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Predictors of *Plasmodium falciparum* Infection in the First Trimester Among Nulliparous Women From Kenya, Zambia, and the Democratic Republic of the Congo

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Background. Malaria can have deleterious effects early in pregnancy, during placentation. However, malaria testing and treatment are rarely initiated until the second trimester, leaving pregnancies unprotected in the first trimester. To inform potential early intervention approaches, we sought to identify clinical and demographic predictors of first-trimester malaria.

Methods. We prospectively recruited women from sites in the Democratic Republic of the Congo (DRC), Kenya, and Zambia who participated in the ASPIRIN (Aspirin Supplementation for Pregnancy Indicated risk Reduction In Nulliparas) trial. Nulliparous women were tested for first-trimester *Plasmodium falciparum* infection by quantitative polymerase chain reaction. We evaluated predictors using descriptive statistics.

Results. First-trimester malaria prevalence among 1513 nulliparous pregnant women was 6.3% (95% confidence interval [CI], 3.7%–8.8%] in the Zambian site, 37.8% (95% CI, 34.2%–41.5%) in the Kenyan site, and 62.9% (95% CI, 58.6%–67.2%) in the DRC site. First-trimester malaria was associated with shorter height and younger age in Kenyan women in site-stratified analyses, and with lower educational attainment in analyses combining all 3 sites. No other predictors were identified.

Conclusions. First-trimester malaria prevalence varied by study site in sub-Saharan Africa. The absence of consistent predictors suggests that routine parasite screening in early pregnancy may be needed to mitigate first-trimester malaria in high-prevalence settings.

Keywords. malaria; pregnancy; first-trimester; predictors; early pregnancy; factors; prevalence.

Malaria is a serious global health issue, with an estimated 228 million cases annually and 411 000 associated deaths worldwide in 2018 [1]. Nearly 85% of malaria deaths globally occurred in 21 countries, mostly in children younger than 5 years in sub-Saharan Africa [2]. In addition to children, pregnant women constitute a high-risk group, and in sub-Saharan Africa, 29% of all pregnancies are exposed to malaria infection [2]. Malaria infection in pregnancy can contribute to maternal anemia, preterm birth, stillbirth, and low birth weight [3]. Despite the known perinatal complications of malaria infection in pregnancy, most studies identifying predictors of malaria infection in pregnancy have only been conducted after 20 weeks of gestation. These studies show that factors associated with malaria infection in pregnancy include lower gravidity, duration of pregnancy spent in the rainy season, younger age, shorter stature, human immunodeficiency virus (HIV) infection, maternal anemia, lower body-mass index (BMI), lower education, and lower socioeconomic status [4–6]. With few studies assessing predictors of first-trimester malaria, our understanding of the epidemiology of *Plasmodium falciparum* infections in pregnancy is incomplete.

The first trimester represents a potential target for intervention to prevent the negative consequences of malaria in pregnancy. First-trimester malaria infection could impact the placentation process by inhibiting trophoblast invasion and disrupting placentation, thus affecting fetal growth [7–11]. It has been suggested that adverse changes in placentation could be even more pronounced among nulliparous women (women who have never given birth), especially those living in high

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malaria transmission areas [12]. Pregnant women living in high-transmission areas acquire resistance to malaria by developing antibodies that inhibit binding of *P. falciparum*-infected erythrocytes to the placenta in subsequent pregnancies [13, 14]. Thus, women who lack this parity-dependent immunity are unable to clear placental parasites quickly, leading to higher placental and peripheral parasitemia and chronic placental infection, which is associated with low birth weight and maternal anemia [14–18].

Despite the potentially deleterious effects of malaria during placentation, current treatment and prevention strategies leave women largely unprotected from malaria in the first trimester. To treat and prevent incident malaria infections in pregnancy, the World Health Organization recommends prompt diagnosis and treatment of malaria, intermittent preventive therapy in pregnancy with sulfadoxine-pyrimethamine (IPT-SP), and use of insecticide-treated nets (ITNs) [2]. However, in many malaria-endemic regions, diagnosing malaria in pregnancy is difficult because pregnant women frequently have parasite densities below the detection limit of common diagnostic tools. In addition, IPT-SP is not initiated until the second trimester because antenatal care typically begins around 20 weeks of pregnancy in many malaria-endemic regions, and IPT-SP is not recommended earlier in pregnancy because of a concern for teratogenic effects [19]. Discomfort, lack of ITN ownership, and difficulties hanging nets collectively undermine ITN adherence by women in their first pregnancy in malaria-endemic settings, where up to 75% of women do not regularly use ITNs prior to their first pregnancy [20, 21]. These problems with diagnosing, treating, and preventing malaria leave women underprotected from first-trimester malaria.

Only 2 studies have assessed predictors of malaria in early pregnancy [22, 23]. These studies did not find a relationship between malaria in early pregnancy and maternal factors such as age, height, BMI, socioeconomic status, or season that coincided with early pregnancy, but one found that women with lower overall educational attainment (no secondary education) were more likely to have malaria in early pregnancy [22, 23]. These single-site studies employed different study designs, limiting generalizability; one used a wide interval to define early pregnancy (less than 120 days), and both included mostly multigravid women [22, 23]. No study has assessed first-trimester malaria among nulliparous women across multiple settings.

In this work, we leverage a large clinical trial [24] to efficiently conduct the first multicountry study of first-trimester malaria among nulliparous women. With the overarching goal of informing malaria screening and prevention strategies among pregnant women, our study objectives were to determine the prevalence of first-trimester malaria across multiple settings in sub-Saharan Africa, and to assess potential predictors of first-trimester malaria.

METHODS

Parent Study Design and Population

We conducted a substudy of the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development Global Network for Women's and Children's Health Research trial of low-dose aspirin for the prevention of preterm delivery in nulliparous women with a singleton pregnancy (the ASPIRIN Clinical Trials Number NCT02409680) [24, 25]. Briefly, the ASPIRIN trial was a prospective, randomized, multinational clinical trial that tested the hypothesis that low-dose acetylsalicylic acid reduces the risk of preterm birth when administered in the first trimester [24]. The trial recruited 11 976 nulliparous women between 23 March 2016 and 11 April 2019 at 7 research sites in 6 countries (Democratic Republic of the Congo [DRC], Zambia, Guatemala, Pakistan, Kenya, and 2 sites in India) [24]. Pregnant women were recruited from primary healthcare centers and hospital-based clinics, and through community-based recruitment led by community health workers [25].

The inclusion criteria in the ASPIRIN trial included nulliparous women between 18 and 40 years of age (minors 14 years old or older could be enrolled if allowed by the country's guidelines) who were residents of the study area and had no more than 2 previous first-trimester pregnancy losses [24]. In addition, all women must have had a single live intrauterine pregnancy that was between 6 weeks and 0 days to 13 weeks and 6 days in gestational age that was confirmed by an early dating ultrasound [24].

Women were excluded if they had already been taking daily acetylsalicylic acid for more than a week or had a multiple gestation pregnancy [24]. Women were also excluded if a fetal anomaly was detected by ultrasound at screening, severe maternal anemia was present at screening (hemoglobin < 7.0 g/dL), systolic blood pressure ≥ 140 mmHg or diastolic ≥ 90 mmHg was present at screening, or if women had any medical condition that could be a contraindication to receiving acetylsalicylic acid (eg, type 1 diabetes, lupus, hypertension, or other significant disease), as evaluated by the site investigator [24].

The sub-Saharan African study sites were located in Nord-Ubangi and Sud-Ubangi provinces in the DRC, where malaria transmission is mostly hyperendemic (defined as estimated parasite rate 50%–75% among children aged 2–10 years); Bungoma, Busia, and Kakamega counties in western Kenya where malaria transmission is mesoendemic (estimated parasite rate 10%–50% among children aged 2–10 years); and Kafue and Chongwe districts in Zambia where malaria transmission is mostly hypoendemic (estimated parasite rate 1%–10% among children aged 2–10 years) (Figure 1) [26, 27]. Each site had multiple recruitment locations.

Participant Data, Substudy Selection, and Sample Collection and Processing

At trial enrollment, information was collected on demographics (including years of maternal age and education),

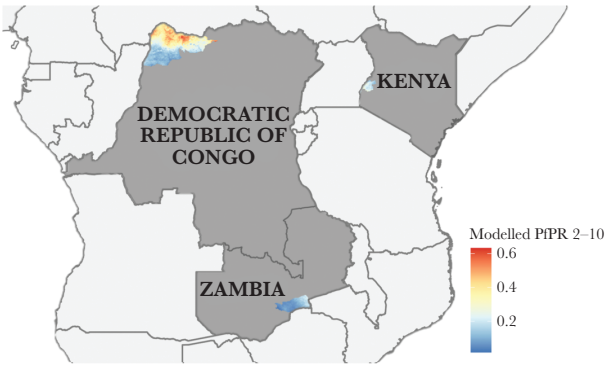

Figure 1. Map of malaria transmission intensity based on study site. The study locations are located in Nord-Ubangi and Sud-Ubangi provinces in the Democratic Republic of the Congo, in Bungoma, Busia, and Kakamega counties in Kenya, and in Kafue and Chongwe districts in Lusaka province in Zambia. Malaria transmission is modeled from the *Plasmodium falciparum* parasite rate among children aged 2–10 years (PfPR 2–10) in 2015 and ranges from 0.6 in Nord-Ubangi and Sud-Ubangi provinces in the Democratic Republic of the Congo to 0.0 in Lusaka province in Zambia. Data on the modeled PfPR 2–10 was obtained from the Malaria Atlas Project [26].

pregnancy and medical history, housing conditions, household assets, and current medical information, including height in centimeters, weight in kilograms, blood pressure, heart rate, and diabetes history [24]. Our malaria substudy was conducted among a convenience sample of women who enrolled from January 2016 to April 2018, consented to dried blood spot (DBS) collection, and had malaria testing performed. This convenience sample was selected based on factors such as the availability of sample collection supplies at the research site, timing of sample transport, and other practical laboratory considerations.

Blood samples were obtained on DBS cards by pricking the substudy participant's finger and placing 3 blood spots on filter paper, which were then completely dried before storage in plastic bags with desiccant. The DBS cards were shipped to the Meshnick Laboratory at the University of North Carolina at Chapel Hill in Chapel Hill, North Carolina for testing in duplicate for *P. falciparum* lactate dehydrogenase (*pfldh*) DNA using quantitative polymerase chain reaction (qPCR), a sensitive detection method [28, 29]. A *P. falciparum*-positive sample was defined as a sample in which fluorescence for both replicates crossed the threshold prior to the 39th cycle, or when 1 replicate did not amplify and the other crossed the threshold prior to the 39th cycle. Discordant results between duplicates were excluded from analysis. To assess the potential for selection bias arising from this sampling process, we compared the demographics of individuals included in the convenience sample to those not included.

Predictor and Outcome Assessment

Based on previous studies of malaria infection in pregnancy, we examined the following potential predictors: maternal age, maternal height, maternal BMI, maternal education, season that coincided with the first trimester of pregnancy, and socioeconomic status. For predictors of maternal age, height, BMI, and socioeconomic status that were measured as continuous variables, we created 2 categories by dividing at the country-specific median based on data from this substudy. We used dichotomous variables for straightforward interpretation of our results, and used country-specific median cutoffs to ensure balanced groups within countries. Maternal education was categorized into 2 levels: lower (no formal schooling and primary education [1–6 years of schooling]), and higher (secondary education [7–12 years of schooling], university, or further education [≥13 years of education]).

We used the Global Network Socioeconomic Status Index [30] to assess socioeconomic status. Developed from approximately 50 000 households, all of which included pregnant women in the Global Network research sites, the Socioeconomic Status Index was determined by taking the sum score of 10 specific items (finished floor material, flush toilet, improved source of drinking water, electricity, television, smartphone, car, motorbike, use of liquefied petroleum gas or electricity for cooking fuel, and refrigerator) owned within the household. The scores were converted to a country-specific socioeconomic status score that ranged from 0 to 100, with higher scores indicating better housing conditions and more household assets [30]. Compared to Demographic and Health Survey indices, which are not designed to be compared between countries, the Global Network Socioeconomic Status Index can be used to compare socioeconomic status within and between sites [30]. In addition, this Socioeconomic Status Index is easy to administer, covers the socioeconomic status continuum, and was validated within the Global Network research sites [30].

The season that coincided with the first trimester of pregnancy was classified as rainy or not rainy, with the specific months defined as rainy varying across countries: April through October in DRC [31], April through June and October through November in Kenya [32], and November through April in Zambia [33]. To examine in greater depth the relationships between seasonality and first-trimester malaria, we plotted the proportion of positive first-trimester malaria tests for each month over the calendar year for each site. The primary outcome of first-trimester malaria was defined as *P. falciparum*–positive status measured by qPCR in a dried blood spot sample obtained in the first trimester.

Statistical Analyses

We calculated crude prevalence ratios (PRs) and prevalence differences (PDs) for first-trimester malaria infection by each variable, using separate 2 × 2 tables for each country. We calculated 99% confidence intervals (CIs) in predictor analyses to account for multiple comparisons and limit inflation of noncoverage rates, and used these CIs to assess the statistical significance of relationships between first-trimester malaria and each variable.

To assess heterogeneity in PD and PR estimates across countries, we calculated the I^2 value for each measure and predictor. If I^2 exceeded a prespecified threshold of 40% [34], we did not pool results across countries to calculate a summary estimate. If the I^2 value was ≤ 40%, we used the DerSimonian and Laird inverse variance method to calculate the summary estimate [34]. Comparisons were limited to observations without missing data for each variable. All analyses were conducted using the R statistical platform (version 4.0.2).

Ethics

The ASPIRIN trial protocol was approved by all the sites' and partner institutions' ethics review committees [25]. Research personnel obtained informed consent from all participants [25].

RESULTS

The ASPIRIN trial enrolled 11 976 nulliparous pregnant women in the first trimester; 3800 of these were enrolled from sub-Saharan sites: 1362 from DRC, 1400 from Kenya, and 1038 from Zambia. For the malaria substudy, we analyzed a convenience sample of 1513 women (485 from DRC, 677 from Kenya, and 351 from Zambia; Figure 2). Among these women, there were no missing demographic data except for socioeconomic status as determined by the Socioeconomic Status Index (missing n = 53, 3.5%); women missing this information were excluded from our socioeconomic status comparisons. We did not see any meaningful differences in the demographics of the trial participants included in the malaria substudy convenience sample compared to those who were not included (Supplementary Table 1).

Overall, most women included in this substudy were younger than 20 years, recruited before 12 weeks of gestation, and had an education level of secondary or university and beyond, although these characteristics varied by site (Table 1). Compared to Kenyan or Zambian women, Congolese women were younger, slightly shorter, had lower BMI, and were considerably more likely to have lower overall educational attainment (no secondary education).

The overall prevalence of first-trimester *P. falciparum* by qPCR was 38.5% (583/1513), with considerable variation in prevalence by site: 62.9% (305/485 [95% CI, 58.6%–67.2%]) at the DRC site; 37.8% (256/677 [95% CI, 34.2%–41.5%]) at the Kenya site; and 6.3% (22/351 [95% CI, 3.7%–8.8%]) at the Zambia site.

In Kenya, women younger than 20 years were more likely to have first-trimester malaria compared to women aged 20 years or older (PR = 1.57 [99% CI, 1.21–2.03]), with a corresponding PD of 0.17 (99% CI, .07–.26) (Table 2). Also, Kenyan women who were 157 cm or shorter were more likely to have first-trimester malaria than women taller than 157 cm (PR = 1.35 [99% CI, 1.04–1.75]; PD = 0.11 [99% CI, .02–.21]). There were no statistically significant relationships

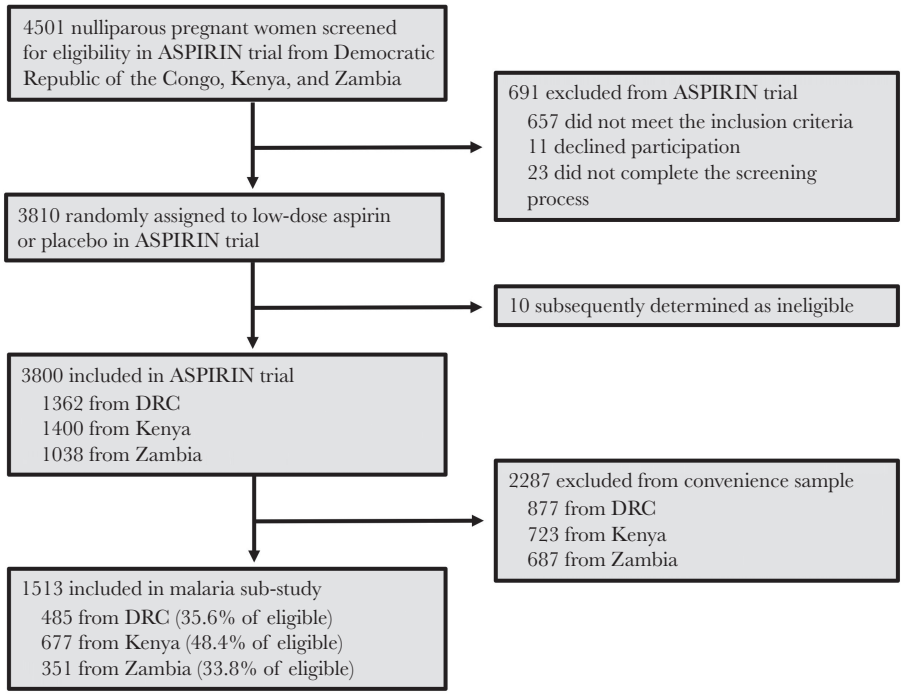

Figure 2. Study population of malaria substudy. The Aspirin Supplementation for Pregnancy Indicated risk Reduction In Nulliparas (ASPIRIN) trial included 3800 women from the Democratic Republic of the Congo (DRC), Kenya, and Zambia. From these women, we took a convenience sample of 1513 women for the malaria substudy: 485 from DRC, 677 from Kenya, and 351 from Zambia.

Table 1. Characteristics of the Study Participant Population, Stratified by Country

Variable	DRC	KENYA	ZAMBIA
Included, No.	485	677	351
Maternal age, y, No. (%)			
< 20	410 (84.5)	316 (46.7)	204 (58.1)
20–29	68 (14.0)	356 (52.6)	144 (41.0)
> 29	7 (1.4)	5 (0.7)	3 (0.9)
Median (P25, P75)	18.0 (17.0, 18.0)	20.0 (18.0, 22.0)	19.0 (18.0, 21.0)
Projected gestation age at enrollment, wk, d, No. (%)[a]			
6, 0–7, 6	50 (10.3)	115 (17.0)	40 (11.4)
8, 0–9, 6	133 (27.4)	216 (31.9)	80 (22.8)
10, 0–10, 6	69 (14.2)	89 (13.1)	35 (10.0)
11, 0–11, 6	82 (16.9)	89 (13.1)	50 (14.2)
12, 0–13, 6	151 (31.1)	168 (24.8)	146 (41.6)
Median (P25, P75)	10.7 (9.0, 12.3)	10.0 (8.3, 11.9)	11.4 (9.1, 12.7)
Maternal height, cm, mean (SD)	155.8 (6.6)	156.1 (8.9)	157.5 (6.4)
Maternal BMI, kg/m^2, mean (SD)	20.8 (2.2)	23.3 (3.5)	22.0 (3.3)
Maternal education, No. (%)			
Lower	310 (63.9)	43 (6.4)	44 (12.5)
Higher	175 (36.1)	634 (93.6)	307 (87.5)
Season coincident with first trimester of pregnancy, No. (%)			
Rainy	244 (50.3)	211 (31.2)	174 (49.6)
Not rainy	241 (49.7)	466 (68.8)	177 (50.4)
Socioeconomic status, median (P25, P75)[b]	16.2 (6.2, 16.2)	8.4 (8.4, 24.6)	38.9 (25.6, 66.3)

Abbreviations: BMI, body-mass index; DRC, Democratic Republic of the Congo; P25, 25th percentile; P75, 75th percentile.

[a]Projected gestational age at enrollment developed from algorithm described in Hoffman et al 2020 [24].

[b]Socioeconomic status developed using Global Network Socioeconomic Status Index described in Patel et al 2020 [30]. Socioeconomic status was calculated by determining the sum score of the number of 10 specific items owned by the household that was converted to a country-specific socioeconomic status score that ranged from 0 to 100 [30].

between maternal BMI, maternal education, season that coincided with the first trimester, or socioeconomic status and prevalence of first-trimester malaria in Kenyan women. In the DRC and Zambia, there were no statistically significant associations between predictors examined and prevalence of first-trimester malaria. There was no clear relationship between the proportion of tests positive for first-trimester malaria in the rainy season versus not rainy seasons (Supplementary Figure 1).

For maternal education, the results of the heterogeneity assessment supported pooling across countries (for PD, I^2 = 0% [99% CI, 0%–93%]; for PR, I^2 = 33% [99% CI, 0%–97%]; Figure 3). Across all 3 study countries, the summary PD and 99% CI indicated that lower versus higher educational attainment was associated with higher prevalence of first-trimester malaria (summary PD = 0.09 [99% CI, .01–.17]). Although not statistically significant, we also observed a consistent elevated summary PR and 99% CI (summary PR = 1.28 [99% CI, .97–1.70]). For season that coincided with the first trimester, the heterogeneity assessment also supported pooling across countries (for PD, I^2 = 27% [99% CI, 0%–96%]; for PR, I^2 = 0% [99% CI, 0%–94%]), revealing no statistically significant association between season and prevalence of first-trimester malaria (summary PD = 0.00 [99% CI, –.06 to .06]; summary PR = 1.06 [99% CI, .91–1.23]).

DISCUSSION

We describe the first known study to assess malarial parasite prevalence in the first trimester among a large number of nulliparous women from sites representing multiple transmission settings. The first-trimester malaria prevalence among 485 Congolese women was 62.9%, among 677 Kenyan women was 37.8%, and among 351 Zambian women was 6.3%. We determined that lower overall educational attainment was associated with higher prevalence of first-trimester malaria in pooled estimates across women in all 3 countries, and, among Kenyan women, we found that younger age and shorter height were associated with higher prevalence of first-trimester malaria. The lack of association between parasite prevalence and other hypothesized predictors suggests that earlier antenatal care and routine (rather than predictor-guided) screening for malaria with sensitive tools may be necessary to identify and treat early infections.

We found no significant association between maternal age or maternal height and first-trimester malaria among Congolese or Zambian women, but did find that younger or shorter western Kenyan women had higher prevalence of first-trimester malaria. In contrast to our findings in Kenya, previous studies conducted in Tanzania and Benin did not find an association between age or height (studied only among Tanzanian women) and malaria prevalence in early pregnancy [22, 23]. These studies included 121 Tanzanian women, of whom 48 were primigravidae, and 387 Beninese women, of whom 30 were primigravidae [22, 23]. Compared to these previous studies, we had a larger sample size in Kenya (n = 677) and focused on nulliparous women, which may have allowed us to detect the relationship between younger age or shorter height and higher prevalence of first-trimester malaria.

Across all 3 countries, we saw elevated prevalences of first-trimester malaria among women with lower overall educational attainment (ie, no secondary education). When we combined data from all 3 countries to calculate pooled estimates, we found that lower overall educational attainment was significantly associated with higher prevalence of first-trimester malaria. These findings are consistent with those of Schmiegelow et al, who found a similar relationship between education and early-pregnancy malaria among Tanzanian women with the same education level comparison (ie, primary education level or less vs higher educational attainment), a much smaller sample (121 vs 1513 women), and a less precise time period for first-trimester malaria (cutoff of 17 weeks vs 14 weeks of gestation) [22].

Table 2. Associations Between Factors and First-Trimester Malaria Among Nulliparous Women in the Democratic Republic of Congo, Kenya, and Zambia

Factor	DRC			Kenya			Zambia		
	Prevalence, % (n/N)	PR (99% CI)	PD (99% CI)	Prevalence, % (n/N)	PR (99% CI)	PD (99% CI)	Prevalence, % (n/N)	PR (99% CI)	PD (99% CI)
Maternal age[a]									
Younger	65.0 (139/214)	1.06 (.89 to 1.27)	0.04 (−.08 to .15)	46.8 (148/316)	1.57 (1.21 to 2.03)	0.17 (.07 to .26)	8.0 (11/138)	1.54 (.53 to 4.46)	0.03 (−.04 to .10)
Older	61.3 (166/271)	Ref	Ref	29.9 (108/361)	Ref	Ref	5.2 (11/213)	Ref	Ref
Maternal height[b]									
Shorter	64.6 (177/274)	1.06 (.89 to 1.28)	0.04 (−.07 to .15)	43.4 (148/341)	1.35 (1.04 to 1.75)	0.11 (.02 to .21)	6.7 (12/180)	1.14 (.39 to 3.32)	0.01 (−.06 to .07)
Taller	60.7 (128/211)	Ref	Ref	32.1 (108/336)	Ref	Ref	5.8 (10/171)	Ref	Ref
Maternal BMI[c]									
Lower	67.5 (166/246)	1.16 (.97 to 1.39)	0.09 (−.02 to .21)	33.7 (112/332)	0.81 (.63 to 1.05)	−0.08 (−.18 to .02)	8.0 (14/174)	1.78 (.59 to 5.39)	0.04 (−.03 to .10)
Higher	58.2 (139/239)	Ref	Ref	41.7 (144/345)	Ref	Ref	4.5 (8/177)	Ref	Ref
Maternal education									
Lower	66.1 (205/310)	1.16 (.95 to 1.41)	0.09 (−.03 to .21)	53.5 (23/43)	1.46 (.99 to 2.15)	0.17 (−.03 to .37)	11.4 (5/44)	2.05 (.59 to 7.11)	0.06 (−.07 to .19)
Higher	57.1 (100/175)	Ref	Ref	36.8 (233/634)	Ref	Ref	5.5 (17/307)	Ref	Ref
Season coincident with first trimester of pregnancy									
Rainy	66.0 (161/244)	1.10 (.92 to 1.32)	0.06 (−.05 to .18)	37.4 (79/211)	0.99 (.75 to 1.30)	−0.01 (−.11 to .10)	5.2 (9/174)	0.70 (.24 to 2.08)	−0.02 (−.09 to .04)
Not rainy	59.8 (144/241)	Ref	Ref	38.0 (177/466)	Ref	Ref	7.3 (13/177)	Ref	Ref
Socioeconomic status[d]									
Lower	62.4 (131/210)	0.97 (.81 to 1.16)	−0.02 (−.14 to .10)	41.8 (177/423)	1.28 (.96 to 1.71)	0.09 (−.01 to .19)	6.1 (11/181)	0.93 (.32 to 2.70)	0.00 (−.07 to .06)
Higher	64.4 (163/253)	Ref	Ref	32.6 (73/224)	Ref	Ref	6.5 (11/169)	Ref	Ref

Abbreviations: BMI, body-mass index; CI, confidence interval; DRC, Democratic Republic of the Congo; n, number with malaria; N, total number with characteristic; PD, crude prevalence difference; PR, crude prevalence ratio; Ref, reference.

[a]Median maternal age cut points (in years, younger vs older): DRC: <18 vs ≥18, Kenya: <20 vs ≥20, Zambia: <19 vs ≥19.

[b]Median maternal height cut points (in cm, shorter vs taller): DRC: ≤156 vs >156, Kenya: ≤157 vs >157, Zambia: ≤157 vs >157.

[c]Median maternal BMI cut points (in kg/m^2, lower vs higher): DRC: ≤20.6 vs >20.6, Kenya: ≤22.8 vs >22.8, Zambia: ≤21.5 vs >21.5.

[d]Median socioeconomic status cut points (lower vs higher): DRC: <16.18 vs ≥16.18, Kenya: ≤8.38 vs >8.38, Zambia: ≤38.92 vs >38.92.

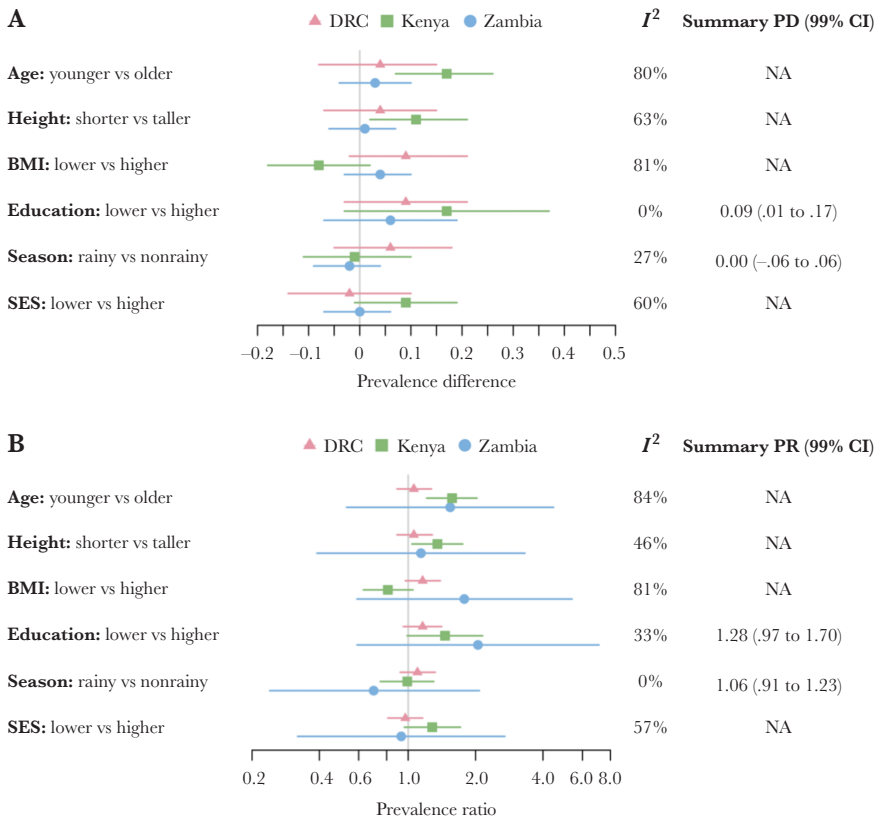

Figure 3. Comparisons of predictors for first-trimester malaria among nulliparous women from the DRC (triangles), Kenya (squares), and Zambia (circles) using prevalence differences (*A*) or prevalence ratios (*B*). The error bars are 99% CI. Summary estimates were only presented if the I^2 value was less than 40% and are provided with 99% CI. The vertical gray line is the null value: 0 for prevalence difference in (*A*), 1 for prevalence ratio in (*B*). Abbreviations: BMI, body mass index; CI, confidence interval; DRC, Democratic Republic of the Congo; NA, not available; PD, prevalence difference; PR, prevalence ratio; SES, socioeconomic status.

In analyses stratified by country, we did not find a statistically significant relationship between lower maternal BMI, lower socioeconomic status, or rainy season coincident with the first trimester and higher prevalence of first-trimester malaria. Previous studies also did not find any significant relationship between these predictors and malaria in early pregnancy [22, 23]. In contrast, these factors have been associated with higher prevalence of malaria in later pregnancy [4, 5].

Most studies examining the relationship between age, education, and malaria in later pregnancy report an association between younger age or lower overall educational attainment and higher prevalence of malaria in later pregnancy [4, 5, 35]. Consistent with findings from malaria in later pregnancy, we found that younger age was associated with higher prevalence of first-trimester malaria among Kenyan women in a moderate malaria transmission setting, and lower overall educational attainment (no secondary education) was associated with higher malaria prevalence in estimates pooled across women from all 3 sites. In addition, shorter stature has been associated with higher prevalence of malaria in late pregnancy, and we found that shorter height was associated with higher first-trimester malaria prevalence among Kenyan women [6, 36]. As our study only included nulliparous women who lack parity-dependent immunity that reduces placental and peripheral parasitemia, our study findings were among those who have the highest risk of malaria in pregnancy and consequent negative health outcomes [12].

We were limited to data collected by the ASPIRIN trial, which focused on low-dose aspirin and pregnancy outcomes, and thus did not collect data to examine several potential malaria predictors, such as ITN use or HIV infection. While our study represents the largest first-trimester malaria study of nulliparous pregnant women to date, we may have been underpowered to detect some associations between hypothesized predictors and malaria prevalence. Because pregnant women frequently have parasite densities below the detection limit of common diagnostic tools, we used qPCR to detect malaria infection during pregnancy; however, the clinical significance of these submicroscopic infections is a matter of some debate [29, 36]. The ASPIRIN trial recruited women starting at 6 weeks of gestation, and thus we did not have any data on women and their malaria prevalence prior to conception or during the earliest stages of pregnancy [24]. While women with severe anemia (hemoglobin < 7 g/dL) were excluded from enrollment, only 1 Congolese woman was screened and excluded because of severe anemia [3]. We also note that our use of historical data to

classify rainy and not rainy seasons may be less accurate in the era of climate change, resulting in some misclassification. While this was a convenience sample, we did not see any meaningful demographic differences between our convenience sample and the ASPIRIN study participants. Furthermore, our sample was chosen for practical reasons that were independent of any demographic considerations. Finally, as our study population was restricted to nulliparous women, our results on predictors of first-trimester malaria should not be generalized to all pregnant women.

Determining the prevalence of first-trimester malaria is useful for defining the magnitude of the potential problem, and identifying predictors of first-trimester infection could be useful for directing preventive strategies toward women at highest risk. We leveraged a multinational clinical trial and used qPCR, a highly sensitive form of detection of malaria infection during pregnancy, to determine parasite prevalence and predictors of first-trimester malaria in a large number of nulliparous women in 3 countries and across multiple transmission settings. We found that first-trimester malaria infections are common in DRC and Kenya, and that infection was more prevalent among those without a secondary education across countries. The differences across countries in our findings on other predictors suggest that more research is needed to identify actionable predictors. In the absence of consistent and practical predictors of first-trimester malaria, earlier antenatal care and routine screening for parasites with sensitive tools among all pregnant women should be considered in high-prevalence settings as a tool to detect and treat these infections.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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