

Asymptomatic parasitaemia as a risk factor for symptomatic malaria in a cohort of Ugandan children

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Summary

OBJECTIVES To assess the prevalence of asymptomatic parasitaemia, determine its association with symptomatic malaria, and identify independent predictors of asymptomatic parasitaemia in a cohort of children from Kampala, Uganda.

METHODS A total of 316 children aged 6 months to 5 years were recruited from the community. The prevalence of asymptomatic parasitaemia was assessed at enrolment and approximately every 30 days during follow-up. Participants received all of their health care in our clinic, including a standardized approach to the diagnosis and treatment of symptomatic malaria.

RESULTS A total of 283 (90%) subjects completed the full 1-year follow-up and were included in this study, yielding 2557 routine smears. The prevalence of asymptomatic parasitaemia was 17% at enrolment, but 5–8% for the remainder of the study. The risk of developing symptomatic malaria within 30 days was significantly higher in those with a positive routine than in those with a negative one (50% vs. 9%, $P < 0.001$). Higher parasite densities were associated with increased odds of developing symptomatic malaria within 30 days ($P = 0.003$). Only 11% of episodes of asymptomatic parasitaemia, involving 6% of subjects, arose and cleared without therapy. In multivariate analysis the only significant risk factor for asymptomatic parasitaemia was whether a child had any episode of symptomatic malaria during the course of the study (OR = 3.0, $P = 0.02$).

CONCLUSION In our cohort of children from an urban meso-endemic environment, asymptomatic parasitaemia was uncommon and frequently followed by symptomatic malaria. This suggests that presumptive treatment of asymptomatic parasitaemia in such settings would be an efficient means of preventing symptomatic malaria.

keywords asymptomatic parasitaemia, Uganda, symptomatic malaria, cohort

Introduction

Asymptomatic parasitaemia, the presence of malaria parasites in the blood in the absence of symptoms, is prevalent in highly endemic areas of Africa, reaching over 90% in children (Bottius *et al.* 1996), with only a small percentage of individuals ever exhibiting clinical symptoms. The clinical consequences of asymptomatic malaria may vary across different epidemiological settings and are not fully understood. It is generally assumed that in endemic areas asymptomatic parasitaemia is involved in the development of partial immunity (Staalsoe & Hviid 1998) and may protect against clinical disease from new infections (Farnert *et al.* 1999). On the other hand, asymptomatic parasitaemia provides a reservoir for transmission and may be a precursor in the progression to symptomatic disease.

Prior studies of asymptomatic parasitaemia have largely been limited by cross-sectional design or longitudinal follow-up in a small number of selected patients. Parasitaemia at a single point in time may represent persistent parasitaemia after an episode of clinical malaria or the early stages on the pathway to symptomatic disease. Asymptomatic *Plasmodium falciparum* infections of varying duration and resolving without therapy have also been reported (Missinou *et al.* 2003). Clearly, longitudinally designed studies with close follow-up would best ascertain the true significance of a single episode of asymptomatic parasitaemia.

To better understand the clinical consequences of asymptomatic parasitaemia, we prospectively followed a cohort of healthy children living in Kampala, Uganda, for 1 year. They were tested for asymptomatic parasitaemia at regular intervals, and all episodes of

symptomatic malaria were identified using a standardized approach to diagnosis and treatment. Our aims were to ascertain the period prevalence of asymptomatic parasitaemia, assess its association with symptomatic malaria, and identify independent predictors of asymptomatic parasitaemia.

Materials and methods

Study site

The study took place in the Kawempe Division of Kampala, Uganda. Kawempe is largely an urban slum with high population density, unemployment, and rural-to-urban migration. Malaria is meso-endemic, occurring perennially with peaks during the two rainy seasons. *Plasmodium falciparum* is responsible for 92% of malaria infections, with *Anopheles gambiae* and *A. funestus* acting as the two main vectors (Ugandan Ministry of Health, unpublished data). Ethical approval was obtained from the Makerere University Clinical Epidemiology Unit, the Faculty Research and Ethics Committee, the Uganda National Council of Science and Technology and the Makerere University and University of California San Francisco Institutional Review Boards.

Study population and follow-up

We used records from a longitudinal randomized trial of antimalarial therapy (Dorsey *et al.* 2002). Briefly, the primary study took place between July 2000 and August 2001 at the outpatient department of Mulago Hospital in Kampala, Uganda. We recruited 316 healthy children from the community using convenience sampling if they fulfilled the following criteria: age 6–59 months; no history of treatment for malaria in the previous 2 weeks or fever in the previous 48 h; no history of adverse reactions to any of the study medications; no history of sickle cell disease; haemoglobin ≥ 5 g/dl; willingness to remain in the city of Kampala and follow the study protocol for the next 12 months; willingness of parent or guardian to provide informed consent. Upon enrolment, participants were randomized to one of three treatment regimens for all future episodes of uncomplicated malaria and followed for 1 year at our clinic, which remained open every day. Parents/guardians were instructed to bring their children to the clinic anytime they required medical attention and to avoid using any medications not administered or approved by a study physician. To control for differences in calendar time, only participants who completed the full 1-year of follow-up were included.

Assessment of asymptomatic parasitaemia

At enrolment all children were tested for asymptomatic parasitaemia. During follow-up, if more than approximately 30 days elapsed without a child being seen in the clinic, he/she was visited at home for a routine assessment. If children were symptomatic (history of fever in the past 48 h) they were taken to the clinic for a full evaluation. If children were asymptomatic, a routine blood smear was performed for the assessment of parasitaemia. If a child was seen at the clinic for a non-febrile illness and a routine smear had not been done in the previous 30 days, he/she was tested for asymptomatic parasitaemia at the clinic. Children with asymptomatic parasitaemia were not given antimalarial therapy.

Assessment of symptomatic malaria

Each time a child presented to the clinic with a new history of fever (previous 48 h) or temperature ≥ 38 °C (tympanic), a thick blood smear was performed. Patients were diagnosed with malaria if they fulfilled any of the following criteria: complicated malaria [defined as the presence of severe malaria (Warrell *et al.* 1990) or danger signs (WHO 1996)] and any parasitaemia; temperature ≥ 38 °C (tympanic) and any parasitaemia; history of fever (not documented) and ≥ 500 asexual parasites/ μ l blood. Patients with symptomatic malaria were treated with their assigned treatment regimen and followed for 14 days using a standardized WHO protocol (Dorsey *et al.* 2002). All patients classified as clinical failures were treated with standard doses of quinine. Any case of malaria diagnosed more than 14 days after a previous episode was considered a new event (for treatment purposes).

Laboratory studies

Thick and thin blood smears were stained with 2% Giemsa for 30 min. An experienced microscopist, blinded to the patient's clinical status, read all smears. Parasite densities were determined from thick blood smears by counting the number of asexual parasites per 200 WBCs and calculating parasites/ μ l assuming a WBC count of 8000/ μ l. A smear was considered negative if no parasites were seen after review of 100 high-powered fields. Thin smears were used to determine the parasite species. A second experienced microscopist, blinded to the first reading, read all thick smears and any discrepancies (positive *vs.* negative; results that would change outcome classification; $>25\%$ difference in parasite density) were resolved by a third microscopist. Haemoglobin measurements were performed in the study clinic using a portable spectrophotometer (HemoCue, Anglholm,

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Sweden). For all recurrent episodes of symptomatic malaria, molecular genotyping was done to distinguish recrudescence (true treatment failure) from new infections as previously described (Cattamanchi *et al.* 2003).

Statistical analysis

Data was double entered and validated using Epi-Info version 6.04 software (Centers for Disease Control and Prevention, Atlanta, GA, USA). Statistical analysis was performed using SPSS version 10.0 (SPSS, Inc., Chicago, IL, USA) and STATA version 7 (STATA Corporation, College Station, TX, USA) statistical software programs. We compared the prevalence of asymptomatic parasitaemia at enrolment with the prevalence during follow-up, controlling for repeated measurements, in the same study subjects using generalized estimating equations with exchangeable correlation and robust standard errors (Liang & Zeger 1986). The risk of developing symptomatic malaria after a routine smear was estimated using the Kaplan–Meier product limit formula. Time at risk was limited to the first 30 days after a routine smear, as this was the designated interval period for assessment of routine smears. Data was censored for subjects who had another routine smear or the study ended prior to the full 30-day interval period. Hypothesis testing for associations between the results of individual routine smears and the odds of developing symptomatic malaria within 30 days used generalized estimating equations as described above. Parasite densities were normalized using log-transformation when including this as an explanatory variable in our model. Independent predictors of asymptomatic parasitaemia and the level of the individual study subject were identified using multivariate logistic regression. A *P*-value of <0.05 was considered statistically significant in all analyses.

Results**Characteristics of the study subjects**

Of 316 children initially enrolled, 283 (90%) completed the full follow-up and were included in this study. Pertinent characteristics of the study subjects are presented in Table 1. Over the 1-year follow-up period 108 subjects (38%) had no episodes of symptomatic malaria diagnosed, and 598 first-line treatments were given to the remaining 175 subjects (median = 2, range: 1–11).

Prevalence of asymptomatic parasitaemia

We collected 2557 routine smears from 283 children during the follow-up period (mean = 8.5, range: 1–14).

Table 1 Characteristics of study subjects (*N* = 283)

Mean age at enrolment, years (SD)	2.6 (1.3)
Mean haemoglobin at enrolment, g/dl (SD)	11.1 (1.8)
Gender, % female	53
Use of preventative measures*, <i>n</i> (%)	82 (29)
Distance of residence from potential mosquito-breeding site, <i>n</i> (%)	
Living within a swamp	156 (55)
Residence 1–100 m from a swamp	42 (15)
Residence >100 m from a swamp	85 (30)
Number of treatments for malaria†, <i>n</i> (%)	
Subjects with no symptomatic malaria	108 (38)
Subjects with at least one episode of malaria, median (range)	175 (62), 2 (1–11)

* Bednets or chemoprophylaxis.

† Not including second-line therapy for treatment failures.

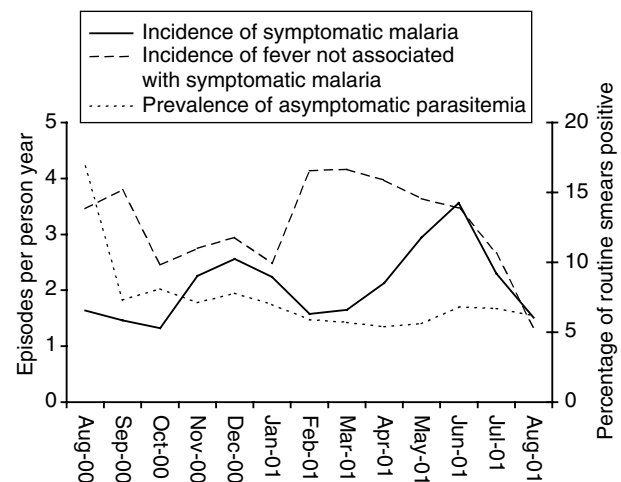


Figure 1 Monthly period prevalence of asymptomatic parasitaemia (number of positive routine smears/total number of routine smears performed) and monthly incidence of symptomatic malaria (number of new episodes of symptomatic malaria diagnosed/time at risk) during the enrolment and 1-year follow-up period.

Overall 205 (8%) routine smears were positive for asymptomatic parasitaemia. The range of parasite densities was 16–592 800 parasites/μl with a geometric mean parasite density of 2987 parasites/μl. The monthly prevalence of asymptomatic parasitaemia ranged from 5.4% to 16.9% (Figure 1). The prevalence of asymptomatic parasitaemia at the time of enrolment was significantly higher than during the follow-up period (16.3% *vs.* 7.0%, OR = 2.0, 95% CI: 1.6–2.6, *P* < 0.001). The incidence of symptomatic malaria peaked in correlation with the two rainy seasons (Figure 1). The incidence of fever not associated with symptomatic malaria peaked in correlation with the two dry seasons (Figure 1). In contrast, the

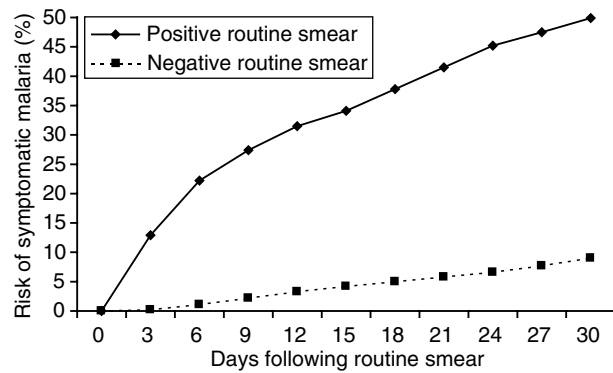


Figure 2 Comparing the 30-day cumulative risk of developing symptomatic malaria for those with positive *vs.* negative routine smears. Cumulative risk of symptomatic malaria estimated as 1 – survival probability using the Kaplan–Meier product limit formula.

prevalence of asymptomatic parasitaemia was relatively constant and low during the entire 1-year follow-up period (5.4–8.1%).

The risk of developing symptomatic malaria

The risk of developing symptomatic malaria within 30 days was significantly higher in those with a positive routine smear than in those with a negative one (50% *vs.* 9%) (Figure 2). For the purpose of hypothesis testing, the odds of developing symptomatic malaria within 30 days were significantly higher for children with asymptomatic parasitaemia than those with negative routine smears (OR = 4.7, 95% CI: 3.7–6.0, $P < 0.001$), controlling for repeated measures in the same patient. In a sensitivity analysis, the association between asymptomatic parasitaemia and the development of symptomatic malaria within 30 days was consistent when the case definition for

symptomatic malaria was restricted to patients with a parasite density ≥ 2000 asexual parasites/ μl (OR = 4.6, 95% CI: 3.6–5.9, $P < 0.001$) or ≥ 5000 asexual parasites/ μl (OR = 4.5, 95% CI: 3.5–5.8, $P < 0.001$). Among those subjects with asymptomatic parasitaemia, higher parasite densities were associated with increased odds of developing symptomatic malaria within 30 days (OR = 1.09 for each log increase in parasite density, 95% CI: 1.03–1.16, $P = 0.003$).

Outcomes of episodes of asymptomatic parasitaemia

The outcomes for each positive routine smear either alone or in series are presented in Table 2. Asymptomatic parasitaemia directly followed by symptomatic malaria occurred in 74% of cases. In 84% of these episodes, symptomatic malaria was diagnosed before the next routine smear. About 92% of cases of symptomatic malaria directly following the diagnosis of asymptomatic parasitaemia were due to new infections, with the remainder due to recrudescence following a previous treatment failure. Asymptomatic parasitaemia directly followed by a negative smear occurred in 26% of cases. About 74% of these episodes were single positive smears followed by a negative smear. Excluding episodes of asymptomatic parasitaemia diagnosed at enrolment or immediately following symptomatic malaria, only 17 (11%) episodes of asymptomatic parasitaemia arose spontaneously and cleared without documented treatment.

Episodes of asymptomatic parasitaemia were further categorized at the level of the individual study subject (Table 3). All routine smears were negative for the duration of the follow-up period in 188 study subjects (66%). About half (53%) these patients without asymptomatic parasitaemia were diagnosed with at least one episode of symptomatic malaria. In 63 study subjects (23%), all episodes of spontaneously occurring asymptomatic parasitaemia were followed directly by symptomatic

Table 2 Outcomes for all episodes of asymptomatic parasitaemia ($N = 185$)*

Category	n (%)
Single positive smear followed by symptomatic malaria	91 (63)
Two consecutive positive smears followed by symptomatic malaria	13 (9)
Three consecutive positive smears followed by symptomatic malaria	3 (2)
Four consecutive positive smears followed by symptomatic malaria	1 (1)
Single positive smear followed by a negative smear	27 (19)
Excluding those done at enrolment or preceded by episode of malaria	15 (10)
Two or more consecutive positive smears followed by a negative smear	10 (7)
Excluding those done at enrolment or preceded by episode of malaria	2 (1)

* Excluding 20 episodes of asymptomatic parasitaemia not followed by any additional blood smears (end of the study period).

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Individual study subject category according to the results of all routine smears assessed during the 1-year follow-up period*	<i>n</i> (%)
All smears negative	188 (66)
Negative smear → single positive smear → symptomatic malaria or the end of the study	50 (18)
Negative smear → two or more consecutive positive smears → symptomatic malaria or the end of the study	13 (5)
Symptomatic malaria or start of the study → single or consecutive positive smear(s) → negative smear	15 (5)
Negative smear → single or consecutive positive smear(s) → negative smear	17 (6)

* Participants classified according to fulfilment of highest order category.

malaria or the end of the follow-up period. The longest period of asymptomatic parasitaemia directly followed by symptomatic malaria was 107 days covering four consecutive positive routine smears. Asymptomatic parasitaemia followed by a negative smear in the absence of documented treatment occurred in 32 study subjects (11%). Asymptomatic parasitaemia directly preceded and followed by negative smears in the absence of therapy occurred in only 17 patients (6%). The longest period of asymptomatic parasitaemia followed by a negative smear in the absence of treatment was 125 days covering five consecutive positive routine smears. One study subject had seven consecutive positive routine smears covering 210 days followed by the end of the follow-up period.

Risk factors associated with asymptomatic parasitaemia

We explored independent associations between various host characteristics and asymptomatic parasitaemia. Study subjects were dichotomized into those with and without at least one episode of asymptomatic malaria followed by a negative smear in the absence of therapy. Potential explanatory variables included gender, age, primary water source, whether a subject had any episodes of symptomatic malaria, use of preventive measures, distance of residence from potential mosquito breeding site, and assigned treatment group. The only statistically significant risk factor for any episode of asymptomatic parasitaemia that resolved without therapy was whether the subject had any episode of symptomatic malaria during follow-up (OR 3.0, 95% CI: 1.2–7.5, $P = 0.02$). When symptomatic malaria was removed from the model, none of the remaining explanatory variables were significantly associated with asymptomatic malaria.

Discussion

Asymptomatic parasitaemia was relatively uncommon in our cohort of children from a meso-endemic, urban area of

Africa. Children with asymptomatic parasitaemia had a much higher risk of developing symptomatic malaria within 30 days than those with no parasitaemia. Indeed, most cases of asymptomatic parasitaemia were followed by episodes of symptomatic malaria and spontaneous clearance of asymptomatic parasitaemia without treatment was rare. The only identifiable risk factor for having an episode of asymptomatic parasitaemia resolving without therapy was having an episode of symptomatic malaria at any other time during follow-up, highlighting our inability to separate these two entities in our study population.

The relatively low prevalence of asymptomatic parasitaemia in our population of children aged 6 months to 5 years is consistent with the classification of Kampala as a meso-endemic area. The prevalence of asymptomatic parasitaemia was highest at enrolment and decreased significantly during the study period. This difference was likely due to improved case management, i.e. availability of prompt and effective therapy throughout the course of the study. The prevalence and age distribution of asymptomatic parasitaemia may vary widely and has been associated with transmission intensity and the development of partial immunity. It has been proposed that the development of partial immunity occurs in two stages: first, anti-disease immunity develops protecting against symptomatic malaria, followed by anti-parasite immunity responsible for reduction of parasite density (Rogier & Trape 1993). While the risk of infection increases with transmission intensity, the risk of symptomatic disease per infection is inversely related to the intensity of transmission and age-dependent acquisition of partial immunity (Rogier *et al.* 1999).

Studies of asymptomatic parasitaemia have often been limited by a single measurement and the use of a cross-sectional study design to assess correlation with other variables. However, the true impact of asymptomatic parasitaemia can only be assessed through longitudinal follow-up. Asymptomatic parasitaemia may represent only one step in a heterogeneous set of disease pathways

Table 3 Asymptomatic parasitaemia at the level of the study subject ($n = 283$)

including the slow or incomplete clearance of parasites after ineffective therapy; the early stages in the progression to symptomatic disease; asymptomatic parasitaemia followed by symptomatic disease due to infection with a new parasite strain(s) and asymptomatic parasitaemia followed by eventual clearance in the absence of treatment. For each of these entities the duration of asymptomatic parasitaemia may vary from a few days to many months. We attempted to assess the distribution of these entities using a population-based cohort of young children. Durations of asymptomatic parasitaemia and outcomes varied, but most episodes were directly followed by the development of symptomatic malaria. Only 11% of episodes of asymptomatic parasitaemia, involving 6% of subjects, arose and cleared without therapy. These findings are similar to a longitudinal study of 200 children followed for 1 year from a hyperendemic area from Gabon, where only four cases of asymptomatic parasitaemia cleared without treatment and asymptomatic parasitaemia of more than 30 days duration occurred in only 5% of patients (Missinou *et al.* 2003).

A significant limitation of our study was the lack of genotyping to distinguish whether the progression from asymptomatic parasitaemia to symptomatic malaria was due to the same parasites or newly acquired infections. However, we found a strong temporal correlation between asymptomatic parasitaemia and symptomatic malaria and there was also an association between asymptomatic parasitaemia of higher parasite density and a higher risk of symptomatic malaria. We recognized the potential explanatory role of environmental risk factors and included the distance of residence from potential mosquito breeding sites in our analytical model when analysing for the risk of having any episode of asymptomatic parasitaemia. The only statistically significant risk factor associated with an episode of asymptomatic parasitaemia was whether the subject had any episode of symptomatic malaria. When symptomatic malaria was removed from the model, none of the remaining explanatory variables (including environmental) were significantly associated with asymptomatic malaria. This evidence strongly suggests that the progression from asymptomatic parasitaemia to symptomatic malaria was more likely due to the same parasites and not newly acquired infections.

In a small study of 10 children from Gabon, the transition phase from asymptomatic parasitaemia to symptomatic malaria was always associated with the appearance of new genotypes (Kun *et al.* 2002). Genotyping was used to study chronic asymptomatic parasitaemia from 26 patients followed for 15 months in a highly seasonal transmission area of Eastern Sudan (Babiker 1998). Some infections were characterized by

multiple strains with turnover in composition, while in others a single strain persisted for months. Progression to symptomatic malaria was associated with both persistent strains and the appearance of new strains. In our study, evidence suggesting that the progression from asymptomatic parasitaemia to symptomatic malaria was more likely due to the same parasites and not newly acquired infections includes the strong temporal correlation between asymptomatic parasitaemia and symptomatic malaria and the association between asymptomatic parasitaemia of higher parasite density and a higher risk of symptomatic malaria.

Assessments for asymptomatic malaria were made at 1-month intervals and did not use polymerase chain reaction (PCR). Thus we could have missed parasitaemia of short duration or at subpatent levels. Multiple studies in Africa have shown a higher prevalence of asymptomatic parasitaemia using PCR compared with microscopy (Bot-tius *et al.* 1996; Owusu-Agyei *et al.* 2002) and rapid turnover of genetically complex infections (Daubersies *et al.* 1996). However, our primary aim was to investigate the clinical consequences of asymptomatic parasitaemia using routine blood smears and to ask how this finding may affect patient management in our study population. Most remarkable was the fact that 50% of children with asymptomatic parasitaemia required treatment for symptomatic malaria within 30 days compared with only 9% of those with negative routine blood smears. This finding suggests that presumptive therapy of asymptomatic parasitaemia in our study population would be an efficient means of preventing symptomatic disease. Recently it has been shown that wide-scale intermittent presumptive therapy for malaria in young children can significantly reduce malaria-associated morbidity (Desai *et al.* 2003; Massaga *et al.* 2003). These studies were conducted in high-transmission intensity settings where the prevalence of asymptomatic parasitaemia is high. In lower-transmission settings a potentially more cost-effective approach, one that would also reduce drug pressure and possibly the selection of resistant parasites, would be to only provide presumptive therapy to those children with a positive routine blood smear.

In summary, asymptomatic parasitaemia can lead to a wide range of clinical outcomes that can best be studied using a longitudinal design. In our study population from a meso-endemic area of Africa, asymptomatic parasitaemia was relatively uncommon and strongly associated with the progression to symptomatic malaria. Further studies in varying epidemiological settings are needed to improve our understanding of the relationship between infection, symptomatic disease, and immunity in order to help plan strategies for intervention.

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