Dynamics of asymptomatic infections and symptomatic malaria show different temporal profiles among infants living in a high transmission area of Ghana

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Abstract

Background Although infants are vulnerable to malaria, the characteristics of their patterns of infections are not well described. This study aimed to examine the longitudinal profiles of asymptomatic infections and symptomatic malaria in the first year of life. **Methods** A birth cohort in Kintampo, Ghana (N = 1855) was followed actively with monthly blood sampling and passively for any febrile illness between 2008 and 2011. Malaria parasites were detected by light microscopy and infants who were infected or uninfected were identified. Infections were classified as either symptomatic or asymptomatic using fever and temperature readings over twelve months of follow up. The longitudinal infection profiles in all infants were then compared. **Results:** Asymptomatic infections and symptomatic malaria were observed at all ages but were rare the first months of life and the proportion of symptomatic malaria increased after six months. Among 1264 infants having microscopy data for at least eight monthly visits, four patterns were observed: parasite negative at all visits (36%), always asymptomatic (7%), always symptomatic (35%) and alternating between asymptomatic infections and symptomatic malaria (22%). The cumulative incidence of infection was highest in the alternating group, and many different profiles (87 different combinations) of asymptomatic infections and symptomatic malaria were observed in this group. Parasite densities were significantly low for the always asymptomatic group and highest for always symptomatic group. **Conclusion** Infants in malaria endemic areas experience highly different infection profiles over the first year of life despite living in the same area. In-depth investigations of why some infants are parasite free and others have repeated symptomatic malaria or maintain asymptomatic infections or alternate between asymptomatic infections and symptomatic malaria can contribute to understanding malaria susceptibility during infancy.

Background

Malaria is often uncomplicated, however complications from severe symptoms can become fatal, and children below five years of age are most vulnerable (1-4). Infections with malaria parasites can be asymptomatic in partly immune individuals living in endemic areas (5, 6). Asymptomatic infections outnumber symptomatic malaria in both high and low transmission settings and as potential reservoirs for transmission can impede efforts to control and eliminate malaria (6, 7).

Infants experience both asymptomatic infections and symptomatic malaria (8, 9). However, during the first six months of life, infections are reported to be mainly asymptomatic, while between six and twelve months of age the incidence of both asymptomatic and symptomatic malaria infections increase (8, 10-13). The low incidence of symptomatic malaria below six months of age has been attributed to presence of fetal hemoglobin (14, 15) and passively acquired maternal IgG (16). Also, malaria-specific antibodies at birth (in maternal and/or cord blood) have been associated with protection against some malaria parasite antigens but not others (16-21).

Studying the temporal patterns of asymptomatic infections and symptomatic malaria in infants is important for understanding relationships between susceptibility and immunity, and to guide control

interventions such as presumptive treatment and vaccines. In addition, the challenge of identifying correlates of protection (22) can be overcome by studying large cohorts of exposed and non-exposed infants who undergo frequent sampling over longer periods. Nonetheless, previous studies assessing malaria morbidity in the first year of life are either based on large cohorts with few cross-sectional samplings (9, 23), or with frequent sampling in small cohorts (16, 19). Consequently, the longitudinal profiles of malaria outcomes and age-specific patterns of infections have not been studied on a large scale within the first year of life (16). Hence the characteristics and determinants of continuous infections in infancy are not well described, there is lack of clarity on the correlates of protection during infancy and the transition from asymptomatic infection to symptomatic malaria or the reverse, through the first year of life is poorly understood.

We conducted a study in a birth cohort living in a high transmission area of Ghana where blood sampling of infants for malaria parasitaemia was performed monthly and at passive/sick visits (24). This study provided a platform to identify exposed and unexposed infants and to examine age-specific infection patterns among infants exhibiting similar longitudinal profiles of asymptomatic infection and symptomatic malaria through their first year of life.

Methods

Study location and population

Kintampo North Municipality (KNM) and Kintampo South District (KSD) consist together of 156 communities and covers an area of 7 162 km² in Ghana. Malaria transmission in this area is high and perennial, peaking between April and October (25, 26).

In October 2008, a birth cohort study enrolled pregnant women from the study area (24). Infants were included in the study at birth between November 2008 and January 2011 and followed up actively every month until 12 months of age or to the end of the study in May 2011 or exit due to death, migration or loss to follow up (24).

Collection of samples and identification of parasites

Each child was visited monthly at home (active visits), by trained field staff who collected blood samples and completed standard questionnaires. In-between active visits, care-givers were encouraged to seek care from a study clinician, and for blood sampling if their infant was unwell (passive visits). Infants with fever and/or parasites were treated with artesunate/amodiaquine according to the national health policies. All infants were given free health insurance, paid for by the study.

Blood samples (500µl – 1ml) were drawn on active and passive visits. Blood smears were examined for malaria parasites by light microscopy. Parasite densities were estimated per microlitre (µl) of blood, by assuming 8000 leukocytes per µl of blood, counting the number of parasites per 200 leukocytes and multiplying counts by 40 for the fraction of leukocytes examined. Parasite counts per 500 leukocytes and

multiplied by 16 were performed if parasites were less than 100/μl. Microscopy test was not carried out on the passive visit if the infant had no fever in the past 48 hours or axillary temperature below 37.5°C. Details including enrollment and follow up visits have been previously described (24).

Definitions of symptomatic malaria and asymptomatic infection

Symptomatic malaria was defined as parasite positive by microscopy, a history of fever, illnesses or axillary temperature $\geq 37.5^{\circ}\text{C}$ (elevated temperature) in the past 48 hours or subsequent seven days at either the active or passive visit. Asymptomatic infections were defined as parasite positive by microscopy at the monthly active visits with temperature $< 37.5^{\circ}\text{C}$ and neither fever, illnesses nor passive visits in the preceding 48 hours or subsequent seven days.

Statistical analyses and profiling of symptomatic malaria and asymptomatic infections

Data were analyzed using Stata Version 14 (STATA Corporation, College Station, TX) and JMP statistical software. The number of infants with microscopy results from birth to one year of age was determined in all visits. Active and passive visits for which variables including temperature, fever or parasitaemia were missing were excluded as these were necessary for classification.

For the temporal profiling of asymptomatic infections and symptomatic malaria, infants having eight or more active and passive visits were included. Supplementary Figures 1a and 1b shows this in more detail.

The frequency distribution and mean of infections, cumulative incidence of infections, age at infection and medians of parasite densities were described for infants in the entire cohort as well as the groups with longitudinal asymptomatic infections or symptomatic malaria or mixed profiles. Infants from the different profiled groups having two or more infections were identified and the time (in days) intervals between any two infections were examined.

The Kruskal Wallis test was used to assess differences in age (in months), infections and parasite densities between groups, while Wilcoxon signed-rank tests were performed for within group analyses. Estimates with p-values ≤ 0.05 were considered statistically significant.

Results

Characteristics of the cohort

The birth cohort included 1855 infants and the second of 36 twin births were excluded to prevent clustering effects during analyses (Figure 1). Table 1 summarizes characteristics of the birth cohort data examined. The 1819 infants included in the analyses had in total 19231 scheduled monthly (active) visits, corresponding to a compliance rate (observed visits/expected visits $\times 100$) of 88% (Table 1). For infants who reported illness between any two successive active visits, 74% (1341/1819) had a corresponding passive visit record.

Malaria microscopy test was performed for 92% (1674/1819) of infants at their active or passive visits (Table 1 and Figure 1). The infants had a mean follow up time of 6 (SD: $\pm$ 3) visits.

Malaria parasites were detected among 58% (971/1674) of infants at least once during the follow up period. In total, 55% (919/1674) of infants had malaria parasites and with corresponding temperature test results and/or history of fever, and these were used to define symptomatic or asymptomatic outcomes.

Malaria parasites, symptomatic malaria and asymptomatic infections in the cohort

Out of a total of 12926 malaria microscopy tests performed during the follow up for 1674 infants, 19% (2394/12926 from 971 infants) were positive for malaria parasites. Majority of the infections were *P. falciparum* (96%; 2293/2394), followed by *P. malariae* (2%; 41/2394) and 'other species' (*P. ovale*, or *P. vivax*: 1%; 21/2394). Mixed species infections were rare (1%; 27/2394) and mainly *P. falciparum* and *P. malariae* co-infections (70%; 19/27). Gametocytes were detected in 1.6% (39/2394) of all infections. *P. falciparum* contributed 85% (33/39), *P. malariae* 5% (2/39) and other species 10% (4/39) of all gametocytes.

Of 2394 malaria parasite infections, 92% (2194/2394 - among 919 infants) had corresponding temperature tests and/or history of fever and were classified as asymptomatic or symptomatic. On both active and passive visits, proportion of parasite negative tests decreased while the proportion of asymptomatic infections and symptomatic malaria increased (Figures 2a and 2b). Of all infections classified, 61% were accompanied with symptoms (1340/2194); and 68% (913/1340) of the symptomatic malaria were detected at passive ill visits while 32% (427/1340) were detected at active visits.

Prevalence of infections (symptomatic or asymptomatic) increased from birth to twelve months of age and was highest (20%, 261 infants infected/1314 total number of infants) at seven months of age (Figure 2c). From birth to five months of age, infections were predominantly asymptomatic and from six to twelve months of age both symptomatic malaria and asymptomatic infections became more frequent among the infants (Figure 2c). This trend was also reflected in the cumulative incidence of symptomatic malaria which was similar to asymptomatic infections until five months of age, after which most infections were symptomatic (Figure 2d).

For both symptomatic malaria episodes and asymptomatic infections, the median *P. falciparum* parasite density was significantly lower from birth to five months of age compared to six months of age and above (Supplementary Figure 2).

Profiles of longitudinal symptomatic malaria and asymptomatic infections

For detailed description of infection dynamics throughout the first year of life, infants (70%, 1264/1819) who had microscopy test results and recorded temperature and/or fever results on eight or more active

visits and any passive visits were analyzed (Supplementary Figure 1a and 1b), to identify the temporal occurrence of asymptomatic infections and symptomatic malaria.

Overall temporal profiles

Four main groups were identified from the longitudinal profiling of asymptomatic infections and symptomatic malaria among the 1264 infants: i) “parasite negative” [36% (459/1264)], if an infant did not have malaria parasitemia during all follow up visits, ii) “Always symptomatic” [35% (444/1264)], if an infant had symptomatic malaria on all occasions parasites were detected. iii) “Always asymptomatic” [7% (87/1264)], if an infant had asymptomatic infection on all occasions parasites were detected, and iv) “Alternating” [22% (274/1264)], if both asymptomatic infection and symptomatic malaria were detected over the first year of life.

In infants with malaria parasites, infections occurred intermittently throughout the year on both passive visits (where microscopy was performed on the same day and infants were treated) and active visits (where infants were ‘healthy’, and microscopy was performed at a later date and no efforts were made to treat) (Figure 3). Infections among infants in the always asymptomatic group were identified during active visits only, unlike the other groups whose infections were identified during both active and passive visits (Figure 3).

Infection profiles among groups of infants

Malaria parasites were detected among 64% (805/1264) of infants in this sub-cohort at least once during the active and passive visits. A proportion [37% (299/805)] of infants belonging to the always symptomatic and always asymptomatic groups had only one detectable infection over the twelve months of follow up (Figure 4). While 55% (246/444) of infants within the always symptomatic group had only one symptomatic malaria episode, 61% (53/87) of infants in the always asymptomatic group had only one asymptomatic infection.

Among the parasite positive infants, 63% (506/805) had two or more separate infections over the one year period (Table 2). Of these, 54% (274/506) had both symptomatic and asymptomatic infections i.e. alternating group, 39% (198/506) were in the always symptomatic group and 7% (34/506) in the always asymptomatic group (Table 2).

The cumulative incidence of infections from birth to twelve months of age was highest in the alternating group, followed by always asymptomatic group – where it was similar to the always symptomatic group from eight months of age onwards (Figure 5a).

The mean number of infections were similar in the always symptomatic [1.75 infections/child (779/444)] and always asymptomatic groups [1.85 infections/child (161/87)] ($P = 0.715$). Within the alternating group, the mean number of symptomatic malaria episodes was 2.17 infections/child (595/274) and asymptomatic infections was 1.86 infections/child (509/274). Per the default definition, single infections were not determined for infants in alternating group (Figure 4).

When comparing infants with two or more infections, the alternating group had the highest mean number of multiple infections [4.03 infections/child (1104/274)] compared to the always symptomatic [2.69 infections/child (533/198)] and always asymptomatic [3.18 infections/child (108/34)] groups ($P = 0.001$).

Analysis of the number as well as the sequence of infections in the alternating group showed 87 different combinations of asymptomatic infections and symptomatic malaria (Table 3). Half (57%; 50/87) of these combinations of alternating asymptomatic infections and symptomatic malaria were unique to individuals (Table 3). Assessing the number of times asymptomatic infections and symptomatic malaria alternated in any of the 87 combinations showed three alternating infections [observed in six different sequences among 25% (68/274) of infants] were frequent, while ten alternations at the most occurred (Table 3). The most common alternating sequence was to first have asymptomatic infection(s) followed by a symptomatic episode(s) (46%; 125/274). The second most common sequence was first symptomatic episode(s) followed by asymptomatic infection(s) (19%; 51/274) (Table 3).

The time span between any two infections peaked between 27 and 38 days for all groups. Infections which were six months (180 days) or more apart were rarely observed in all groups (Supplementary Figure 3).

Age at infections among groups of infants

First infections were detected from one to twelve months of age [median = 6 months, (IQR: 5 – 8)]. Age at first infection was lowest in the alternating group [median = 5 months (IQR: 4-7)] compared to the always asymptomatic group [median = 7 months (IQR: 4-9)] or always symptomatic group [median = 7 months (IQR: 5-9)], all $P < 0.001$.

In the alternating group, first infections which were asymptomatic [61% (167/274) of infants] were more frequent than first infections which were symptomatic [39% (107/274) of infants] ($P = 0.003$).

Whereas the first symptomatic malaria episodes begun from two to twelve months of age for infants in the always symptomatic group (Figure 5b), first infections were detected from one to eleven months of age for infants in the alternating group (Figure 5c). For infants in the always asymptomatic group the first infections occurred between one and twelve months of age (Figure 5d).

Although infections were detected at all ages, only few infants (5%; 36/805) had infections at two months of age, and they were mainly from the alternating group [69% (25/36)]. For all groups with malaria parasites, the number of times infants were infected (on separate occasions) increased as they aged (Figure 5b, 5c and 5d), but the proportion of infants infected decreased with age (Table 2).

The median age of infection was seven months (IQR: 6 – 10) for the alternating group and eight months for both always asymptomatic (IQR: 5 – 9) and always symptomatic groups (IQR: 6 – 10).

Parasite densities among groups of infants

The range of *P. falciparum* asexual parasite densities overall was 2 – 974759 parasite/ul. Parasite densities increased with age in all groups (Figure 6), and the median parasite density at the first infection was highest in the always symptomatic group, followed by the alternating group and lowest in the always asymptomatic group ($P < 0.001$) (Table 4). Compared to other groups, the always symptomatic group had the highest median parasite density from one to six months of age ($P < 0.001$) as well as from seven to twelve months of age ($P < 0.001$) (Table 4).

Within the alternating group, the median parasite density [14812 parasite/ul (IQR: 2847 – 77843)] was higher when infants were symptomatic compared to when they were asymptomatic [2848 parasite/ul (IQR: 858 – 8228)] ($P < 0.001$).

In all groups, the parasite densities were lower the first six months of age compared to seven months of age and above (all $P < 0.001$).

Discussion

The longitudinal profiles and age-specific patterns of asymptomatic infections and symptomatic malaria were examined in a birth cohort from a high malaria transmission area of Ghana. Despite living in the same area, infants were found to experience highly diverse infection patterns the first year of life. Based on microscopy defined asymptomatic infections and symptomatic malaria, four main groups of infants were identified. A third of infants studied were parasite negative throughout, and of those having parasites, half had only symptomatic malaria, less than a quarter remained asymptomatic while a little over a quarter alternated between asymptomatic infections and symptomatic malaria at any time infections were detected. In total, 87 different patterns of alternating asymptomatic infection and symptomatic malaria (over half unique to individuals) were detected among 805 infants with *P. falciparum* parasites, suggesting that although there may be factors facilitating the transitioning from asymptomatic infections to symptomatic malaria, there is no uniform sequence of going between being either symptomatic or asymptomatic.

Given the development of naturally acquired immunity against malaria at intermittent time periods (27, 28) and the observations made, the natural course of malaria in the first year of life may follow the different profiles. Perhaps, each infection time point may have been influenced by factors (placental malaria, decreasing maternal antibodies, use of medications, ITN use or host genetic factors) that could modulate the manifestation of symptoms or morbidity. The modifications resulting out of interactions between the infected child and combinations of such factors is thought to have led to infections which were always accompanied with symptoms for some infants or without symptoms for others through the first year of life.

When comparing asymptomatic infections to symptomatic malaria in the entire cohort, the cumulative incidence of symptomatic malaria was higher from six to twelve months of age. However, when comparing the groups of infants, the cumulative incidence of infection was lowest in always symptomatic group and highest in the alternating group, indicating relatively poor protection against

asymptomatic infections and symptomatic malaria among infants in alternating group. Importantly, given the higher number/cumulative incidence of infection, the alternating pattern may be observed among infants more frequently, compared to the other infection patterns. For instance, asymptomatic infections were frequently followed with symptomatic malaria among children aged six months to five years of age in urban Uganda (29). The frequent observation of infants with alternating infection patterns can diminish the detection of other infection patterns and their contribution to malaria the first year of life. Nevertheless, the findings in this study provide new approaches for analyzing data from cohort studies to yield a better understanding of immunity and susceptibility to malaria in infancy. For example, comparing individuals who are repeatedly infected and asymptomatic versus those symptomatic, or studying the same individuals when they are asymptomatic versus when they are symptomatic.

Assessing all infections from the three groups, two or more discrete infections (mostly detected 27 to 38 days apart) were more frequent than one infection. A previous study conducted in 2003/2004 showed children below three years of age and living in Kintampo had as many as seven malaria attacks/child/year (30). In this current study conducted in 2009/2010, the maximum mean number of infections was four, observed within the alternating group, indicating that although two or more infections were frequent than one, there has been a considerable drop in malaria among infants in Kintampo between 2003 and 2010, a reflection of global trends (1).

The age at first infection was lower for the alternating group (seven months) than for both always asymptomatic and always symptomatic groups (eight months). On the contrary, Wagner and others (16) reported a median age at first infection in infants at ten months in coastal Ghana although ITN usage was absent in the era of their research. However, their sample size was small (71 infants) and their definitions for malaria were based on microscopy/PCR and temperature $\geq 37.5^{\circ}\text{C}$ at the time of sample collection (16). Clearly, differences in sample size, definitions and study design/location may have contributed to the dis-similar age at first infection we observed.

Having asymptomatic infections first have been shown to reduce the risk of subsequent symptomatic malaria among two to ten year old children in rural Mali (31) but increase the risk among children who are six months to five years of age living in urban Uganda (29). In this study, infants in the alternating group whose first infections were asymptomatic were more compared to infants whose first infections were symptomatic. Also, in the always symptomatic group, malaria was observed from two months of age onwards. Although this study did not aim to model for risk of symptomatic malaria, the results indicate that after birth, asymptomatic infections occurred first before symptomatic malaria, possibly due to the influence of host genetics and/or immunity.

Consistent with the classical age distribution of infections in infants (12, 32, 33), we observed frequent asymptomatic infections between birth and five months of age and increased asymptomatic infections and symptomatic malaria from six to twelve months of age in the entire cohort and in the groups of infants studied. As infants aged, the number of times they were infected increased in all groups. However, the proportion of infants accumulating multiple infections as they aged decreased in all groups. These

observations support the notion that there are mechanisms which reduce risk of symptoms but not parasite infections within the first six months of life (16, 19). Nevertheless, the significantly lower age at first infection but higher cumulative incidence of infection for alternating group compared to other groups, suggest possible differences between the groups of infants regarding mechanisms (that may include host genetics, bed-net usage, exposure to maternal antibodies or parasite parameters) which may reduce the risk of symptoms.

Similar to observations in a hyperendemic area of Burkina Faso (9) and a meso-endemic area of Uganda (29), in this study, parasite densities increased with age and was highest in always symptomatic group. Generally, studies in malaria endemic areas show low parasite density in both asymptomatic infections and symptomatic malaria for infants below six months of age (9, 13). High parasite density during symptomatic malaria in infants below six months of age is reported to be influenced by placental malaria (34). Whether low parasite densities lead to protection against symptoms is however unclear. Nevertheless, this study showed significantly lower parasite densities for below six months of age (where infections were predominantly asymptomatic) than above when the longitudinal patterns of infections was either considered or not. In addition, significantly lower parasite densities during the asymptomatic periods compared to the symptomatic periods were observed among infants in alternating group, suggesting that protection against symptoms could be influenced by low parasite densities. However, the significantly lower parasite densities throughout the entire year for always asymptomatic group compared to the other groups suggest that other mechanisms in addition to parasite densities may influence the manifestation of symptoms through the first year of life.

The proportions of asexual stages *Plasmodium falciparum* (96%) and *Plasmodium malariae* (2%) detected were similar to observations made in other malaria endemic areas of Africa (35). Sexual stages of the malaria parasites were of relatively low frequency and this observation is also consistent with that made in other endemic areas (36, 37).

There may be underlying factors that influenced assigning infants to the always asymptomatic or always symptomatic group and the use of microscopy for parasite detection could be one major factor, therefore it is important to restrict interpretations to microscopy. All infants could have submicroscopic infections (that may be asymptomatic or symptomatic) detectable with methods which are more sensitive than microscopy e.g. polymerase chain reaction (PCR) (16, 38). In this regard, carriage of submicroscopic infections would be important for examining low-grade parasitaemia among infants identified as parasite negative, an analyses beyond the detection limit of this study.

Moreover, a universally accepted or precise definition for asymptomatic malaria is lacking currently, and this may have influenced the assigning of an infection a symptomatic or an asymptomatic status. While seasonality and parasite density have been considered in some studies (39-41), antimalarial usage, microscopy or polymerase chain reaction detection methods and varying duration between initial detection of parasites and onset of symptoms ranging from three to twenty-one days, have also been used to define malaria in other studies (5, 21, 42). Consequently, the profiles obtained for infants in this

study could change if our definitions followed any of the above. However upon infection, infants show symptoms, compared to remaining asymptomatic (8, 11, 29, 43), and this informed our expanded search for passive/hospital visits, elevated temperature, illnesses or fever, 48 hours before and seven days after an infection was detected. Further, the susceptibility of infants to symptomatic malaria enabled us to classify any infection unaccompanied with symptoms or morbidity within the set time frame as truly asymptomatic. Nevertheless, if sampling intervals, definitions and parasite detection methods which can influence outcomes are standardized, (for various populations in different transmission settings) accurate interpretations and better understanding of transitioning from asymptomatic infection to symptomatic malaria can be achieved. In summary, the limitation of this study mainly lies in the use of microscopy detection methods, while the strength lies in the ability to analyze a large number of monthly samples.

Given the complex heterogeneity of infection patterns during infancy which has been observed, it is thought that studies based on single cross-sectional samples would capture the effects of exposure only briefly and consequently restrict the understanding of malaria susceptibility and immunity in infancy. Nevertheless, grouping infants on the bases of their temporal malaria infections can facilitate the understanding of the immunological mechanisms of protection against malaria. Importantly, the results obtained opens up new opportunities for the concise analysis of host (genetic and immunology) and external determinants (risk factors and parasite parameters) of malaria and can guide the design of future studies aimed at malaria control and elimination in endemic areas.

In conclusion, different infection patterns were observed in the first year of life. In-depth investigations to determine why within the same transmission area some infants remain parasite free while others have repeated symptomatic malaria or asymptomatic infections or alternate, can contribute to understanding malaria susceptibility during infancy.

Declarations

Ethical approval and consent to participate

This study was granted ethical approval from the Kintampo Health Research Centre (KHRC) Institutional Ethics Committee (Office for Human Research Protections federal wide assurance number is 00011103 and Institutional Research Board registration number is 0004854). Additional approval for this study (Dnr 2018/1967-32) was from the Regional Ethics Board of Stockholm. Ethical approvals for the original birth cohort study were from KHRC, Ghana Health Service and London School of Hygiene and Tropical Medicine. Mothers gave consent for themselves and their infants to participate in the birth cohort study.

Consent for publications

Not applicable

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

All authors declare no competing interest

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Authors' Contributions

AKB contributed to the design, data analyses and writing of this manuscript, SOA contributed to the design and writing, MA contributed to data analyses and writing, UH contributed to the data analyses, FBO contributed to the data analyses and writing, SG contributed to data analyses, DD contributed to the field work and laboratory analyses, GJ contributed to the laboratory analyses and writing, MFK contributed to the data analyses, EBK contributed to the field work, FO contributed to the design and writing, AF contributed to the design, data analyses and writing, and KPA contributed to the design, data analyses and writing of this manuscript.

All authors read and approved the final manuscript

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Tables

Table 1. Characteristics of infants and visits

Characteristic	Level	Active visits		*Passive visits	
		Value	Proportion (%)	Value	Proportion (%)
Number of births	All	1819	100	-	-
Transmission season at birth	High	1308	71.9		
	Low	511	28.1	-	-
Infants visited at least once	Yes	1819	100	1488	81.8
	No	-	-	331	18.2
Infants having microscopy records	Yes	1674	92	1437	96.6
	No	145	8	51	3.4
Infants having ≥ 8 microscopy records/visits	Yes	1264	69.5	1177	93
	No	0	0	87	7
Number of visits for 1819 infants		19231	78.5	5 254	21.5
Expected active visits for 1819 infants		21828	88.1	-	-

*Active visits on which infant was febrile and taken to hospital were regarded as passive visits. Microscopy test was not carried out on passive visit if infant had no history of fever or temperature $< 37.5^{\circ}\text{C}$.

Table 2. Proportion of infants per number of times infected

	Number of infants infected n (%)										Number of infections n (%)
	Once or more	Twice or more	Thrice or more	Four or more	Five or more	Six or more	Seven or more	Eight or more	Nine or more	Ten	
Infants with active infection	444 (100)	198 (45)	92 (21)	31 (7)	13 (3)	5 (1)	0	0	0	0	779 (38)
Infants with passive infection	274 (100)	274 (100)	217 (79)	147 (54)	93 (34)	52 (19)	29 (11)	13 (5)	9 (3)	1 (0.4)	1104 (54)
Infants with no infection	87 (100)	34 (39)	22 (25)	10 (11)	5 (6)	3 (3)	1 (1)	0	0	0	161 (8)
Total	805	506	331	188	111	60	30	13	9	1	2044

Table 3. Patterns of infections in always asymptomatic, always symptomatic or alternating groups

Always asymptomatic		Alternating patterns with asymptomatic first infections							
1	N=87	2	N=125	3	N=19	4	N=15	≥5	N=8
A	53	AS	42	ASA	5	ASAS	2		
AA	12	ASS	23	ASSA	4	AASAS	2	5	
AAA	13	AAS	17	AASA	1	ASAAS	1	ASASA	2
AAAA	4	ASSS	8	AASAA	1	ASSASS	2	AASASA	1
AAAAA	2	AASS	7	ASSAA	1	AAASAS	1	ASASSSA	1
AAAAAA	2	AAAS	5	ASAAA	1	ASASSS	1	ASASSAA	1
AAAAAAA	1	ASSSS	6	AAASSA	1	AASSASS	1	AAASSASAA	1
		AAAAS	4	AAAASA	1	ASSSAAS	1		
		AAASS	2	ASSSAA	1	ASAAASS	1	6	
		AASSS	2	AAAAASA	1	ASSASSS	1	ASASSAASS	1
		AAAAAS	3	AASAAAA	1	AAAASAS	1		
		ASSSSS	1	ASSSSSSA	1			8	
		AASSSS	1					ASASAASAS	1
		AAASSS	1						
		ASSSSSS	2						
		AASSSSS	1						
Always symptomatic		Alternating patterns with symptomatic first infections							
1	N=444	2	N=51	3	N=42	4	N=7	≥5	N=7
S	246	SA	16	SAS	7	SASA	2		
SS	107	SAA	8	SASS	4	SASAA	1	5	
SSS	61	SSA	8	SSAS	7	SSASA	1	SASASS	2
SSSS	19	SAAA	4	SAAS	5	SSSASA	1	SASSSAS	1
SSSSS	6	SSAA	4	SASSS	2	SASSSSSA	1	SAASSASS	1
SSSSSS	5	SSSA	3	SAAAS	2	SAAASSAA	1		
		SSSSA	2	SAASS	3			6	
		SAAAA	2	SSSAS	3			SASASA	1
		SSSSAA	1	SSAAS	2			SSSASASSA	1
		SSSAAA	1	SSASS	1				
		SSSSAAA	1	SSSASS	1			7	
		SAAAAAA	1	SAAASS	1			SSASASASS	1
				SSASSS	1				
				SAAAASS	1				
				SSSSSSSAS	1				
				SASSSSSSS	1				

A=asymptomatic infection and S=symptomatic malaria. Number of infants with alternating infections who first experienced an asymptomatic infection (8 types of sequences) or symptomatic malaria (7 types of sequences)

with in total 87 different combinations are shown above and below respectively.

Table 4. Distribution of asexual *P. falciparum* parasite densities by group

ensity (μ l)	Level	Always symptomatic	Alternating	Always asymptomatic	P-value
	Range	22 – 974759	2 – 932795	19 – 190959	-
	Median (IQR)	23177 (4354 – 101733)	5421 (1404 – 26355)	1702 (544 – 5748)	< 0.001
ections	Median (IQR)	16717 (2912 – 72055)	2674 (755 – 12570)	1569 (388 – 5748)	< 0.001
ths of age	Median (IQR)	10623 (2107 – 35137)	4088 (1032 – 16275)	1143 (388 – 4020)	< 0.001
nths of	Median (IQR)	38302 (7281 – 132975)	6843 (1794 – 36948)	1929 (608 – 6854)	< 0.001

Figures

Figure 1

Flow chart of the selection of infants for profiling

Figure 2

Distribution of parasite negative and postivity among 1674 infants

Figure 3

Temporality of infections in active and passive visits the first year of life

Figure 4

Overall number of infections by group

Figure 5

Age specific number of infections during the first year of life

Figure 6

Age specific distribution of parasite densities

Supplementary Files

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