by placing a drop of blood at the center of the slide and another clean slide was used as a spreader. The slides were then placed horizontally on a rack to air dry for 30–45 min. After which they were fixed in absolute methanol for 30 seconds and then air-dried again. The slides were then stained with 3% Giemsa for 30 min and then examined at ×100 oil immersion. All asexual forms of the *Plasmodium* and the developmental stages were checked and recorded. The species were identified as *P. falciparum, P. vivax, P. malariae, P. ovale,* and *P. knowlesi*.

Parasite density determination

A thick smear for each participant was prepared and parasite densities were calculated as parasites per microliter of whole blood (parasites/µl) determined by the number of parasites counted × 8000 = parasites/ µl. The mean parasite densities (MPD) per µl were determined by counting the number of all parasite forms (trophozoites, schizonts, and gametocytes) to the number of WBCs in the field of view and scaled up to the WHO assumption of 8000 WBC/ µl.

Inclusion criteria

i Pregnant women aged above 18 and within the reproductive bracket.
ii Residents of Kakamega County for at least 6 months.
iii HIV seronegative
iv Willing to sign consent forms and take part in the study for the duration of the study.

Exclusion criteria

i Pregnant women aged below 18 years and over 45 years.
ii Non-residents of Kakamega County.
iii HIV positive pregnant women
iv Unwillingness to participate in the study and sign the consent forms.

Limitations of the study

The limitation of this study was the inability to collect data all year round across the four seasons and the inability to use other diagnostic tools such as polymerase chain reaction (PCR) due to limited financial resources.

Data management and analysis

Collected data were entered into Microsoft Excel spreadsheets and cleaned. The data were analyzed using SPSS ver 16.0 in which descriptive statistics were carried out to determine relative frequencies and proportions. Tables and Graphs (by MS Excel) were generated to show the distribution of relative frequencies of various variables. The Pearson's Chi-square test was used to test for statistical significance between *Plasmodium* species prevalence across study sites, age, education, parity, and gestation status of the participants. The malaria parasite densities (MPD) across different variables for pregnant women were calculated and tested using the Kruskal Wallis test and the Mann Whitney U test was used to analyze the difference between groups. Association between variables was considered significant at a 95% confidence interval (CI) and p-value ≤ 0.05.

Data quality control and management

The data collectors were clinical officers trained by the principal investigator (PI) on expectations of the study particularly consent seeking and filling out the questionnaires. The questions of the questionnaire were made in a clear, understandable, and flowing format which was regularly reviewed by the PI and other researchers. Experienced clinical officers were used to collect blood samples, prepare and read the slides. A single type of microscope was used throughout to minimize error. For quality assurance, slides with disagreement in reading, a third microscopist or the PI rechecked, and their reading was final.

Table 1
Baseline characteristics of pregnant women attending antenatal clinic in Kakamega County.

Variable		Navakholo	Malava	Iguhu	KCTRH	Total	
		N (%)	N (%)	N (%)	N (%)	N (%)	*p*-value
Age (years)	Median (IQR)	28 (25– 35)	28 (25 -35)	27(25-35)	27 (25– 35)	28(25–35)	0.162
Education	Primary	23 (30.3)	18 (23.7)	19 (25)	16 (21)	76 (25)	0.530
	Secondary	36 (47.4)	36 (47.4)	35 (46.1)	31 (40.8)	138 (45.4)	
	Tertiary	17 (22.3)	22 (28.9)	22 (28.9)	29 (38.2)	90 (29.6)	
Marital status	Single	18 (23.7)	24 (31.6)	22 (28.9)	29 (38.2)	93 (30.6)	0.683
	Married	51 (68)	46 (60.5)	47 (62.8)	41 (53.9)	185 (60.9)	
	Widowed/divorced	7 (8.3)	6 (7.9)	7 (8.3)	6 (7.9)	26 (8.5)	
Parity	Primigravidae	13 (17.1)	16 (21.1)	14 (18.4)	21 (27.6)	64 (21.1)	0.534
	Secundagravidae	20 (26.3)	21 (27.6)	27 (35.5)	20 (26.3)	88 (28.9)	
	Multigravidae	43 (56.4)	39 (51.3)	35 (46.1)	35 (46.1)	152 (50)	
Gestation	1st trimester	15 (19.7)	18 (23.7)	15 (19.7)	22 (28.9)	70 (23)	0.419
	2nd trimester	29 (38.2)	30 (39.5)	32 (42.1)	35 (46.1)	126 (41.4)	
	3rd trimester	32 (42.1)	28 (36.7)	28 (38.2)	19 (25)	108 (35.6)	
Residence	Rural	61 (80.3)	55 (72.5)	40 (52.6)	33 (43.4)	189 (62.2)	0.0001
	Urban	15 (19.7)	21 (27.6)	36 (47.3)	43 (56.6)	115 (37.8)	
Use ITNs	Yes	51 (67.1)	54 (71.1)	62 (81.2)	67 (88.2)	234 (77)	0.0075
	No	25 (32.9)	22 (28.9)	14 (18.8)	9 (11.8)	70 (23)	
Use IRS	Yes	29 (38.2)	32 (42.1)	19 (25)	18 (23.7)	98 (32.2)	0.0001
	No	47 (61.8)	44 (57.9)	57 (75)	58 (76.3)	206 (67.8)	

IQR – interquartile range, ITNs – insecticide-treated nets, IRS – indoor residual spraying

Results

Socio-demographic characteristics of the participants

The baseline socio-demographic characteristics of the participants were as shown in Table 1. The mean age was 29 years while the median was 28 years. The majority of the women had a post-primary education (75%), were married (60.9%), multigravidae (50%), second trimester (41.4%), residing in rural areas (62.2%), used ITNs (77%), and did not practice IRS (67.8%) as a malaria prevention method. Expectant women attending KCTRH used the ITNs (88.2%) more than the county average (77%), while those of Malava (42.1%) used IRS more than the other women in the county (32.2%). The use of ITNs was significantly higher among the participants while the use of IRS was significantly lower. This study reported that the pregnant women of the county have knowledge about and used the ITNs and IRS to control malaria transmission.

Malaria parasite prevalence in pregnant women in Kakamega County

A total of 74 asymptomatic pregnant women were detected to be infected with *Plasmodium* parasites representing a *Plasmodium* species infection prevalence of 24.34% (95% CI, 19.52–29.16). The prevalence of malaria parasites was presented in table 2. Iguhu (36.8%) had the highest prevalence while Malava had the least prevalence. There was no significant difference in *Plasmodium* parasites prevalence ($p = 0.1068$) across the study sites. The age group 21–25 years (40.6%) had the highest prevalence while 36–40 years had the least prevalence. There was no significant difference in *Plasmodium* parasites prevalence ($p = 0.1039$) in the four age groups. Pregnant women with primary education (57.9%) had the highest prevalence while those with tertiary education (10.0%) had the least prevalence. There was a significant difference in the prevalence of *Plasmodium* parasites prevalence ($p = 0.0001$) across the education levels. Primigravidae women (54.7%) had the highest prevalence while multigravidae women (8.6%) had the least prevalence. There was a significant difference in the prevalence of *Plasmodium* parasites ($p = 0.0001$) in the parity groups. Women in their first trimester of gestation (60%) had the highest prevalence while those in the third trimester (11.1%) had the least prevalence. There was a significant difference in the prevalence of *Plasmodium* parasites ($p = 0.0001$) across the gestation periods. This study reported that the study site and age have no significance on malaria parasites but *Plasmodium* parasites prevalence reduces significantly with level of education, parity status, and gestation period.

Plasmodium *species diversity in pregnant women in Kakamega County*

The malaria parasite species diversity was as shown in Fig. 2 All the four malaria parasites were observed during microscopy. *P. falciparum* (82.4%, 95% CI 73.72–91.08) was the most prevalent species while *P. knowlesi* was not observed in any of the slides. There was a significant difference in the prevalence of the *Plasmodium* parasites in the study participants ($p = 0.026$). Across the study sites, Navakholo had three species *P. falciparum, P. vivax,* and *P. malariae*, Malava had two species *P. falciparum* and *P. malariae*, Iguhu had all the four species while KCTRH had two species *P. falciparum* and *P. ovale*. Age groups below 30 years, women with secondary education and below, primigravidae, and those in the first trimester showed a prevalence of all the *Plasmodium* species.

Table 2
Prevalence of *Plasmodium* parasites across study sites, age groups, education levels, parity, and gestation.

		NE (NI)	Prevalence (%)	95% CI	*p*-value
Study site	Navakholo	76 (19)	25.0	15.26–34.74	0.1068
	Malava	76 (12)	15.8		
	Iguhu	76 (28)	36.8		
	KCTRH	76 (15)	19.7		
Age group	15–20	37 (10)	27.0	14.07–33.60	0.1040
	21–25	69 (28)	40.6		
	26–30	85 (15)	17.6		
	31–35	53 (11)	20.8		
	36–40	41 (6)	14.6		
	41–45	19 (4)	21.1		
Education	Primary	76 (44)	57.9	9.47–31.85	0.0001
	Secondary	138 (21)	15.2		
	Tertiary	90 (9)	10.0		
Parity	Primigravidae	64 (35)	54.7	13.33–37.25	0.0001
	Secundagravidae	88 (26)	29.5		
	Multigravidae	152 (13)	8.6		
Gestation	1st trimester	70 (42)	60	7.61–27.42	0.0001
	2nd trimester	126 (19)	15.1		
	3rd trimester	108 (12)	11.1		

NE – number examined, NI – number infected

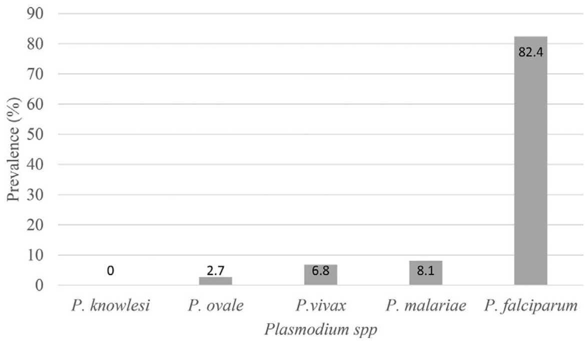

Fig. 2. Prevalence of *Plasmodium* species in pregnant women attending ante-natal clinics in Kakamega County

Plasmodium *parasite densities in pregnant women in Kakamega County*

The MPD based on the site, age, education, parity, and gestation was as shown in Table 3 Navakholo had the highest MPD (585 parasites/µl) while KCTRH had the least (495 parasites/µl). However, MPD was not significantly different ($p = 0.932$) across the four study sites. The age group 15–20 years had the highest MPD (670 parasites/µl) while 40–45 years had the least MPD (387 parasites/µl). It was observed that MPD values decrease with an increase in age. However, this was not significantly different ($p = 0.701$). Level of education did not have significance on MPD because pregnant women with secondary education had the highest MPD (538 parasites/µl) while those with tertiary education had the least (465 parasites/µl). Parity ($p = 0.0001$) and gestation ($p = 0.0001$) status were both had significantly different MPD. Therefore, this study reported that the pregnancy status had an impact on the MPD of the woman.

Discussion

This study reported an API prevalence of 24.34% which is among the highest in the country as well as in this sub-region of western Kenya. We found gestation period and parity status as some of the leading risk factors for *Plasmodium* infection. Pregnant women within the county may likely act as reservoirs that sustained malaria transmission. Additionally, it may be an index that the consumption of malaria transmission mitigation strategies is yet to fully bear fruits within the region or the pregnant women sub-set. Mothers who seek antenatal care in health centers have low health-seeking behaviors concerning malaria. Unless known malaria signs and symptoms are presented, they do not seek health care. This could be a hindrance to the malaria elimination strategy in the region.

The microscopy detection of an API of 24.34% among pregnant women should call for continuous screening of women attending antenatal clinics to enable them to make proper decisions about the malaria disease. The women would be taught about the complication that may arise from malaria infection and hence institute appropriate protective methods. The findings are consistent with those of a study in Ouagadougou, Burkina Faso 24% [9]. These findings are lower than a study done

Table 3
Mean parasite densities in pregnant women in Kakamega County.

		NE (NI)	MPD (Number/µl)	95% CI	
Site	Navakholo	76 (19)	585	256–914	0.932
	Malava	76 (12)	534	184–885	
	Iguhu	76 (28)	495	227–765	
	KCTRH	76 (15)	466	Ref	
Age (years)	15–20	37 (10)	670	356–1124	0.701
	21–25	69 (28)	576	409–1100	
	26–30	85 (15)	523	94–673	
	31–35	53 (11)	506	1–442	
	36–40	41 (6)	489	3–515	
	41–45	19 (4)	387	Ref	
Education	Primary	76 (44)	500	383–771	0.583
	Secondary	138 (21)	538	166–867	
	Tertiary	90 (9)	465	Ref	
Parity	Primigravidae	64 (35)	640	450–1561	0.0001
	Secundagravidae	88 (26)	536	126–694	
	Multigravidae	152 (13)	384	Ref	
Gestation	1st trimester	70 (42)	600	108–1089	0.0001
	2nd trimester	126 (19)	471	60–889	
	3rd trimester	108 (12)	493	Ref	

NE – number examined, NI – number infected, MPD – mean *Plasmodium* densities

in Nigeria 40.0%. However, the results are higher than the findings of studies done in Kinshasa in Congo at 21.6% [21], Nigeria teaching hospital at 3.1% [14], and Jawi District, Northwest Ethiopia [34]. Studies in other parts of Kenya showed lower API prevalence, 12.9% in Kwale County [26] and 21.6% in Bungoma County [35] except a study in the endemic lake region that showed a 31.0% prevalence [11]. Therefore, there must be a need for proper diagnosis and treatment of all asymptomatic pregnant women attending ante-natal clinics in Kakamega County to prevent them from acting as reservoirs and forestall transmission.

This study reported that infections of *P. falciparum* (82.4%) were the most common followed by *P. malariae* (8.1%). All the *Plasmodium* species were represented across the county except *P. knowlesi* which is known to infect monkeys. This finding was crucial in instituting diagnostic treatment procedures in pregnancy. The findings supported studies in Kwale, Kenya [26], West Ethiopia [12], in which *P. falciparum* had been reported to cause 80–95% of all malaria infections in pregnant women and the general population. However, the WHO health malaria report of 2017 placed infections by *P. falciparum* to be 99.0% [27]. Reasons for these variations range from climatic, environmental to human factors [12]. *Falciparum* malaria is the most severe form of malaria associated with major complications and death in the patients. This called for the need for a more aggressive drive in protecting the pregnant mother from the disease and its effects. This could be achieved through educational campaigns besides the provision of the ITNs, promoting IRS, and intermittent treatment of pregnant women.

Residence, use of ITNs and IRS were risk factors significantly associated with API with a pregnant woman in a rural area, who does not use ITN and does not practice IRS is more likely to suffer from a malaria bout. This result supported findings of other studies in which residing in a rural environment exposed pregnant women to *Plasmodium* infection more [34,7], some pregnant women had misgivings about the use of ITNs during pregnancy and thus suffered API more [31] and placental malaria was reported more in women who did not employ the use of IRS in Uganda [23]. The ITNs and IRS have been strongly advocated for use in pregnancy, but are still hindered by cost, availability, perception, politics, and environmental issues [20], and that insufficient IRS coverage does not offer protection [30]. These risk factors can be countered through EIC spread to the most vulnerable members such as pregnant women.

The parity and gestation status of the pregnant women were found to have a significant association with API. Primigravida women and those in their first trimester had a higher probability of API than the other statuses. This finding sends a critical piece of information to managers of ANC about where to channel more energy and attention concerning malaria infection and prevention. This result supported other findings in Northwest Ethiopia [12] and Kwale, Kenya [16], and [26].

Conclusions and recommendations

This study showed that a significant number of women in Kakamega County attending ANCs were asymptomatic for *Plasmodium* malaria. Women in their first pregnancy and those in their first trimester had a higher likelihood of API infections. Therefore, it was recommended that malaria screening through microscopy and treatment should be incorporated into maternal health within the county.

Author contribution

DMW conceptualized the study as partial fulfillment of the requirements of the award of M.Sc. and was the PI, DHM and JKC refined the concept and accepted to be supervisors of the project, DMW and NLK acted as field guides, DHM, DMW, and

KDM carried out data management and analysis and prepared the manuscript, and all the authors assisted in editing the article and gave authority for it to be presented for consideration for public action.

Author information

DMW is an M. Sc Medical Parasitology student, KDM is a Ph.D. student, NLK is a lecturer, and DHM and JKC are senior lecturers, MMUST. All authors belong to the Department of Biological Sciences, MMUST, Kenya.

Ethical consideration

This study was approved by the MMUST Institutional review committee vide approval number MMUST/IERC/01/18 while the National Council for Science, Technology, and Innovation (NACOSTI) gave the permit to conduct the research vide license number NACOSTI/P/18/19968/26534. The study was equally reviewd and apprioved by the Kakamega County Teaching and Referral Hospital ethical review committee. The study participants were provided with all the information to enable them to make an informed decision concerning their participation. Oral and written consent in either English, Kiswahili, or Luhya was obtained before involvement. The study was strictly voluntary and the privacy and confidentiality of the participants were highly respected. Unique codes were used for the study, while courtesy and polite language were used when addressing the participants.

Availability of data and material

Data and supplementary material are available from the corresponding author upon request.

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Declaration of Competing Interest

The authors wish to confirm that there are no known conflicts of interest associated with this manuscript and that there has been no financial support for this work that could have influenced its outcome.

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