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ASSOCIATION OF HELMINTH INFECTIONS WITH INCREASED GAMETOCYTE CARRIAGE DURING MILD FALCIPARUM MALARIA IN THAILAND

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Abstract. The objective of this study was to determine whether pre-existing helminth infections could affect sexual forms of *Plasmodium falciparum.* A cross-sectional case record study compared 120 mild *P. falciparum* malaria cases with patent gametocyte carriage and 187 without gametocytes for helminth exposure. Relevant crude odds ratios and potential confounders were included in a logistic regression model. Helminth infections were associated with the presence of gametocytes with a crude odds ratio of 1.9 (95% confidence interval = 1.1–3.3) ($P = 0.01$). A positive linear trend was observed between the odds of having patent gametocytemia and the number of different helminth species ($P = 0.003$). However, when adjusting for hemoglobin concentration the significance of the association between helminths and gametocytes disappeared ($P = 0.15$). Pre-existing helminth infections may increase the severity of malarial anemia and therefore increase the likelihood of carrying gametocytes. At a population level, helminth infections may thus have a significant influence on malaria transmission.

INTRODUCTION

Malaria transmission from host to vector relies on the presence of infective gametocytes in peripheral blood. The triggering of gametocytogenesis is still unclear.[1,2] It is influenced by parasite stress and multiple environmental factors.[3–6] Among these, low hemoglobin concentrations are associated with gametocyte carriage.[6] Gametocytogenesis occurs over a period of 10 days for *Plasmodium falciparum* and involves five intracellular stages. Immature gametocytes from stages I to IV are sequestered in the spleen and bone marrow capillaries before re-entering the circulation. The absorption of viable gametocytes by anopheline mosquitoes allows *P. falciparum* to perpetuate its life cycle. Helminth infections are widespread in malaria-endemic areas. They have immunomodulating effects on their hosts and may, in some cases, lead to iron deficiency and malnutrition.

We recently observed an association of helminth infections with protection from cerebral malaria,[7] with indirect elements suggesting decreased cytoadherence in helminth-infected patients. We also observed decreased hemoglobin concentrations in helminth-infected mild *falciparum* malaria cases. Thus, considering that immature *Plasmodium falciparum* gametocytes are sequestered and given the lower hemoglobin concentrations of helminth-infected patients during mild *falciparum* malaria, we aimed to determine if gametocyte carriage was affected by preexisting helminth infections.

PATIENTS, MATERIALS, AND METHODS

The study took place at The Hospital for Tropical Diseases in Bangkok, Thailand. A cross-section of 307 case records of patients with mild *P. falciparum* malaria was reviewed. These patients all received artesunate plus mefloquine for mild *P. falciparum* malaria between 1993 and 1997. Patients from western Thailand with mild malaria often go to the Hospital for Tropical Diseases in Bangkok because treatment there is free. The socioeconomic level of these patients is very poor (< US$750 per year) and they prefer to travel to Bangkok than to pay for treatment in local health facilities. This study was approved by the Ethical Committee of Mahidol University's Faculty of Tropical Medicine. All patients were informed and agreed that the information concerning their illness be used for malaria research.

Inclusion and exclusion criteria. Patients had mild *falciparum* malaria, defined as absence of any severity criteria.[8] One hundred twenty patients had circulating gametocytes and 187 had no circulating gametocytes. The presence of gametocyte forms defined Gf+ cases and the absence of gametocytes defined Gf− controls. To eliminate potential confounding factors, patients with hemoglobinopathies, glucose-6-phosphate dehydrogenase deficiency, recrudescent infections (infections previously treated during the same hospitalization, in which parasitemia was cleared, but subsequently reappeared during follow-up), mixed *vivax-falciparum* infections,[6] or circulating schizonts were excluded.

Screening for helminths. At the Hospital for Tropical Diseases, patients are systematically screened for intestinal parasites. All included patients systematically had one stool examination to screen for intestinal parasites by a concentration technique and a simple smear technique. Helminth eggs were counted per 1.5 mg of stool. Although this may not have been the most sensitive procedure, we believed this lack of sensitivity was randomly distributed and was not preferentially related to our dependent variable. Thus, since the error was random, it would not affect the validity of observed differences.

Variables. Thick and thin blood smears were prepared daily. Gametocytes were counted per 200 white blood cells and gametocyte clearance time and the peak gametocyte count was recorded. Clinical examination was performed on admission. The symptoms' duration before admission was recorded. A complete blood count was performed for all patients on admission. Reticulocyte counts on admission were available only for 120 patients. Height and weight were recorded to calculate the body mass index (weight/height^2).

Statistical analysis. Analysis was performed using STATA 6.0 software (STATA corporation, College Station, TX). Helminth infection 'exposure' in patients with and without

gametocyte carriage was determined. To determine the influence of the worm burden, an ordinal variable was created allowing the division of the patients into three categories: no helminths, low helminth burden (*Ascaris* eggs > 0 and < 5,000/gram, *Trichuris, Strongyloides,* and hookworm > 0 and < 1,000 eggs or larvae/gram), or high helminth burden (*Ascaris* eggs > 5,000/gram, *Trichuris, Strongyloides,* and hookworm > 1,000 eggs or larvae/gram). We also created a variable reflecting the number of different helminth species infecting each patient. Relevant crude odds ratios and potential confounders were included in logistic regression models. The quantitative adjustment variables were included in the reported model as continuous variables. However, during analysis, they were also transformed as categorical variables and included in the model to avoid missing a non-linear relationship. Interaction terms were generated between relevant variables and added to the models.

Normally distributed variables were compared using Student's unpaired *t*-test. In non-Gaussian distributions, the Wilcoxon Rank-sum test was used. Multiple linear regression or quantile regression (non-parametric test) were used to adjust for other relevant variables. To quantify the relationship between variables of non-Gaussian distribution, we used Spearman's correlation.

RESULTS

Age. The median age was 25 years (range = 15–62 years). There was no median age difference between Gf+ and Gf− patients (24.5 versus 25 years, interquartile range [IQR] = 19–23 years versus 20–23 years; $P = 0.5$).

Different helminth infections. One hundred forty-six patients (47%) were infected by *Trichuris trichiura,* 143 (46%) by hookworms, 146 (34%) by *Ascaris lumbricoides,* 63 (20%) by *Strongyloides stercoralis,* and 30 (10%) by *Opistorchis viverini.* The most frequent association was *Ascaris* and *Trichuris* in 85 patients (27%). Twenty-two percent of the patients had one helminth species, 20% had two different helminths, 21% had three different helminths, and 8% had four or five different helminths.

Symptoms duration. The median symptoms duration was higher in Gf+ patients than Gf− patients (5 versus 3 days, IQR = 3–7 days versus 2–5 days; $P < 0.0001$). There was no significant difference of median symptoms' duration between patients with or without helminth infections (4 versus 4 days, IQR = 3–7 days versus 3–6 days; $P = 0.6$).

Biological differences between Gf+ and Gf− patients. The median asexual parasitemia was significantly lower in Gf+ patients than in Gf− patients (11,680/μl versus 19,120/μl, IQR = 4,160–25,920/μl versus 5,500–53,280/μl; $P = 0.007$). There was a negative correlation between asexual parasitemia and duration of symptoms (Spearman's $\rho = -17.5$, $P = 0.002$). Conversely, there was a positive correlation between asexual parasitemia and hemoglobin concentration (Spearman's $\rho = 0.21$, $P = 0.0002$). Thus, after adjusting for hemoglobin concentrations and symptoms' duration, there was no significant difference between median parasitemia in Gf+ patients and Gf− patients. There was a negative correlation between asexual parasitemia and reticulocyte percentage (Spearman's $\rho = -26.4$, $P = 0.003$). The median reticulocyte percentage was higher in Gf+ patients than in Gf− patients (0.7% versus 0.3%, IQR = 0.2–1.8% versus 0.2–0.6%; $P = 0.001$). The median reticulocyte percentage was higher in patients with splenomegaly (n = 15) than those without splenomegaly (n = 105) (0.8% versus 0.3%, IQR = 0.4–2.4% versus 0.2–0.7%; $P = 0.007$). We observed a positive correlation between gametocyte counts and reticulocyte percentage (Spearman's $\rho = 0.47$, $P = 0.0001$). This remained significant after adjusting for hemoglobin concentrations, symptoms' duration and splenomegaly using median multiple regression (quantile regression). The mean hemoglobin concentrations were lower in Gf+ patients than in Gf− patients (9.9 ± 2 g/dl versus 12.6 ± 2 g/dl; $P < 0.0001$). After adjusting for symptoms' duration before admission and parasitemia this remained significant ($P < 0.0001$). Table 1 shows that the lower the hemoglobin concentration, the higher the proportion of patients with gametocyte carriage. The geometric mean platelet count was significantly higher in Gf+ patients than in Gf− patients (127,695/μl versus 93,320/μl; $P < 0.0001$). This remained significant after adjusting for mean corpuscular volume, symptoms' duration, hemoglobin concentration, parasitemia and spleen size ($P = 0.01$).

TABLE 1
Stratification of gametocyte carriage per hemoglobin levels

Hemoglobin level (g/dl)	Gametocytes	No gametocytes	Odds	*P*
5.6–6.5	11	1	11	Test of homogeneity
6.6–8	14	1	14	$P < 0.0001$
8.1–9.9	35	14	2.5	
10–12	40	63	0.6	Score test for trend of odds
12.1–18	20	108	0.18	$P < 0.0001$

Peak gametocyte density. There was a negative correlation between the peak gametocyte density and hemoglobin concentrations (Spearman's $\rho = -31.4$, $P = 0.0005$), but not the duration of symptoms (Spearman's $\rho = 0.04$, $P = 0.66$).

Gametocyte clearance time. The median gametocyte clearance time was 144 hr (IQR = 72–312 hr). Among patients with gametocyte carriage, there was no significant correlation between gametocyte clearance time and hemoglobin concentrations (n = 120, Spearman's $\rho = -0.11$, $P = 0.23$). Conversely, there was a positive correlation between gametocyte clearance time and the symptoms' duration (Spearman's $\rho = 0.23$, $P = 0.01$).

Hemoglobin and helminths. The mean hemoglobin concentrations were significantly lower among helminth-infected patients (n = 222) compared to those without helminths (n = 85) (11.3 ± 2.5 g/dl versus 12.2 ± 2.4 g/dl; $P = 0.004$). This association persisted ($P = 0.005$) after adjusting for parasitemia, splenomegaly, symptoms duration, age, sex, and body mass index. When excluding hookworm infections, helminth-infected patients (n = 79) still had significantly lower mean hemoglobin concentrations than those without helminths (n = 85) (11.1 ± 2.6 g/dl versus 12.2 ± 2.3 g/dl; $P = 0.005$). Platelets counts were negatively correlated with hemoglobin concentrations (Spearman's $\rho = -29.5$, $P < 0.0001$). There was no significant difference in median corpuscular volume between Gf+ and Gf− patients (82 fl versus 85 fl, IQR = 76–89 fl versus 79–90 fl; $P = 0.12$). Helminth-infected patients had a lower median mean corpus-

TABLE 2

Crude and adjusted odds ratios comparing helminth infection exposure between patients with mild *Plasmodium falciparum* malaria with and without circulating gametocytes

	Gametocyte+ (%)	Gametocyte− (%)	Crude odds ratio*	*P*	Adjusted odds ratio*	*P*
Helminths						
Yes	96 (80)	126 (67)	1.93	0.01	1.63	0.15
No	24 (20)	61 (33)	(1.1–3.3)		(0.84–3.2)†	
Number of different helminths			Odds		1.27 (1.02–1.58)‡	0.029
0	26 (20)	63 (33)	0.41	χ^2 for trend = 8.52		
1	25 (21)	44 (24)	0.57			
2	22 (18)	39 (21)	0.56			
3	37 (31)	28 (15)	1.32	0.003		
4	10 (12)	12 (6)	0.83			
5	0 (0)	1 (0)	0			

* Values in parentheses are 95% confidence intervals (CIs).

† Best model included helminths and adjustment for hemoglobin concentration in g/dl (0.6, 95% CI = 0.5–0.7, $P < 0.0001$), symptoms' duration in days (1.25, 95% CI = 1.1–1.4, $P < 0.0001$), and splenomegaly (1.3, 95% CI = 0.9–1.9, $P = 0.13$). Before adding hemoglobin concentrations, helminth infections were significantly associated with gametocyte carriage: adjusted odds ratio = 2.36, 95% CI = 1.34.3, $P = 0.006$.

‡ Best model included number of different helminth eggs found in the patients' stool and adjustment for hemoglobin concentration in g/dl (0.6, 95% CI = 0.5–0.7, $P < 0.0001$), symptoms' duration in days (1.25, 95% CI = 1.1–1.4, $P < 0.0001$), and splenomegaly (1.3, 95% CI = 0.9–1.9, $P = 0.10$).

cular volume than those without helminths (83 fl versus 86 fl, IQR = 78–89 fl versus 79–91 fl; $P = 0.049$). This was of borderline significance after excluding observations with hookworm infections ($P = 0.048$). There was no significant difference in the median percentage of reticulocytes between helminth-infected patients and non-infected patients (0.4% versus 0.4%, IQR = 0.2–0.8% versus 0.2–1.1%; $P = 0.18$). However, when adjusting for symptoms duration, presence of splenomegaly, and hemoglobin concentrations, the median reticulocyte counts were lower in patients with helminths than in patients without helminths ($P = 0.027$).

Admission temperature and helminths. Helminth-infected patients had a lower temperature on admission (37.8 ± 0.9°C versus 38.1 ± 0.86°C; $P = 0.008$). This remained significant ($P = 0.01$) after adjusting for age, sex, body mass index, symptoms duration, parasite counts, and hemoglobin concentration. When we removed hookworm infections, this was no longer significant (38 ± 0.9°C versus 38.1 ± 0.86°C; $P = 0.36$).

Helminths and gametocytes. Table 2 shows that helminth infections were associated with increased gametocyte carriage, and that this increase was linked to the lower hemoglobin concentrations in helminth-infected patients. The quantity of eggs in the patients' stool was not significantly linked to gametocyte carriage (score for trend of odds, $\chi^2 = 2.87$, $P = 0.09$). However, the logistic model using the number of different helminths found in the patients stool showed that the risk of gametocyte carriage increased with the number of different helminth species, despite adjustment for hemoglobin concentrations. In the model without hemoglobin concentration, adjusting for the body mass index did not alter the association between helminths and gametocyte carriage (adjusted odds ratio = 2, 95% confidence interval [CI] = 1.03–3.8, $P = 0.039$). To search for a potential confounder linked to socioeconomic status, we excluded Thai patients and kept Mon and Karen ethnic groups, who are refugees from Burma, and have a more homogenous socioeconomic status. In this subgroup, after adjusting for evolution duration the association between helminths and gametocyte carriage remained significant (adjusted odds ratio = 3.2, 95% CI = 1.3–8, $P = 0.01$). The logistic regression models also showed that the two most important determinant of gametocyte carriage were decreased hemoglobin concentrations and the duration of the malarial symptoms. Neither of the interaction terms between hemoglobin concentrations and helminth infections nor between hemoglobin concentrations and symptoms duration were significant ($P = 0.6$ and $P = 0.2$).

DISCUSSION

During mild *falciparum* malaria, helminth-infected patients were more likely to carry gametocytes. The odds of gametocyte carriage increased with the number of different helminth species. The association between decreased hemoglobin concentrations and gametocytes could have reflected older infections in patients with gametocyte carriage, but controlling for the duration of symptoms suggested that decreased hemoglobin concentrations had an effect on gametocytogenesis. The association between gametocyte carriage and helminths was related to helminth-infected patients' lower hemoglobin concentrations on admission. There was also a linear trend to this relationship suggesting that low hemoglobin concentrations may have triggered gametocytogenesis. The lower hemoglobin concentrations among helminth-infected patients may have resulted from hematologic stress during malaria, possibly revealing or aggravating subclinical deficiencies in these patients. The lower mean corpuscular volume in helminth-infected patients could support the suspicion of latent iron deficiency. Differences in the patients' nutritional status, as reflected by the body mass index, did not seem to affect the association between helminths and decreased hemoglobin concentrations. The persistence of the linear association between the number of different helminths and gametocyte carriage despite adjustments for hemoglobin concentrations suggested that other immunologic mechanisms, such as a bias towards T_H2 cytokines, might have been involved. Increased platelet counts in patients with gametocyte carriage could have reflected decreased splenic clearance,[9] which could have favored sexual conversion.[10] A second explanation for the increased platelet counts in patients with gametocyte carriage could be

iron deficiency or increased erythropoietin secretion.[11] Finally, one could question a direct relation between platelets and gametocytogenesis.

Although we did not observe any significant differences between the presence of helminths and the peak gametocyte counts and the duration of gametocyte carriage, helminths were related to variables that could influence both. The peak gametocyte counts were negatively correlated to hemoglobin concentrations, which were lower in helminth-infected patients. The gametocyte clearance time was proportional to the duration of the infection. The fact that helminth-infected patients had lower fever on admission could, in some cases, delay the time of consultation. Our patients, consulting in Bangkok, often had longer evolution durations than patients treated on site by Price and others.[6] Therefore, this may have explained the higher proportion of patients with patent gametocyte carriage in our group (39% versus 2% in the group receiving artemisinin derivatives[6]). However, a complementary explanation would be that the prevalence of helminth-infected patients in the refugee settlements was lower ($<$ 10%, White N., Nosten F., unpublished data) than in the patients we observed in Bangkok.

Whether the gametocytes in helminth-infected patients were viable remains to be answered. However, given the high prevalence of helminths in areas with high transmission of *P. falciparum,* we speculate that gametocytes must be viable in helminth-infected patients to sustain such high transmissions.

The association of decreased hemoglobin concentrations with gametocyte carriage suggests another perverse consequence of tropical anemias in general, which could create a vicious circle by increasing transmission of one of the major causes of anemia in the tropics: malaria. *P. falciparum* and helminths are among the most common tropical pathogens. Therefore, regardless of the underlying mechanisms, at a population level, the increase in malaria transmission in helminth-infected patients could be highly significant. Helminth infections, which are most frequent in children, could constitute a complementary explanation to age-related fluctuations of malaria transmission indicators.[12] From an evolutionary perspective, a mutualist equilibrium may have developed between different parasite species: *P. falciparum* increasing its transmission and helminths benefiting by protecting their host,[7] therefore surviving and continuing to reproduce. Virulence and reproduction are closely linked,[13] and helminths by favoring gametocytogenesis and protecting against severe malaria could be an illustration of this. In remote areas, where malaria treatment is likely to be delayed, treating helminths could theoretically result in less anemia and gametocyte carriage during *falciparum* malaria, but it could also increase the risk of cerebral malaria.[7] Thus the extent of harmful[14] and beneficial consequences[7] of helminth infections may need further investigations in order to define malaria and helminth control strategies.

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