

High Iron Levels Are Associated with Increased Malaria Risk in Infants during the First Year of Life in Benin

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Abstract. The World Health Organization (WHO) estimates that 40% of children in low-income countries are anemic. Therefore, iron supplements are recommended by WHO in areas with high anemia rates. However, some studies have set into question the benefits of iron supplementation in malaria-endemic regions. In Benin, a west African country with high prevalence of anemia and malaria, no iron supplements are given systematically to infants so far despite the WHO recommendations. In this context, we wanted to investigate the effect of iron levels during the first year of life on malarial risk in Benin considering complementary risk factors. We followed 400 women and their offspring between January 2010 and June 2012 in Allada (Benin). Environmental, obstetric, and numerous clinical, maternal, and infant risk factors were considered. In multilevel models, high iron levels were significantly associated with the risk of a positive blood smear (adjusted odds ratio = 2.90, $P < 0.001$) and *Plasmodium falciparum* parasitemia (beta estimate = 0.38, $P < 0.001$). Infants with iron levels in the lowest quartile were less likely to have a positive blood smear ($P < 0.001$), and the risk increased with higher iron levels. Our results appeal for additional evaluation of the effect of different doses of iron supplements on the infant health status, including malaria incidence. Thus, the health status of infants should be compared between cohorts where iron is given either for prevention or anemia treatment, to better understand the effect of iron supplements on infant health.

INTRODUCTION

Infant health morbidity in sub-Saharan Africa is mainly driven by infectious diseases and nutritional deficiencies.¹ Indeed, malaria and anemia (mainly due to iron deficiency [ID]) contribute to enhance significantly the disease burden among African infants.² The World Health Organization (WHO) estimates indicate there were over 214 million new malaria cases and 438,000 deaths in 2015.³ In addition, malaria causes anemia, which entails severe long-term consequences for the development of the children.⁴ Anemia is the second leading cause of disability worldwide,⁵ and both malaria and anemia harm mainly children under 5 years of age. For these reasons, public health strategies have been developed to tackle both diseases simultaneously.

WHO recommends the administration of 12.5 mg iron and 50 µg folic acid daily between 6 and 12 months of age to tackle anemia.⁶ However, in Benin, this policy has not been implemented so far. In general, Beninese pediatricians give a preventive treatment consisting of 10 mg/kg iron per day starting at 6 months of age until 5 years of age. These supplements are given during 2 months followed by a 4-month period without treatment. With regard to malaria in children, WHO recommends at present the use of insecticide-treated nets and/or indoor residual spraying for vector control, and prompt access to diagnostic testing of suspected malaria and treatment of confirmed cases.

As both diseases overlap geographically and they harm mainly children under 5 years of age, it is essential to analyze the association between iron levels and malaria risk in infants. Old clinical trials reported increased susceptibility among iron-supplemented children,^{7–9} and ID has been associated with reduced malaria odds among pregnant women and infants.^{10,11} However, iron supplements are not significantly associated with increased malaria risk in recent clinical trials or in the 2016 Cochrane review.¹² Furthermore, one of the most recent Cochrane reviews outlines the scarcity of prospective cohorts analyzing the iron–malaria association during infancy.¹¹

For these reasons, we investigated the effect of the infants' iron levels during the first year of life on malarial risk in infants taking into account complementary information on pregnancy-associated malaria (PAM), environmental, socio-economic, and clinical indicators and comorbidities to better understand malaria risk factors in the context of the present malaria control strategies.

MATERIALS AND METHODS

A prospective cohort of 400 infants was followed from birth to 12 months of age in the context of the Anemia in Pregnancy: Etiology and Consequences (APEC) study. The APEC study is an ancillary survey nested within the Malaria in Pregnancy Preventive Alternative Drugs (MiPPAD) trial in Benin (<http://clinicaltrials.gov/ct2/show/NCT00811421>). This study was conducted in three clinics in the district of Allada, between January 2010 and May 2012. Allada is a semirural area of 91,778 inhabitants located 50 km north of Cotonou (Benin). *Plasmodium falciparum* is the species responsible for the majority of infections.

Complete details of MiPPAD are presented elsewhere,¹³ but briefly, MiPPAD was a randomized trial comparing the

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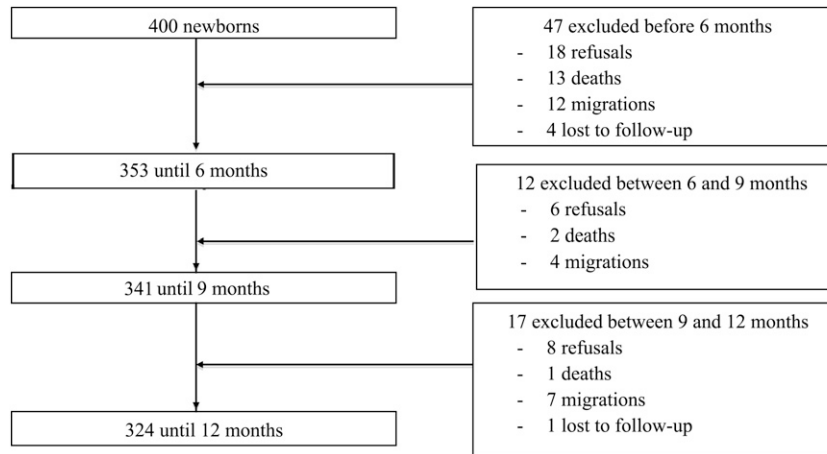


FIGURE 1. Study profile.

efficacy and safety of intermittent preventive treatment in pregnancy (IPTp) with sulfadoxine–pyrimethamine (SP; 1,500/75 mg per dose) and mefloquine (MQ; 15 mg/kg per dose). At delivery, placenta was examined to analyze *P. falciparum* parasite infestation. Clinical data of the infants were collected during systematic visits at 6, 9, and 12 months of age. In case of sickness, infants were evaluated and, if necessary, treated according to Beninese guidelines (artemether–lumefantrine for uncomplicated malaria; parenteral artesunate or quinine for complicated malaria).

In the unscheduled visits, clinical and biological examinations were performed following the same protocol as systematic visits. All drugs prescribed to the infants during the follow-up were free of charge.

According to the protocol of the APEC substudy, anthropometric measures and an extensive clinical examination were performed during the visits. In addition, 8 mL of venous blood were collected at each visit. Hemoglobin, serum ferritin, C-reactive protein (CRP), vitamin B12, and folate levels were assessed. A container was also given to the women to collect the infant's stools in search of intestinal helminths. The containers were collected the following day by the study nurses within the first 6 hours after emission. Microbiological examinations were performed as follows: Lambaréné technique was used to assess malaria infection.¹⁴ It consists of spreading a calibrated 10 μ L amount of blood on a slide's rectangular area of 1.8 cm² (1.8 \times 1 cm). The slide was stained with Giemsa and read at a magnification of 1,000 \times with an oil immersion lens. To assess parasite density (in parasites/ μ L), a multiplication factor was applied to the average parasitemia/field. A malaria-infected placenta was defined as a placenta with a positive smear at birth. Helminthic infestations were assessed using the Kato-Katz concentration method (VestergaardFrandsen kit[®], New Delhi, India). In case of inflammation (CRP > 5 mg/L), serum ferritin was adjusted following the corrections recommended by Thurnham and others in their meta-analysis,¹⁵ to avoid the extrinsic effect of inflammation on serum ferritin levels. More precisely, we multiplied serum ferritin by 0.76 in the presence of *Plasmodium* without inflammation, and we multiplied serum ferritin by 0.53 in case of concurrent *Plasmodium* infection and inflammation.

We used rain quantity as a surrogate for the risk of exposure to anopheline bites. In the semirural area of Allada,

malaria has a perennial transmission pattern with two transmission peaks corresponding to the rainy seasons in April–July and October–November. According to literature, rainfall can be a valid surrogate for anopheline risk.^{16–18} Because of the anopheline timeliness, rainfall quantity was calculated as the mean rain volume of the 7 days prior to the 2 weeks before the consultation.¹⁹ Even if the clinics were close to each other, rainfall quantity was independently assessed for each visit and each clinic.

Socioeconomic status was assessed using a socioeconomic index created in a two-step process. First, socioeconomic items (home possession of latrines, electricity, a refrigerator, a television, a vehicle with at least two wheels, the mother being married, and the mother working outside the home) were plotted into a multiple correspondence analysis.^{20,21} Then, two predictors were created to synthesize the information, and as the first captured the large majority of the information, it was withheld as the socioeconomic index. We used this approach because it allows us to create a synthetic objective index of socioeconomic items without any a priori on the weight of each of the elements of the index.

Data were double entered and analyzed with ACCESS 2003, and STATA 12.0 Software (Stata Corp, College Station, TX). Then, exploratory and univariate analyses were performed to assess the association of all variables with both infant positive smear and peripheral *P. falciparum* density at each visit (systematic or unscheduled visit). χ^2 and

TABLE 1
Clinical and biologic results of the infants at birth

	Mean or proportion (95% CI)
Sex of the infants	Male: 183 (46.9%) Female: 207 (53.1%)
Gestational age at birth (weeks) (Ballard score)	38.1 (37.8; 38.4)
Weight (g)	3,033.5 (2,992; 3,075)
Low birthweight (%) (birthweight < 2,500 g)	9 (6.2; 11.9)
<i>Plasmodium falciparum</i> -infected placenta (%)	10.9 (7.8; 13.9)
Hemoglobin (g/L)	139 (136.9; 141)
Serum ferritin (μ g/L)	182.6 (165.5; 199.7)
Folate (ng/L)	16.5 (12.6; 20.4)

CI = confidence interval.

TABLE 2
Clinical and biologic results of the infants during the follow-up period (6, 9, and 12 months)

Characteristics	6 months (N = 327)	9 months (N = 325)	12 months (N = 324)
	Mean or proportion (95% CI)	Mean or proportion (95% CI)	Mean or proportion (95% CI)
<i>Plasmodium falciparum</i> infection (%)	12.06 (8.45; 15.68)	12.00 (8.28; 15.52)	12.34 (8.70; 15.99)
Parasite density (nb/mm ³)	6,960.862 (1,869.05; 12,052.19)	18,392.52 (4,791.55; 31,993.49)	9,794.40 (2,764.46; 16,824.35)
Hemoglobin (g/L)	102.22 (100.55; 103.88)	102.91 (101.32; 104.50)	103.80 (102.14; 105.47)
Anemia (%) (Hb < 110 g/L)	66.99 (61.74; 72.23)	69.81 (64.65; 74.96)	64.86 (59.54; 70.17)
Mild anemia (%) (Hb = 100–109 g/L)	31.41 (26.23; 36.59)	34.09 (28.77; 39.41)	36.42 (31.06; 41.78)
Moderate anemia (%) (Hb = 80–109 g/L)	28.53 (23.48; 33.56)	30.52 (25.34; 35.69)	21.73 (17.13; 26.32)
Severe anemia (%) (Hb < 80 g/L)	7.05 (4.19; 9.90)	5.19 (2.70; 7.69)	6.70 (3.92; 9.50)
Corrected serum ferritin (µg/L)	604.58 (507.64; 701.52)	455.37 (384.27; 526.48)	436.16 (350.42; 521.90)
Iron deficiency (%) (corrected SF < 15 µg/L)	16.09 (8.21; 23.97)	29.63 (20.88; 38.38)	34.28 (25.06; 43.52)

CI = confidence interval; Hb = hemoglobin; SF = serum ferritin.

Kruskal–Wallis tests were used in the univariate analyses. For time-dependent variables, univariate analyses were performed using a random intercept model at the infant level. Then, all variables with *P* values < 0.2 were included in either a logistic or a linear multivariate multilevel model with a random intercept and slope at the infant level including all visits (systematic and unscheduled visits) for each infant, to explore the determinants of the probability of having a positive smear or peripheral *P. falciparum* parasitemia, respectively. More precisely, a random slope was applied to the infant age, as the effect of the variables might differ between the infants. The statistical significance in the final multivariate models was set to *P* < 0.05 (two-sided tests). To evaluate the possible diverse effect of different iron levels on malaria risk, we ran the same multilevel model considering the different quartiles of corrected ferritin.

The study was conducted in the context of a clinical trial. According to the International Committee of Medical Journal Editors guidelines, our clinical trial was registered as follows: EDCTP-IP.07.31080.002, MiPPAD study “Malaria in Pregnancy Preventive Alternative Drugs,” (<http://clinicaltrials.gov/ct2/show/NCT00811421>). This study was approved by the Ethics Committee of the Faculty of Medicine of Cotonou. It was explained in the local language to the mothers and their voluntary consent was obtained before enrollment.

RESULTS

Between January 2010 and June 2012, 400 mother–infant pairs were included in the cohort. In all, 353 infants continued to be followed up until the first systematic visit at 6 months, 341 until the second visit at 9 months, and 324

completed the 12 month follow-up (Figure 1). Even though 10.9% of the placentas were infected by *Plasmodium*, no cord blood infection by *Plasmodium* was detected at the microscopic examination. The main characteristics of the infants at birth are presented in Table 1.

During the first year of life, 159 infants (40%) had at least one malarial episode, with a range of 0–4 positive smears taking into account both systematic and unscheduled visits. More precisely, 241 infants (60.25%) had no positive blood smear during the entire follow-up, 88 (22%) had one, 50 (12.50%) had two, 18 (4.5%) had three, and three (0.75%) had four positive blood smears during follow-up. The clinical and biological characteristics of the infants at the systematic visits are summarized in Table 2. The proportion of infants with a positive smear at the systematic visits remained constant along the follow-up (around 12% of the infants were infected at each systematic visit). However, *P. falciparum* parasitemia did change significantly during the first year of life. Among infants with at least one positive smear, the mean *P. falciparum* density was 57,699.87 parasites/mm³ (95% confidence interval [CI] = 17,585; 97,815) at 6 months, 154,596.6 parasites/mm³ (95% CI = 45,882; 263,311) at 9 months, and 81,190.45 parasites/mm³ (95% CI = 26,020; 136,361) at 12 months of age. The geometric mean values of *P. falciparum* density were 8,250.784 parasites/mm³ (95% CI = 3,723; 18,285) at 6 months, 18,595.95 parasites/mm³ (95% CI = 8,676; 39,859) at 9 months, and 11,448.56 (95% CI = 5,491; 23,869) at 12 months of age among infants with a positive smear. In parallel, the mean hemoglobin values increased slightly, though not significantly, through the follow-up (102.1, 102.9, and 103.6 g/L at the 6-, 9-, and 12-month systematic visits, respectively).

TABLE 3
Multilevel model on factors associated with having positive blood smears during the first year of life

Factor	aOR (95% CI)	<i>P</i> value
Infant factors		
Ferritin corrected on inflammation (logarithm of µg/L)	2.90 (1.68; 4.98)	< 0.001
Inflammatory process (CRP > 5 mg/L)	4.41 (2.51; 7.74)	< 0.001
Age 1–4 months (reference)		
Age 4–8 months	4.63 (0.65; 33.16)	0.13
Age 8–12 months	3.52 (0.41; 30.04)	0.25
Demographic and environmental factors		
Low socioeconomic index	1.14 (0.88; 1.47)	0.33
Rain volume (mm)	1.04 (0.99; 1.10)	0.14

aOR = adjusted odds ratio; CI = confidence interval; CRP = C-reactive protein. Random intercept at the infant level. Random slope for the age of the infants. Analysis on 906 blood smears from 344 infants.

TABLE 4
Multilevel model on factors associated with *Plasmodium falciparum* parasitemia (in logarithm) during the first year of life

Factor	Beta estimate (95% CI)	P value
Infant factors		
Ferritin corrected on inflammation (logarithm of µg/L)	0.38 (0.21; 0.55)	< 0.001
Inflammatory process	0.69 (0.50; 0.88)	< 0.001
Age of the infant (1–4 months (reference))		
Age of the infant 4–8 months	0.27 (0.01; 0.52)	0.04
Age of the infant 8–12 months	0.07 (–0.18; 0.32)	0.58
Demographic and environmental factors		
Low socioeconomic index	0.13 (–0.08; 0.11)	0.78
Rain volume (mm)	0.01 (–0.01; 0.04)	0.28

CI = confidence interval. Random intercept at the infant level. Random slope for age of the infant. Analysis on 905 blood smears of 344 infants.

Iron indicators decreased through the follow-up. The mean ferritin levels decreased after the 6-month visit from 605 µg/L (95% CI = 508; 702) to 455 µg/L (95% CI = 384; 526) at 9 months, and then decreased again to 436 µg/L (95% CI = 350; 522) at 12 months. ID increased, from 16% at 6 months, to 29% at 9 months, and up to 34% at 12 months of age.

During the first year of life, malaria rates and *P. falciparum* parasitemia were determined by clinical, environmental, and socioeconomic factors. The risk factors for malaria and *P. falciparum* parasite density are presented in Table 3 and Table 4, respectively.

There were no statistical differences in the number of positive smears or in *P. falciparum* density during the first year of life of the infant depending on the placental malaria status. The IPTp regimen of the mothers (either SP or MQ) was not associated with the number of positive smears or with the parasite density of the infants.

The infant iron levels (log of ferritin corrected on inflammation) were significantly associated with the risk of a positive blood smear (adjusted odds ratio [aOR] = 2.90, 95% CI = 1.68; 4.98, $P < 0.001$) and *P. falciparum* parasite density (beta estimate = 0.38, 95% CI = 0.21; 0.55, $P < 0.001$) during the first year of life. Infants with ID were significantly less likely to have a positive blood smear and a high *P. falciparum* density ($P = 0.01$ in both cases). Thus, the ongoing inflammatory status of the infant (CRP > 5 mg/L) was significantly associated with an increased risk of a positive blood smear (aOR = 4.41, 95% CI = 2.51; 7.74, $P < 0.001$) and to a higher *P. falciparum* parasite density (beta estimate = 0.69,

95% CI = 0.50; 0.88, $P < 0.001$). The presence of other parasites such as intestinal helminths was not significantly associated with increased risk of a positive smear or with *P. falciparum* parasitemia. Infants between 4 and 8 months of age had a significantly higher *P. falciparum* parasitemia compared with the other age periods (infants between 0 and 4 months, and infants between 8 and 12 months). The rain quantity (representing the anopheline risk) was not associated with increased risk of a positive smear or with increased *P. falciparum* parasitemia.

Finally, we further investigated the differences in malaria risk factors considering the different quartiles of iron levels in infants to evaluate the possible different effects of iron on malaria risk depending on the different iron levels. Indeed, infants with iron levels in the three upper quartiles had significantly higher risk of having malaria during the first year of life (Table 5). Infants with iron levels in the upper quartiles had significantly higher *P. falciparum* parasite density.

DISCUSSION

In this study, high iron levels were significantly associated with malaria incidence and with parasite density in infants during the first year of life in a prospective longitudinal cohort, considering environmental, socioeconomic, and PAM factors. Iron levels, measured by ferritin adjusted on inflammation, a consistent indicator of iron,^{22,23} were significantly associated with a positive blood smear and *P. falciparum* parasitemia. Furthermore, this association was

TABLE 5

Multilevel model on factors associated with malaria risk during the first year of life depending on the different iron levels (905 blood smears from 344 infants)

Factor	Multilevel model on the positive blood smear		Multilevel model on <i>Plasmodium falciparum</i> parasitemia	
	aOR (95% CI)	P value	Beta estimate (95% CI)	P value
Infant factors				
Ferritin corrected on inflammation (logarithm of µg/L)	(Reference)			
1st quartile				
Ferritin corrected on inflammation 2nd quartile	2.33 (1.04; 5.21)	0.04	0.16 (–0.08; 0.40)	0.19
Ferritin corrected on inflammation 3rd quartile	2.88 (1.30; 6.37)	< 0.01	0.26 (0.02; 0.50)	0.03
Ferritin corrected on inflammation 4th quartile	4.15 (1.89; 9.15)	< 0.001	0.45 (0.21; 0.70)	< 0.001
Inflammatory process	4.21 (2.46; 7.21)	< 0.001	0.69 (0.50; 0.88)	< 0.001
Age of the infant (1–4 months (reference))				
Age of the infant 4–8 months	3.75 (0.53; 26.68)	0.19	0.28 (0.02; 0.53)	0.04
Age of the infant 8–12 months	3.13 (0.37; 26.81)	0.30	0.08 (–0.17; 0.33)	0.52
Demographic and environmental factors				
Low socioeconomic index	1.16 (0.87; 1.55)	0.35	0.01 (–0.08; 0.11)	0.77
Rain volume (mm)	1.04 (0.99; 1.10)	0.16	0.01 (–0.01; 0.04)	0.30

aOR = adjusted odds ratio; CI = confidence interval.

TABLE 6
Univariate analyses on factors associated with having positive blood smears during the first year of life

Factor	aOR (95% CI)	P value
Infant factors		
Ferritin corrected on inflammation (logarithm of µg/L)	2.53 (1.61; 3.99)	< 0.001
Inflammatory process (CRP > 5 mg/L)	4.11 (2.52; 6.70)	< 0.001
Age 1–4 months (reference)		
Age 4–8 months	1.96 (1.13; 3.41)	0.02
Age 8–12 months	2.02 (1.02; 3.98)	0.04
Demographic and environmental factors		
Low socioeconomic index	1.09 (0.90; 1.33)	0.38
Rain volume (mm)	1.03 (0.99; 1.07)	0.08

aOR = adjusted odds ratio; CI = confidence interval; CRP = C-reactive protein. Random intercept at the infant level. Random slope for the age of the infants. Analysis on 906 blood smears from 344 infants.

significant even after adjustment on inflammatory status. ID was associated with a significant protection against malaria through the entire follow-up. More precisely, infants with iron levels in the first quartile seemed to be significantly protected against malaria.

The mothers of these infants were also followed during pregnancy. We assessed their malarial risk taking into account pregnancy parameters, comorbidities, environmental and socioeconomic indicators, and their IPTp regime.²⁴ Among these women, high iron levels (measured by the log₁₀ of ferritin corrected on inflammation) were also significantly associated with increased risk of a positive blood smear (aOR = 1.75; 95% CI [1.46; 2.11]; *P* < 0.001) and high *P. falciparum* density (beta estimate = 0.22; 95% CI [0.18; 0.27]; *P* < 0.001) during the entire pregnancy follow-up period. Furthermore, iron-deficient women were significantly less likely to have a positive blood smear and high *P. falciparum* density (*P* < 0.001 in both cases).

Indeed, ID was associated with a significant degree of protection from episodes of clinical malaria in a cohort of young children living on the Kenyan coast.²⁵ Nevertheless, results on the effect of iron levels on malaria differ in the context of clinical trials with iron supplements. In a specific Cochrane review,¹² no significant difference in clinical malaria episodes was detected between children supplemented with iron alone and those receiving a placebo (risk ratio [RR] = 0.93, 95% CI = 0.87; 1.00). However, the effect of ID was not assessed, and solid preventive measures against malaria were implemented in the clinical trials. Indeed, an increased risk of malaria with high iron levels was observed in trials that did not provide malaria surveillance and treatment, and the risk of malaria parasitemia was higher with high iron levels in a previous version of the Cochrane review (RR = 1.13, 95% CI = 1.01; 1.26).¹¹ Furthermore, in numerous studies

included in the meta-analysis, iron was seldom determined longitudinally.

Malaria physiopathology could explain the increased malarial risk associated with elevated iron levels. In effect, iron inhibits the synthesis of nitric oxide by inhibiting the expression of inducible nitric oxide synthase at the host level, and thereby interferes with macrophage-mediated cytotoxicity against *Plasmodium*.²⁶ Furthermore, non-transferrin bound iron is associated with the severity of malaria,^{27–29} With regard to the parasite itself, *Plasmodium* has the capacity of acquiring iron in a transferrin-independent pathway,³⁰ and it has a vacuolar iron-transporter homologue that acts as a detoxifier.³¹ Indeed, in in vitro parasite cultures, *P. falciparum* infects iron-deficient erythrocytes less efficiently compared with iron-replete human erythrocytes.³²

In any case, it is essential to consider that iron supplements have undeniable benefits for infants. A 2013 meta-analysis showed supplementation was associated with a reduced risk of anemia, of ID, and of iron deficiency anemia.³³ Epidemiological studies have linked ID to several adverse consequences of child development, including impairments in cognitive, emotional, and motor development.⁴ As pondering the advantages and risk of iron supplements is daunting because they are not epidemiologically quantifiable, the implementation of malaria protective strategies should be seriously encouraged.

CONCLUSIONS

Malaria risk during the first year of life is also associated with high ferritin levels in a prospective longitudinal cohort considering complementary risk factors. Our data also suggest that malaria risk increases with higher ferritin levels. Indeed, the interaction between iron and malaria is complex because of the iron requirements during infancy and the fact

TABLE 7
Univariate analyses on factors associated with *Plasmodium falciparum* parasitemia (in logarithm) during the first year of life

Factor	Beta estimate (95% CI)	P value
Infant factors		
Ferritin corrected on inflammation (logarithm of µg/L)	0.38 (0.22; 0.57)	< 0.001
Inflammatory process	0.71 (0.52; 0.90)	< 0.001
Age of the infant (1–4 months [reference])		
Age of the infant 4–8 months	0.19 (–0.02; 0.41)	0.08
Age of the infant 8–12 months	0.10 (–0.10; 0.31)	0.33
Demographic and environmental factors		
Low socioeconomic index	0.03 (–0.08; 0.13)	0.62
Rain volume (mm)	0.02 (–0.01; 0.04)	0.09

CI = confidence interval. Random intercept at the infant level. Random slope for age of the infant. Analysis on 905 blood smears of 344 infants.

that iron contributes to the parasite growth. These results appeal for additional epidemiological studies to evaluate the effect of different doses of iron supplements on the infant infectious and hematological outcomes. Complementary interventional data are needed to determine the benefits and risks of differently dosed iron supplements to ascertain their impact on infant health in malaria-endemic regions. Finally, the epidemiological comparison between cohorts in which iron is given as preventive intervention, and cohorts in which iron is given for anemia or ID treatment should also be analyzed.

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