Determinants of Infant Susceptibility to Malaria During the First Year of Life in South Western Cameroon

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Background. Falciparum malaria is an important pediatric infectious disease that frequently affects pregnant women and alters infant morbidity. However, the impact of some prenatal and perinatal risk factors such as season and intermittent preventive treatment during pregnancy (IPTp) on neonatal susceptibility has not been fully elucidated.

Methods. A cohort of 415 infants born to women who were positive and negative for malaria was monitored in a longitudinal study in Southwestern Cameroon. The clinical and malaria statuses were assessed throughout, whereas paired maternal-cord and 1-year-old antimalarial antibodies were assayed by enzyme-linked immunosorbent assay. Infant susceptibility to malaria was ascertained after accounting for IPTp and season in the statistical analysis.

Results. Malaria prevalence was higher in women ($P = .039$) who delivered during the rainy season and their infants ($P = .030$) compared with their dry season counterparts. Infants born to women who were positive for malaria (6.40 ± 2.83 months) were older ($P = .028$) than their counterparts who were negative for malaria (5.52 ± 2.85 months) when they experienced their first malaria episode. Infants born in September–November (adjusted odds ratio [OR] = 0.31, 95% confidence interval [CI] = 0.13–0.72) and to mothers on 1 or no IPTp-sulfadoxine/pyrimethamine (SP) dose (adjusted OR = 0.51, 95% CI = 0.28–0.91) were protected, whereas those born in the rainy season (adjusted OR = 2.82, 95% CI = 1.21–6.55) were susceptible to malaria.

Conclusions. Intermittent preventive treatment during pregnancy and month of birth have important implications for infant susceptibility to malaria, with 2 or more IPTp-SP dosage possibly reducing immunoglobulin M production.

Keywords. antibodies; infant susceptibility; IPTp; malaria; pregnancy; season.

Falciparum malaria is the most important pediatric infectious disease in sub-Saharan Africa, where it is estimated to kill mainly children less than 5 years of age [1]. Pregnant women constitute a major risk group, partly due to the transient depression of cell-mediated immunity that allows fetal allograft retention but interferes with resistance to infectious diseases [2]. With at least 50 million mothers at risk annually [3], malaria in pregnancy (MiP) due to *Plasmodium falciparum*, represents a major public health burden [4, 5].

Although newborns in areas of high malaria transmission are relatively protected from malaria in early life [6], the time to first malaria infections and the intensity of symptoms vary among infants [4, 7]. Identifying the factors underlying these differential susceptibilities is critical for malaria control in this vulnerable group. Reports indicate that MiP affects symptomatic infant morbidity, although its exact role remains unknown. Although studies have documented increased susceptibility to malaria [4, 7–10] and nonmalarial

fevers [11] in infants born to mothers with *P falciparum* infection at delivery, others [12, 13] have reported improved resistance to malaria in infancy.

The administration of intermittent preventive treatment during pregnancy (IPTp) with at least 2 therapeutic doses of sulfadoxine-pyrimethamine (SP) [14] has been shown to be effective in reducing malaria-associated adverse events [15, 16] and placental infection in the study area [17]. Nevertheless, the reduction in exposure to *P falciparum* might affect exposure intensity in utero, interfere with the development of malaria-specific immunity [18], and consequently alter infant susceptibility to malaria. However, little is known about the potential effect of IPTp on maternal and fetal immunity [19] and neonatal susceptibility to disease.

In spite of the high seasonality of malaria transmission in the study area [20–22], its impact on placental malaria (PM), pregnancy outcome, and newborns' susceptibility has not been fully elucidated. The effect of some risk factors on the infant's antibody profile and their susceptibility to malaria is poorly understood. In this study, the clinical and malaria status of infants was assessed from birth to 12 months, in an attempt to evaluate the relationship between perinatal (season and month of birth) and prenatal (maternal malaria, IPTp, maternal- cord antibodies at parturition) risk factors on the infants' antibodies and malaria susceptibility. Knowledge of the immune mechanisms underlying changes in susceptibility may have implications, not only for the understanding of human malaria immunobiology but also for the evaluation of future vaccine efficacy in this age group.

MATERIALS AND METHODS

Ethics Statement
The study was approved by the Institutional Review Board of the University of Buea, whereas administrative authorization was obtained from the South West Regional Delegation of Public Health. Written informed consent was obtained from all the study participants. We followed the US Department of Health and Human Services Human Experimentation guidelines throughout the study.

Study Area
The study was conducted in the Mutengene Health Centre from June 2008 to July 2010. Mutengene is a semiurban setting located at approximately 220 m along the slope of Mount Cameroon in the South West region. The population of the area is highly heterogeneous, consisting of individuals from almost all the ethnic groups of Cameroon and some parts of neighboring Nigeria [20, 21]. The climate consists of a short dry season (December–February) and long rainy season (March–November), whose peak period (June–August) is interspersed by early moderate (March–May) and late moderate (September–November) rains. The minimal temperature varies between 18°C in August

to 20°C in December, whereas maximal temperature ranges from 30°C in March to 35°C in February [20]. Malaria transmission is perennial but especially intense during the rainy season. *Anopheles gambiae*, *Anopheles Funetus*, and *Anopheles Hancocki* are the main vectors with the highest infectivity rate (entomological inoculation rate = 9.3%) in the region. *Plasmodium falciparum*, the predominant parasite species, accounts for up to 96.0% of malaria infections [20]. Intermittent preventive treatment during pregnancy with SP from 4 months was implemented in Mutengene since 2006, in line with the World Health Organization guidelines for the delivery of IPTp at each scheduled visit after "quickening" (16 weeks) to ensure that a high proportion of women receive a minimum of 2 doses of IPT [14].

Study Population and Sample Survey
Volunteer mothers who had been resident in the study area for at least 2 years were enrolled through a cross-sectional survey during the third trimester (≥35 weeks) of gestation at the antenatal clinic (ANC) in the health center. A standard questionnaire was used to document information relating to maternal age, last menstrual period, gravidity, parity, gestational age, history of fever attack during gestation, use of IPTp- sulfadoxine/pyrimethamine (SP) and dosage as well as infant birth weight and Apgar score at enrollment, and delivery from ANC cards, patient's medical record book, and health center maternal care register. The cohort of newborns was then evaluated through a longitudinal, community-based approach during the first year of life.

Sample Collection at Delivery
Blood samples (3–5 mL) were collected from the umbilical cord at delivery in the health center and from the mother by venipucture within 24 hours of parturition for parasitological, hematological, and immunological analysis. After the delivery of the placenta, a piece ($5 \times 5 \times 2$ cm) was excised from the paracentric area [17].

Longitudinal Surveys
The health status of the infants was monitored bimonthly at home using a structured morbidity questionnaire, and febrile infants (axillary temperature >37.5°C) were clinically examined and treated at the health center. Finger prick blood samples were collected from all febrile infants for hemoglobin (Hb) and malaria parasitemia determination. An episode of malaria was defined as fever and presence of malaria parasites. Infants presenting with acute malaria were given Artesunate-Lumefantrine, the first-line combination currently recommended for use in the treatment of uncomplicated malaria in Cameroon. Infants with severe malaria were treated using a uniform protocol based on standard recommendations [23] consisting of parenteral quinine at 20 mg/kg loading dose followed by 10 mg/kg maintenance dose every 12 hours for 3 days.

Scheduled clinic visits were also undertaken on a quarterly basis from birth until 1 year. During such visits, infants were clinically examined and anthropometric measurements and axillary temperature were recorded. Finger prick blood samples were then collected for parasitological and hematological analyses. At 1 year of age, venous blood (3–5 mL) was also collected for analysis.

Malaria Parasitemia Determination

This test was done using thin and thick smears of maternal and cord blood stained with 10% Giemsa (Sigma-Aldrich, St. Louis, MO) as well as in placental impressions and biopsies as described previously [17]. The malaria parasitemia status and density were determined under oil immersion with the $100 \times$ objective, $10 \times$ eyepiece of a binocular Olympus microscope, whereas the *Plasmodium* species was identified on the thin blood smear fixed for 10 seconds in methanol prior to staining. A smear was only considered negative if no malaria parasites were seen in 50 high-power fields. Freshly collected placenta biopsies were fixed in 10% neutral-buffered formalin for a maximum of 3 months, embedded in paraffin wax, sectioned, and then stained with hematoxylin and eosin. Impression smears and biopsies were examined for malaria parasites and pigments using polarized light microscopy at $\times 1000$, and the infection was classified as active or past based on the presence of parasites in erythrocytes or malaria pigment in fibrin or monocyte/macrophage, respectively [24]. Placental histological sections were examined without knowledge of peripheral blood film and impression smear results. Overall, a mother was considered positive for malaria parasite at delivery if any parasites or malaria pigments were seen in a peripheral blood, placental blood, or tissue.

Hematological Analysis

Hemoglobin concentrations were determined at delivery (maternal and cord), during morbidity visits (if axillary temperature $\geq 37.5°C$), and during quarterly clinic visits using a hemoglobinometer (HemoCue, Angelholm, Sweden). Anemia was defined as Hb <11.0 g/dL and further classified as severe (Hb <5 g/dL), moderate (Hb <7.0, 7.0–10.0 g/dL), and mild (Hb >10.0–11.0 g/dL) [22]. Ferrous sulphate syrup was given to all children with Hb <10 g/dL, per Integrated Management of Childhood Illness guidelines.

Hemoglobin Genotyping

Infant Hb genotype was determined by cellulose acetate electrophoresis using packed red cells. The AA, AS, and SS genotypes were deduced by comparison to positive controls run alongside the samples in an electric field (350 V) for 25 minutes.

Antibody Measurement

Paired maternal cord and 1-year infant plasma samples were assayed for *P falciparum*-specific antibodies of the immunoglobulin (Ig)G, IgG1, IgG2, IgG3, IgG4, IgE, and IgM isotypes by indirect enzyme-linked immunosorbent assay (ELISA). All antibodies were measured using an extract of (schizont-enriched) blood-stage parasites (F32) as the capture antigen [25], and goat antihuman-IgG (Sigma-Aldrich) was used as capture antibody. From checkerboard titrations, it was determined that the optimal concentration for coating plates with the antigen for all isotypes or subclasses was 10 μg/mL.

Each test plasma was screened at a 1:1000 for IgG, 1:20 for IgG2/IgG4, and 1:40 dilution for IgG1/IgG3, with phosphate-buffered saline (PBS) containing 0.5% (v/v) Tween 20, 20% sodium azide, and 0.5% bovine serum albumin (BSA) as the diluent.

For the ELISA, the microtiter plates (Costar, Cambridge, MA) were incubated overnight at 4°C with the crude antigen in carbonate buffer (50 μL/well) and then blocked for 2 hours at 37°C, with PBS containing 0.5% BSA. Duplicate wells were subsequently incubated for 1 hour at 37°C, with 50 μL diluted test plasma/well, washed, and then probed with the relevant isotype-specific antiserum-conjugated to alkaline phosphatase (ALP) (Mabtech, Sweden). The IgG2-4 assays were enhanced using biotinylated mouse anti-human IgG and then ALP-conjugated streptavidin (Mabtech, Stockholm) as described elsewhere [26]. After incubation with the ALP conjugates, para-nitrophenol phosphate (Sigma-Aldrich) was added as substrate before the optical density at 405 nm of each well was read on an ELISA plate reader (Tecan, Menlo Park, CA). In each assay, test samples giving optical densities (OD) that exceeded the corresponding mean + 2 standard deviation for 25 nonimmune European donors were considered seropositive for the antibody.

Statistical Analyses

All data were analyzed using SPSS Statistics 20 (SPSS Inc., Chicago, IL). Antibody OD were log transformed before analysis. The significance of differences in prevalence and seropositivity were explored using the Pearson's χ^2 test, whereas the differences in group means were assessed using Student t test or analyses of variance. Association analysis of clinical episodes and risk factors was undertaken by logistic regression. A P value $\leq .05$ was considered statistically significant.

RESULTS

Four hundred forty-two volunteer pregnant women were enrolled into the study (Table 1). However, maternal cord blood and placental biopsies were only collected for 415 women due to voluntary termination, referral before or after delivery due to complications, prompt discharge, and change of hospital. An additional 16 cord blood samples could not be analyzed due to clotting. Two hundred eighty-three infants successfully completed the follow-up, and loss to follow-up was mainly due to relocation of parents, voluntary termination of participation, and death.

Table 1. Baseline Characteristics of the Placental Malaria-Positive and Malaria-Negative Parturient Mothers and Their Infants From Mutengene, South Western Cameroona[a]

| Parameter	Subclass	% of Study Participants (n)	Maternal Malaria Status		P Value
			Positive	Negative	
Maternal Characteristics (Mean ± SD)					
Age (years)		100 (415)	23.8 ± 5.4 (166)	24.2 ± 5.1 (247)	.472
Hb (g/dL)		99.0 (411)	10.5 ± 1.8 (165)	10.9 ± 1.7 (246)	**.046**
Maternal characteristics, % (n)					
Age group (years)	≤20	28.3 (117)	45.3 (53)	54.7 (64)	.382
	21–35	68.0 (281)	38.4 (108)	61.6 (173)	
	>35	3.6 (15)	33.3 (5)	66.7 (10)	
Gravidity	Primigravid	32.0 (132)	46.2 (61)	53.8 (71)	.231
	Secundigravid	27.4 (113)	37.2 (42)	62.8 (71)	
	Multigravid	40.1 (168)	37.5 (63)	62.5 (105)	
IPTp-SP Usage	Yes	92.2 (379)	39.6 (150)	60.4 (229)	.347
	No	7.8 (32)	48.5 (16)	51.5 (17)	
IPTp-SP Dosage	One or less	41.4 (170)	41.2 (70)	58.8 (100)	.720
	Two or more	58.6 (241)	39.4 (95)	60.6 (146)	
History of Fever	Yes	50.6 (207)	43.0 (89)	57.0 (118)	.268
	No	49.4 (202)	37.6 (76)	62.4 (126)	
Anemia Status	Anemic	50.4 (207)	45.4 (94)	54.6 (113)	**.028**
	Nonanemic	49.6 (204)	34.8 (71)	65.2 (133)	
Infant characteristics, mean ± SD (n)					
Birth weight (g)		99.8 (414)	3214 ± 574 (166)	3353 ± 542 (248)	**.014**
Apgar		97.8 (406)	8.95 ± 1.0 (162)	9.10 ± 0.9 (244)	.126
Cord Hb (g/dL)		88.2 (366)	15.5 ± 2.1 (146)	15.4 ± 2.1 (220)	.674
Infant characteristics [% (n)]					
Low birth weight (<2500 g)		5.6 (23)	65.2 (15)	34.8 (8)	**.012**
Anemia status	Anemic	1.6 (6)	16.7 (1)	83.3 (5)	.233
	Nonanemic	98.4 (360)	40.3 (145)	59.7 (215)	
Gender	Male	47.2 (195)	40.0 (78)	60.0 (117)	.939
	Female	52.8 (218)	40.4 (88)	59.6 (130)	
Hb genotype	AA	83.6 (235)	42.6 (100)	57.4 (135)	.466
	AS	15.4 (43)	44.2 (19)	55.8 (24)	
	SS	0.7 (2)	0.0 (0)	100.0 (2)	
Season of birth	Rainy	79.7 (314)	42.7 (134)	57.3 (180)	**.039**
	Dry	20.3 (80)	30.0 (24)	70.0 (56)	
Month of birth	Mar–May	25.1 (99)	61.6 (61)	38.4 (38)	**<.001**
	Jun–Aug	39.3 (155)	38.1 (59)	61.9 (96)	
	Sep–Nov	21.6 (85)	21.2 (18)	78.8 (67)	
	Dec–Feb	14.0 (55)	36.4 (20)	63.6 (35)	

Abbreviations: Hb, hemoglobin; IPTp, intermittent presumptive treatment for malaria during pregnancy; SD, standard deviation; SP, sulfadoxine/pyrimethamine; AA, normal Hb; AS, sickle cell trait; SS, sickle cell disease.

[a] Values in parentheses denote total participants with valid values for this variable. A mother was considered positive for malaria parasite at delivery if any parasites or malaria pigment were seen in a peripheral blood, placental blood, or tissue; The possible association between maternal malaria prevalence with age groups, gravidity, anemia status, reported fever history, IPTp-SP dosage, birth weight category, gender, Hb genotype, season of birth, and period of birth were explored using the Pearson's χ^2 test, whereas the differences in mean age, Hb level, birth weight, and Apgar between malaria-positive and malaria-negative women were assessed using the Student t test. Bold text indicate significant P values.

Basic Characteristics of Malaria-Positive and Malaria-Negative Women

The mean age of the mothers was 24.05 ± 5.2 (range, 15–41) years. Most of the women were between 21 and 35 years old, multigravida, and had taken at least 2 doses of IPTp-SP (Table 1).

Cord blood malaria parasitemia was rare because it was only detected in 1.5% (6 of 399) of study participants. The most sensitive method for the determination of maternal malaria infection was placenta histology with a malaria parasite prevalence of 37.2 (141 of 379). Maternal peripheral malaria parasitemia
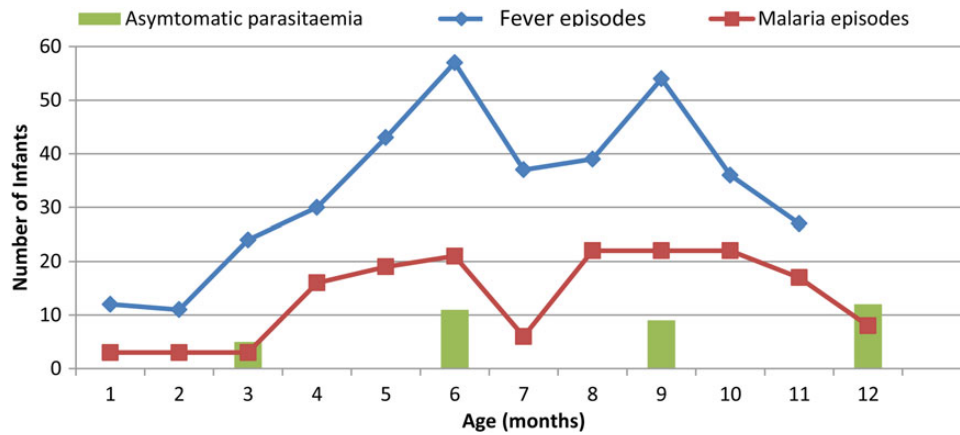
Figure 1. Relative morbidity of 283 infants from Mutengene, South Western Cameroon within the first year of life. Asymptomatic parasitemia was only assessed during the quarterly surveys, whereas fever and malaria episodes were recorded throughout the morbidity visits to the health center.

screens could only detect 7.5% (30 of 400), and placenta impressions detected only 15% (62 of 352), suggesting that some infected mothers could not be detected with these methods. Overall, active maternal malaria parasite infection at delivery was detected in 40.2% (166 of 415) of the study women, whereas active plus past infection prevalence was 62.7% (259 of 413).

The proportion of women with malaria was higher ($P = .039$) in the rainy season (42.7%) compared to the dry season (30.0%); the March–May (61.6%) was higher ($P < .001$) than other months. Anemic mothers were also more ($P = .028$) malaria positive (45.4%) compared with their nonanemic counterparts (34.8%). Twelve women (5.8%) had severe anemia, 97 (46.9%) had moderate anemia, and 98 (47.3%) had mild anemia. Women who were positive for malaria also had lower Hb levels ($P = .046$) and lower birth weight babies ($P = .014$) compared with their negative counterparts (Table 1).

Most of the infants were born in the rainy season (79.7%), during the months of June–August (39.3%). The proportion of low birth weight (5.6%), anemia (1.6%), and sickle cell disease (0.7%) in neonates was quite low. The proportion of infants with low birth weight (65.2%) and delivered in the rainy season (42.7%) from March to May (61.6%) was higher in malaria-positive women compared with their negative counterparts. However, there was no association between maternal malaria status and infant Hb genotype.

Incidence of Malaria Infections Among Study Infants During Their First Year of Life

One hundred twenty-six (44.5%) infants who completed the survey experienced at least 1 episode of malaria. Malaria incidence in infants was recorded from the 1st to the 12th month, although they were most vulnerable between 4 and 11 months (Figure 1). The mean number of malaria attacks per infant per year was 0.66 ± 0.91. The mean ages and relative incidence of

malaria in the infants are shown in Table 2. Although these sick infants tended to be more from mothers without PM (46.5%) compared with their PM-positive counterparts (41.9%), there was no significant ($P = .265$) association between PM at delivery and infant susceptibility to malaria during the first year of life. Infants born to PM-positive women were older ($P = .028$) than their PM-negative counterparts when they experienced their first malaria episode (Table 3). The age of first malaria episode also varied ($P = .006$) with month of birth.

All 5 infants with malaria parasitemia at 3 months were born to mothers with anemia at delivery ($P = .023$). Nevertheless, no association was observed between maternal anemia and infant malaria parasitemia during the second, third, and fourth quarter of the year. Malaria prevalence in infants during the 1-year period was higher ($P = .030$) in those born during the rainy season compared with the dry season (Table 3). Note that infants born to mothers who had taken 1 or no IPTp-SP dose (adjusted OR = 0.51, 95% CI = 0.28–0.91; $P = .024$) in September–November (adjusted OR = 0.31, 95% CI = 0.13–0.72; $P = .007$) were protected from malaria during the first year of life after multivariate analysis. In contrast, infants born in the rainy

Table 2. Malaria Parasitemia Prevalence and Mean (±Standard Deviation [SD]) Age (Months) of the Study Infants According to Frequency of Fever Attacks During the First Year of Life

Frequency of Morbidity	No. of Infants	Malaria Parasitemia Prevalence (%)	Age (Months) Mean ± SD	Range
1	162	59.3	6.47 ± 2.84	0–12
2	75	57.3	7.80 ± 2.22	1–12
3	34	64.7	9.70 ± 1.86	5–12
4	8	50.0	11.20 ± 0.86	10–12

Table 3. Effect of Perinatal and Prenatal Risk Factors on Infant Susceptibility to Fever and Malaria in the First Year of Life[a]

Parameter	Subclass	AFFA (Months) Mean ± SD (n)	P Value	Prevalence of Malaria Episodes % (n)	Unadjusted P Value	OR	95% CI	Adjusted P Value
Maternal characteristics								
Age group (years)	≤20	5.4 ± 2.8 (57)	.213	38.7 (29)	.223	0.55	0.21–1.44	.224
	21–25	6.3 ± 3.0 (71)		42.1 (45)		0.53	0.26–1.10	.087
	>25	5.9 ± 2.8 (86)		51.0 (51)		REF		
Gravidity	Primigravidae	5.8 ± 2.9 (63)	.638	40.9 (36)	.717	1.47	0.60–3.60	.402
	Secundigravidae	5.7 ± 2.5 (66)		45.8 (33)		1.64	0.76–3.52	.205
	Multigravidae	6.1 ± 3.1 (84)		46.3 (56)		REF		
Malaria parasite status	Positive	6.4 ± 2.8 (83)	**.028**	41.9 (49)	.453	0.72	0.40–1.28	.262
	Negative	5.5 ± 2.8 (131)		46.4 (77)		REF		
Anemia status	Anemic	5.7 ± 2.9 (99)	.533	44.4 (63)	.915	0.81	0.46–1.42	.462
	Nonanemic	6.0 ± 2.8 (113)		45.0 (63)		REF		
Reported fever history	Yes	5.8 ± 2.9 (115)	.901	49.3 (67)	.089	1.61	0.92–2.83	.095
	No	5.9 ± 2.8 (96)		39.2 (56)		REF		
IPTp-SP dosage	One or less	5.7 ± 3.1 (75)	.440	35.1 (34)	**.021**	0.51	0.28–0.91	**.024**
	Two or more	6.0 ± 2.8 (139)		49.5 (92)		REF		
Infant characteristics								
Birth weight	Low	4.9 ± 3.0 (10)	.279	25.0 (2)	.473	0.49	0.08–2.98	.439
	Normal	5.9 ± 2.9 (203)		44.7 (122)		REF		
Anemia status	Anemic	4.2 ± 0.9 (2)	.392	0.0 (0)	.131	–	–	–
	Nonanemic	5.9 ± 2.9 (188)		45.2 (113)		REF		
Gender	Female	5.9 ± 2.9 (121)	.660	47.6 (70)	.276	1.30	0.75–2.26	.349
	Male	5.8 ± 2.8 (93)		41.2 (56)		REF		
Hb genotype	AS	6.2 ± 3.2 (32)	.617	41.5 (17)	.437	0.77	0.36–1.23	.490
	AA	6.0 ± 2.9 (148)		44.2 (99)		REF		
Season of birth	Rainy	6.1 ± 2.9 (169)	.069	48.2 (105)	**.030**	2.82	1.21–6.55	**.016**
	Dry	5.1 ± 2.8 (38)		31.4 (16)		REF		
Month of birth	Mar–May	7.3 ± 2.7[a] (38)	**.006**	46.4 (32)	**.003**	0.57	0.24–1.34	.197
	Jun–Aug	5.6 ± 3.0 (94)		56.5 (61)		0.55	0.24–1.26	.157
	Sep–Nov	5.7 ± 2.3 (53)		32.8 (19)		0.31	0.13–0.72	**.007**
	Dec–Feb	5.1 ± 3.1 (22)		26.5 (9)		REF		

Abbreviations: CI, confidence interval; AFFA, age of first fever attack; Hb, hemoglobin; IPTp, intermittent presumptive treatment for malaria during pregnancy; OR, odds ratio; REF, reference group; SD, standard deviation; SP, sulfadoxine/pyrimethamine; AS, sickle cell trait; SS, sickle cell disease.

[a] Significantly older than the corresponding values in June–August, September–November, and December–February. The significant differences in mean AFMA between age group, gravidity, and period of birth were assessed using analyses of variance and post hoc Turkey tests, whereas the Student t test was used to compare mean AFMA with malaria parasite status, anemia status, reported fever history, IPTp-SP dosage, birth weight category, gender, Hb genotype, and season of birth. Association analysis of clinical episodes and risk factors was undertaken by logistic regression. Bold text indicate significant P values.

season (adjusted OR = 2.82, 95% CI = 1.21–6.55; P = .016) were more susceptible to malaria (Table 3).

Antibody Responses

The OD and seropositivities to *P falciparum*-specific IgG, IgG1, IgG2, IgG3, IgG4, IgE, and IgM in malaria-exposed and malaria-unexposed pregnant women and their offspring is shown on Figure 2. Antibody levels were influenced by maternal parasitemia, maternal anemia status, IPTp dosage, and season and months of delivery (Table 4). Malaria-positive women at delivery had higher maternal IgG (P = .049) and higher maternal-cord IgG2 compared with their malaria-negative counterparts.

Infants born to malaria-positive women also had higher IgE (P = .035), IgM (P = .005) and IgG2 (P = .015) compared with malaria-negative women.

Anemic women had lower peripheral (P = .006) and cord (P = .047) blood IgG1 levels (Table 4). In addition, women who had taken the recommended 2 or more IPTp-SP doses (P = .061) and their corresponding 1 year olds (P = .049) had lower IgM compared with those who had taken 1 or no IPT dose.

The rainy season was associated with lower (P < .001 each) maternal-cord and the corresponding 1-year-old infant IgE levels (Table 4). Likewise, maternal (P = .001) and cord (P = .056)
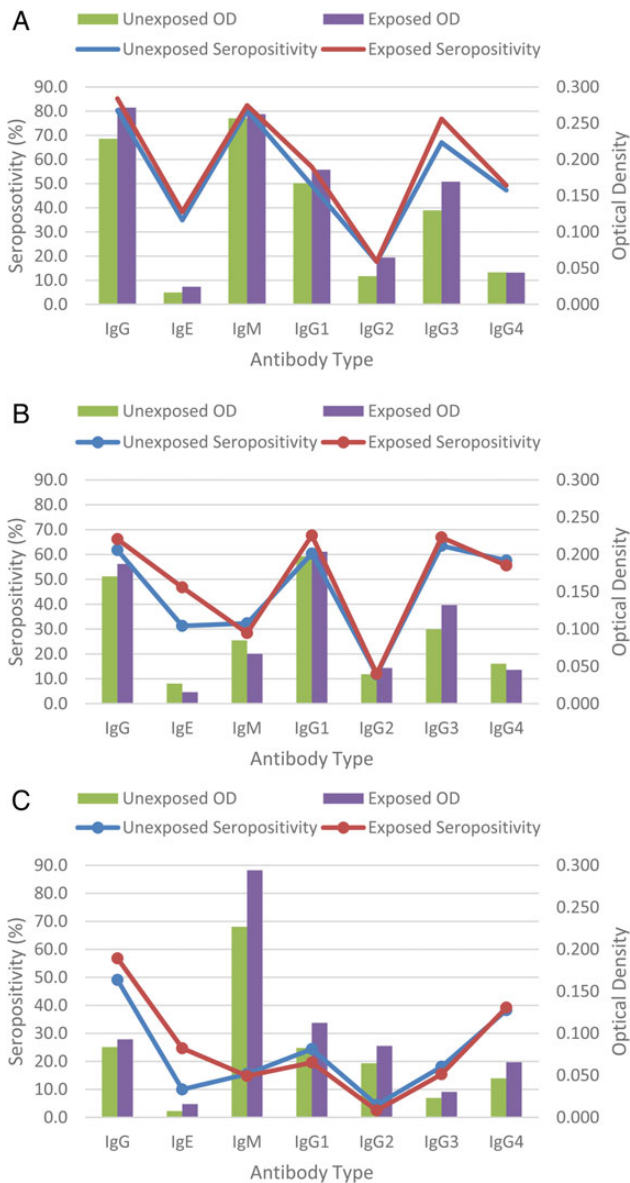
Figure 2. *Plasmodium falciparum*-specific immunoglobulin (Ig)G, IgG1–4, IgE, and IgM optical densities (OD) and seropositivity in malaria-exposed and unexposed pregnant women and their offspring from South Western Cameroon. A, maternal blood; B, cord blood; C, 1-year-old children.

blood IgG3 levels were lower in the rainy season compared with the dry season. Furthermore, maternal IgG4 ($P < .001$) as well as 1-year-old infant IgG ($P = .008$), IgG2 ($P < .001$), and IgG3 ($P < .001$) levels were lower in the rainy season months of September–November compared with June–August. However, cord IgG ($P = .028$) and IgG4 ($P = .021$) as well as 1-year-old infant IgG ($P = .004$), IgG2 ($P < .001$), IgG3 ($P = .011$), and IgG4 ($P = .034$) levels were higher in the rainy compared with the dry season. In addition, 1-year-old infants had higher IgE levels ($P = .009$) in the rainy season months of September–November compared with June–August.

It is interesting to note that infants who did not experience any malaria episode in the first year of life were born to mothers with lower *P falciparum* IgG ($P = .022$), IgM ($P = .027$), and IgG2 ($P = .005$) levels (Table 5). Nevertheless, no maternal antibody was predictive of infant susceptibility to malaria in the first year of life after adjusting for maternal age, gravidity, IPTp-SP dosage, malaria parasitemia, anemia, and season of delivery.

DISCUSSION

Although in utero exposure to *P falciparum* has been demonstrated in South Western Cameroon [17, 21], altered susceptibility of infants to *P falciparum* infection has not been reported. This study assessed the effect of perinatal and prenatal risk factors on malaria in 283 infants from birth to 12 months of age. The maternal malaria parasitemia recorded at parturition in this study was still as high as previously reported in the area [17] despite the implementation of malaria-control strategies (free insecticide-treated bednets, IPTp-SP, and constant health education). This high prevalence parallels the intense perennial transmission of *Plasmodium* spp [20] and suggests that intense monitoring and evaluation is needed to ensure effective utilization of the control tools in place.

As previously reported elsewhere in the country [4], there was no significant relationship between either PM or gravidity and malaria prevalence in infancy in this study. Nevertheless, infants born to PM-negative mothers tended to be more sick and experienced their first malaria episode at a significantly younger age compared with their PM-positive counterparts. This finding is in contrast to the documented increased susceptibility to malaria [7–10] and nonmalarial fever episodes [11] in infants born to mothers with *P falciparum* infection detected at delivery. Although it is possible that the malaria parasitemia prevalence in the infants was underestimated because molecular diagnosis was not used [27], these findings suggest that exposure to *P falciparum* in utero may alter fetal and neonatal immune development. Parasite antigens may prime the immune system [12, 13], resulting in improved resistance to malaria in infancy.

The recent implementation of IPTp in the study area has undoubtedly improved antimalarial protection, clearing most placentas of parasites [28, 29]. However, the fact that infants born to mothers on the recommended 2 or more doses of IPTp were more vulnerable to clinical episodes is worrisome. Nevertheless, the lower IgM levels in these mothers and their 1-year-old infants suggest that the reduced protection of these infants to malaria may be linked to the reduced maternal exposure to the parasite. It is possible that IPTp might affect infant immune response (reducing their antibodies) by hindering transplacental passage of maternal antibodies [19] or fetal in utero exposure to malaria antigens [30, 31].

Table 4. Effect of Perinatal and Prenatal Risk Factors on Paired Maternal-Cord and 1-Year-Old *Plasmodium falciparum*-Specific IgE, IgM, IgG, IgG1, IgG2, IgG3, and IgG4 Levels[a]

Parameter	Maternal [Mean ± SD]							Cord [Mean ± SD]							One Year [Mean ± SD]						
	IgG	IgE	IgM	IgG1	IgG2	IgG3	IgG4	IgG	IgE	IgM	IgG1	IgG2	IgG3	IgG4	IgG	IgE	IgM	IgG1	IgG2	IgG3	IgG4
Maternal malaria parasitemia																					
Positive (n = 142)	0.271 ± 0.21	0.024 ± 0.08	0.262 ± 0.20	0.186 ± 0.16	0.065 ± 0.09	0.169 ± 0.20	0.044 ± 0.04	0.187 ± 0.16	0.015 ± 0.04	0.067 ± 0.14	0.204 ± 0.16	0.048 ± 0.06	0.132 ± 0.15	0.045 ± 0.03	0.093 ± 0.06	0.016 ± 0.03	0.294 ± 0.18	0.112 ± 0.14	0.085 ± 0.91	0.030 ± 0.07	0.066 ± 0.09
Negative (n = 203)	0.228 ± 0.18	0.016 ± 0.04	0.257 ± 0.27	0.167 ± 0.15	0.039 ± 0.06	0.130 ± 0.14	0.044 ± 0.04	0.171 ± 0.16	0.027 ± 0.08	0.085 ± 0.20	0.198 ± 0.19	0.039 ± 0.08	0.100 ± 0.11	0.054 ± 0.06	0.084 ± 0.06	0.008 ± 0.01	0.227 ± 0.15	0.083 ± 0.09	0.064 ± 0.10	0.023 ± 0.04	0.047 ± 0.06
P Value	.049	NS	NS	NS	**.001**	NS	NS	NS	NS	NS	NS	**.026**	NS	NS	NS	**.035**	**.005**	NS	**.013**	NS	NS
Maternal anemia status																					
Anemic (n = 112)	0.247 ± 0.20	0.020 ± 0.07	0.249 ± 0.19	0.150 ± 0.14	0.048 ± 0.07	0.151 ± 0.17	0.044 ± 0.04	0.176 ± 0.15	0.021 ± 0.06	0.069 ± 0.15	0.181 ± 0.17	0.046 ± 0.08	0.113 ± 0.13	0.051 ± 0.05	0.090 ± 0.07	0.010 ± 0.02	0.266 ± 0.18	0.096 ± 0.12	0.071 ± 0.09	0.029 ± 0.07	0.057 ± 0.09
Nonanemic (n = 96)	0.246 ± 0.19	0.019 ± 0.05	0.269 ± 0.29	0.201 ± 0.17	0.051 ± 0.08	0.141 ± 0.17	0.045 ± 0.04	0.179 ± 0.17	0.023 ± 0.07	0.087 ± 0.21	0.222 ± 0.18	0.039 ± 0.07	0.114 ± 0.14	0.050 ± 0.05	0.085 ± 0.06	0.012 ± 0.03	0.244 ± 0.14	0.093 ± 0.11	0.075 ± 0.11	0.023 ± 0.04	0.051 ± 0.06
P value	NS	NS	NS	**.006**	NS	NS	NS	NS	NS	NS	**.047**	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
IPTp dosage																					
≤1 (n = 115)	0.248 ± 0.22	0.018 ± 0.04	0.293 ± 0.32	0.190 ± 0.07	0.060 ± 0.10	0.151 ± 0.20	0.047 ± 0.04	0.167 ± 0.17	0.018 ± 0.05	0.076 ± 0.15	0.202 ± 0.17	0.044 ± 0.06	0.113 ± 0.14	0.044 ± 0.03	0.089 ± 0.06	0.012 ± 0.03	0.289 ± 0.19	0.105 ± 0.13	0.074 ± 0.10	0.020 ± 0.03	0.051 ± 0.07
≥2 (n = 158)	0.247 ± 0.18	0.021 ± 0.07	0.236 ± 0.18	0.162 ± 0.14	0.043 ± 0.06	0.140 ± 0.15	0.042 ± 0.04	0.186 ± 0.05	0.024 ± 0.07	0.080 ± 0.20	0.194 ± 0.18	0.043 ± 0.08	0.115 ± 0.13	0.054 ± 0.06	0.087 ± 0.06	0.011 ± 0.02	0.238 ± 0.15	0.090 ± 0.11	0.073 ± 0.10	0.030 ± 0.07	0.057 ± 0.08
P value	NS	NS	.061	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	**.049**	NS	NS	NS	NS
Season of birth																					
Dry (n = 75)	0.232 ± 0.19	0.039 ± 0.10	0.282 ± 0.19	0.183 ± 0.16	0.041 ± 0.06	0.188 ± 0.19	0.040 ± 0.04	0.170 ± 0.16	0.054 ± 0.12	0.074 ± 0.13	0.237 ± 0.20	0.053 ± 0.11	0.148 ± 0.15	0.045 ± 0.05	0.068 ± 0.07	0.008 ± 0.01	0.208 ± 0.10	0.096 ± 0.13	0.025 ± 0.02	0.025 ± 0.08	0.045 ± 0.08
Rainy (n = 250)	0.253 ± 0.20	0.012 ± 0.03	0.255 ± 0.26	0.166 ± 0.15	0.053 ± 0.08	0.139 ± 0.16	0.046 ± 0.04	0.189 ± 0.16	0.010 ± 0.02	0.064 ± 0.13	0.183 ± 0.17	0.041 ± 0.06	0.108 ± 0.13	0.053 ± 0.05	0.095 ± 0.06	0.012 ± 0.03	0.267 ± 0.18	0.085 ± 0.10	0.085 ± 0.11	0.026 ± 0.05	0.057 ± 0.07
P value	NS	**.001**	NS	NS	NS	**.001**	NS	**.028**	**<.001**	NS	NS	NS	.056	**.021**	**.004**	**<.001**	NS	NS	**<.001**	**.011**	**.034**
Month of birth																					
Mar–May (n = 75)	0.250 ± 0.21	0.018 ± 0.04	0.286 ± 0.16	0.150 ± 0.12	0.054 ± 0.07	0.133 ± 0.14	0.047 ± 0.04	0.186 ± 0.15	0.014 ± 0.01	0.037 ± 0.08	0.190 ± 0.15	0.045 ± 0.05[Ω]	0.106 ± 0.10	0.056 ± 0.06	0.130 ± 0.06[b]	0.032 ± 0.03[c]	0.405 ± 0.19[e]	0.114 ± 0.15	0.161 ± 0.13[f]	0.029 ± 0.04[h]	0.103 ± 0.09[i]
Jun–Aug (n = 116)	0.260 ± 0.19	0.004 ± 0.02	0.247 ± 0.25[¥]	0.199 ± 0.17	0.059 ± 0.09	0.159 ± 0.19	0.054 ± 0.05	0.197 ± 0.15	0.007 ± 0.02[π]	0.106 ± 0.27	0.197 ± 0.16	0.036 ± 0.05	0.116 ± 0.16	0.058 ± 0.06	0.086 ± 0.06[a]	0.0001 ± 0.01	0.195 ± 0.12	0.075 ± 0.07	0.050 ± 0.07[g]	0.032 ± 0.06[i]	0.035 ± 0.05
Sep–Nov (n = 82)	0.228 ± 0.21	0.017 ± 0.03	0.252 ± 0.31	0.155 ± 0.14	0.038 ± 0.06[$]	0.127 ± 0.13	0.029 ± 0.03[&]	0.174 ± 0.18	0.030 ± 0.09	0.027 ± 0.04	0.207 ± 0.20	0.053 ± 0.12	0.102 ± 0.11	0.043 ± 0.04	0.060 ± 0.05	0.004 ± 0.01[d]	0.191 ± 0.10	0.060 ± 0.05	0.031 ± 0.03	0.015 ± 0.03	0.035 ± 0.05
Dec–Feb (n = 52)	0.248 ± 0.20	0.047 ± 0.12	0.309 ± 0.21	0.151 ± 0.13	0.046 ± 0.06	0.196 ± 0.21	0.043 ± 0.03	0.173 ± 0.17[≠]	0.047 ± 0.10	0.148 ± 0.15	0.191 ± 0.18	0.044 ± 0.05	0.161 ± 0.16	0.043 ± 0.02	0.062 ± 0.07	0.008 ± 0.01	0.216 ± 0.10	0.119 ± 0.15	0.025 ± 0.02	0.026 ± 0.09	0.052 ± 0.10
P value	NS	NS	**.020**	**.046**	**.019**	NS	**<.001**	**.025**	**.020**	NS	NS	**.001**	NS	NS	**<.001**	**<.001**	**<.001**	NS	**<.001**	**.001**	**<.001**

Significantly higher than the corresponding values for Dec–Feb (*P* = 0.029) and Sep–Nov (*P* = 0.008).

Abbreviations: ANOVA, analysis of variance; Ig, immunoglobulin; IPTp, intermittent presumptive treatment for malaria during pregnancy; NS, not significant; OD, optical density; SD, standard deviation; SP, sulfadoxine/pyrimethamine.

[a] Boldface indicates significant results. The significance of differences in mean OD with maternal malaria parasite status, anemia status, IPTp-SP dosage groups, and season were explored using the Student *t* test, whereas mean OD between months of delivery were compared using ANOVA. Bold text indicate significant *P* values.

[b] Significantly higher than the corresponding values for Dec–Feb (*P* < .001) and Sep–Nov (*P* < .001).

[c] Significantly higher than the corresponding values for Dec–Feb (*P* < .001), Sep–Nov (*P* < .001), and Jun–Aug (*P* = .001).

[d] Significantly higher than the corresponding values for Jun–Aug (*P* = .009).

[e] Significantly higher than the corresponding values for Dec–Feb (*P* < .001), Sep–Nov (*P* < .001), and Jun–Aug (*P* < .001).

[f] Significantly higher than the corresponding values for Dec–Feb (*P* < .001), Sep–Nov (*P* < .001), and Jun–Aug (*P* < .001).

[g] Significantly higher than the corresponding values for Sep–Nov (*P* < .001).

[h] Significantly higher than the corresponding values for Dec–Feb (*P* = .003) and Sep–Nov (*P* = .001).

[i] Significantly higher than the corresponding values for Dec–Feb (*P* < .001) and Sep–Nov (*P* < .001).

[j] Significantly higher than the corresponding values for Dec–Feb (*P* = .001), Sep–Nov (*P* < .001), and Jun–Aug (*P* = .002).

[¥] Significantly lower (*P* = .029) than the corresponding values for Mar–May.

[$] Significantly lower (*P* = .011) than the corresponding values for Mar–May.

[&] Significantly lower than the corresponding values for Dec–Feb (*P* = .001), Mar–May (*P* < .001), and Jun–Aug (*P* < .001).

[≠] Significantly lower than the corresponding values for Mar–May (*P* = .040) and Jun–Aug (*P* = .040).

[π] Significantly lower than the corresponding values for Dec–Feb (*P* = .013).

[Ω] Significantly higher than the corresponding values for Sep–Nov (*P* = .006) and Jun–Aug (*P* = .003).

Table 5. Effect of Maternal Antibodies on Infant Susceptibility to Malaria in the First Year of Life[a]

Antibody Type	Optical Density [Mean ± SD]				Unadjusted P Value	Multivariate Analysis		
	n	Nonsusceptible	n	Susceptible		OR	95% CI	Adjusted P Value
IgG	139	0.225 ± 0.193	103	0.286 ± 0.210	**.022**	107.08	0.23–498.65	.136
IgE	84	0.017 ± 0.050	66	0.026 ± 0.090	.212	2.712	0.15–47.75	.495
IgM	116	0.218 ± 0.173	76	0.299 ± 0.255	**.027**	153.20	0.27–866.48	.120
IgG1	91	0.172 ± 0.167	53	0.180 ± 0.145	.335	0.075	0.00–17.16	.350
IgG2	139	0.039 ± 0.055	103	0.060 ± 0.076	**.005**	0.407	0.012–9.82	.580
IgG3	139	0.115 ± 0.156	103	0.171 ± 0.181	.081	0.10	0.01–1.97	.130
IgG4	139	0.046 ± 0.043	103	0.049 ± 0.047	.719	7.892	0.16–397.13	.301

Abbreviations: CI, confidence interval; Ig, immunoglobulin; OR, odds ratio; SD, standard deviation.

[a] The significance of differences in mean antibody levels between susceptible and nonsusceptible infants was assessed using the Student t test. Multivariate analysis was undertaken using logistic regression, with the following covariates: maternal age, gravidity, IPTp-SP dosage, maternal malaria parasite status, maternal anemia status, and season of delivery. Bold text indicate significant P values.

Consistent with previous reports [6, 32], infants under 5 months were protected from infection in this study. This protection is related to the nonexposure of the neonate to the malaria vector (free insecticide-treated nets usage and cultural factors such as swaddling), innate mechanisms that limit parasite growth (fetal Hb and para-aminobenzoic acid-deficient breast milk) [33], transplacental transfer of the mother's protective antibody [34, 35], and neonatal immune priming by transplacental transfer of parasites or their products [13].

Maternal antibodies persist in the child's blood for a few months, but this does not seem to be related to the increased resistance to malaria of infants born to placenta-infected mothers [35]. Indeed, the umbilical cord blood levels of anti-*Pf*IgG, IgM, IgE, IgG1, IgG3, and IgG4 antibodies were similar in both groups of infants. Furthermore, the susceptibility of infants to malaria within the first year was independent of maternal antibody levels because OD were similar in both susceptible and nonsusceptible infants as reported previously [32]. This lack of association between antibodies malaria in infants suggests that other immune mechanisms might be involved in protection of infants against malaria.

The seasonal variation of malaria prevalence is well established with highest prevalence during the rainy season in the South Western region [22, 32]. Consequently, maternal and infant malaria morbidity was higher in the rainy season compared with the dry season as reported previously [8]. Because the area is flat (and warm), there are more standing water and breeding sites for, and faster breeding by, *A gambiae sensu stricto* and thus larger vector populations during this season. In June–August, when repeated infective bites are highest [20], the highest levels of infant morbidity, peripheral maternal parasitemia prevalence, placental infection, and the lowest age of first malaria episode were recorded. This accrues to the increased chance of both mother and child succumbing to clinical manifestation of the disease during this period.

In general, maternal seropositivity to all antimalarial antibodies except IgE and IgG2 was quite high. The raised antibody levels in pregnant women reflect maternal infection and, thus, an increased risk of fetal exposure to *P falciparum* antigens [4, 8]. In this study, the presence of IgM in cord (an indicator of in utero exposure) was not associated with an increased risk of infant malaria. The observed positive association between maternal antibody levels and the risk of infant infection might reflect similar risk of exposure to the parasites in mothers and infants living in the same household [36].

The seasonal variation in malaria prevalence has consequences for both the maternal-cord and infant humoral immunity, suggesting that IgM, IgE, IgG, IgG1, IgG2, IgG3, and IgG4 may be involved in the control of malaria infection. The low IgG3 in the rainy season is consistent with several studies [37–40] implicating cytophilic antibodies in protection against malaria. These antibodies mediate the opsonization of infected erythrocytes [41]. Immunoglobulin G2 has also been shown to mediate protection against malaria infection, possibly as a cytophilic antibody [42], and, as such, its low seropositivity in the rainy season reflects diminished protection against infection. Immunoglobulin E is thought to play a role in protection against malaria [43, 44], and thus the higher levels in the dry season may contribute to its lower malaria prevalence. In fact, previous studies in the region [22] have shown that children at low altitude, where malaria transmission is most intense, were more likely to be seropositive for anti-*P falciparum* IgE than those at high altitude and that those with high-grade parasitemia had lower levels of such parasite-specific IgE than the other parasitemics, indicating a protective role for such antibodies. Alternatively, the raised cord IgG4 levels in the rainy season may be contributing to malaria susceptibility as previously reported [42, 45]. Immunoglobulin G4 inhibits the protection provided by IgG2 [45] and the action of cytophilic antibodies in vitro [41]. Immunoglobulin G4 may thus neutralize the cellular

cytotoxicity mediated by monocytes or other effector cells that depend on IgG2.

In the present study, antibody seropositivity was common at 1 year in all seasons and months of delivery. This result suggests a gradual acquisition of immunity with exposure.

CONCLUSIONS

Intermittent preventive treatment during pregnancy and month of birth have important implications for infant susceptibility to malaria. The lower IgM levels in mothers who had taken the recommended 2 or more IPTp-SP doses and their 1-year-old infants suggests that the reduced protection of these infants to malaria may be linked to the reduced maternal exposure to the parasite.

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