

Effects of trimethoprim-sulfamethoxazole and insecticide-treated bednets on malaria among HIV-infected Ugandan children

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Background: Trimethoprim-sulfamethoxazole (TMP/SMX) prophylaxis and insecticide-treated bednets reduce malaria risk among HIV-infected adults. The efficacy of TMP/SMX may be diminished where antifolate resistance to malaria is high. We evaluated the efficacy of these interventions for malaria prevention among Ugandan children.

Methods: We concurrently followed 300 HIV-infected children aged 1–10 years and a community-based cohort of 561 healthy children aged 1–11 years over 11 months in Kampala, Uganda. The HIV-infected children received TMP/SMX prophylaxis and insecticide treated bednets. In the community cohort, insecticide-treated bednets were introduced during the observation period. Children from both cohorts were followed using a standardized protocol to measure the incidence of malaria.

Results: Only nine episodes of malaria were diagnosed among HIV-infected children (incidence = 0.07/person-year) in comparison with 440 episodes among children from the community (incidence = 0.90/person-year; $P < 0.0001$). The use of insecticide-treated bednets was associated with a 43% reduction in malaria incidence ($P < 0.001$), and a combination of TMP/SMX and use of insecticide-treated bednets with a 97% reduction in malaria incidence ($P < 0.001$). The prevalence of five mutations associated with antifolate resistance was high among malaria cases detected in both the HIV (100%) and community cohorts (75%). Malaria accounted for only 4% of febrile episodes in the HIV cohort in comparison with 33% in the community-based cohort ($P < 0.0001$).

Conclusion: In a malaria endemic area with a high level of molecular markers of antifolate resistance, the combined use of TMP/SMX prophylaxis and insecticide-treated bednets was associated with a dramatic reduction in malaria incidence among HIV-infected children.

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Introduction

Africa has heavy burdens of both HIV and malaria. Important current initiatives are expanding access to optimal regimens for the control of both infections [1,2].

Studies from Africa have shown that HIV infection is associated with an increased risk of malaria among adults [3–5]; however, less is known about interactions between malaria and HIV in children, the population at greatest risk of malaria [6].

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Trimethoprim–sulfamethoxazole (TMP/SMX) prophylaxis is associated with decreased morbidity and mortality [7–10] and is now widely recommended for HIV-infected children and adults in resource-limited settings [11]. Among HIV-infected adults, a benefit of TMP/SMX prophylaxis is a decreased risk of malaria [10,12]. There is, however, limited data available concerning the benefits of TMP/SMX and on the combined effect of this intervention plus insecticide-treated bednets (ITNs) in children, particularly in East Africa, where antifolate resistance in malaria parasites is common [13].

We studied a cohort of HIV-infected children in Kampala, Uganda and a parallel community-based cohort of healthy children to estimate the protective efficacy of TMP/SMX prophylaxis and ITNs on the incidence of malaria in HIV-infected children and to investigate the prevalence of molecular markers associated with antifolate resistance in incident malaria cases.

Methods

Overall design and recruitment of study participants

The present study compared the incidence of malaria between two cohorts of children followed concurrently at separate study clinics at Mulago Hospital, which serves a largely poor urban population in Kampala, Uganda.

From October 2005 to August 2006 we enrolled 300 HIV-infected children from the Mulago Hospital Pediatric Infectious Disease Clinic using convenience sampling. Eligibility criteria were: (1) documented HIV-1 infection; (2) age 1 to 10 years; (3) living within a 20 km radius of the clinic; (4) weight \geq 5 kg; (5) agreement to come to the study clinic for any febrile episode or other illness; (6) agreement to remain in Kampala for the duration of the study; (7) agreement to avoid medications administered outside the study protocol; and (8) willingness of parents or guardians to provide informed consent.

We compared malaria incidence of the HIV-infected cohort simultaneously with a cohort of healthy children who were followed longitudinally in a malaria drug efficacy trial [14]. These healthy children came from a community-based cohort of 601 children recruited from November 2004 to April 2005 [15]. Children were recruited using probability sampling and eligibility criteria that were similar to those for the HIV-infected cohort [14].

Baseline assessment and follow-up of study participants

Children from both cohorts are being followed for 3 years for all of their healthcare needs at clinics that are open 7 days a week, with no cost for care, reimbursement for transport, and after-hours care at Mulago Hospital. The

data for this study come from an 11-month period (October 2005 to August 2006) during which the HIV-infected cohort was being recruited. Children who presented to the study clinics with new medical problems underwent a standardized medical evaluation and treatment. Medications with antimalarial activity were avoided for the treatment of nonmalaria illnesses, including tetracyclines, antifolates (with the exception of TMP/SMX prophylaxis), and macrolide antibiotics, when acceptable alternatives were available. Participants not seen in clinic over any 30-day period were visited at home to document their continued participation in the study. Patients were withdrawn from the studies for: (1) movement out of the study area; (2) inability to locate for any consecutive 60-day period; (3) withdrawal of informed consent; and (4) death.

Trimethoprim–sulfamethoxazole prophylaxis, insecticide-treated bednets use, and antiretroviral therapy

All HIV-infected cohort participants were prescribed TMP/SMX prophylaxis prior to recruitment into this study in accordance with Pediatric Infectious Diseases Clinic guidelines. None of the community-based cohort received TMP/SMX prophylaxis. The policy of the HIV clinic was for all children to use ITNs. We assessed ITN use at home visits during the study, and provided a new net for those for whom a net was not available. We assessed ITN use in the community-based cohort at enrollment; however, no intervention was made at that time. Due to changes in the standard of care, ITNs were provided to all participants in the community-based cohort in May–June 2006. All children in the HIV-infected cohort who met standardized WHO eligibility criteria were provided with antiretroviral therapy.

Diagnosis and treatment of malaria

Subjects who presented with a documented fever (tympanic temperature \geq 38.0°C) or history of fever in the previous 24 h had a fingerprick for thick blood smear. If the smear was positive, the patient was diagnosed with malaria regardless of parasite density. If the smear was negative the patient was not given antimalarial therapy and was managed at the discretion of the study physicians. Patients with uncomplicated malaria received directly observed therapy according to weight-based guidelines. Patients from the community-based cohort were treated with one of three combination antimalarial regimens (amodiaquine plus sulfadoxine–pyrimethamine, amodiaquine plus artesunate, or artemether–lumefantrine). Patients from the HIV-infected cohort were all treated with amodiaquine plus artesunate. Patients from both cohorts with severe malaria or danger signs [16] were treated with quinine in accordance with national guidelines. Patients treated for malaria were asked to return on days 1, 2, 3, 7, 14 and 28 or any other day they felt ill. Follow-up evaluation consisted of a standardized history and physical examination. Blood was obtained by

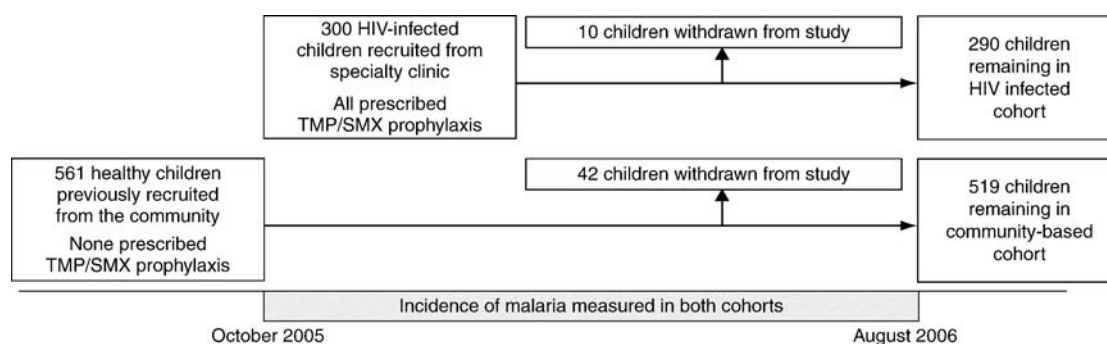


Fig. 1. Study profile.

finger prick for thick blood smears on all follow-up days, except day 1. For data analysis, episodes of malaria that occurred within 14 days of initiation of antimalarial therapy were not considered new episodes.

Laboratory procedures

Blood smears were taken at enrollment to assess the prevalence of asymptomatic parasitemia. Thick smears were stained with 2% Giemsa for 30 min. Parasite density (asexual parasites per μl) was estimated by counting the number of asexual parasites per 200 white blood cells and multiplying this value by the white blood cell count on the day malaria was diagnosed. A smear was considered negative if no parasites were seen in 100 high powered fields. Thin blood smears were done on the day malaria was diagnosed to determine parasite species. Final microscopy results were determined after a second microscopist reading of all smears and resolution of any discrepancies by a third microscopist. Parasite DNA from blood samples collected on filter paper the day malaria was diagnosed was studied for key mutations associated with antifolate resistance (dihydrofolate reductase (dhfr) N51I, C59R, S108N, and I164L, and dihydropteroate synthase (dhps) A437G and K540E). Mutations were identified using nested PCR followed by restriction enzyme digestion, as previously described [17]. CD4+ lymphocyte measurements were done using a FacsCalibur instrument (Becton Dickinson San Jose, California, USA).

Statistical analysis

Data were double-entered in Access (Microsoft Corporation, Redmond, Washington, USA) and statistical analysis was performed using Stata version 8 (Stata, College Station, Texas, USA). Categorical variables were compared using the chi-squared test or Fisher exact test. Continuous variables were compared using the Wilcoxon rank-sum test (age) or the two-sample *t*-test (log-transformed parasite densities and temperature). Comparisons of malaria incidence were expressed as incident rate ratios (IRR) using a negative binomial regression model controlling for age differences between the two cohorts and adjustment for clustering of

participants in the same household. Time at risk only included only that accrued during the observation period, when the cohorts were being followed concurrently (Fig. 1), with censoring for study withdrawals. For participants not using an ITN at the beginning of their observation period, exposure began 28 days after the ITN was given to account for an expected lag in protective effect. Our statistical model for comparison of malaria incidence included number of episodes of malaria as the dependent variable; TMP/SMX prophylaxis, ITN use, and an interaction term for the two interventions combined as independent variables; time at risk; and adjustment for participants living in the same household, specifying that these observations may not have been independent. The significance level was 5%.

Role of the funding source and ethical approval

The study sponsors had no role in study design, data collection, analysis, and interpretation, or writing this report. Ethical approvals were obtained from the Uganda National Council of Science and Technology, the Makerere University Research and Ethics Committee, and the University of California, San Francisco Committee on Human Research.

Results

Participants and baseline characteristics

A total of 561 community cohort patients who were actively followed as of October 2005 were compared with 300 children enrolled in the HIV-infected cohort between that time and August 2006 (Fig. 1). Prior to the observation period, the incidence of malaria in the community-based cohort was identical in the 40 children withdrawn compared with the 561 children not withdrawn from the cohort (1.16 episodes per person-year in each group). In comparison with the community cohort, the HIV-infected children were significantly younger (mean age 5.6 vs. 6.5 years; $P < 0.0001$), more likely to use ITNs (88 vs. 6%; $P < 0.0001$), and less likely to have asymptomatic parasitemia at enrollment (0 vs. 20%;

Table 1. Baseline characteristics of study participants.

	HIV infected (N = 300)	Community-based (N = 561)	P-value
Female	162 (54%)	266 (47%)	0.07
Mean age in years (SD)	5.6 (2.6)	6.5 (2.6)	<0.0001
ITN use	265 (88%)	35 (6%)	<0.0001
Parasite prevalence ^a	0 (0%)	113 (20%)	<0.0001
CD4 percentage distribution			
Median (IQR)	21% (15–28)	N/A	N/A
< 15%	74 (25%)		
15–20%	64 (21%)		
> 20%	162 (54%)		
On antiretroviral therapy	35 (12%)	N/A	N/A

IQR, interquartile range; ITN, insecticide-treated bednets.

^aPositive blood smear at enrollment into cohort.

$P < 0.0001$) (Table 1). The median CD4+ lymphocyte percentage in the HIV-infected cohort was 21% and 12% of these children were taking antiretroviral drugs at enrollment.

Follow-up

Overall 42 (7%) out of 561 children in the healthy community cohort were withdrawn during the observation period due to movement out of Kampala for more than 60 days ($n = 23$), inability to locate the child for more than 60 consecutive days ($n = 9$), withdrawal of consent ($n = 7$), or diagnosis of serious chronic disease ($n = 3$). Ten (3%) of the 300 HIV-infected children were withdrawn during the observation period due to death ($n = 6$), withdrawal of consent ($n = 3$), or movement out of Kampala for more than 60 days ($n = 1$). Cumulative follow-up was 490 person-years for the community-based and 125 person-years for the HIV-infected cohort.

Study clinic visits for new medical problems

A total of 411 visits by children in the HIV-infected cohort and 3452 by children in the community-based cohort were made to the study clinic for new medical problems (Fig. 2). Among these, 228 (55%) by HIV-infected children and 1325 (38%) by children in the community-based cohort were for febrile illnesses (Fig. 2). Remarkably, among evaluations for episodes of new febrile illnesses, only nine (4%) of the HIV-infected children compared with 440 (33%) of the community-cohort children included a positive blood smear leading to diagnosis and treatment for malaria ($P < 0.0001$).

The effect of trimethoprim-sulfamethoxazole prophylaxis and insecticide-treated bednets on malaria incidence

Only nine episodes of malaria were diagnosed in the HIV-infected cohort (0.07 episodes/person-year) in comparison with 440 episodes in the community-based cohort (0.90 episodes/person-year, $P < 0.0001$). In comparison with children not taking TMP/SMX and not using an ITN, the use of ITNs alone was associated with a 43% reduction [IRR = 0.57 (95% CI, 0.46–0.71); $P < 0.001$], and a combination of TMP/SMX and ITN use with a 97% reduction in the incidence of malaria [IRR = 0.03 (95% CI, 0.01–0.10); $P < 0.001$] (Table 2). As almost all HIV-infected children were using both ITN and TMP/SMX, only 7.8 person-years of follow-up for children prescribed TMP/SMX alone were available for analysis. In comparison with no intervention, TMP/SMX alone was associated with a 39% reduction [IRR = 0.61 (95% CI, 0.25–1.51); $P = 0.29$] in the incidence of malaria. Similar results were seen in the 110 HIV-infected children who would not have met current WHO

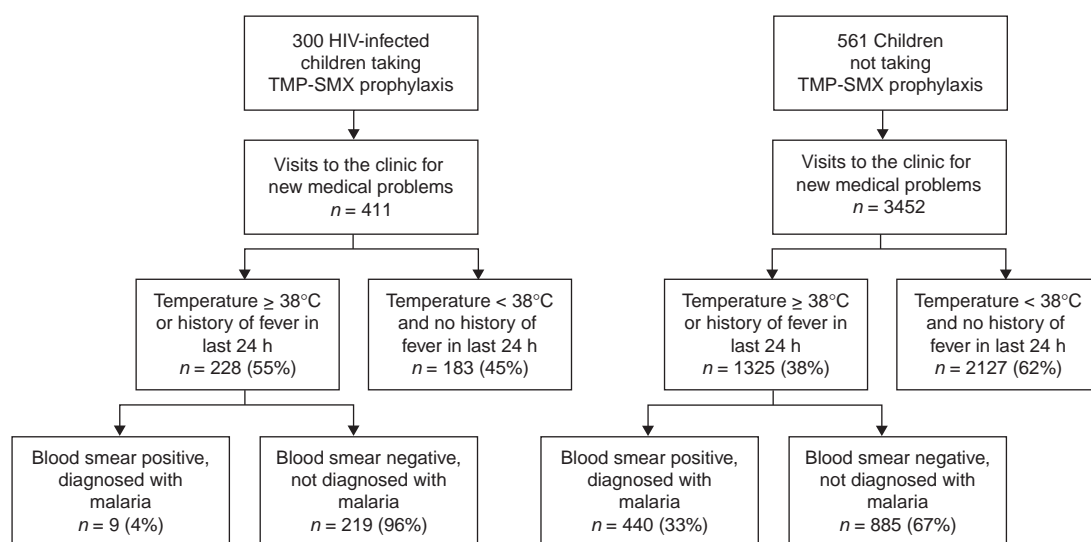


Fig. 2. Profile of visits made to the study clinic for new medical problems and proportion of children diagnosed with malaria in either cohort out of the total number children evaluated for episodes of new febrile illnesses. TMP/SMX, trimethoprim-sulfamethoxazole.

Table 2. Effect of trimethoprim–sulfamethoxazole (TMP/SMX) prophylaxis and insecticide-treated bednet (ITN) use on malaria incidence.

Cohort	Exposure group	Episodes of malaria	Observation time (person-years)	Incidence any malaria per 100 person-years (95% CI)	Incidence first episode of malaria per 100 person-years (95% CI)
Community-based (N = 561)	Not on TMP/SMX prophylaxis, no ITN	356	340.3	104.6 (94.0–116.1)	74.6 (64.7–85.6)
	Not on TMP/SMX prophylaxis, ITN	84	150.0	56.0 (44.7–69.3)	52.6 (40.9–66.1)
HIV infected (N = 300)	On TMP/SMX prophylaxis, no ITN	5	7.8	64.3 (20.8–149.6)	68.2 (22.2–159.3)
	On TMP/SMX prophylaxis, ITN	4	117.2	3.4 (0.9–8.7)	2.6 (0.5–7.6)
Comparative results					
Exposure group ^a			Incident rate ratio (95% CI) ^b	P-value	
TMP/SMX prophylaxis alone			0.61 (0.25–1.51)	0.29	
ITN alone			0.57 (0.46–0.71)	< 0.001	
Both TMP/SMX and ITN			0.03 (0.01–0.10)	< 0.001	

CI, confidence interval.

^aComparison group no TMP/SMX prophylaxis, no ITN.

^bEstimated using negative binomial regression model controlling for age and adjustment for children living in the same household.

recommendations for starting TMP/SMX at enrollment (WHO stage 1 and CD4 cell count $\geq 25\%$ if age 1–4 years or ≥ 350 cells/ μL if age 5 years or older). Considering these children, ITN use alone was associated with a 43% reduction [IRR = 0.57 (95% CI, 0.46–0.71); $P < 0.001$], and a combination of TMP/SMX and ITN use with a 95% reduction in the incidence of malaria [IRR = 0.05 (95% CI, 0.01–0.15); $P < 0.001$] compared with children in the community cohort not using TMP/SMX or an ITN. Of note, prior to distribution of ITNs to the community-based cohort, participants reporting the use of a noninsecticide-treated bednet had a similar incidence of malaria compared with participants who reported no bednet use [IRR = 1.10 (95% CI, 0.83–1.47); $P = 0.51$].

Effect of immune suppression on malaria incidence

Among the HIV-infected cohort, advanced immune suppression, as measured by percentage CD4 cells at enrollment, was associated with an increased incidence of malaria after controlling for ITN use [IRR = 0.66 per 5% increase in percentage CD4 cells, (95%CI, 0.43–1.00); $P = 0.05$]. The incidence of malaria was significantly higher among children with a CD4 percentage $< 15\%$ at enrollment in comparison with those with a higher percentage (0.17 episodes/person year vs. 0.03 episodes/person year; $P = 0.02$).

Characteristics of malaria episodes and molecular markers of antifolate resistance

Patient age at the time of malaria diagnosis, the species of infecting parasite, and temperature at presentation were similar between the two cohorts (Table 3). Parasite density was higher in the community-based cohort than in the HIV-infected cohort ($P = 0.05$). We determined the prevalence of polymorphisms in the genes that encode *dhfr* and *dhps*, the targets of trimethoprim and sulfamethoxazole, respectively, from parasites infecting all nine

patients with malaria from the HIV-infected cohort and 80 patients with malaria randomly selected from 440 cases in the community-based cohort. The prevalences of three *dhfr* and two *dhps* mutations that are commonly associated with sulfadoxine–pyrimethamine (SP)-resistant malaria in East Africa [17] were extremely high in parasites infecting children from both cohorts (Table 3). Parasites from one child with malaria from the HIV-infected cohort, but none of 80 from the community cohort also demonstrated the *dhfr* 164L mutation that is associated with high-level resistance to SP in Asia and South America, but remains rare in Africa [18].

Discussion

Our results show that the incidence of malaria can be markedly decreased in HIV-infected children living in a malaria endemic area through the combined use of TMP/SMX prophylaxis and ITNs. Advanced immune

Table 3. Baseline characteristics of malaria episodes.^a

	HIV-infected (n = 9)	Community-based (n = 440)
Mean age (SD)	6.8 (2.6)	6.8 (2.7)
Infection with <i>P. falciparum</i>	9 (100%)	419 (95%)
Geometric mean parasite density	2769/ μL	11791/ μL
Mean temperature °C (SD)	37.3 (1.0)	37.7 (1.3)
Prevalence of <i>dhfr/dhps</i> mutations ^b		
<i>dhfr</i> 51I	9/9 (100%)	79/80 (99%)
<i>dhfr</i> 59R	9/9 (100%)	65/80 (81%)
<i>dhfr</i> 108N	9/9 (100%)	80/80 (100%)
<i>dhfr</i> 164L	1/9 (11%)	0/80 (0%)
<i>dhps</i> 437G	9/9 (100%)	77/80 (96%)
<i>dhps</i> 540E	9/9 (100%)	76/80 (95%)

^aNone of the comparisons in baseline characteristics of malaria episodes between the two cohorts reached statistical significance (P -value ≥ 0.05).

^bEighty random samples selected from malaria episodes in patients not on trimethoprim–sulfamethoxazole prophylaxis.

suppression was associated with an increased incidence of malaria; however, the protective effect of TMP/SMX plus ITNs was achieved in children at all stages of HIV infection. Protection was seen even though the study was conducted in an area where the prevalence of molecular markers of antifolate resistance among malaria parasites is high. This study provides the first data to quantify the benefit of TMP/SMX and use of ITNs for the prevention of malaria among HIV-infected children, extending recent findings from a study of HIV-infected adults [12]. The impact of the two simple interventions was profound, with combined use of TMP/SMX prophylaxis and ITNs providing a 97% reduction in the incidence of malaria in comparison with that in healthy community-based controls.

The use of ITNs was associated with a 43% reduction in the incidence of malaria. This reduction was consistent with prior studies in which ITNs reduced the incidence of clinical episodes of malaria by about 50% [19,20]. We had limited power to quantify the contribution of TMP/SMX to malaria prevention because the combined use of TMP/SMX and ITNs is now the standard of care in the clinic where our study was conducted. Nevertheless, considering available data, this intervention appears to have played a key role in the reduction of malaria in our population. Similarly, in an observational study of HIV-infected Ugandan adults, TMP/SMX prophylaxis reduced the incidence of malaria by 72% [10].

In Africa, the diagnosis of malaria is often empiric, with fever in the absence of any localizing symptoms attributed to malaria, and treatment administered without smear confirmation. In our study, only 4% of fevers among HIV-infected children receiving TMP/SMX and ITNs was attributable to malaria, compared to 33% among children not receiving TMP/SMX. Thus, among HIV-infected children receiving TMP/SMX and using ITNs, malaria was rarely the cause of fever, and the empiric treatment of all fevers as malaria would result in extensive inappropriate use of expensive and potentially toxic antimalarial therapies and hinder prompt diagnosis of other causes of fever. In this new era of artemisinin-based combination therapy, there has been an increased emphasis on antimalarial treatment based on laboratory confirmation. The results of this study support this policy, especially in relatively low transmission areas.

Of interest, TMP/SMX prophylaxis offered protection against malaria even though the parasites infecting patients in Kampala carried mutations associated with resistance to the related antifolate SP. Five *dhfr* and *dhps* mutations known to be common in Uganda and well correlated with SP treatment failure [13] were present among all isolates from HIV-infected children and notably, in 75% of isolates infecting children in the community cohort. Antifolate resistance among malaria

parasites appears to be increasing in Kampala, as an analysis of drug resistance markers among parasites captured in one of our previous cohort studies of healthy children in 2000–2001 showed that 56% of the parasites had the five key mutations [17]. This increase in the prevalence of molecular markers of antifolate resistance corresponds to the 2001 implementation of a national policy change from chloroquine (CQ) to CQ plus SP as the recommended first-line treatment for malaria in Uganda [21]. Evidence exists for cross-resistance between TMP/SMX and SP [22] but whether the use of TMP/SMX is creating additional selection pressure and higher level antifolate resistance remains an open question. It could be anticipated that the addition the *dhfr* 1164L mutation, which is associated with high-level resistance to SP [23], but remains rare in Africa, might prevent protective efficacy of TMP/SMX. In this regard, it is concerning that one of 9 episodes of malaria in our HIV-infected cohort was caused by a strain with the 164L mutation. Although only a single isolate, this finding might presage a worrisome loss of the antimalarial benefits of TMP/SMX prophylaxis.

Although this study found a strong antimalarial protective effect of TMP/SMX and ITN use, its observational nature merits some caution in interpreting the effect estimates of these interventions. A randomized, controlled trial of TMP/SMX prophylaxis and/or ITN use was deemed to be unethical, however, as these two interventions are now the standard of care for HIV-infected individuals in Uganda. When we examined the characteristics of the two cohorts for evidence of selection bias, little difference between the cohorts was detected, with the HIV-infected cohort slightly younger (which would be expected to produce a conservative bias). Although it is possible that some subjects in the community cohort were HIV-infected resulting in misclassification, the HIV prevalence in this age group in Uganda is estimated to be less than 2%, and therefore such misclassification would tend to minimize the observed difference between the two cohorts (a conservative bias). Some geographic bias in malaria incidence may have influenced the observed differences between the cohorts, with the community cohort subjects residing within a limited region, and the HIV cohort subjects residing in a larger area. The variation in malaria incidence over the larger geographic region is, however, unlikely to be large enough to account for the large differences found in this study. Although malaria treatment regimens differed between the two cohorts, it is unlikely that differences in malaria incidence could be explained by differences in antimalarial treatment efficacy. In the community-based cohort the incidence of malaria in patients randomized to amodiaquine plus artesunate was not significantly different from that in patients randomized to amodiaquine plus SP or artemether-lumefantrine (1.34 episodes per person year compared

with 1.49 episodes per person year; $P=0.50$) [14]. In summary, the necessary methodological limitations of this study may have led to bias in our estimates of the protective effect of TMP/SMX prophylaxis and ITN use. Given the dramatic differences in malaria incidence it is clear, however, that these two interventions were highly effective in preventing malaria when used together.

The results from our study have important policy implications for the treatment of HIV-infected children living in malaria endemic areas. Our data support the use of ITNs and TMP/SMX prophylaxis for all HIV-infected children. This recommendation would broaden current World Health Organization (WHO) guidelines for children aged 1 year or older, which currently recommend TMP/SMX only for children at WHO stage 2 or higher or when the CD4 cell count is less than 25% (age 1–4 years) or 350 cells/ μl (age 5 years or older) [11]. In determining policies for prophylaxis, however, one must also consider other factors such as the prevention of other diseases (e.g. bacterial infections), the potential for adverse events due to TMP/SMX and the selection of drug resistance. Clearly, more research is needed to determine whether continuing TMP/SMX prophylaxis for children living in areas in which malaria is highly endemic may be beneficial even among children who have experienced immune recovery in response to antiretroviral therapy, and whether there are any risks of increased rates of malaria if TMP/SMX is discontinued after an extended period of use.

There are many opportunities for synergism, in particular at a time of growing political and financial commitment to reduce the burden of HIV/AIDS, tuberculosis and malaria. Providing integrated health services in areas heavily affected by malaria and HIV is crucial for reducing the burden of the two diseases. Our study demonstrates the profound benefit that coordinated efforts on HIV and malaria can have in reducing disease burden.

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Conflict of interest: none.

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