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Associations between Helminth Infections, *Plasmodium falciparum* Parasite Carriage and Antibody Responses to Sexual and Asexual Stage Malarial Antigens

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Abstract. Infections with helminths and *Plasmodium* spp. overlap in their geographical distribution. It has been postulated that helminth infections may influence malarial transmission by altering *Plasmodium falciparum* gametocytogenesis. This cross-sectional study assessed the effect of helminth infections on *P. falciparum* gametocyte carriage and on humoral immune responses to sexual stage antigens in Gabon. *Schistosoma haematobium* and filarial infections as well as *P. falciparum* asexual forms and gametocyte carriage were determined. The antibody responses measured were to sexual (Pfs230, Pfs48/45) and asexual *P. falciparum* antigens (AMA1, MSP1, and GLURP). A total of 287 subjects were included. The prevalence of microscopically detectable *P. falciparum* asexual parasites was higher in *S. haematobium*–infected subjects in comparison to their uninfected counterparts (47% versus 26%, $P = 0.003$), but this was not different when filarial infections were considered. *Plasmodium falciparum* gametocyte carriage was similar between *Schistosoma*- or filaria-infected and uninfected subjects. We observed a significant decrease of Pfs48/45 immunoglobulin G titer in *S. haematobium*–infected subjects ($P = 0.037$), whereas no difference was seen for Pfs230 antibody titer, nor for antibodies to AMA1, MSP1, or GLURP. Our findings suggest an effect of *S. haematobium* on antibody responses to some *P. falciparum* gametocyte antigens that may have consequences for transmission-blocking immunity.

INTRODUCTION

In many malaria-endemic regions, helminth infections are also prevalent, thereby affecting the same population.[1,2] There is some evidence suggesting an interaction between helminth and *Plasmodium* spp.; however, this has not been consistent. For example, the prevalence and severity of malaria as well as *Plasmodium falciparum* parasitemia density has been reported to be higher[3,4] in some but lower[5,6] in other studies comparing helminth-infected subjects with those uninfected. Similarly, at the immunological level, there are conflicting reports regarding the effect of chronic helminth infections on the immune responses to *Plasmodium* spp.[7,8]

The immunological protection against clinical malarial episodes is associated with a more pronounced Th1 response[9] and with the production of cytophilic antibodies (immunoglobulin G [IgG]1 and IgG3).[10,11] However, the immune phenotype of helminth-infected subjects is generally characterized by a Th2 skewed response[12,13] and is marked by the production of noncytophilic IgG (IgG4) and immunoglobulin E antibodies.[14] Helminth infections have also been shown to induce a strong regulatory network that can dampen the immune response to unrelated antigens like those from *Plasmodium* spp. parasites.[15,16] One may, therefore, speculate that malaria-specific immune responses may be impaired in subjects chronically infected with helminths. However, studies that have assessed this question have yielded conflicting results indicating that larger and better designed studies are needed.[17–20]

To date, studies assessing the coinfection of helminths and malaria have mainly focused on the asexual forms of *P. falciparum* (reviewed in reference 21). However, there are indications that helminth infections may also influence the prevalence or density of *P. falciparum* gametocytes, the parasite stage responsible for transmission of infections to mosquitoes.[22] Studies in Africa and Asia have reported an increased prevalence of *P. falciparum* gametocyte carriage in helminth-infected subjects.[22,23] Interestingly using a murine model of coinfection, Noland and others[24] showed that transmission of gametocytes from mice to mosquitoes was higher when mosquitoes were fed on helminth- and malaria-coinfected mice. Taken together, these findings might indicate a role for helminths in sustaining malarial transmission in coendemic areas.

Toward understanding the effect of helminths on malarial immunity and transmissibility, we have conducted a cross-sectional study in an area endemic for both helminths and malaria. In this study, we have assessed the effect of *Schistosoma haematobium* and filarial parasites on the prevalence of sexual and asexual forms of *P. falciparum* parasite. Finally we determined the association between helminth and malarial coinfections on the humoral responses to sexual stage antigens Pfs230 and Pfs48/45, along with a panel of asexual stage antigens (apical membrane antigen [AMA1], merozoite surface protein [MSP1], glutamate rich protein [GLURP]).

METHODS

Study population, study area, and study procedure. The study participants were selected among the population of the Zilé village in the Moyen-Ogooué Province (Gabon). This village is endemic for *S. haematobium*, *Loa loa*, and *Mansonella perstans* as well as for various geohelminths.[25–27] Malaria is also endemic in the area with *P. falciparum* reported as dominant species.[28] Inclusion of participants in the study was not random but based on their willingness to participate. Participants were recruited at home during field visits of the study team. Urine and blood samples collected in ethylenediaminetetraacetic acid tubes were taken for all the subjects to assess for *Schistosoma*, filarial, and malarial infection.

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Venous blood was drawn and serum was obtained for enzyme-linked immunosorbent assay (ELISA).

Parasitological diagnosis. *Plasmodium* spp. infection was determined by microscopic examination of thick blood smears. Asexual forms of the parasite were detected by the Lambaréné method as described elsewhere.[29] The presence of *P. falciparum* gametocytes was established using the World Health Organization method after counting 1,000 leukocytes. DNA extraction and real-time polymerase chain reaction (PCR) (quantitative PCR) was carried on to detect submicroscopic infection by asexual stage *P. falciparum* as previously described.[30]

Schistosoma haematobium infection was determined before inclusion in the study. *Schistosoma* eggs were sought in 10 mL of fresh urine passed through a 12-μm pore-size filter. Absence of infection was set after the negativity of three urines samples collected after three consecutive days. A subject was classified as infected if at least one egg was detected in the urine sample.

Loa loa and *M. perstans* microfilaria was detected by a modified Knott method.[31] Microfilarial count was determined by microscopy, and difference between species was established based on the presence of the sheath of *L. loa*.

Enzyme-linked immunosorbent assay. Pfs48/45-10C was obtained from the chimeric R0-10C vaccine protein.[32] R0 was cleaved from Pfs48/45-10C, and successful removal of GLURP-R0 was confirmed by testing plasma samples from GLURP-vaccinated volunteers.[33] Pfs230-230CMB was obtained from Fraunhofer USA Center for Molecular Biotechnology. Apical membrane antigen (AMA-1 3D7, Biomedical Primate Research Center, Rijswijk, The Netherlands), merozoite surface protein 119 (MSP-119 Wellcome allele, provided by Patrick Corran, London School of Hygiene and Tropical Medicine with permission of Tony Holder) and R2 region of GLURP, provided by Michael Theisen, Statens Serum Institut, Copenhagen, Denmark, were used.

AMA-1, MSP-1, and GLURP ELISAs were performed as described previously.[34] Pfs48/45 and Pfs230 antibodies were quantified as follows: 96-well Maxisorp NUNC plates (Nalge Nunc International Corp., Naperville, IL) were coated overnight at 4°C with 100 μL per well of 0.1 μg/mL of antigen diluted in phosphate-buffered saline (PBS). Plates were blocked for 30 minutes with 150 μL of 5% nonfat skim milk (Marvel; Premier International Foods Ltd., Spalding, United Kingdom) in PBS. Following this, plates were washed three times with PBS and 100 μL of test serum was diluted to 1/500 in PBS (with 1% milk and 0.05% Tween 20) and incubated on the plates for 4 hours at room temperature. Plates were then washed three times as before and incubated with 100 μL per well of human IgG horseradish peroxidase (Pierce Biotechnology Inc., Rockford, IL) diluted to 1/40,000 in PBS with 0.05% Tween 20 for 2 hours at room temperature. Next, plates were washed four times, then 100 μL of tetramethylbenzidine substrate solution was added per well, and incubated for 20 minutes. Reactions were stopped using 50 μL per well of 0.2 M sulfuric acid and optical densities (ODs) were measured at 450 nm (Bio-Rad iMark Microplate Reader; Bio-Rad Laboratories, Hertfordshire, United Kingdom). For all assays, averaged sample ODs were normalized (using the midpoint dilution as reference) against a titration curve fitted to the positive control sample by least squares minimization using a three-variable sigmoid model.[34,35] The mixture model was used to distinguish positive and negative samples by fitting test sample ODs to two Gaussian distributions using maximum likelihood methods in STATA (Version 11; StataCorp, College Station, TX). The mean OD of the seronegative (the test samples with low ODs) population plus three standard deviations was used as the cutoff value. Antibody densities were expressed as percentage of the reference value (i.e., the normalized value).

Statistical analysis. The statistical analysis was conducted using STATA (Version 11, StataCorp) and R (Version 3.0.1; R core team, Vienna, Austria). Chi-squared test was used for the comparison of proportion. Continuous data that were not normally distributed were transformed either using a log10 transformation or a Box–Cox transformation when appropriate. Comparison of mean was carried out using the Student's *t* test or the analysis of variance test for normally distributed data or the Mann–Whitney and the Kruskal–Wallis test otherwise. Multivariable linear regression analysis was performed to assess the relationship between infectious status and the antibody response specific to *P. falciparum* gametocyte antigens. Significance level was set for a P value < 0.05.

Ethics. The study was approved by the "Comité d'éthique Régional Indépendant de Lambaréné". Informed consent was obtained from each participant and in case they had less than 18 years old from their parents or legal guardians. Appropriate treatment was given to children found with *P. falciparum* or *S. haematobium* infection as per the local guidelines.

RESULTS

A total of 287 participants were included in this study. Among them, 229 (81%) had either *S. haematobium* or one of the filarial infections, *M. perstans* or *L. loa*, whereas 197 (75%) carried *P. falciparum* parasite as determined by PCR or 120 (42%) as determined by microscopy. None of the participants carrying *P. falciparum* were symptomatic for malaria. Coinfection with *Plasmodium* and with one or more helminth species was found in 155 (55%) subjects (Table 1). The prevalence of microscopic *P. falciparum* asexual parasites was higher in *S. haematobium*–infected subjects in comparison to those free of *S. haematobium* infection (47% versus 26%, $P = 0.003$; Table 2), whereas *P. falciparum* gametocyte carriage was similar between helminth-infected and helminth-uninfected

TABLE 1
Characteristics of the study population

Characteristics	% (*n*/*N*)
Age (in years): median (IQR)	11 (8–15)
Sex: M/F	153/134
Hemoglobin level (in g/dL): median (IQR)	11.3 (10.5–12.2)
Subjects with *Plasmodium falciparum* asexual stage infection (diagnosed by microscopy)	42 (120/285)
Subjects with *P. falciparum* asexual stage infection (diagnosed by PCR)	75 (197/262)
P. falciparum gametocyte carriers	19 (52/275)
Subjects with *Schistosoma haematobium* infection	75 (214/284)
Subjects with filarial infection*	28 (81/287)
Helminth infection status	
Subjects with no helminth infection	20 (55/284)
Subjects infected with one helminth species	57 (163/284)
Subjects infected with more than one helminth species	23 (66/284)
Subjects with *P. falciparum* and helminth coinfection	55 (155/284)

F = female; IQR = interquartile range; M = male; PCR = polymerase chain reaction.
* *Loa loa* and *Mansonella perstans*.

TABLE 2
Characteristics of the study population stratified by helminth infections

	Schistosoma haematobium infection status			Filarial infection status*		
	Uninfected subjects	Infected subjects	*P*	Uninfected subjects	Infected subjects	*P*
Age in years: median (IQR)	10 (6)	11 (6.7)	0.9	10 (5)	14 (8)	< 0.001
Sex (M/F)	38/32	112/102	0.2	113/93	40/41	0.4
Hemoglobin level (g/dL), mean (SD)	11.6 (1.3)	11.2 (1.45)	0.06	11.3 (1.3)	11.5 (1.6)	0.3
Subjects with *Plasmodium falciparum* asexual stage infection (diagnosed by microscopy), *n* (%)	18/70 (26%)	100/214 (47%)	0.003	42% (86/206)	43% (34/81)	1
Subjects with *P. falciparum* asexual stage infection (diagnosed by PCR), *n* (%)	45 (66.2%)	146 (78%)	0.07	136 (74%)	58 (78%)	0.55
P. falciparum gametocyte carriers, *n* (%)	14 (20.1%)	36 (18%)	0.8	41 (21%)	11 (14%)	0.2

F = female; IQR = interquartile range; M = male; PCR = polymerase chain reaction; SD, standard deviation.
* *Loa loa* and *Mansonella perstans*.

subjects (Table 2). Also when malarial parasites were detected by PCR, we observed a higher malarial prevalence in *S. haematobium*–infected individuals, albeit not statistically significant ($P = 0.07$; Table 2). We did not detect any differences when comparing those with and without filarial infections (Table 2). The absence of association between filaria and carriage of sexual and asexual forms of *P. falciparum* remained even after correction for age in a multivariable analysis (data not shown).

In this study, we measured the total IgG response of the participants to three asexual (MSP1, AMA1, and GLURP) and two sexual (Pfs48/45 and Pfs230) *P. falciparum* antigens. We did not observe a statistically significant effect of age or gender on the level of the different antibodies (data not shown). Asexual *P. falciparum* parasites, as determined by microscopy, did not influence the level of the five antibodies measured. However, when *P. falciparum* asexual form was determined by PCR, higher antibody concentrations were observed in infected subjects compared with uninfected for Pfs230 (65.7 [95% confidence interval {CI}: 59.3–72.5] versus 51.1 [95% CI: 42.7–60], $P = 0.01$) and AMA1 (1430.8 [95% CI: 1064.3–1904.9] versus 689.9 [95% CI: 391.7–1147.3], $P = 0.01$) but not for other antibodies, as shown in Table 3. Moreover, we found that carriage of *P. falciparum* gametocytes was associated with a trend toward increased antibodies to Pfs48/45 in gametocyte-positive (55.1 [95% CI: 46.2–66.2] compared with gametocyte-negative individuals (44.7 [95% CI: 40.4–49.3], $P = 0.056$). The same trend was observed for Pfs230 (72 [95% CI: 60.5–84.4] in gametocyte-positive and 59.8 [95% CI: 54.1–65.6] in gametocyte-negative individuals, $P = 0.068$) as shown in Table 3.

Regarding helminth infections, we observed a significant decrease of Pfs48/45 IgG titers in *S. haematobium*–infected subjects compared with those uninfected (44.2 [95% CI: 39.7–49.2] versus 53.2 [95% CI: 46.3–60.7], $P = 0.037$), whereas no difference was seen for Pfs230 antibody titers, nor for the other antibodies as shown in Table 3. In contrast to *S. haematobium*, filarial infection was not associated with a significant effect on the concentration of antibodies to sexual or asexual *P. falciparum* stage antigens.

To further assess how schistosome infection affects the humoral responses to *P. falciparum* sexual and asexual stage antigens, we performed a multivariable analysis on the titer of antibodies against the five antigens. In this analysis, asexual *P. falciparum* infection diagnosed by PCR, *P. falciparum* gametocyte carriage, hemoglobin concentration, and participant age was used as predictor variables. These predictor variables were selected based on their reported effect on gametocyte carriage and on antibodies specific to *P. falciparum* asexual or sexual stage antigens. We fitted the model for the total population as well as for *S. haematobium*–uninfected and –infected individuals, separately. In the total population, we observed no apparent association between Pfs230, AMA1, GLURP, or MSP1 antibody responses and *S. haematobium* infection (Table 4), but we did find a significant decrease of Pfs48/45 antibody titer in *Schistosoma*-infected subjects compared with their uninfected counterparts ($\beta = 0.68$, 95% CI: 0.48–0.97, $P = 0.035$; Table 4). When restricting the analysis to subjects

TABLE 3
Effect of malarial and helminth infection as well as malarial exposure on the level of total IgG specific to Pfs48/45, Pfs230, GLURP, MSP1, and AMA1

		Pfs48/45, mean (±SD)	*P*	Pfs230, mean (±SD)	*P*	GLURP, mean (±SD)	*P*	MSP1, mean (±SD)	*P*	AMA1, mean (±SD)	*P*
Plasmodium falciparum asexual stage infection status (diagnosed by microscopy)	Uninfected	46 (6.3)	0.8	59.5 (14.3)	0.3	55.9 (5.9)	0.25	54.8 (6.7)	0.09	1064.7 (12.5)	0.14
	Infected	47 (6.5)		65.1 (15.6)		71.7 (5.9)		36.9 (7.2)		1533.8 (17.9)	
P. falciparum asexual stage infection status (diagnosed by PCR)	Uninfected	40.2 (5.5)	0.068	51.1 (11.5)	0.01	48.6 (5.7)	0.16	53 (7)	0.38	689.9 (10)	0.01
	Infected	48.7 (6.6)		65.7 (16.2)		69.2 (6)		41.3 (7)		1430.8 (17)	
P. falciparum gametocyte carriage status	Non carriers	44.7 (6.4)	0.056	59.8 (15.1)	0.068	58.2 (6)	0.36	47.6 (6.8)	0.64	1226 (16.4)	0.6
	carriers	55.1 (5.7)		72 (13)		74 (5)		41.2 (7.7)		1439 (10.5)	
Schistosoma haematobium infection status	Uninfected	53.2 (4.3)	0.037	63.3 (12.3)	0.7	69 (4.7)	0.53	55.3 (5.2)	0.33	1372.2 (11.4)	0.6
	Infected	44.2 (7)		61.3 (15.7)		60 (6.4)		43.7 (7.3)		1205.8 (16.2)	
Filarial infection status*	Uninfected	46.5 (6.4)	0.9	62.2 (15.4)	0.8	61 (5.4)	0.79	47.8 (7)	0.68	1169.1 (15.9)	0.4
	Infected	46.2 (6.2)		60.9 (13.5)		65 (7.5)		43.1 (7)		1455.3 (12.4)	

IgG = immunoglobulin G; PCR = polymerase chain reaction; SD, standard deviation; GLURP = glutamate rich protein; AMA1 = apical membrane antigen 1; MSP1 = merozoite surface protein 1.
* *Loa loa* and *Mansonella perstans*.

TABLE 4

Multivariable linear regression analysis assessing the effect of *Schistosoma haematobium* infection, *Plasmodium falciparum* infection as detected by PCR, hemoglobin level as well as age on antibodies to *P. falciparum* sexual and asexual stage antigens

		S. haematobium infection status					
		All subjects		*S. haematobium*–uninfected subjects		*S. haematobium*–infected subjects	
Antibody	Covariates	β (95% CI)	*P* value	β (95% CI)	*P* value	β (95% CI)	*P* value
Pfs48/45	*S. haematobium* infection	0.68 (0.48–0.97)	0.0352	–	–	–	–
	Infection with asexual forms of *P. falciparum*	1.14 (0.78–1.67)	0.487	1.85 (1.25–2.74)	0.003	0.93 (0.56–1.55)	0.773
	Carriage of sexual forms of *P. falciparum*	1.41 (0.93–2.15)	0.106	1.16 (0.73–1.85)	0.529	1.5 (0.87–2.6)	0.142
	Hemoglobin level	1 (0.89–1.13)	0.95	1.02 (0.89–1.18)	0.748	1.01 (0.87–1.17)	0.946
	Age	1.01 (0.99–1.03)	0.125	1.02 (1–1.03)	0.03	1.01 (0.99–1.04)	0.316
Pfs230	*S. haematobium* infection	0.79 (0.54–1.17)	0.237	–	–	–	–
	Infection with asexual forms of *P. falciparum*	1.19 (0.78–1.81)	0.412	1.91 (1.1–3.34)	0.023	0.93 (0.54–1.61)	0.792
	Carriage of sexual forms of *P. falciparum*	1.30 (0.82–2.07)	0.264	1.37 (0.71–2.66)	0.348	1.26 (0.7–2.27)	0.431
	Hemoglobin level	0.98 (0.86–1.12)	0.785	0.97 (0.79–1.19)	0.757	0.98 (0.84–1.16)	0.86
	Age	1.01 (0.99–1.03)	0.261	1.01 (0.99–1.03)	0.345	1.01 (0.99–1.04)	0.293
AMA1	*S. haematobium* infection	0.64 (0.3–1.35)	0.242	–	–	–	–
	Infection with asexual forms of *P. falciparum*	2.02 (0.9–4.53)	0.085	3.74 (1.08–12.93)	0.038	1.56 (0.55–4.38)	0.4
	Carriage of sexual forms of *P. falciparum*	1.25 (0.51–3.05)	0.629	1.41 (0.32–6.17)	0.645	1.16 (0.38–3.52)	0.786
	Hemoglobin level	1.03 (0.8–1.32)	0.846	1.24 (0.79–1.95)	0.344	0.97 (0.72–1.32)	0.86
	Age	1.03 (0.99–1.07)	0.085	1.03 (0.98–1.08)	0.258	1.04 (0.99–1.09)	0.144
MSP1	*S. haematobium* infection	0.76 (0.43–1.36)	0.357	–	–	–	–
	Infection with asexual forms of *P. falciparum*	0.69 (0.37–1.29)	0.248	0.79 (0.3–2.1)	0.633	0.66 (0.3–1.47)	0.312
	Carriage of sexual forms of *P. falciparum*	0.92 (0.46–1.85)	0.822	0.61 (0.19–1.96)	0.4	1.06 (0.45–2.48)	0.9
	Hemoglobin level	0.91 (0.75–0.11)	0.363	0.99 (0.69–1.41)	0.95	0.89 (0.71–1.13)	0.341
	Age	1.01 (0.98–1.04)	0.492	0.998 (0.96–1.04)	0.928	1.02 (0.98–1.06)	0.4
GLURP	*S. haematobium* infection	0.81 (0.45–1.32)	0.399	–	–	–	–
	Infection with asexual forms of *P. falciparum*	1.52 (0.9–2.56)	0.116	2.17 (0.9–5.21)	0.082	1.34 (0.69–2.56)	0.386
	Carriage of sexual forms of *P. falciparum*	1.36 (0.76–2.42)	0.303	1.37 (0.48–3.89)	0.551	1.33 (0.66–2.68)	0.427
	Hemoglobin level	0.99 (0.84–1.16)	0.878	1.19 (0.87–1.64)	0.276	0.94 (0.77–1.14)	0.522
	Age	1.03 (1–1.05)	0.027	1.02 (0.99–1.06)	0.186	1.03 (0.99–1.06)	0.068

Three models were considered for this analysis. In the first model, all subjects were included regardless of whether or not they were infected with *S. haematobium*. Subsequent analysis focus on *S. haematobium*–uninfected (model 2) or –infected subjects (model 3). CI = confidence interval; PCR = polymerase chain reaction.

not infected with *S. haematobium*, we observed that *P. falciparum* infection as determined by PCR was associated with a significant increase of Pfs48/45 ($\beta = 1.84$, 95% CI: 1.2–2.7, $P = 0.003$; Table 4) and Pfs230 specific antibodies ($\beta = 1.9$, 95% CI: 1.2–3.3, $P = 0.02$) as shown in Table 4. This association was not seen when the model was applied to those infected with *S. haematobium* (Table 4).

DISCUSSION

To our knowledge, this is the first study to assess the effect of helminth infections on both the carriage and the humoral immune response to *P. falciparum* sexual stage antigens. Our primary interest was to determine if gametocyte production, and antibody responses to gametocyte antigens, was higher in malaria-schistosome coinfected subjects. What we observed is that while gametocyte carriage as determined by microscopy did not differ between individuals infected with malaria alone and with malaria and *S. haematobium*, antibody levels to Pfs48/45 sexual stage antigen but not to Pfs230 were lower in coinfected individuals. Our initial hypothesis, based on the literature, was that a higher prevalence of *P. falciparum* gametocytes and markers of gametocyte exposure would be found in *S. haematobium* coinfected individuals.[22,23] Contrary to this hypothesis, we observed no apparent effect of *S. haematobium* coinfection on gametocyte carriage. This could be explained by the fact that we used microscopy to detect gametocytes, which is less sensitive than molecular methods.[36] It is possible that molecular detection methods for gametocytes or functional assays (e.g., standard membrane feeding) could have given a different picture in line with what has been described in two studies in the literature showing that helminth infections increase gametocyte carriage.[22,23] A more sensitive marker of gametocyte exposure than microscopy might be antibodies to two gametocyte antigens, Pfs230 and Pfs48/45.[37,38] Antibody responses to these molecules indeed appeared to be elevated in patent gametocyte carriers, suggesting that they may serve as specific markers of gametocyte exposure. Interestingly, in the current study, lower levels of antibodies to Pfs48/45 but not to Pfs230 were seen in subjects with schistosome infection. This suggests either a spurious finding, a selective suppressory effect of *Schistosoma* coinfection on antibody responses against Pfs48/45 or lower immunogenicity of Pfs48/45 and amenable to modulation. In line with our finding, a study in Papua New Guinea with high transmission of malaria showed that if sera recognized the gametocyte surface antigens, the response was dominated by antibodies to Pfs230 with fewer people showing a response to pfs48/45.[39] However, helminth infections, which are highly prevalent in the area were not considered and therefore it is not possible to delineate whether the low response to Pfs48/45 is due to the presence of helminth infections.

In our study, we noted that a higher percentage of *Schistosoma*-infected participants was infected with *P. falciparum*, and this was statistically significant when parasites were detected by microscopy but fell short of significance when PCR was used to detect malarial parasites. One way to interpret this is that schistosome infections are associated with higher burden of malarial parasites. The higher carriage of *P. falciparum* suggests a possible interaction between helminths and *P. falciparum*, which is unlikely to be explained by structural features of the houses, an important factor in

malarial transmission, as houses in our study area were very similar to each other. Other factors such as nutritional status or proximity to water bodies could explain such an interaction but we did not collect data on these parameters.

We did not observe an effect of schistosomiasis on the antibody titers to *P. falciparum* asexual stage antigens, AMA1, GLURP, or MSP1. This is in line with the results from Lyke and others[7] in Mali but it contrasts to the observations of Diallo and others[8] in Senegal who reported a significant increase of the humoral response to *Plasmodium* antigens in *Schistosoma*-infected subjects. A plausible explanation of these differences might lie in the characteristics of the populations studied as well as in the epidemiological feature of malaria and schistosomiasis in the different study areas. For instance, in the study in Mali, all participants came from an area where, like in our study area, both malaria and schistosomiasis show intense seasonal transmission.[7] In contrast, the study in Senegal was conducted in an area of low malarial transmission and helminth-free subjects were recruited from a village, where *S. haematobium* had never been reported before and was absent at the time of the study.[8] Because exposure to these parasites is expected to be very different in the two studies, this could affect the immune response profiles measured. Finally, it is also important to emphasize that Diallo and others measured the level of the different IgG subclasses, whereas Lyke and others and ourselves studied total IgG. Future studies should examine the effect of helminths on IgG subclasses since protection to malaria is linked with an increase of cytophilic antibodies of IgG1 and IgG3 subclasses. Why filarial infections did not affect the antibody responses to malarial antigens is not clear. Species-dependent effect of helminth on malariometric indices and on malaria-specific immune response has already been reported.[40,41] It is possible that schistosomiasis has a more pronounced effect on the host immune response and/or metabolism than filarial infections. In this study, we observed, for example, that *Schistosoma* but not filaria-infected subjects were more likely to be anemic. Future studies will need to expand and further assess the effect of filaria or other helminths species on malarial transmission.

The present study should be regarded as hypothesis generating and calls for additional work to assess the association between helminths and malarial transmission. Future studies should ideally have a longitudinal design and include the collection of material for sensitive gametocyte detection by RNA-based methods.[42] These studies will equally need to take into consideration the possible confounding effect of other helminths infection such as intestinal nematodes. Future studies should also determine the functional importance of the observed decrease of Pfs48/45 specific IgG in *Schistosoma*-infected subjects using mosquito-feeding assays.

In summary, this study suggests a selective effect of *S. haematobium* on the humoral response to an important *P. falciparum* sexual stage antigen. It indicates an association between helminths and malarial transmission and may form a starting point for more detailed studies on the consequences of dual infections for disease transmission.

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