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Associations between Peripheral *Plasmodium falciparum* Malaria Parasitemia, Human Immunodeficiency Virus, and Concurrent Helminthic Infection among Pregnant Women in Malawi

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Abstract. Approximately 2 billion persons worldwide are infected with schistosomiasis and soil-transmitted helminthes (STH), many in areas where endemic malaria transmission coexists. Few data exist on associations between these infections. Nested within a larger clinical trial, primigravid and secundigravid women provided blood samples for human immunodeficiency virus (HIV) testing and peripheral malaria films and stool and urine for evaluation of STH and *Schistosoma* spp. during their initial antenatal clinic visit. The most common parasitic infections were malaria (37.6%), *S. haematobium* (32.3%), and hookworm (14.4%); 14.2% of women were HIV-infected. *S. haematobium* infection was associated with lower malarial parasite densities (344 versus 557 parasites/μL blood; $P < 0.05$). In multivariate analysis, HIV and hookworm infection were independently associated with malaria infection (adjusted odds ratio = 1.9 and 95% confidence interval = 1.2–3.0 for HIV; adjusted odds ratio = 1.9 and 95% confidence interval = 1.03–3.5 for hookworm). Concurrent helminthic infection had both positive and negative effects on malaria parasitemia among pregnant women in Malawi.

BACKGROUND

Malaria accounts for substantial morbidity and mortality throughout Africa. The World Health Organization (WHO) estimates that approximately 300 million persons become infected and 1 million people die of malaria each year.[1] In areas of stable malaria transmission, partial immunity to *Plasmodium falciparum* is acquired with repeated exposure. However in contrast to non-pregnant women of similar age, pregnant women have diminished immunity to *P. falciparum*. This is especially manifest during the first pregnancy, where primigravid women are 2–3 times more likely to have peripheral malaria and placental malaria than multigravid women.[2–5] Pregnancy increases the risk of malarial infection by uncertain mechanisms, but it may be partly because of shifts in cellular immunity from T helper (Th) type 1 to Th2 responses. During normal pregnancy, the woman's cellular immunity favors more Th2 cellular responses in the placenta, because the proinflammatory Th1 cytokines have adverse effects on the developing fetus.[6] However, experimental evidence suggests this Th1 response is needed for non-specific control of malarial infection.[7–9]

In response to a variety of helminthic infections, the non-pregnant host develops a strong Th2/regulatory T cell (Treg) response with interleukin-4 (IL-4), IL-5, IL-10, and IL-13 synthesis. Similar to pregnancy, the Th2 cytokine production from chronic helminthic infection could also downgrade the initial Th1 responsiveness required for control of malaria infection.[10,11] However, epidemiologic studies evaluating helminthic coinfection with malaria among non-pregnant adults and children have provided conflicting results, suggesting that immune responses may be helminth-dependent, with other factors, such as stage and intensity of infection and age of the host, playing an important role.[12–17] These complex relationships remain even less clear among pregnant or human immunodeficiency virus (HIV)-infected patients.

Because an estimated 1–2 billion persons worldwide are infected with soil-transmitted helminths and another 200 million are infected with *Schistosoma* spp., many in areas where malaria infection coexists, concomitant helminthic infection could theoretically play a significant role in the overall burden of malaria infection among susceptible individuals.[18–20] Despite high levels of mixed malarial and soil-transmitted helminthic infections in sub-Saharan Africa,[21,22] only recently has there been renewed interest in the interactions between these infections during pregnancy.[23] Therefore, during a randomized controlled trial in southern Malawi evaluating intermittent preventive treatment regimens for malaria during pregnancy, we conducted a nested substudy investigating interactions between malaria and concurrent helminthic infection among primigravid and secundigravid women.[24]

MATERIAL AND METHODS

As part of the larger clinical trial at Machinga District Hospital in Liwonde, between November 2002 and September 2004, we screened and consented primigravid and secundigravid women during their second trimester. Eligible participants completed surveys containing questions on demographic characteristics, prior medical history, socioeconomic status (SES), and education. In addition, study nurse midwives provided pre- and post-test human HIV counseling.

Participants provided blood samples for rapid HIV testing and peripheral malaria blood films and midday urine for *Schistosoma haematobium.* When possible during the study visit, participants also provided stool for hookworm, *Ascaris*, *Trichuris*, and *Schistosoma mansoni*. Malaria blood films were stained with 3% Giemsa for 30 minutes and examined by two independent expert microscopists for *P. falciparum*. The microscopists counted the total number of asexual malaria forms per 300 white blood cells (WBCs). We multiplied this count by 20 to estimate the number of malarial parasites per 6,000 WBCs to approximate 1 μL blood. To assess *S. haematobium* infection, we centrifuged 10 mL urine and resuspended the pellet. We then placed the suspension on a glass slide (with glass coverslip) and counted the total number of *S. haematobium* eggs. For stool samples, we used the Kato–Katz method published previously.[25] A trained microscopist counted the total number of hookworm eggs less than 2 hours after collection. Other helminth eggs

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(including *S. mansoni*, *Trichuris*, and *Ascaris*) were counted 4–8 hours after collection. Egg counts are reported as eggs per gram (epg) of stool (for hookworm, *S. mansoni*, *Trichuris*, and *Ascaris*) or eggs per 10 mL (for *S. haematobium*). We defined light, moderate, and heavy infections according to WHO classifications as follows: hookworm, 1–1,999, 2,000–3,999, and ≥ 4,000 epg; *Ascaris*, 1–4,999, 5,000–49,999, and ≥ 50,000 epg; *Trichuris*, 1–999, 1,000–9,999, and ≥ 10,000 epg; *S. mansoni*, 1–99, 100–399, and ≥ 400 epg.[26] Light and heavy intensity *S. haematobium* infections were defined as < 50 and ≥ 50 eggs/10 mL, respectively.[26] We performed HIV tests on all consenting participants using Determine HIV-1/2 (Abbott Diagnostics, Abbott Park, IL) and Unigold (Trinity Biotech, Bray, Ireland) rapid test kits in parallel. We considered two positive or negative results as being confirmed positive or negative, respectively. In the event of discordant results, we performed a third rapid test (HemaStrip; Saliva Diagnostics System, Brooklyn, NY); all discordant results were confirmed using enzyme-linked immunosorbent assay (ELISA) antibody testing.

We clinically managed all participants in accordance with Malawi Ministry of Health and Population (Malawi MOHP) guidelines at the time. Specifically, we screened all participants for anemia (using calibrated Hemocue equipment; Mission Viejo, CA) and syphilis by Venereal Disease Research Laboratory (VDRL) testing. All participants received ferrous sulfate and folic acid if noted to be anemic (hemoglobin < 11 g/dL). Participants who were VDRL positive received intramuscular benzathine penicillin. In addition, we provided all HIV-1–infected participants and their exposed infants with single-dose nevirapine administered as per the HIVNET012 protocol.[27] All participants noted to have soil-transmitted helminthes (STH) or schistosomiasis at any point during the study received a treatment course of mebendazole (500 mg) or praziquantel (600 mg), respectively, soon after delivery as recommended by the Malawi MOHP.

We entered all data locally using Epi Info version 6.03 (Centers for Disease Control and Prevention) and used SAS version 9.1 (SAS Institute Inc., Cary, NC) for all analyses. We normalized data on parasite and helminth infection intensity using log transformation and compared differences in geometric means using Student *t* test and one-way analysis of variance. In univariate analysis, we used Cochran-Mantel-Haenszel χ^2 statistics to characterize factors associated with the most common parasitic infections (malaria, hookworm, and *S. haematobium*). To ensure that mixed helminthic infections did not mask significant findings from individual helminthic infections, we repeated analyses with mixed hookworm and *S. haematobium* infection being categorized separately. We subsequently used multivariate logistic regression to characterize factors associated with malaria infection, including in our model all variables potentially associated with infection on univariate analysis ($P \leq 0.20$). We restricted the model to cases for which information on all variables was available (including only women who provided blood, urine, and stool at the initial visit) and checked for two-way interactions. For presentation of final results, we considered *P* values < 0.05 to be statistically significant.

This protocol was reviewed and approved by the Institutional Review Board of the Centers for Disease Control and Prevention (Atlanta, GA) and the Ethics Review Committee for Malawi (Lilongwe, Malawi).

RESULTS

We enrolled a total of 1,892 participants during the overall randomized clinical trial. Of these, 1,772 (93.7%) provided urine and a blood sample at the initial visit, of whom 848 (44.8%) also provided stool samples. The baseline characteristics of these participants are shown in Table 1. There was no significant difference in baseline characteristics among

TABLE 1
Baseline characteristics of pregnant women in southern Malawi providing blood, urine, and stool samples

Characteristic	Participants providing blood and urine samples (*N* = 1,772)	Subset also providing stool (*N* = 848)
Maternal age in years (mean ± SD)	20.0 ± 2.8	20.1 ± 2.9
Gestational age in weeks (mean ± SD)	22.2 ± 3.6	22.1 ± 3.5
Maternal weight in kilograms (mean ± SD)	53.5 ± 6.8	53.4 ± 6.7
Maternal MUAC* in centimeters (mean ± SD)	25.4 ± 2.0	25.4 ± 2.1
Primigravida (%)	51.6	48.6
Ethnicity		
Yao (%)	46.3	44.7
Lomwe (%)	21.7	25.0
Chewa (%)	12.2	11.4
Other (%)	19.8	18.9
Secondary education or greater (%)	22.7	22.9
Housing characteristics		
Earth/sand/dung floor (%)	84.2	85.8
Borehole for drinking water (%)	59.5	59.2
Pit latrine (%)	92.9	92.0
Property ownership		
Own a bicycle (%)	44.0	41.9
Own land (%)	14.6	16.3
Occasional exposure to lakes/rivers (%)	60.2	63.5
Married (%)	91.1	89.6
Slept under an ITN† the previous night (%)	11.9	10.7
Other pre-existing medical conditions‡ (%)	2.6	4.1
HIV positive (%)	14.2	14.6

* MUAC = mid-upper arm circumference.
† ITN = insecticide-treated bed net.
‡ Includes hypertension, diabetes, tuberculosis, asthma, epilepsy, sickle cell disease, and psychiatric disorders.

participants providing only blood and urine samples and those providing blood, urine, and stool. Of the 1,772 participants providing blood, 251 (14.2%) were HIV infected. A total of 667 (37.6%) participants were infected with *P. falciparum*; the geometric mean malaria parasite density was 484 parasites/μL (range = 20–106,000 parasites/μL). Among the 848 participants providing blood, stool, and urine, the most commonly identified helminthic infection was *S. haematobium* (*N* = 274; 32.3%). An additional 122 (14.4%) and 21 (2.5%) participants were infected with hookworm and *S. mansoni*, respectively. *Ascaris* and *Trichuris* infections occurred rarely (< 0.5%). Sixty-three (7.4%) participants had two or more helminthic infections. The most common combination was hookworm and *S. haematobium* coinfection (*N* = 46). Two participants had three or more helminthic infections. The geometric mean egg count was 163 epg for hookworm (range = 24–5,208 epg), 93 epg for *S. mansoni* (range = 24–600 epg), and 22 eggs/10 mL for *S. haematobium* (range = 2–252 eggs/10 mL). Among those with hookworm, 95% (*N* = 116) had light infections; only six (4.9%) infected participants had moderate or heavy hookworm infections. Similarly, most *S. haematobium* (75.9%) or *S. mansoni* (57.1%) infections were light.

Although mild anemia (hemoglobin < 11 g/dL) was commonly observed (77.5%), severe anemia (hemoglobin < 7 g/dL) was identified in only 34 (4%) participants. Presence of mild anemia was not associated with any of the three most common helminthic infections (*S. haematobium*, hookworm, and *S. mansoni*). On univariate analysis, severe anemia was more common among participants infected with hookworm (7.4% versus 3.4%; $P < 0.05$). However, when other variables associated with anemia were incorporated into a multivariate logistic regression model, only malaria infection, first pregnancy, and elevated temperature (> 37.5°C) at presentation were associated with severe anemia (adjusted odds ratio [aOR] = 2.7, 95% confidence interval [CI] = 1.2–6.1; aOR = 3.7, 95% CI = 1.5–9.1; aOR = 3.3, 95% CI = 1.6–6.8, respectively]; neither helminthic nor HIV infection were significant.

The demographic and clinical characteristics associated with having *P. falciparum*, hookworm, or *S. haematobium* infection on univariate analysis are shown in Table 2. The characteristics

TABLE 2

Demographic and clinical characteristics associated with prevalence of malarial, hookworm, or *Schistosoma haematobium* infection among 1,772 primi- and secundigravid women in southern Malawi

Characteristic	Malaria infection		Hookworm infection*		*S. haematobium* infection	
	N (%)	RR (95% CI)	*N* (%)	RR (95% CI)	*N* (%)	RR (95% CI)
Age						
< 20 years	392 (46.3)	1.6 (1.4–1.8)†	55 (14.1)	1.0 (0.7–1.3)	322 (38.1)	1.5 (1.3–1.7)†
≥ 20 years	275 (29.7)	Ref	67 (14.6)	Ref	241 (26.0)	Ref
Weight						
< 50 kg	220 (44.9)	1.3 (1.1–1.5)†	43 (18.2)	1.4 (1.00–2.0)†	180 (36.7)	1.3 (1.1–1.4)†
≥ 50 kg	445 (34.9)	Ref	79 (13.0)	Ref	381 (29.9)	Ref
MUAC						
≤ 25 cm	394 (44.3)	1.4 (1.3–1.6)†	73 (17.1)	1.5 (1.04–2.0)†	296 (33.3)	1.1 (0.97–1.3)
> 25 cm	270 (30.8)	Ref	49 (11.7)	Ref	264 (30.1)	Ref
Pre-existing medical problems						
Yes	20 (43.5)	1.2 (0.8–1.6)	5 (14.3)	1.0 (0.4–2.3)	15 (32.6)	1.0 (0.7–1.6)
No	647 (37.5)	Ref	117 (14.4)	Ref	548 (31.7)	Ref
Gravidity						
Primigravida	444 (48.5)	1.9 (1.6–2.1)†	58 (14.1)	1.0 (0.7–1.3)	306 (33.4)	1.1 (0.97–1.3)
Secundigravida	223 (26.0)	Ref	64 (14.7)	Ref	257 (30.0)	Ref
Marital status						
Married	607 (37.7)	1.0 (0.8–1.2)	116 (15.3)	2.2 (1.02–4.9)†	517 (32.1)	1.1 (0.9–1.5)
Single/divorced/widowed	59 (37.3)	Ref	6 (6.8)	Ref	45 (28.5)	Ref
Education						
Primary or less	549 (40.1)	1.4 (1.2–1.6)†	102 (15.7)	1.6 (1.01–2.6)†	496 (36.3)	2.2 (1.7–2.7)
Secondary or greater	117 (29.1)	Ref	19 (9.7)	Ref	67 (16.7)	Ref
Has a job						
Yes	12 (17.9)	0.5 (0.3–0.8)†	3 (9.1)	0.6 (0.2–1.9)	5 (7.5)	0.2 (0.1–0.5)†
No	655 (38.4)	Ref	119 (14.6)	Ref	558 (32.7)	Ref
Flooring in home						
Earth/sand/dung	601 (40.3)	1.7 (1.4–2.1)†	107 (14.7)	1.2 (0.7–1.9)	520 (34.9)	2.3 (1.7–3.0)†
Cement	66 (23.6)	Ref	15 (12.5)	Ref	43 (15.4)	Ref
Sanitation						
Pit latrine or no facility	652 (38.4)	1.8 (1.2–2.9)†	121 (14.9)	5.7 (0.8–39.5)	552 (32.5)	2.1 (1.3–3.7)†
Flush toilet	15 (20.8)	Ref	1 (2.6)	Ref	11 (15.3)	Ref
Exposure to lakes/rivers						
At least occasionally	429 (42.3)	1.4 (1.3–1.7)†	65 (13.0)	1.3 (0.9–2.0)	358 (35.3)	1.4 (1.2–1.6)†
None	197 (29.4)	Ref	28 (9.7)	Ref	174 (25.9)	Ref
Slept under an ITN treated < 6 months ago						
Yes	54 (25.6)	0.7 (0.5–0.8)†	6 (6.6)	0.4 (0.2–0.9)†	64 (30.3)	1.0 (0.8–1.2)
No	613 (39.3)	Ref	116 (15.3)	Ref	498 (31.9)	Ref

Ref = reference.
* Includes only the 848 participants who provided stool, urine, and malaria smear.
† Significant results.

associated with malaria infection in order of the strength of association are first pregnancy, having a pit latrine or no sanitary facility, earthen flooring at home, age < 20 years, exposure to lakes and rivers, mid-upper arm circumference (MUAC) ≤ 25 cm, having a primary school education or less, and weight < 50 kg. Having a job and sleeping under an insecticide-treated bed net (ITN) were associated with a significant reduction in the likelihood of having malaria infection (relative risk [RR] = 0.5, 95% CI = 0.3–0.8; RR = 0.7, 95% CI = 0.5–0.8, respectively). Although 26.9% of participants reported fever during pregnancy, there was no association between reporting fever and malaria parasitemia (47.1% with subjective fever versus 38.2% without subjective fever had malaria parasitemia; $P = 0.36$). Only 3 of 332 (0.9%) participants with malaria parasitemia had a temperature ≥ 37.5°C.

Associations in prevalence and parasite densities among those with coinfections are shown in Tables 3 and 4. HIV-infected participants were more likely to have malaria parasitemia (RR = 1.2, 95% CI = 1.00–1.4) and had higher geometric mean malarial parasite densities (721 versus 449 parasites/μL blood; $P < 0.005$) than those without HIV. However, HIV-positive participants were less likely to have *S. haematobium* infection (RR = 0.7, 95% = CI 0.5–0.9) and had significantly lower *S. haematobium* egg counts (17 versus 24 eggs/mL urine; $P < 0.05$). Malaria infection was more common among participants with hookworm coinfection (RR = 1.6, 95% CI = 1.4–1.9). However, hookworm coinfection was not associated with differences in malaria parasite density (557 versus 473 parasites/μL blood; $P = 0.5$). In contrast, participants with *S. haematobium* coinfection had lower malaria parasite densities (410 versus 528 parasites/μL blood; $P < 0.05$) but were not more likely to be infected with malaria (RR = 1.1, 95% CI = 0.99–1.3).

We further categorized heminthic infections into single or mixed infections. Hookworm infection alone remained associated with malaria infection (RR = 1.6, 95% CI = 1.3–2.1) compared with no helminthic infection. This same effect was noted even with very light infections (< 100 epg; RR = 1.6, 95% CI = 1.2–2.3). In addition, *S. haematobium* infection alone or mixed hookworm/*S. haematobium* infection was associated with greater odds of having malaria infection (RR = 1.2, 95% CI = 1.02–1.5; RR = 1.9, 95% CI = 1.5–2.4, respectively) compared with no helminthic infection.

Hookworm infection alone or mixed hookworm/*S. haematobium* infection had no apparent association with geometric mean malarial parasite densities compared with no helminthic infection (524 versus 557 parasites/μL blood; $P = 0.85$; 625 versus 557 parasites/μL blood; $P = 0.75$, respectively). However, *S. haematobium* infection alone remained significantly associated with lower geometric mean malarial parasite densities (344 versus 557 parasites/μL blood; $P < 0.05$) compared with no helminthic infection. No difference in mean malaria parasite density was identified between light and heavy *S. haematobium* infections.

On multivariate logistic regression analysis (Table 5), the characteristics associated with malaria infection in order of the strength of association included mixed hookworm/*S. haematobium* infection, first pregnancy, earthen flooring at home, hookworm infection alone, HIV infection, exposure to lakes and rivers, and age < 20 years. *S. haematobium* infection alone was no longer significant when the variable exposure to lakes and rivers was added to the model. As might be expected, sleeping under an ITN remained significantly associated with reductions in malaria infection.

DISCUSSION

In this large cohort of primi- and secundigravid pregnant women in Malawi, we found both positive and negative associations between concurrent helminthic and malaria infections. Although malaria prevalence was higher among pregnant women coinfected with hookworm, no such effect was noted with *S. haematobium* or *S. mansoni*. However, malaria parasite density was significantly lower among those coinfected with *S. haematobium*; this was not observed with hookworm and *S. mansoni*.

Our results provide further evidence to show that *S. haematobium* coinfection may be associated with reductions in

TABLE 3

Coinfections associated with prevalence of malarial, hookworm, or *Schistosoma haematobium* infection among 1,772 primi- and secundigravid women in southern Malawi

	Malaria infection		Hookworm infection*		*S. haematobium* infection	
Concurrent infection	*N* (%)	RR (95% CI)	*N* (%)	RR (95% CI)	*N* (%)	RR (95% CI)
HIV serostatus						
Positive	108 (43.0)	1.2 (1.00–1.4)†	13 (10.5)	0.7 (0.4–1.2)	58 (23.1)	0.7 (0.5–0.9)†
Negative	559 (36.8)	Ref	109 (15.1)	Ref	505 (33.2)	Ref
Malaria						
Present	–	–	71 (21.4)	2.2 (1.6–3.0)†	229 (34.3)	1.1 (0.99–1.3)
Absent			51 (9.9)	Ref	334 (30.2)	Ref
Hookworm*						
Present	71 (58.2)	1.6 (1.4–1.9)†	–	–	48 (39.3)	1.3 (0.99–1.6)
Absent	261 (36.0)	Ref			226 (31.1)	Ref
S. haematobium						
Present	229 (40.7)	1.1 (0.99–1.3)	48 (17.5)	1.4 (0.97–1.9)	–	–
Absent	438 (36.2)	Ref	74 (12.9)	Ref		
*S. mansoni**						
Present	11 (52.4)	1.3 (0.9–2.0)	8 (38.1)	2.8 (1.6–4.9)†	11 (52.4)	1.6 (1.1–2.5)†
Absent	321 (38.8)	Ref	114 (13.8)	Ref	263 (31.8)	Ref

Ref = reference.
* Includes only the 848 participants who provided stool, urine, and malaria smear.
† Significant results.

TABLE 4

Coinfections associated with malarial, hookworm, or *Schistosoma haematobium* parasite density among 1,772 primi- and secundigravid women in southern Malawi

	Malaria infection		Hookworm infection*		*S. haematobium* infection	
Concurrent infection	Parasite density†	*P* value	Parasite density‡	*P* value	Parasite density‡	*P* value
HIV serostatus						
Positive	721	< 0.005§	125	0.45	17	< 0.05§
Negative	449		168		24	
Malaria						
Present	–	–	168	0.7	25	0.15
Absent			155		22	
Hookworm*						
Present	547	0.5	–	–	35	< 0.001§
Absent	473				19	
S. haematobium						
Present	410	< 0.05§	158	0.8	–	–
Absent	528		166			
*S. mansoni**						
Present	472	0.95	193	0.7	23	0.8
Absent	488		161		21	

* Includes only the 848 participants who provided stool, urine, and malaria smear.
† Geometric mean parasite density per microliter of blood.
‡ Geometric mean helminth eggs per 1 g stool or 10 mL urine.
§ Significant results.

malaria parasite density in pregnant women.[13,14] In addition, consistent with prior studies, our findings suggest that hookworm infection may exacerbate malaria infections among pregnant women.[23,28] Hillier and others[23] showed that hookworm and *Mansonella perstans* infections were significantly associated with higher prevalence of asymptomatic malaria parasitemia, although this difference depended on the geographic location of residence in Entebbe, Uganda. Yatich and others[28] also showed that hookworm coinfection was associated with increased prevalence of malaria infection among pregnant Ghanaian women, although women with *Ascaris* coinfection had an even greater likelihood of malaria infection. In contrast, van Eijk and others[29] showed that Kenyan pregnant women in their second and third pregnancy were less likely to have malaria infection if infected with *Ascaris*.

TABLE 5

Results of multivariate logistic regression analysis of factors associated with malarial infection among 779 primi- and secundigravid women in southern Malawi*

Characteristic	Adjusted odds ratio (95% CI)
Gravidity	
Primigravida	2.8 (1.9–4.0)
Secundigravida	Reference
HIV serostatus	
Positive	1.9 (1.2–3.0)
Negative	Reference
Slept under an ITN treated less than 6 months ago	
Yes	0.5 (0.3–0.9)
No	Reference
Age	
< 20 years	1.5 (1.04–2.1)
≥ 20 years	Reference
Flooring in home	
Earth/sand/dung	2.1 (1.3–3.6)
Cement	Reference
Exposure to lakes/rivers	
At least occasionally	1.6 (1.2–2.3)
None	Reference
Helminthic infection	
Hookworm alone	1.9 (1.03–3.5)
Schistosoma haematobium alone	1.3 (0.9–1.9)
Mixed hookworm/*S. haematobium* infection	3.9 (1.8–8.5)
Other combinations of helminthes	1.4 (0.5–4.1)
None	Reference

* Includes only participants with complete information for the following variables: gravidity (first or second pregnancy), age, weight, height, mid-upper arm circumference, education level, flooring in home, source of drinking water, type of sanitation, land or bicycle ownership, employment, contact with surface water, optimal use of bed net (slept under a bed net treated with insecticide in the previous 6 months), HIV infection, and helminthic infection.

The reasons for these conflicting results remain uncertain but are likely multifactorial. In animal models, the stage and intensity of helminthic infection affect host response.[30] In human epidemiologic studies, other factors, such as spatial variations in helminth density (even over short distances), have been suggested as significant contributors to malaria–helminthic interactions.[23] Clearly, the host response may also depend on the specific type of helminthic infection and the age of the host. Although the vast majority of helminths and their antigens are able to stimulate a Th2 host response, tissue-dwelling helminths such as *Schistosoma* spp. may require additional mechanisms to modulate the host immune system given that infection may persist for years. Of late, research has focused on helminth-derived compounds as effectors of the host immune system. Omega-1, a glycoprotein secreted by *S. mansoni*, has recently been identified as a potent inducer of Th2 immune responses, even in the absence of IL-4 signaling.[31] Although additional research is needed, potent compounds such as omega-1 could contribute to the protective effect of *Schistosomiasis* on malaria infection.

However, all the above-mentioned epidemiologic studies (ours included) are observational studies and therefore, are limited in determining cause and effect between these coinfections. Thus far, only one randomized clinical trial has looked at the effect of helminthic treatment on malaria infection.[32] Although this clinical trial noted that treatment of *Ascaris* was associated with a two-fold increase in malaria parasitemia in adults (suggesting a protective effect of the *Ascaris* coinfection), one must interpret these results with some caution, because the medication used (levamisole) has intrinsic immunomodulatory properties.[33] Of note, *Ascaris* treatment in children showed neither a beneficial nor detrimental effect on

malaria infection. Given these limited results, additional clinical trials are needed.

Although this substudy was designed to evaluate the impact of helminthic coinfection on malaria, we also showed that HIV infection was associated with increased malarial prevalence and geometric mean malaria parasite density, but it reduced *S. haematobium* prevalence and egg counts. Neither of these findings was unexpected. A recent meta-analysis of 12 clinical trials showed that HIV-infected pregnant women are 1.6 times more likely to have malaria peripheral and placental parasitemia than those who are uninfected. Two- to three-fold increases in malaria peripheral parasitemia were also reported.[34] In contrast, prior observational studies have consistently shown reduced excretion of *S. haematobium* and *S. mansoni* eggs among non-pregnant adults coinfected with HIV, potentially leading to underdiagnosis of these infections among HIV-infected persons. This may be related to findings from animal studies that have shown that maturation of adult worms and transposition of *S. mansoni* eggs to the intestine are T-cell–dependent processes.[35]

However, our study has several limitations. As with all observational studies, the results can only describe potential associations and not detail cause and effect of these coinfections. In addition, because host immune responses vary throughout pregnancy and with additional pregnancies, these findings might be different in multigravid pregnant women or those in their first or third trimesters. Similarly, because CD4 counts were not available from the HIV-infected women in this study, we cannot confirm that these results can be extrapolated across all varying levels of immunosuppression. Although we inquired about a multitude of potential socioeconomic factors that could potentially confound these results, it is possible that there were additional factors about which participants were not asked but that might explain this effect. However, despite the finding that type of floor in the home was significantly associated with malarial infection (suggesting SES did have a role in malaria infection), hookworm infection still remained statistically significant on multiple logistic regression analysis. Because we did not receive stool samples from all women providing blood and urine samples, it is also possible that unidentified STH infection could potentially confound the results. Given the infrequent occurrence of other STH infections, we are also unable to conduct further analysis of *Ascaris* or *Trichuris* coinfections. However, this was not unexpected, because *Ascaris* and *Trichuris* infections are rarely found in Malawi.[36] In addition, it is possible that our results were biased to those who were able to provide stool samples (such as those with gastrointestinal illness), although the majority of stool samples were formed. Lastly, both Kato–Katz stool examination and midday urine collection for *S. haematobium* have low sensitivity for detecting helminthiasis on a single sample, and therefore, some infections may be missed. However, this would suggest that the prevalence of STH and *S. haematobium* in this population is likely underestimated.

In summary, the potential effect of concurrent helminthic infection on malaria is of great importance given the high frequency and morbidity of each in sub-Saharan Africa. However, the data remain complicated and at times, conflicting. Only through well-conceived placebo-controlled randomized clinical trials in different populations, such as the trial ongoing in Uganda, will we know whether treatment of helminthic infections will have a beneficial or detrimental effect on the morbidity from malaria infection.[37,38]

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