Age-related Changes in Malaria Clinical Phenotypes During Infancy are Modified by Sickle Cell Trait

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Article Summary: In a birth cohort of 678 Ugandan infants, more than 1000 malaria episodes were observed through 12 months of age. Age-dependent changes in HbAS protective efficacy were accompanied by differential loss of anti-parasite and anti-disease protection among HbAS and HbAA infants.

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ABSTRACT

Background

Young infants are protected against Plasmodium falciparum malaria. Mechanisms driving this protection remain unclear due to a poor understanding of malaria clinical phenotypes during infancy.

Methods

We enrolled a birth cohort of 678 infants in Busia, Uganda, an area of high malaria transmission. We followed infants through 12 months of age, and quantified protection against parasitemia and clinical disease.

Results

Symptomatic malaria incidence increased from 1.2 to 2.6 episodes per person year between 0-<6 months and 6-12 months of age, while the monthly probability of asymptomatic parasitemia given infection decreased from 32% to 21%. Sickle cell trait (HbAS) was protective against symptomatic malaria (incidence rate ratio (IRR) = 0.57 comparing HbAS vs. HbAA, 95% CI 0.44-0.74, p<0.001), but age modified this relationship (P_int=<0.001), with non-linear protection that waned between 0-9 months of age before increasing. Increasing age was associated with higher parasite densities at the time of infection, and, in infants with HbAS, a reduced ability to tolerate high parasite densities without fever.
Conclusions

Age-dependent changes in HbAS protective efficacy in infancy were accompanied by differential loss of anti-parasite and anti-disease protection among HbAS and HbAA infants. This provides a framework for investigating mechanisms underlying infant protection against malaria.

**Key words:** malaria in infancy, asymptomatic parasitemia, sickle cell trait

**Clinical Trials Registration:** NCT02793622
INTRODUCTION

Malaria is responsible for 228 million clinical cases and 405,000 deaths annually, with morbidity and mortality highest in young African children due to *Plasmodium falciparum* [1]. Infants living in highly endemic areas are thought to be partially protected against symptomatic malaria [2-4], though studies assessing the burden of malaria during the first year of life have been limited [5]. An improved understanding of anti-malarial protection in infancy may motivate novel interventions to prevent adverse malaria outcomes in children.

Epidemiologic studies suggest that detection of microscopic parasitemia may be uncommon in the first 6 months of life [2]. This has partially been ascribed to reduced vector exposure (e.g. swaddling), leading to a lower risk of infection [6]. However, once infected, infants also present with lower parasite densities [7] and asymptomatic infection [4, 8], findings which typically occur only after years of repeated infection [9]. Several potential mechanisms for protection against high density parasitemia and symptomatic malaria in infants have been proposed, including the presence of fetal hemoglobin (HbF), which may be less susceptible to *Plasmodium* infection [10, 11], and maternally-acquired antibodies [12], although definitive evidence for these mechanisms has proven elusive. One reason it has been challenging to define protective mechanisms in infants is that few available birth cohorts have independently detailed the risk of infection and the risk of disease once infected [9, 13]. Furthermore, it is unclear whether infant characteristics, such as sickle cell trait [14-17], modify these risks.

To better understand malaria clinical phenotypes among infants, we followed a birth cohort of infants living in a high transmission setting in south-eastern Uganda. We quantified age-related changes in malaria incidence and the probability of symptoms given infection in infancy and
determined the relative protective effects of both individual and household-level factors. We then focus on two specific types of protection – anti-parasite (i.e., the ability to control parasite densities upon infection), and anti-disease (the ability to tolerate higher parasite densities without developing fever), as these have been defined as independent components of acquired anti-malarial immunity[9, 18].

METHODS

Study setting and participants

This study was conducted from September 2016 to December 2018, in Busia district, Uganda, an area of high malaria transmission intensity. Liveborn infants included in this study were born to women enrolled in a trial of intermittent preventive treatment of malaria in pregnancy (IPTp); inclusion and exclusion criteria for the parent trial have been published[19]. Briefly, pregnant women were enrolled between 12-20 weeks of gestation, randomized to receive IPTp with monthly sulfadoxine pyrimethamine (SP) or monthly dihydroartemisinin piperaquine (DP), and followed through delivery. The study was approved by ethics committees at Makerere University School of Biomedical Sciences, University of California San Francisco, and Stanford University.

Study procedures

All women were provided long-lasting insecticide treated bednets at enrollment and a household survey was conducted to collect socioeconomic and house construction data[20]. Handheld Global Positioning System Navigators (GARMIN eTrex, Olathe, K) were used to record coordinates of participants’ homes, and ArcGIS used for map projection.
Mothers were encouraged to bring their infants to a study clinic for all care. Routine assessments were conducted every 4 weeks including collection of blood for detection of malaria parasites. Infants who presented with a history of fever in the past 24 hours, or were found to have a tympanic temperature $>38.0^\circ C$ on clinical assessment, had a blood smear performed for detection of malaria parasites. If the smear was positive, infants were treated for malaria according to the Uganda Ministry of Health guidelines. For uncomplicated malaria, infants were treated with artemether-lumefantrine. For complicated malaria (malaria with danger signs) and severe malaria, infants were treated with intravenous artesunate. Asymptomatic parasitemia was not treated, and children diagnosed with sickle cell disease were provided weekly chloroquine prophylaxis, as per Uganda guidelines. Infants were followed to 12 months of age. Criteria for premature study withdrawal included 1) movement out of study area, 2) inability to be located for $>60$ days, 3) withdrawal of consent, and 4) death.

**Laboratory procedures**

Blood smears were stained with 2% Giemsa and read by experienced microscopists. Blood smears were considered negative when the examination of 100 high power fields did not reveal asexual parasites. All slides were read by a second microscopist and a third reader settled any discrepancies. Hemoglobin (Hb) genotype was ascertained by PCR-based detection[21] using DNA extracted from dried blood spots collected during clinic visits.

**Statistical analysis**

Analyses were conducted using Stata version 14 and R version 3.6.1. Incident outcomes included symptomatic malaria (malaria episode requiring treatment and not preceded by another episode in the prior 14 days), and complicated malaria (malaria with danger signs or severe malaria).
monthly prevalence measures, we considered a 28-day window around each routine visit for the presence/absence of malaria parasitemia. If parasitemic, monthly periods were further characterized as 1) Symptomatic malaria (from 21 days prior to 7 days after the routine visit); or 2) Asymptomatic parasitemia (positive routine blood smear in the absence of fever, without symptomatic malaria 21 days prior to 7 days after the visit). We also considered the peak parasite density and objective temperature measured while parasitemic during this 4-week window.

Exposure variables included infant characteristics at birth (sex, birthweight, gestational age at delivery, and Hb genotype) and household characteristics (household wealth, maternal education level, distance from clinic, and housing construction). Principal components analysis was used to generate a wealth index based on ownership of common household items[20], with households grouped into tertiles. Distance from clinic was categorized as households being <5 or >=5 km from the study clinic. Housing construction types were classified as modern (plaster or cement walls, metal or wooden roofs, and closed eaves) or traditional (all other houses)[22].

Follow-up started at birth and ended on the day the infant was 12 months old or prematurely withdrawn. Incident outcomes were compared using negative binomial regression models. Repeated prevalence measures were compared using log-binomial, log-Poisson, or gaussian models with robust standard errors and generalized estimating equations to adjust for clustering. Exposure variables found to be significant on univariate analyses were included in multivariate models; these models also included maternal IPTp regimen. We assessed for interaction between exposure variables and age. In all analyses, two tailed p-values of <0.05 were considered statistically significant.
Generalized additive models (Gams) were used to model and visualize associations between age, parasite density, and probability of symptoms if infected (Supplemental Methods). For anti-parasite protection, the outcome was the parasite density recorded at each parasite positive study visit[9]. For anti-disease protection, the outcome was the objective temperature recorded during parasite positive visits, conditional on the parasite density.

RESULTS

Study participants and household characteristics

Between December 2016 and December 2017, 678 live infants were born to 666 mothers. Maternal households ranged from 0.1 to 26 km from the study clinic (Fig 1a). The majority of mothers (76.6%) had a primary level education or less, and most households (77.5%) were built with traditional materials (Table 1).

Hb genotype was ascertained in 650/678 (95.5%) of infants with available DNA for testing, of which 79.2% were HbAA, 18.8% HbAS, and 2.0% HbSS. Pediatric characteristics at birth were similar between Hb genotype groups (Table 1). Of 97 infants prematurely withdrawn before one year of age, 39 were unable to be located for >60 days, 32 moved out of the study area, 16 died, and 10 withdrew consent. Of 16 deaths, 12 occurred within the first 2 months of life, with asphyxia (n=3, Figure 1b) and sepsis (n=3) the most common causes of death.
Symptomatic malaria in infancy

Overall, 1131 incident episodes of symptomatic malaria were observed; incidence increased from 1.15 episodes per person year (ppy) between 0-<6 months of age to 2.58 episodes ppy between 6-12 months of age (Fig 2a). Infants with HbAS had 39% less symptomatic malaria than HbAA infants (Table 2, Supplementary Figure 1), but we observed non-linear interaction between Hb genotype and age (P_int=0.001, Figure 2b). Infants with HbAS had 66% less symptomatic malaria between 0-3 months (Incidence Rate Ratio (IRR) 0.34, 95% CI 0.17-0.68, P=0.002) and 51% less symptomatic malaria between 3-6 months of age (IRR 0.49, 95% CI 0.31-0.77, P=0.002) compared with HbAA infants. Between 6-9 months of age, there was no significant difference between HbAS vs HbAA infants (IRR 0.84, 95% CI 0.62-1.15, P=0.27). From 9-12 months of age, infants with HbAS again had 49% less symptomatic malaria than HbAA infants (IRR 0.51, 95% CI 0.37-0.69, P<0.001). Infants with sickle cell disease (HbSS) had 69% less symptomatic malaria than HbAA infants (aIRR 0.31, 95% CI 0.16-0.61, p=0.001), without significant interaction with age (P_int=0.26), although this may have been due (in part) to these infants receiving chloroquine prophylaxis, given evidence of a return of parasite susceptibility to chloroquine in Uganda[23]. Other infant characteristics were not associated with symptomatic malaria incidence (Table 2).

Regarding maternal and household characteristics, infants born to mothers with O level of education or higher had 38% less symptomatic malaria than infants born to mothers with none or primary level education (aIRR 0.62, 95% CI 0.48-0.78, p<0.001). Infants living in houses constructed with modern materials had 27% less symptomatic malaria than infants living in traditional households (aIRR 0.73, 95% CI 0.58-0.92, p=0.008). No significant interaction was observed between infant age, symptomatic malaria, and maternal education (P_int=0.92) nor housetype (P_int=0.40).
Complicated malaria in infancy

There were 65 incident episodes of complicated malaria (0.11 episodes ppy, Table 1); 57 episodes of malaria with danger signs (47 severe emesis, 6 inability to breastfeed, 2 convulsions, 2 lethargy) and 8 episodes of severe malaria (7 respiratory distress, 1 severe anemia). All cases of severe malaria were among HbAA infants, and no child had severe malaria more than once. One case of severe malaria resulted in the death of a child, a 9-month-old who presented with fever, multiple seizures, respiratory distress, a parasite density of ~150,000 parasites/μl, and Hb of 6.4 gm/dl. She died a day after presentation despite being hospitalized and treated with IV artesunate and antibiotics. This was the child’s third episode of malaria; her previous two episodes were uncomplicated.

The incidence of complicated malaria was nearly 7 times higher between 6-12 months of age compared with 0-6 months of age (aIRR 6.81, 95% CI 3.23-14.4, P<0.001, Fig 2a). Infants with HbAS had 83% less complicated malaria compared with HbAA infants (aIRR 0.17, 95% CI 0.04-0.68, p=0.01), with no significant interaction observed between age and Hb genotype (P_int=0.27). Other infant characteristics were not significantly associated with the incidence of complicated malaria (Table 3).

Infants whose mothers were given IPTp with DP had 44% less complicated malaria than infants whose mothers were given IPTp with SP (Table 3), as previously reported [24]. Infants living in the wealthiest households had 71% less complicated malaria compared with those living in the poorest households (aIRR 0.29, 95% CI 0.13-0.64, p=0.002). Infants living closer (<5 km) to the study clinic had 57% less complicated malaria compared with those living ≥ 5km from the clinic (aIRR 0.43, 95% CI 0.20-0.93, p=0.03). Neither maternal education nor housetype were associated with protection against complicated malaria (Table 3).
**Parasite prevalence and probability that parasitemia is asymptomatic in infancy**

Microscopic parasite prevalence, measured every 4 weeks and including both symptomatic and asymptomatic infections, increased from 14.3% between 0-6 months of age to 23.7% between 6-12 months of age. Parasite prevalence did not differ significantly by Hb genotype overall (PRR 0.84, 95% CI 0.69-1.03, P=0.10), without significant interaction with age (P_int=0.20, Figure 3a, Supplemental Table 1).

The probability that parasitemia was asymptomatic decreased from 32.1% between 0-6 months to 20.7% between 6-12 months of age (aPRR 0.65, 95% CI 0.54-0.768, P<0.001), and was significantly higher in infants with HbAS (Supplemental Table 2). However, we observed non-linear interaction between Hb genotype, the probability of asymptomatic parasitemia given infection, and age (P_int=0.04, Fig 3b). From 0-<6 months of age, infants with HbAS had ~2-fold higher probability that parasitemia was asymptomatic than HbAA infants (Table 4). From 6-9 months, there was no difference in the probability that parasitemia was asymptomatic (PRR 1.07, 95% CI 0.62-1.85, P=0.81), but from 9-12 months of age, infants with HbAS again had a significantly higher probability that parasitemia was asymptomatic (PRR 1.89, 95% CI 1.32-2.71, P<0.001).

**Anti-parasite and anti-disease protection in infancy**

Finally, we sought to determine whether the rising incidence of symptomatic malaria, and decline in asymptomatic parasitemia, was due to loss of the ability to control parasite densities (i.e. anti-parasite protection) vs. loss of the ability to tolerate higher parasite densities without fever (i.e., anti-disease protection.)
Among infants with HbAA, mean parasite densities at the time of any infection increased from 3.4 log10 parasites/µl from 0-<3 months of age to 4.1 log10 parasites/µl between 6-<12 months of age (Table 4, Fig 3c, Supplementary Figure 3). In contrast, mean temperatures experienced by HbAA infants at parasite densities >10^4 parasites/µl was >38°C across infancy and did not significantly change with age (Table 4, Fig 3d). This suggests that, among HbAA infants, increasing age was associated with loss of anti-parasite protection.

Among infants with HbAS, mean parasite densities at the time of any infection also increased modestly with age, from 3.3 log10 parasites/µl at 0-3 months to 3.6 log10 parasites/µl between 6-12 months of age (Table 4, Fig 3c, Supplementary Figure 3). However, the temperature experienced by HbAS infants at parasite densities >10^4 parasites/µl rose significantly during the first year of age, from <38.0°C from 0-<6 months of age to > 38°C between 6-<12 months of age (Table 4, Fig 3d). These data suggest that, among infants with HbAS, increasing age was associated with loss of predominantly anti-disease protection.

**DISCUSSION**

In this birth cohort, the incidence of symptomatic malaria in infancy was high, rising with age, with the vast majority of cases uncomplicated. HbAS genotype was protective against symptomatic malaria in infancy, but age modified this relationship, with a non-linear protective effect that waned between 0-9 months of age before increasing. Infants with HbAS had a significantly higher probability of asymptomatic parasitemia given infection than HbAA infants, but this was also similarly modified by age. Surprisingly, although increasing age was associated with higher parasite densities in all infants, infants with HbAS had a reduced ability to tolerate high parasite densities.
Consistent with prior reports, infants with HbAS had 40% less symptomatic malaria, and >80% less complicated malaria, than HbAA infants[7, 17]. However, to our knowledge, this is the first study to suggest that HbAS protection against symptomatic malaria wanes in the first 9 months of age before increasing. Mechanisms of protection afforded by HbAS genotype are likely multifactorial, and include: hypoxic inhibition of parasite growth[25, 26], superior clearance of infected RBCs in the spleen[27-29], increased expression of heme oxygenase-1[30], and HbS polymerization-dependent parasite growth inhibition[31]. Furthermore, protection afforded by HbAS genotype increases with age, suggesting an interaction between HbAS genotype and acquired anti-malarial immunity[32-34].

Given the loss of anti-disease protection observed in infants with HbAS, we hypothesize that there likely exists a synergism between HbAS genotype, maternally-acquired antimalarial antibodies, and clearance of parasites[35] that declines in the setting of waning maternal titers. We speculate that increasing HbAS protection later in infancy may be driven by acquisition of infant antimalarial antibodies[32], although this remains to be determined.

Parasite densities at the time of infection rose among all infants, suggesting waning anti-parasite protection in infancy. Although mechanisms driving this loss of anti-parasite protection are unclear, HbF, present early in life, has been thought to be protective against Plasmodium infection[10, 11, 36]. However, a more recent study found that Plasmodium can develop normally in HbF erythrocytes[35]. Many have also hypothesized a role for maternally-derived antibodies in protection against parasitemia in infants. However, others have instead reported that certain malaria-specific antibodies are associated with an increased risk of infection[37, 38], suggesting that antibodies may alternatively be markers of exposure. More systematic investigation of the role of maternally-derived antibodies in anti-parasite protection in infancy is needed.
Importantly, modifiable factors including living within a house constructed of modern materials or having a mother with higher educational achievement were associated with protection against symptomatic malaria, and higher household wealth and closer proximity to the study clinic were protective against complicated malaria. The protection afforded by these modifiable factors was similar in magnitude to sickle cell trait and that potentially offered by the RTS,S/AS02A vaccine[39]. The vast majority of malaria episodes in infants were uncomplicated, with <1% experiencing severe malaria. In a similar study conducted in Tanzania, 11.1% (102/882) experienced at least one episode of severe malaria, despite a similar prevalence of HbAS genotype[7]. Differences in the burden of severe malaria between the two studies may be due to: 1) The Tanzanian study was conducted before widespread implementation of LLINs and ACTs; and 2) Smaller catchment area (731 km\(^2\) vs. 1,498 km\(^2\) in the Tanzanian study[7]), which may have reduced the time to initial treatment[40]. In the absence of a widely available malaria vaccine, public and private investments addressing housing, education, and transportation are actionable and provide a range of benefits beyond malaria prevention.

Our study had limitations. We did not capture episodes of complicated and severe malaria occurring after one year of age. However, prior reports suggest that the risk of severe malaria peaks within the first year of life[7]. Infants may have received medications outside the study clinic. However, self-reported use of outside medications was rare, and unlikely to explain results stratified by age or by Hb genotype. Use of microscopy instead of PCR to diagnose infection may have underestimated the true prevalence of parasitemia. Unmeasured changes in HbF expression by age may have confounded our analyses. Finally, though we observed a relatively low burden of severe malaria and high prevalence of asymptomatic infection, these findings may not be generalizable to lower transmission settings.
By distinguishing between the risk of infection and risk of disease once infected, and assessing interactions between age and sickle cell trait, our study provides a framework for investigating mechanisms underlying protection against malaria in infants. Defining these mechanisms, why they wane, and how they differ between infants with and without sickle cell trait, may spur the development of novel vaccine and/or antibody-based preventive strategies for malaria.
NOTES

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Disclaimer

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Conflicts of Interest

N.Z. reports a Stanford Med Scholars Grant. All other authors declare no conflict of interest.
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Figure Legends

Figure 1. Study site and profile. (A) Distribution of households in Busia District, Uganda. (B) Trial profile.

Figure 2. Incidence of symptomatic malaria in the first year of life and effect modification by sickle cell trait. (A) Overall incidence of uncomplicated and complicated malaria by age. (B) Incidence of symptomatic malaria by age stratified by Hb genotype. Error bars represent 95% confidence intervals.

Figure 3. Probability infection is asymptomatic in infancy is modified by sickle cell trait. (A) Parasite prevalence, stratified by Hb genotype. (B) Probability of asymptomatic parasitemia given infection, stratified by Hb genotype. In A-B, error bars represent standard errors, estimated by generalized estimating equations. (C) Mean log_{10} parasite densities given an infection during the first year of life, stratified by Hb genotype. Shaded area represent 95% confidence intervals. (D) Temperature at a given parasite density during the first year of life, stratified by Hb genotype (HbAA, Left; HbAS, Right.)
Table 1. Baseline characteristics and descriptive statistics through 1 year of age

| Characteristics of mothers and households | Overall | HbAA | HbAS | HbSS |
|------------------------------------------|---------|------|------|------|
| Maternal sulfadoxine-pyrimethamine IPTp  | 330 (49.6%) | 253 (49.9%) | 61 (51.3%) | 4 (30.8%) |
| Maternal education – primary education or less | 510 (76.6%) | 395 (77.9%) | 82 (68.9%) | 11 (84.6%) |
| Traditional housing construction | 516 (77.5%) | 397 (78.3%) | 88 (74%) | 7 (53.9%) |
| House greater than 5km from the clinic, n/N (%) | 521/658 (79.2%) | 396 (78.9%) | 93 (80.2%) | 8 (61.5%) |

| Characteristics of children at birth | Overall | HbAA | HbAS | HbSS |
|--------------------------------------|---------|------|------|------|
| Maternal education – primary education or less | 510 (76.6%) | 395 (77.9%) | 82 (68.9%) | 11 (84.6%) |
| Traditional housing construction | 516 (77.5%) | 397 (78.3%) | 88 (74%) | 7 (53.9%) |
| House greater than 5km from the clinic, n/N (%) | 521/658 (79.2%) | 396 (78.9%) | 93 (80.2%) | 8 (61.5%) |

| Incident outcomes through one year of age |
|-----------------------------------------|
| Total incident episodes of malaria | 1131 | 986 | 127 | 7 |
| Uncomplicated malaria treated with AL | 1066 | 925 | 124 | 6 |
| Complicated malaria (malaria with danger signs) | 57 | 53 | 3 | 1 |
| Severe malaria | 8 | 8 | 0 | 0 |
| Symptomatic malaria incidence, ppy, thru 1 year of age ppy (Range) | 1.84 (0-10) | 2.04 (0-10) | 1.17 (0-10) | 0.58 (0-2) |
| Complicated malaria incidence, ppy, thru 1 year of age ppy (Range) | 0.11 (0-3) | 0.11 (0-3) | 0.03 (0-1) | 0.08 (0-1) |
| Severe malaria incidence, ppy, thru 1 year of age ppy (Range) | 0.01 (0-1) | 0.02 (0-1) | 0 | 0 |
| Total hospitalizations | 27 | 21 | 2 | 1 |
| Total deaths | 16 | 6 | 2 | 0 |

| Prevalence outcomes |
|---------------------|
| Use of LLIN night prior to routine visit | 7803/7829 (99.7%) | 6160/6182 (99.6%) | 1379/1383 (99.7%) | 158/158 (100%) |
| Microscopic parasite prevalence (4-week intervals, n/N (%)) | 1518/7829 (19.4%) | 1264/6182 (20.4%) | 229/1383 (16.6%) | 12/158 (7.6%) |
| Asymptomatic if parasitemic (4 week intervals, n/N (%)) | 392/1518 (25.8%) | 289/1264 (22.9%) | 96/229 (41.9%) | 5/12 (41.7%) |

1Hb genotype not ascertained in 28 infants living in 27 maternal households; 2LLIN compliance was ascertained through self-report at each participant visit, where parents/guardians were asked if child slept under a bednet in the previous night.
| Risk Factor | Category   | Number of children | Episodes of malaria | PY of observation | Incidence of malaria PPY | IRR (95% CI) | p-value | aIRR (95% CI) | p-value |
|------------|------------|--------------------|---------------------|------------------|--------------------------|--------------|---------|----------------|---------|
| Age in months | 0-6       | 366                | 317.4               | 1.15             | reference                | reference    |         |                 |         |
|            | >6-12      | 765                | 296.1               | 2.58             | 2.24 (2.00-2.52)         | <0.001       | 2.19    | (1.95-2.45)    | <0.001  |
| Season     | Aug-Oct    | 193                | 157.2               | 1.23             | reference                | reference    |         |                 |         |
|            | Feb-Mar    | 156                | 97.0                | 1.61             | 1.31 (1.07-1.60)         | 0.009        | 1.00    | (0.82-1.22)    | 0.99    |
|            | Nov-Jan    | 286                | 152.7               | 1.87             | 1.53 (1.30-1.80)         | <0.001       | 1.30    | (1.10-1.53)    | 0.002   |
|            | Apr-Jul    | 496                | 206.6               | 2.40             | 1.96 (1.68-2.28)         | <0.001       | 1.70    | (1.45-1.98)    | <0.001  |
| Infant Hb genotype\(^b\) | HbAA      | 515                | 986                 | 483.7            | 2.04                     | reference    |         |                 |         |
|            | HbAS       | 122                | 127                 | 108.8            | 1.17                     | 0.57 (0.44-0.74) | <0.001 | 0.61 (0.48-0.79) | <0.001 |
|            | HbSS       | 13                 | 7                   | 12.1             | 0.58                     | 0.28 (0.15-0.53) | <0.001 | 0.31 (0.16-0.58) | 0.001  |
| Infant Sex | Male       | 332                | 545                 | 296.2            | 1.84                     | reference    |         |                 |         |
|            | Female     | 346                | 586                 | 317.4            | 1.85                     | 1.01 (0.85-1.21) | 0.88   |                 |         |
| Preterm birth | No        | 634                | 1075                | 578.7            | 1.86                     | reference    |         |                 |         |
|            | Yes        | 44                 | 56                  | 34.9             | 1.60                     | 0.85 (0.58-1.25) | 0.40   |                 |         |
| Low birthweight | No        | 618                | 1059                | 567.6            | 1.87                     | reference    |         |                 |         |
|            | Yes        | 60                 | 72                  | 45.9             | 1.57                     | 0.83 (0.60-1.17) | 0.30   |                 |         |
| Maternal IPTp | SP        | 339                | 602                 | 304.4            | 1.98                     | reference    |         |                 |         |
|            | DP         | 339                | 529                 | 309.2            | 1.71                     | 0.87 (0.73-1.03) | 0.10   |                 |         |
| Education Level | None or primary | 520               | 960                 | 473.0            | 2.03                     | reference    |         |                 |         |
|            | O level or higher | 158               | 171                 | 140.6            | 1.22                     | 0.59 (0.47-0.75) | <0.001 | 0.62 (0.49-0.78) | <0.001 |
| Housing    | Traditional | 525                | 939                 | 472.2            | 1.99                     | reference    |         |                 |         |
| Household wealth | Modern | Poorest | Mid | Highest | Distance from clinic<sup>c</sup> | ≥5 km | <5 km | Reference | Reference | Reference |
|------------------|--------|---------|-----|---------|-----------------------------|-------|-------|-----------|-----------|-----------|
| type             |        |         |     |         |                             |       |       |           |           |           |
| type             | 153    | 236     | 223 | 219     |                             | 528   | 142   |           |           |           |
| type             | 192    | 405     | 386 | 340     |                             | 889   | 230   |           |           |           |
| type             | 141.3  | 208.6   | 203.4 | 201.5 |                             | 473.3 | 132.2 |           |           |           |
| type             | 1.36   | 1.94   | 1.90 | 1.69     |                             | 1.88  | 1.74   |           |           |           |
| type             | 0.68 (0.54-0.86) | reference | 0.98 (0.80-1.21) | 0.87 (0.70-1.08) | reference | 0.93 (0.75-1.15) | 0.50 |
| type             | 0.002  | reference | 0.85 | 0.21     |                             | reference |         |           |           |           |
| type             | 0.73 (0.58-0.92) | reference |         |          |                             | reference |         |           |           |           |
| type             | 0.007  | reference |         |          |                             | reference |         |           |           |           |

<sup>a</sup>Adjusted incidence rate ratio, with adjustment for age, season, infant Hb genotype, maternal education, and household construction type.  
<sup>b</sup>N=650 with Hb genotype;  
<sup>c</sup>n=670 with household distance measured.
Table 3. Factors associated with incidence of complicated malaria

| Risk Factor | Category          | Number of children | Episodes of complicated malaria | PY of observation | Incidence PPY | IRR (95% CI) | p-value | aIRR (95% CI) | p-value |
|-------------|-------------------|--------------------|---------------------------------|-------------------|---------------|--------------|---------|---------------|---------|
| Age in months | 0-6               | 7                  | 317.4                           | 0.02              | reference     | 8.89 (4.11-19.2) | <0.001  | reference     | <0.001  |
|             | >6-12             | 58                 | 296.1                           | 0.20              | reference     |             |         |               |         |
| Season      | Aug-Oct           | 6                  | 157.2                           | 0.04              | reference     |             |         |               |         |
|             | Feb-Mar           | 21                 | 97.0                            | 0.22              | reference     | 5.68 (2.24-14.4) | <0.001  | reference     | 0.006   |
|             | Nov-Jan           | 7                  | 152.7                           | 0.05              |              | 1.20 (0.44-3.29) | 0.72    |              | 0.74    |
|             | Apr-Jul           | 31                 | 206.6                           | 0.15              |              | 3.94 (1.71-9.09) | 0.001   |              | 0.005   |
| Infant Hb genotype | HbAA        | 515                | 483.7                           | 0.12              | reference     |             |         |               |         |
|             | HbAS              | 122                | 108.8                           | 0.03              |              | 0.22 (0.07-0.70) | 0.01    |              | 0.01    |
|             | HbSS              | 13                 | 12.1                            | 0.08              |              | 0.67 (0.10-4.44) | 0.67    |              | 0.85    |
| Infant Sex  | Male              | 332                | 296.2                           | 0.13              | reference     |             |         |               |         |
|             | Female            | 346                | 317.4                           | 0.09              |              | 0.66 (0.39-1.12) | 0.13    |              |         |
| Preterm birth | No               | 634                | 63                              | 0.11              | reference     |             |         |               |         |
|             | Yes               | 44                 | 2                               | 0.06              |              | 0.53 (0.13-2.08) | 0.36    |              |         |
| Low birthweight | No            | 618                | 64                              | 0.11              | reference     |             |         |               |         |
|             | Yes               | 60                 | 1                               | 0.02              |              | 0.19 (0.03-1.37) | 0.10    |              |         |
| Maternal IPTp | SP                | 339                | 42                              | 0.14              | reference     |             |         |               |         |
|             | DP                | 339                | 23                              | 0.07              |              | 0.54 (0.31-0.93) | 0.03    |              | 0.03    |
| Education Level | None or primary  | 520                | 57                              | 0.12              | reference     |             |         |               |         |
|             | O level or higher | 158                | 8                               | 0.06              |              | 0.47 (0.18-1.21) | 0.12    |              |         |
| Housing type | Traditional | 525 | 52 | 472.2 | 0.11 | reference | reference |
|--------------|-------------|-----|----|-------|------|-----------|-----------|
| Modern       | 153         | 13  | 141.3 | 0.09 | 0.84 (0.42-1.68) | 0.61 |
| Household wealth | Poorest | 236 | 29 | 208.6 | 0.14 | reference | reference |
|               | Mid         | 223 | 28 | 203.4 | 0.14 | 0.99 (0.57-1.73) | 0.97 |
|               | Highest     | 219 | 8  | 201.5 | 0.04 | 0.29 (0.13-0.64) | 0.002 |
| Distance from clinic<sup>c</sup> | ≥5 km | 528 | 57 | 473.3 | 0.12 | reference | reference |
|               | <5 km       | 142 | 7  | 132.2 | 0.05 | 0.44 (0.20-0.95) | 0.04 |

*Adjusted incidence rate ratio, with adjustment for age, season, infant Hb genotype, maternal IPTp, household wealth, and distance from clinic. <sup>a</sup>N=650 with Hb genotype; <sup>b</sup>n=670 with household distance measured.
Table 4. Effect modification between sickle cell trait, age, and clinical phenotypes of malaria in infancy

| Age in months | Episodes (Incidence PPY) | Malaria incidence | Prevalence asymptomatic parasitemia given infection<sup>a</sup> | Parasite densities at time of infection (Anti-parasite protection) | Temperature experienced at parasite densities >10<sup>4</sup> parasites/μl (Anti-disease protection) |
|---------------|--------------------------|-------------------|---------------------------------------------------------------|---------------------------------------------------------------|---------------------------------------------------------------|
|               | HbAS | HbAA (Ref) | IRR (95% CI) | p-value | n/N (%) | PRR (95% CI) | p-value | Log<sub>10</sub> Parasite Density, Mean (SE) | Coef (95% CI)<sup>b</sup> | p-value | Log<sub>10</sub> Parasite Density, Mean (SE) | Coef (95% CI)<sup>b</sup> | p-value |
| 0-<3          | 8 (0.27) | 100 (0.78) | 0.34 (0.17-0.68) | 0.002 | 11/22 (50.00%) | 1.79 (1.05-3.07) | 0.03 | 3.27 (0.18) | Reference | 3.40 (0.08) | Reference |
| 3-<6          | 25 (0.92) | 228 (1.87) | 0.49 (0.31-0.77) | 0.002 | 42/62 (67.74%) | 2.42 (1.82-3.21) | <0.001 | 3.13 (0.14) | -0.14 (-0.57-0.29) | 0.52 | 3.86 (0.06) | 0.46 (0.28-0.65) | <0.001 |
| 6-<9          | 52 (1.98) | 279 (2.36) | 0.84 (0.62-1.15) | 0.27 | 14/61 (22.95%) | 1.07 (0.62-1.85) | 0.81 | 3.60 (0.12) | 0.32 (0.01-0.75) | 0.14 | 4.05 (0.06) | 0.65 (0.46-0.85) | <0.001 |
| 9-<12         | 42 (1.65) | 379 (3.27) | 0.51 (0.37-0.69) | <0.001 | 29/84 (34.52%) | 1.89 (1.32-2.70) | <0.001 | 3.59 (0.13) | 0.31 (-0.13-0.75) | 0.16 | 4.09 (0.05) | 0.70 (0.52-0.88) | <0.001 |

<sup>a</sup> Prevalence asymptomatic parasitemia given infection

<sup>b</sup> Coef (95% CI)
| months | Temp, Mean (SE) | Coef (95% CI)\(^b\) | p-value | Temp, Mean (SE) | Coef (95% CI)\(^b\) | p-value |
|--------|----------------|----------------------|---------|----------------|----------------------|---------|
| 0-<3   | 36.89 (0.09)   | Reference            |         | 38.42 (0.12)   | Reference            |         |
| 3-<6   | 37.99 (0.26)   | 1.10 (0.54-1.66)     | <0.001  | 38.30 (0.10)   | -0.13 (-0.42-0.17)  | 0.41    |
| 6-<9   | 38.32 (0.23)   | 1.43 (0.93-1.94)     | <0.001  | 38.51 (0.09)   | 0.09 (-0.18-0.35)   | 0.53    |
| 9-<12  | 38.61 (0.22)   | 1.73 (1.27-2.19)     | <0.001  | 38.63 (0.08)   | 0.21 (-0.05-0.46)   | 0.11    |

\(^a\) Prevalence asymptomatic parasitemia given microscopic infection, as measured during 4 week windows; \(^b\) Coefficients estimated using generalized estimating equations with robust standard errors; IRR: Incidence rate ratio; PRR: Prevalence rate ratio
Figure 1

A.

B.

782 women enrolled

391 women randomized to monthly SP
- 53 withdrawn prior to delivery
- 31 unable to locate for >60 days
- 13 withdrew informed consent
- 6 moved out of study area
- 1 unable to comply with protocol

338 women followed through delivery resulting in 348 infants delivered (10 sets of twins)
- 9 infants excluded
- 4 spontaneous abortions
- 5 stillbirths

391 women randomized to monthly DP
- 42 withdrawn prior to delivery
- 19 unable to locate for >60 days
- 11 withdrew informed consent
- 6 moved out of study area
- 5 unable to comply with protocol
- 1 became HIV-infected

349 women followed through delivery resulting in 352 infants delivered (3 sets of twins)
- 13 infants excluded
- 10 spontaneous abortions
- 3 stillbirths

678 live births included in the analysis

97 infants prematurely withdrawn
- 39 unable to locate for >60 days
- 32 moved out of study area
- 16 died
- 12 within first 2 months
  - 3 asphyxia
  - 2 SIDS
  - 2 unknown
- 1 sepsis
- 1 hypothermia
- 1 severe malnutrition
- 1 congenital malformation
- 1 anemia
- 4 from 2-12 months
  - 2 sepsis
  - 1 malaria
  - 1 unknown
- 10 withdrew informed consent

581 infants reached 12 months of age and completed follow-up
Figure 3

A. Microscopic parasite prevalence

B. Probability of parasitemia

C. Log10 parasite density

D. Heat map showing parasite density over temperature.