Risk of *Plasmodium falciparum* infection during a malaria epidemic in highland Kenya, 1997

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Abstract

Malaria epidemics in highland areas of East Africa have occurred with increasing frequency since the late 1980s, but the actual risk of *Plasmodium falciparum* infection in children and adults during these epidemics has not been well characterized. During a malaria epidemic in a highland area of Kenya, risk of infection was assessed in 50 adults ($\geq$18 years old) and 32 children ($\leq$8 years old) after treatment and parasitologic clearance with sulfadoxine-pyrimethamine treatment. Over a 10-week period, 36 of the 82 study participants (43.9%) became infected. The risk of infection was similar in children and adults (hazard ratio for children = 1.21, 95% CI: 0.63–2.33). These findings contrast with the age-related protection from infection reported in areas of stable, intense transmission, and demonstrate that during malaria epidemics, both children and adults in highland areas of Kenya are at high risk of infection.
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1. Introduction

Malaria epidemics in highland areas of East Africa have resulted in significant morbidity and mortality in the past decade (Malakooti et al., 1998; Shanks et al., 2000; Some, 1994). Highland areas of East Africa have been characterized as having unstable transmission, with similar rates of infection and disease in children and adults (Lindsay and Martens, 1998). However, rates of infection and time to infection in children and adults during epidemics have not been described to date.

In 1997, we performed a treatment-reinfection study in the highland sub-location of Kabobo, Uasin Gishu District, Kenya, as part of an investigation of immune responses to *Plasmodium falciparum* antigens in this area. Anti-malarial treatment was given to 50 adults and 32 children at the end of a long dry season and just prior to a subsequent epidemic. Weekly blood smear testing for *P. falciparum* infection over a 10-week pe-

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riod allowed us to determine prospectively the risk of infection adults and children in this highland area during a malaria epidemic.

2. Materials and methods

2.1. Study site and human participants

Volunteers were recruited from the sub-location of Kabobo in Uasin Gishu district of Kenya. Kabobo is located at an altitude of 2134 m in an isolated rural area where access to health facilities is limited. Most land in this area is used for maize farming and cattle grazing. Transmission of *P. falciparum* is episodic, and local outbreaks of malaria with high morbidity and mortality have occurred in the past (Khan et al., 1992). Both *P. falciparum* and *P. malariae* infection have been documented in Uasin Gishu (Some, 1994).

To minimize the confounding effects of travel and acquisition of infection in nearby lowland areas where malaria is holoendemic, only volunteers who lived year-round in Kabobo were recruited. Clinic records from Kabobo Health Centre of all cases of malaria from 1991 to 2001 were also reviewed. Cases of malaria at this health centre were diagnosed clinically as individuals with symptoms of malaria who were treated with anti-malarial medications, but were not confirmed microscopically.

2.2. Treatment reinfection study

Eighty-four individuals were recruited from 14 villages in the sub-location of Kabobo for antibody testing and a treatment-reinfection study. Full details of this study cohort have been published previously (John et al., 2003). For the treatment-reinfection study, adults were defined as persons ≥18 years old and children as ≤8 years old, and only individuals in these age groups were recruited for this study. Children ranged in age from 6 months to 8 years (two children aged 6 months to 1 year, eight children aged 2–3 years, eight children aged 4–5 years, 14 children aged 6–7 years and two children aged 8 years). Adults ranged in age from 18 to 80 years. All participants were given a single dose of sulfadoxine-pyrimethamine in May 1997 to clear blood-stage infection regardless of whether *P. falciparum* was seen on peripheral blood smear. By 2

weeks after treatment, blood-stage malaria was successfully eliminated in all but two individuals, both children, who were treated with quinine and doxycycline and excluded from follow-up. Thick and thin blood smears were obtained weekly for 10 consecutive weeks in the remaining 82 individuals (32 children, 50 adults). Individuals who missed more than 2 weeks of blood smear testing were included in follow-up to the time of their last blood smear. Blood smears were stained and examined for *Plasmodium* species by trained microscopists from the Division of Vector Borne Diseases, Ministry of Health, Kenya. Each slide was read by two microscopists. Any discrepancies in readings were resolved by a reading from a third microscopist. A smear was deemed negative when no parasites were observed after counting microscopic fields that included at least 200 leukocytes. The density of parasitemia was expressed as the number of asexual *P. falciparum* per μl blood assuming a leukocyte count of 8000 per μl. Malaria species identification was made by examination of thin blood smears, except in a few cases of very low density, where parasites were detected only on thick smear. Presence of *P. falciparum* gametocytes was also assessed on blood smear and gametocyte density calculated as for asexual blood-stage parasites.

Any individual whose blood smear was positive for *P. falciparum* in follow-up was treated with a single dose of sulfadoxine-pyrimethamine. Presence or absence of symptoms and fever (axillary temperature >37.5 °C) were recorded when blood smears were obtained. Time to reinfection was compared to age (adult versus child) and the presence of parasitemia prior to clearance with sulfadoxine-pyrimethamine treatment.

2.3. Informed consent and ethical approval

Written informed consent was obtained from all participants and/or their guardians. Ethical approval for the study was granted by the Kenya Medical Research Institute National Ethical Review Committee and the Institutional Review Board for Human Studies at University Hospitals of Cleveland and Case Western Reserve University.

2.4. Statistical analysis

The presence of an epidemic was defined by the three epidemic detection techniques recommended by

the WHO and modified by Hay et al. (2002b). The WHO quartile estimate terms an epidemic month any month in which the number of cases is above the third quartile of cases for that month, using the previous 5 years of data (Najera et al., 1998). The modified c-sum (cumulative-sum) method defines an epidemic month as a month when the number of cases is above the 95% confidence interval (upper bound) of the number of cases in the same, prior and following months, for the previous 5 years (Centers for Disease Control and Prevention, 1989). The Cullen method considers an epidemic present when the number of cases seen in a month is above the mean + 2 standard deviations of cases seen in the same month over the 5 previous years (Cullen et al., 1984). In the Cullen and c-sum estimates, prior epidemic years are excluded from the calculation.

Children were compared with adults for time to appearance of parasitemia by Kaplan–Meier survival analysis and for relative risk of infection with adjustment for presence of prior parasitemia by Cox proportional hazards analysis. The study had 70% power to detect a 50% difference in infection between children and adults, assuming a cumulative incidence of malaria of 60% in children. Statistical analysis was done with Stata 8.0 (Stata Corporation, College Station, TX).

3. Results

3.1. Clinical malaria in Kabobo, 1992–1998

Kabobo is an area of highly seasonal transmission, as shown in the graph of clinical malaria cases from 1992 to 1998 (Fig. 1). A nursing strike in December 1992 led to the anomaly of no cases reported in that month. In years 1996 and 1997, the increase in sea-
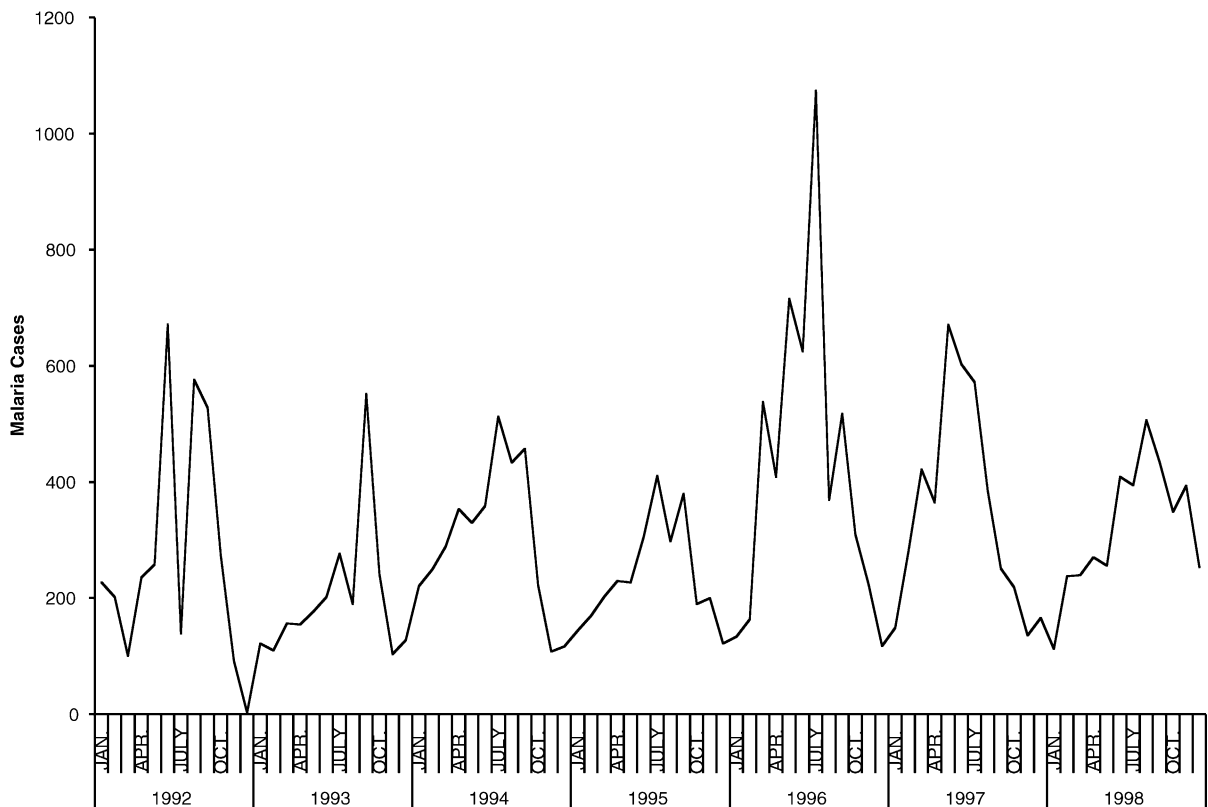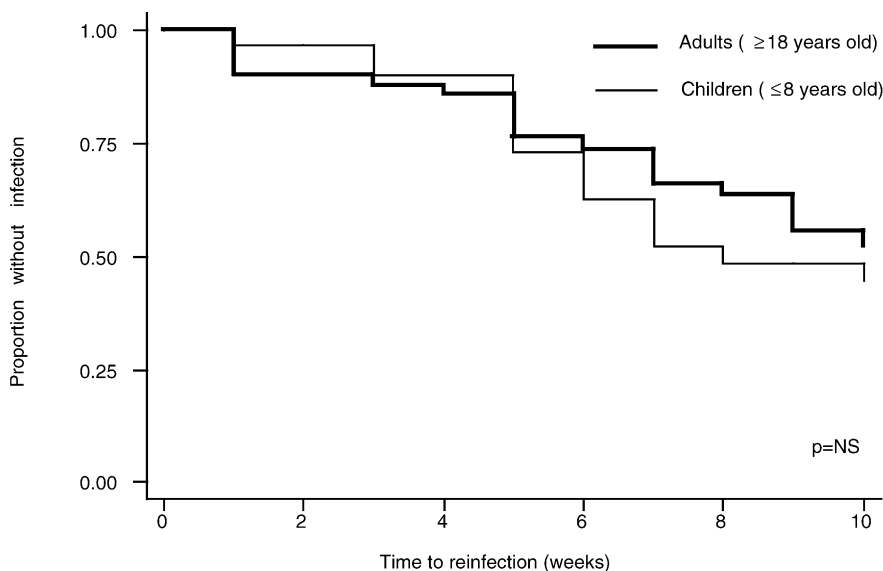

Fig. 1. Cases of clinical malaria, Kabobo sub-location, Uasin Gishu District, Kenya, 1992–1998.

Fig. 2. Time to reinfection in children and adults during a malaria epidemic in Kabobo, Kenya.

sonal cases was high enough to be termed a malaria epidemic by all three of the commonly used methods for defining epidemic thresholds (the WHO quartile estimate, the c-sum estimate with 95% confidence intervals, and the Cullen estimate) (Najera et al., 1998; Centers for Disease Control and Prevention, 1989; Cullen et al., 1984). For example, in May 1997, 671 cases of malaria were seen, greatly exceeding the epidemic threshold for this month as calculated by the WHO, c-sum and Cullen methods (321, 349 and 376 cases, respectively).

3.2. Time to reinfection and risk of infection in children and adults during an epidemic

The treatment-reinfection study was performed during the 1997 epidemic (May–July 1997). Cumulative incidence of *P. falciparum* infection over the 10-week period of study was 43.9%. No *P. vivax*, *P. ovale* or *P. malariae* infections were observed. Median time to reinfection did not differ in children and adults (7 weeks versus 8 weeks, respectively, Fig. 2). Risk of infection also did not differ between children and adults (hazard ratio for infection in children as compared to adults = 1.21, 95% CI: 0.63–2.33). There was no significant difference in risk of infection in individuals with or without *P. falciparum* parasitemia prior to treatment (hazard ratio for those with prior parasitemia = 0.64, 95% CI: 0.27–1.53). Risk of infection remained similar in children and adults when adjusted for prior parasitemia (hazard ratio 1.26, 95% CI: 0.65–2.44). Since protection from infection has been documented to increase with age in adults in malaria holoendemic areas (Bojang et al., 2001; Sokhna et al., 2000), risk of infection was also compared in adults <35 years of age (n = 23) and those ≥35 years of age (n = 27). There was no significant difference in risk for older as compared to younger adults (hazard ratio for individuals ≥35 years versus individuals 18–35 years = 0.75, 95% CI: 0.31–1.80). No interaction was seen between age and *P. falciparum* infection prior to enrollment.

Thirty-three of the 36 individuals infected (91.7%) were asymptomatic at the time infection was diagnosed by microscopy testing. The remaining three individuals had an axillary temperature >37.5 °C but were otherwise asymptomatic. All

smear-positive individuals were treated immediately with sulfadoxine-pyrimethamine, so we were not able to ascertain the percentage of smear-positive individuals who would have developed symptoms of clinical malaria. Parasite densities in all infected individuals were low, ranging from 40 to 600 asexual *P. falciparum* per µl of blood.

4. Discussion

Prior studies of malaria epidemics in highland areas of East Africa have used health center or hospital records of clinical malaria to assess the numbers of adults and children affected by the epidemic (Hay et al., 2002a,b, 2003; Githeko et al., 2000; Lindblade et al., 1999; Shanks et al., 2000, 2002; Some, 1994; Malakooti et al., 1998). These studies have provided much needed information about the epidemiology of malaria in different highland areas of East Africa, but have not documented the actual risk of *P. falciparum* infection in adults and children during epidemics. Microscopy-confirmed diagnosis of malaria was recorded in some studies (Shanks et al., 2000, 2002), but clinical diagnosis of malaria without confirmatory microscopy was relied upon in most others (Hay et al., 2002a,b, 2003; Lindblade et al., 1999; Some, 1994; Githeko et al., 2000). The protean manifestations of clinical malaria and the varying clinical skills of the health care professionals staffing the different health centers and hospitals in these studies likely resulted in varying accuracy of the estimates of malaria cases. Health-care seeking practices, including the relative importance of formal versus informal and retail sector providers, may also affect the accuracy of malaria case estimates (Nuwaha, 2002; Guyatt and Snow, 2004). Infection and disease rates in malaria epidemics in the late-1980s in highland areas of Madagascar were well characterized (Lepers et al., 1988, 1989a,b, 1991), but geographic, vector, climate and social conditions in these areas appear to differ from those in highland areas of East Africa (Albonico et al., 1999; Fontenille et al., 1989, 1990; Fontenille and Campbell, 1992; Lepers et al., 1988, 1990, 1991; Mouchet et al., 1998; Mouchet, 1998; Jambou et al., 2001). The present prospective study of *P. falciparum* infection during an epidemic in the highland area of Kabobo, Kenya, therefore provides for the first time

important new information about rates of infection in a highland area of East Africa during an epidemic. The design of the treatment-infection study, which involved weekly blood-smear testing in a defined study population, allowed calculation of the rate of infection in this area. We documented a frequency of *P. falciparum* infection of 43.9% over a 10-week epidemic period (or an infection rate of 2.28 infections per person year), with similar rates of infection in adults and children.

The frequency of *P. falciparum* infection in adults in the present study at 10 weeks of follow-up ($\sim$40%) approaches that of adults in a treatment-reinfection study in an area of intense, stable transmission at 10 weeks ($\sim$56%) (Sokhna et al., 2000). However, the frequency of *P. falciparum* infection in children ($=$8 years of age) in the present study ($\sim$50%) is significantly lower than that seen in children aged 1–6 years or 7–14 years in the area of stable, intense transmission ($\sim$85–90%). In addition, age-related risk of infection in adults in the present study was different from that seen in adults in an area of the Gambia also characterized as having highly seasonal transmission. In the Gambian study, conducted to assess efficacy of the RTS,S-AS02 vaccine in adults, adults aged >37 years had a 66% decrease in risk of infection as compared to those 18–19 years old, independent of vaccination status (Bojang et al., 2001). In contrast, no significant differences in risk of infection in adults <35 years and $=$35 years of age were seen in the present study, which had 60% power to detect the large decrease in infection seen in the Gambia. The highland area of the present study, an area of highly seasonal transmission, thus differed in age-related infection rates not only from an area of stable, intense transmission but also from another area characterized as having highly seasonal transmission.

Interestingly, most individuals were asymptomatic at the time of initial detection of infection. In the malaria epidemics in highland areas of Madagascar in the 1980s, most individuals became symptomatic with infection, some repeatedly (Lepers et al., 1991). The lack of symptoms in our study participants most likely reflects early detection of infection, since only low-density parasitemia was seen in individuals with positive blood smears, and individuals with positive blood smears were immediately treated. Alternatively, or in addition, a degree of anti-disease immunity may

have been present in this population, particularly in light of the dramatic outbreak in the previous year. It is unclear why the epidemic of 1996 was so much larger than that of 1997. A degree of anti-disease immunity, as discussed above, and the increased availability of anti-malaria medication in the clinic are two possible factors that may have limited the 1997 epidemic.

Study participants were recruited from across 14 different villages in the sub-location of Kabobo, so we have no reason to believe that the study sample reflects a particular bias in collection either toward or away from individuals more likely to develop infection. Similarly, immunoglobulin G antibodies to merozoite surface protein-1, which are short-lived in the absence of recent exposure (Cavanagh et al., 1998), were equally frequent in adults and children, including children under 4 years of age, at the start of the study (unpublished data), suggesting that exposure did not differ significantly in children and adults. Although polymerase chain reaction (PCR) genotyping was not done on parasites to distinguish reinfection from recrudescence, the clearance of parasites after 14 days of treatment and the lack of correlation between infection prior to treatment and reinfection risk increase the likelihood that recrudescent or drug-resistant infections were not the major source of the reinfections detected. The study sample, though not large, had adequate power to detect the large age-related changes seen in prior studies in areas of stable, intense transmission and highly seasonal transmission were present. Thus the study findings appear robust. The study did not have power to detect a difference in risk of infection between adults and children of $<50\%$, so smaller age-associated risk differences in this area cannot be excluded.

Malaria transmission in the highland area of Kabobo appears to be unstable and the area itself epidemic-prone, despite the typical highly seasonal pattern of malaria transmission. Infection rates in this area were high during an epidemic, and rates of malaria infection and disease did not differ significantly by age. The present study highlights the vulnerability of both children and adults in highland areas to *P. falciparum* infection during malaria epidemics and the high rates of infection during these epidemics. There is a clear need for additional research into variations in transmission and epidemiology at both the larger district/province scale and the smaller village/location scale for planning of effective malaria prevention programs in highland areas of East Africa.

Acknowledgements

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